COVID-19: NEW VARIANTS AND HOST DEMOGRAPHY

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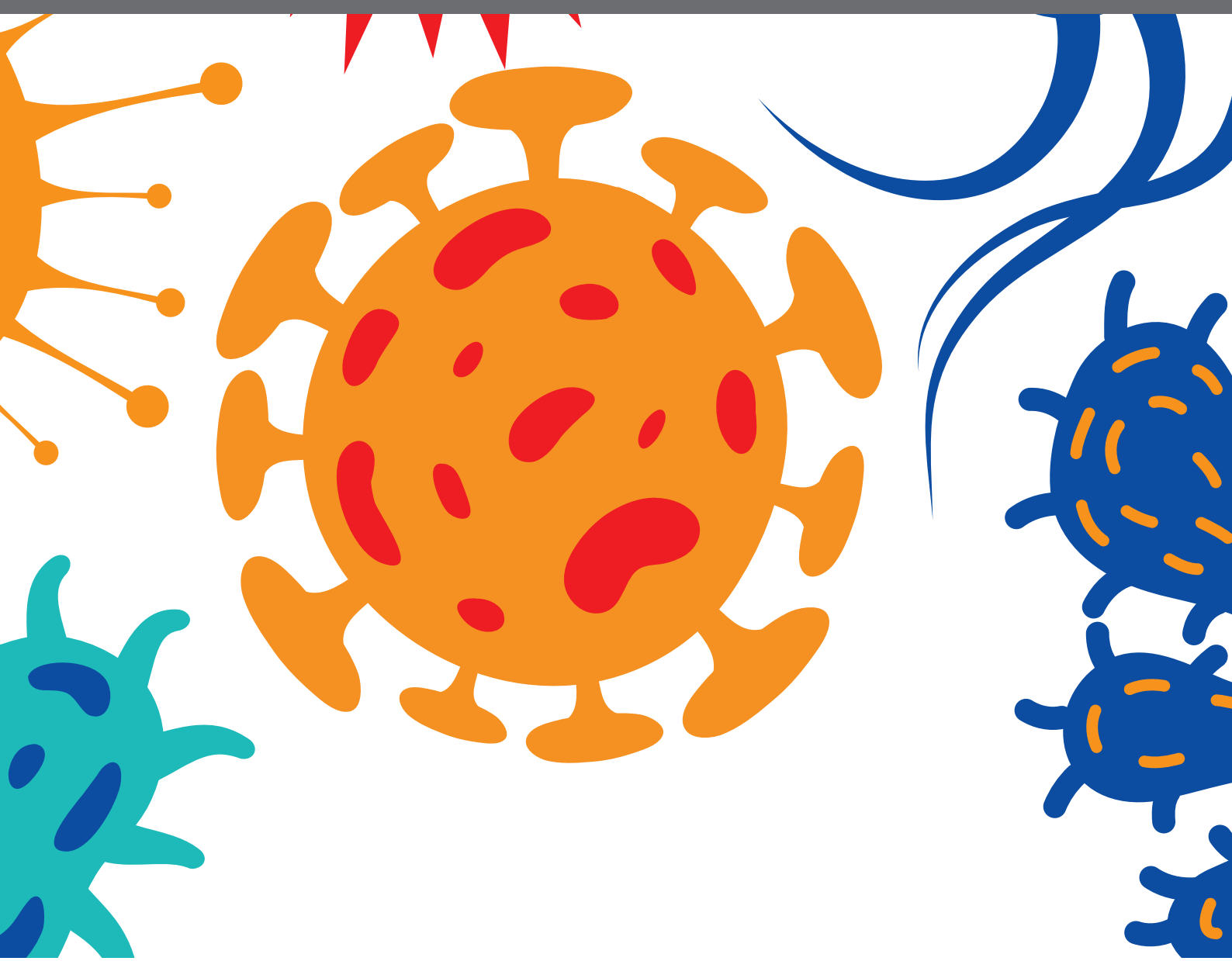

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COVID-19: NEW VARIANTS AND HOST DEMOGRAPHY

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EDITORIAL
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Editorial: COVID 19: New Variants and Host Demography

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Editorial on the Research Topic

COVID-19: New Variants and Host Demography

As the world continues to battle the SARS-Cov-2 virus, the mutants being sequenced suggest a grim chance of avoiding a very possible third wave. The mutation landscape of SARS-CoV-2 has been under constant global scrutiny to understand the effect of these changes on the infectivity and antigenicity of the virus. While most mutations are of little to no consequence, sometimes the virus acquires a mutation that gives it an advantage over other strains. Despite the virus's sluggish mutation rate, researchers have catalogued more than 12,000 mutations in SARS-CoV-2 genomes. Compared with HIV, SARS-CoV-2 changes much more slowly as it spreads. The variants of concern have mostly been identified with a mutation in the gene encoding the spike protein, which helps virus particles to penetrate cells.

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NEW VARIANTS

The D614G mutation took place at the 614th amino-acid position of the spike protein, the amino acid aspartate was regularly being replaced by glycine because of a copying fault that altered a single nucleotide in the virus's 29,903-letter RNA code. All vaccines worked against this which is why the spread and infectivity was controlled. An interesting paper presented by Tuli et al. highlights the molecular evolution of SARS-CoV-2 variants, and effective targeting using vaccines. Mohammad et al. presented an article on the variation of structural proteins, corresponding to new genetic variants, and the adoption of a genomics-based approach to further our understanding of their effects. The 3 new variants that have rapidly become dominant within their respective countries have raised concerns include B.1.1.7 (also known as VOC-202012/01), 501Y.V2 (B.1.351), and P.1 (B.1.1.28.1).All three variants have the N501Y mutation, which changes the amino acid asparagine (N) to tyrosine (Y) at position 501 in the receptor-binding domain of the spike protein.

A new variant has recently been identified in West Bengal, India, popularly being called as 'Triple Mutant Variant' with the E484K, a major immune-escape mutation. The team of Bhat et al. highlighted the importance of mutation surveillance, using biological and computational models. The article by Singh et al. explores the emergence of new variants of SARS-CoV-2, and the effectiveness of vaccines in their management.

TREATMENT OPTIONS FOR THE FUTURE

Variants are going to continue to mutate and at some point its going to happen that more of these will be able to evade the immune system. A few possible ways that are being discussed to mitigate the rate of mutation include a third dose of the same vaccine, a slightly modified booster dose and combinatorial dosing. The article by Rana et al. offers an in-depth insight into the future perspectives and possibilities for newer treatment strategies, moving forward. In addition to this, the article by Kushwaha et al. highlights the identification of natural inhibitors against SARS-CoV-2 and identification of druggable targets, using various approaches. Caldera-Crespo et al. presented an insightful paper on the various experimental models of COVID-19, while Singh et al. provided an enriching look into the future perspectives of COVID-19 management, using antibody-based therapy.

NEW VARIANT DEMOGRAPHY

After spending much of last year affecting elderly patients, healthcare workers are now seeing a demographic shift: young and middle-aged adults are becoming a growing share of the patients in COVID-19 hospital wards. In addition to the mapping of disease progression across different ages, this issue explores the impact of ethnicity on the incidence of the disease. The article by Al Zahmi et al. explains the difference of COVID-19 impact among various ethnicities across the globe. In addition to this, the article by Statsenko et al. highlights the impact of age and sex on the severity of COVID-19, based on evidence obtained from radiologic and clinical data. Nayak et al. aimed to provide an insight into the host response to existing and emerging variants of SARS-CoV-2, in patients with hepatic and gastrointestinal complications. With regards to analysis of the emergence of new variants in the Indian subcontinent, the papers presented by Rana et al. and Kandelwal et al. provided a thorough overview of the severity and extent of spreading of newer variants.

SIDE EFFECTS OF VACCINES

The COVID-19 vaccines can cause mild adverse effects after the first or second dose, including pain, redness or swelling at the site of vaccine shot, fever, fatigue, headache, muscle pain, nausea, vomiting, itching, chills and joint pain, and can also rarely cause anaphylactic shock. The specific cause of the anaphylactic reactions remains unknown. The Moderna and Pfizer–BioNTech vaccines use hollow lipid nanoparticles, linked with PEG, which have been known to cause allergic reactions.

A larger body of studies are now showing an increased incidence of neurological manifestations among patients hospitalized with COVID-19. The complications include: acute encephalopathy, headaches, loss of sense of taste or smell, coma and strokes. An interesting paper submitted by Rustagi et al. maps the effects of COVID-19 vaccination in Asian countries, using machine learning. We hope that you all will enjoy the reading of this thematic issue on "Current Aspects in Chemopreventive Strategies" from Frontiers in Pharmacology.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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Identification of Natural Inhibitors Against SARS-CoV-2 Drugable Targets Using Molecular Docking, Molecular Dynamics Simulation, and MM-PBSA Approach

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The present study explores the SARS-CoV-2 drugable target inhibition efficacy of phytochemicals from Indian medicinal plants using molecular docking, molecular dynamics (MD) simulation, and MM-PBSA analysis. A total of 130 phytochemicals were screened against SARS-CoV-2 Spike (S)-protein, RNA-dependent RNA polymerase (RdRp), and Main protease (M^{pro}). Result of molecular docking showed that Isoquercetin potentially binds with the active site/protein binding site of the Spike, RdRP, and Mpro targets with a docking score of -8.22, -6.86, and -9.73 kcal/mole, respectively. Further, MS 3, 7-Hydroxyaloin B, 10-Hydroxyaloin A, showed -9.57, -7.07, -8.57 kcal/mole docking score against Spike, RdRP, and M^{pro} targets respectively. The MD simulation was performed to study the favorable confirmation and energetically stable complex formation ability of Isoquercetin and 10-Hydroxyaloin A phytochemicals in M^{pro}-unbound/ligand bound/standard inhibitor bound system. The parameters such as RMSD, RMSF, Rg, SASA, Hydrogen-bond formation, energy landscape, principal component analysis showed that the lead phytochemicals form stable and energetically stabilized complex with the target protein. Further, MM-PBSA analysis was performed to compare the Gibbs free energy of the M^{pro}-ligand bound and standard inhibitor bound complexes. The analysis revealed that the His-41, Cys145, Met49, and Leu27 amino acid residues were majorly responsible for the lower free energy of the complex. Drug likeness and physiochemical properties of the test compounds showed satisfactory results. Taken together, the study concludes that that the Isoquercetin and 10-Hydroxyaloin A phytochemical possess significant efficacy to bind SARS-Cov-2 M^{pro} active site. The study necessitates further *in vitro* and *in vivo* experimental validation of these lead phytochemicals to assess their anti-SARS-CoV-2 potential.

Keywords: isoquercetin, 10-hydroxyaloin A, SARS-CoV-2, *in silico*, drugable targets

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INTRODUCTION

SARS-CoV-2 is a single-stranded RNA-enveloped virus whose gene fragments consist of structural and non-structural proteins. Some of the genes (*viz.*, E, M, N, and S) encode structural proteins, whereas some encode important non-structural proteins (*viz.*, papain-like protease- PLpro, 3-chymotrypsin-like/main protease-M^{pro}, Spike-protein, and RNA-dependent RNA polymerase-RdRp) by the ORF region. The Spike-protein possesses two subunits (S1 and S2), which play important roles in the receptor recognition and membrane fusion process. The S1 subunit encompasses a receptor-binding domain (RBD) which interacts with the host receptor protein [such as angiotensin-converting enzyme 2 [ACE2] or TMPRSS2 protein], whereas the S2 subunit negotiates fusion of the virus to the host cellular membrane. These two events are critical to the entry of the viral particle into the host cells. After entry into the host cells, the viral RNA is released into the cell and the polyproteins are processed by the M^{pro} protein. The M^{pro} protease of SARS-CoV-2 is identified as a cysteine protease that possesses a catalytic dyad Cys145-His41 in the active site of the protease, responsible for its activity. M^{pro} plays an important role in the processing of replicase polyprotein and in the maturation of virus. The RNA-dependent RNA polymerase (RdRp) is essentially required for the viral RNA synthesis, which ultimately enhances the viral virulence. Thus, the SARS-CoV-2 Spike-protein, M^{pro}, and RdRp proteins are important drugable target to mitigate the viral entry and virulence of the disease. Several preclinical and clinical studies on the chemically synthesized inhibitors of these drugable targets showed significant toxicity and other adverse effects. For instance, antiviral drugs such as lopinavir and remdesivir exert many side-effects in COIVD-19 patients (Barlow et al., 2020; Cao et al., 2020; Liu et al., 2020). Therefore, there is an urgent need to identify potential natural SARAS-CoV-2 drugable target inhibitors. Plant-based small molecules showed efficacy against different diseases/ailment including antiviral disease in *in silico* and *in vitro* experiments (Gupta et al., 2020; Kushwaha et al., 2020a; Kushwaha et al., 2020b; Kushwaha et al., 2021a). In the present study we selected two Indian medicinal plants (*Azadirachta indica* and *Aloe vera*) based on the rationale reviewed through the literature.

Verma (1974) first time reported antiviral potential (against potato virus X) in *Azadirachta indica* phytochemical (Verma, 1974). After that several antiviral activities have been reported in extract and/or pure isolated compounds from the plant against Coxsackie B group of viruses, Polio virus, Dengue virus type-2, HIV, Herpes simplex virus type 1, etc. Phytochemicals present in *A. indica* has potential to modulate the early event of viral replication cycle (Badam et al., 1999). SaiRam et al. (2000) proposed that neem phytochemicals have the ability to neutralize the viral particle before entering the cells (SaiRam et al., 2000). They also indicate the entry point inhibition potential in neem phytochemical against viral entry into the host cell. Parida et al. (2002) in their study also indicated that the neem phytoconstituents could inhibit the viral entry into the host cells (Parida et al., 2002). In a different study, Udeinya et al. (2004) reported that neem extract ameliorates adverse effects of human immunodeficiency virus (HIV) in human subjects possibly by inhibiting the cellular entry of the virus (Udeinya et al., 2004). In an interesting study, Xu et al. (2012) reported that the phytochemicals present in *A. indica* have the potential to neutralize the viral particle and inhibit the viral entry (Xu et al., 2012). Different studies reported the antiviral efficacy such as disruption of herpes simplex virus type 1 envelope, treatment of genital herpes virus, pigeon paramyxovirus type 1 Replication, anti-influenza activity, porcine epidemic diarrhea anti-viral activity of *Aloe vera* extracts/phytochemicals in various experimental models and clinical study (Sydiskis et al., 1991; Perfect et al., 2005; Dziewulska et al., 2018; Borges-Argáez et al., 2019). Interestingly, *A. vera* is an active ingredient of "Bioaron C" syrup, which is a herbal medicine known to prevent upper respiratory tract infections (URTIs). Besides, the plant has been used as medicine for URTIs for many decades (Glatthaar-Saalmüller et al., 2015). Over all the literature revealed the multimechanism-mediated antiviral potential in *A. Indica* and *A. vera* phytochemicals.

Our research group identified lead *A. Indica* phytochemicals having potential to bind human host protein TMPRSS2 involved in Spike-protein entry into the cell (Senapati et al., 2021). Matveeva et al. (2020) studied selected phytochemicals (n=1,911) from 55 plant species to find anti-SARAS-CoV-2 protein inhibitors. This showed about 36 phytochemicals per plant species. Similarly, few studies reported the main protease binding potential of only *A. Indica* phytochemicals, but they considered less number of phytochemicals (Garg et al., 2020; Adegbola et al., 2021; Umar et al., 2021). Lim et al. (2021) suggested *A. indica* as a potential herbal source of anti-SARS-CoV-2 agent with a multimodal efficacy of antiviral, anti-inflammatory, and immunomodulatory properties. Recently two different randomized controlled trials of *A. indica* extract/capsule on COVID-19 positive patients established the significant anti-SARS-CoV-2 potential in *A. indica* plant (Khan et al., 2020; Nesari et al., 2021). Similarly, few studies reported the *Aloe vera* phytochemicals as SARS-CoV-2 M^{pro} and RdRp binding potential (Mpiana et al., 2020; Abouelela et al., 2021; Balkrishna et al., 2021). These studies either considered fewer compounds or concerned only the *Aloe vera*–specific phytochemicals for the study. In the present study we targeted the three SARS-CoV-2 drugable targets (M^{pro}, Spike-protein, and RdRp) and considered phytochemicals especially reported in *Aloe vera* plant. Keeping all the abovementioned facts, we selected the *A. indica* and *A. vera* phytochemicals (at larger scale) to revisit the identification of potential antiviral inhibitor of plant origin.

MATERIALS AND METHODS

Azadirachta indica and *Aloe vera* (L.) Phytochemical Retrieval and Preparation

Compounds present in *Azadirachta indica* (n=93) and *Aloe vera* (L.) (n=37) were searched from different sources such as Science Direct, PubMed Central Google Scholar, Web of Science,

PubMed, Scopus, Semantic Scholar, Medline, and Google Scholar (Kushwaha et al., 2021b). Marvin Sketch software (https://chemaxon.com/products/marvin) was used to prepare the structures of phytochemicals. Three-dimensional or two-dimensional structures of compounds were retrieved form NCBI PubChem database (Xie, 2010). Two-dimensional structures of compounds were converted into three-dimensional structure by using Open Babel software (O'Boyle et al., 2011). Using PyRx-Python prescription 0.8 for 200 steps, energy minimization of the ligands was performed by using Merck molecular force field (MMFF94) along with conjugate gradient optimization algorithm (Chitrala & Yeguvapalli, 2014).

Protein Retrieval and Preparation

Crystal structures of target proteins, *viz.*, M^{pro} (PDB ID: 5RFS), Spike glycoprotein (PDB ID: 6VSB), RdRp (PDB ID: 6M71), were obtained from Protein Data Bank (https://www.rcsb.org/) with a resolution of 1.70, 3.46, and 2.90 Å, respectively. Three-dimensional structures of the targeted proteins were prepared for molecular docking using UCSF Chimera (Pettersen et al., 2004). All water molecules and ligands present in crystallized structure were removed. Steepest descent protocol with 100 steps and 0.02 step size along with conjugate gradient with 10 steps and step size 0.02 Å was applied for the energy minimization of the obtained protein structures.

Molecular Docking

Auto Dock Tools 1.5.6 (ADT) was used to perform molecular docking of obtained proteins and ligands (Trott and Olson, 2010; Pushpendra et al., 2018). Proteins and ligands were loaded in ADT. Following this, the merging of non-polar hydrogens and torsions was used for the ligands by allowing the rotation of all rotatable bonds. Gestgeiger partial charge was assigned for the ligands. Docking calculations were performed for the all protein models. ADT tools were applied for the assignment of Kollman charges, polar hydrogen atoms, and solvation parameters. To explore the active binding region having differential efficacy, the Lamarckian Genetic Algorithm was used. The whole binding site was used to assign grid boxes of the target proteins to allow sufficient space for the ligands' translational and rotational walk. Then 27,000 GA operations were generated with a single population of 150 individuals for every 30 independent runs. PyMOL software was used for visualization, and Discovery studio visualizer was used for the analysis of interface between receptors and ligands (DeLano, 2002).

Molecular Dynamics Simulation

Following the docking studies, lead compounds obtained from both *Aloe vera* and *Azadirachta indica* were subjected to MD simulation studies for the evaluation of their binding efficacy and to illustrate the effect of lead compound binding on the internal dynamics of M^{pro} protein, along with standard inhibitor and unbound protein (Gupta et al., 2020; Singh et al., 2021). The GROMACS (Version 2020.4) was utilized to perform MD simulation (Abraham et al., 2015). GROMOS54a7 force field and single-point charge (SPC) water model was applied to conduct MD simulation of all complexes. Topologies and parameter files of the lead compounds and the standard inhibitor were generated using PRODRG server (Schüttelkopf and Van Aalten, 2004). All the complexes were simulated inside a cubic box with 1 Å of buffer distance. For the electro neutralization of the complexes, the respective number of ions were added. Bad contacts and clashes in the protein were resolved by energy minimization using 5,000 steps of steepest decent method. Following the energy minimization, all the complexes underwent two steps of equilibration, first 100 ps of NVT equilibration followed by 100 ps of NVT equilibration. In order to avoid the cold solute–hot solvent difficulty, temperature coupling was applied, which was achieved by indexing the system into non-water and water components by using *gmx make_ndx* module of GROMACS (Lemkul, 2019). The temperature of the system was maintained at 300°C by using Berendsen thermostat (Berendsen et al., 1984). Similarly, the pressure of the system was maintained by using Parrinello-Rahman barostat (Parrinello and Rahman, 1981). Long-range interaction present in the system was treated by applying the LINCS method (Hess et al., 1997). MD simulations were performed for 20,000 ps, and the coordinates were saved at every 1 ps for all the system. Structural and conformational analysis of all system was conducted using various analysis modules implemented in GROMACS package.

Trajectory Analysis

Trajectories obtained following the molecular dynamics simulation were studied using tools available in the GROMACS package. The RMSD and RMSF of the lead phytochemical bound, standard inhibitor bound, and non-bound proteins were computed using *gmx rms* and *gmx rmsf* tools, respectively. The SASA and Rg were computed using *gmx sasa* and *gmx gyrate* tools, respectively. The energy calculations were performed using *gmx energy* tool. The change in the secondary structure of the unbound and ligand-bound test protein was analyzed using *gmx do_dssp* tool. The hydrogen bond formation was analyzed using *gmx hbond* tool. Visualization was performed using VMD and PyMol software (Humphrey et al., 1996; DeLano, 2002). Graphical representations were made using Grace Software (https://plasma-gate.weizmann.ac.il/Grace/).

Principal Component Analysis

The PCA is a popular analytical tool to assess the reduction in the dimensionality of large datasets. It is also a widely applied technique in MD simulations for the illustration of the slow/functional movements in biomolecules (Gupta et al., 2020; Singh et al., 2021). The principal components for all three complexes were obtained by diagonalization and solvation of the eigenvalues and eigenvectors for the covariance matrices. The eigenvectors and eigenvalues demonstrate the direction and the magnitude of the motion, respectively. Calculation of covariance matrix was performed using GROMACS tool *gmx covar*. The *gmx covar* tool of the GROMACS package was applied to build and also diagonalize the covariance matrix. Further *gmx anaeig* was applied for the calculation of overlap between the computed principal components and coordinates of the trajectory.

Free Energy Surface and Dynamical Cross-Correlation Matrix

Free energy surface (FES) is used to represent the possible conformation of the proteins in MD simulations. The change in possible conformation and the Gibbs free energy of the test protein (both in unbound and in ligand-bound complex) was studied. FES demonstrates the two variables that illustrate individual properties of the M^{pro}-lead phytochemical bound, standard inhibitor bound, and unbound protein systems. The FES calculations also measured the conformational variability of the test systems. The FES calculation was made by calculating the probability distribution of the first two eigenvectors. For the DCCM analysis, GROMACS trajectory files (.xtc) were converted into Nanoscale Molecular Dynamics (NAMD) format (.dcd) (Phillips et al., 2020). Trajectory conversion was performed using VMD software. Bio3D package was used to calculate DCCM (Grant et al., 2006).

g_mmpbsa Analysis

The *g_mmpbsa* is an important package used with GROMACS for the calculation of binding free energy (BE) of the ligand-bound complexes. The g_mmpbsa applies Molecular Mechanic/Poisson-Boltzmann Surface Area (MM-PBSA) approach for the BE calculation (Gupta et al., 2020). The BE was calculated for the known standard and the *A. indica* and *A. vera* lead phytochemicals bound at the active site of the target protein. The MD simulation trajectories for the last 5 ns (15–20 ns) were utilized to calculate the BE of the test complexes. The representation of the BE ($\Delta G_{binding}$) of the lead *A. indica* and *A. vera* phytochemical-bound protein complex was calculated using the following equation:

$$\Delta G_{binding} = G_{complex} - (G_{protein} + G_{ligand})$$

In above equation, $G_{complex}$ demonstrates energy of the lead phytochemical/standard inhibitor bound test protein complex, and $G_{protein}$ and G_{ligand} demonstrate the protein and ligand energy in water surrounded environment, respectively.

ADMET Parameter and Bioactivity Prediction

Physicochemical properties and pharmacokinetics parameters of the identified compounds were evaluated by using the free web tool SwissADME (http://www.swissadme.ch/) (Daina et al., 2017). Boiled egg and bioavailability radar analysis was performed to evaluate the absorption and bioavailability of identified compounds.

RESULTS

In the present study, a total of 130 phytochemicals present in *Azadirachta indica* and *Aloe vera* were identified in literature-based search. The phytochemicals were docked at SARS-CoV-2 Spike, RdRp, and M^{pro} proteins. Docking score of the *Aloe vera* and *Azadirachta indica* lead phytochemicals (≤−6.0 kcal/mole cut-off value) against the test proteins is tabulated in **Tables 1**, **2**, respectively. The 2D structures of the top lead compounds against various test proteins are depicted in **Figure 1**.

In the present study, Isoquercetin, MS 3, 7-Hydroxyaloin B, and 10-Hydroxyaloin A present in *Azadirachta indica* and *Aloe vera* medicinal plants showed potential binding against SARS-CoV-2 drugable targets, *viz*., Spike-protein, RdRp, and Main protease proteins. The standard inhibitors lopinavir, VE607, and ribinavir were used to compare the docking efficacy of phytochemicals against M^{pro}, Spike, and RdRp proteins, respectively. The 10-Hydroxyaloin A, 7-Hydroxyaloin B, and 10-Hydroxyaloin B 6'-catate *A. vera* compounds showed -8.57, -8.37, and -8.35 kcal/mole docking score against M^{pro} protein. Top three M^{pro} inhibitors present in *A. indica* (Isoquercetin, VEPAOL, and Nimbidiol) showed -9.73, -9.43, and -6.8 kcal/mole binding efficacy at the active site of the M^{pro} protein. The standard inhibitor lopinavir showed -5.33 kcal/mole binding efficacy. The binding pose of the lead M^{pro} inhibitors (Isoquercetin and 10-Hydroxyaloin A) and standard compound is depicted in **Figure 2A**. Amino acids involved in the binding with Isoquercetin, 10-Hydroxyaloin A, and standard inhibitor are depicted in **Figures 2B–D**. Asn142, residue of M^{pro}, was involved in hydrogen bond formation with lopinavir. Besides, amino acid residues Ser46, Thr45, Cys44, Thr25, Thr24, Leu27, Gly143, Thr26, Met49, Arg188, His41, Asp187, Val186, His164, Met165, Ser144, Phe140, Leu141, Cys145, His163, Glu166, and Gln189 were involved in hydrophobic interaction with protease (**Table 3**). Isoquercetin formed hydrogen bonds with residues Glu166, Asn146, and His41. Gly143, Leu27, Thr25, Cys44, Val42, Met49, Cys145, Met165, Arg188, Gln189, Thr190, Pro168, Leu167, Leu141, Ser144, His163, and Phe140 amino acid residues of M^{pro} protein interacted with Isoquercetin by hydrophobic interaction (**Table 3**). 10-Hydroxyaloin A showed hydrogen bonding with His41, Leu141, Gly143, and Cys145 residues and furthermore interacted with Leu27, Arg188, Thr26, Glu166, Ser144, His164, Asn142, His163, Thr25, Thr45, Cys44, Ser46, Met49, Gln189, Met165 amino acid residues *via* hydrophobic interaction. The top three lead molecules from *Azadirachta indica* and *Aloe vera* (10-Hydroxyaloin A, 7-Hydroxyaloin B, 10-Hydroxyaloin B 6'-catate, Isoquercetin, VEPAOL, and Nimbidiol) showed H-bond interaction with His41, Leu141, Gly143, Cys145, Gln189, Glu166, Thr190, His164, Phe140, Asn146, Thr26, Asn142 residues. Moreover, the active phytochemicals showed hydrophobic interaction at the test protein active site (**Figure 2** and **Table 3**).

MS 3, **BB**, and Barbaloin phytochemicals in *A. vera* plant showed -9.57, -8.57, and -8.03 kcal/mole docking score against SARS-CoV-2 Spike-protein. The top three Spike-protein inhibitors present in *A. indica* (Isoquercetin, VEPAOL, and Kaempferol) showed -8.22, -7.71, and -6.24 kcal/mole binding efficacy at the protein binding site (S1) of the Spike protein. The standard inhibitor VE607 showed -7.81 kcal/mole binding efficacy. The binding pose of the lead Spike protein inhibitors (MS 3 and Isoquercetin) and standard compound is depicted in **Figure 3A**. Amino acids involved in the binding with MS 3, Isoquercetin, and standard inhibitor are depicted in

TABLE 1 | Docking score of *Aloe vera* phytochemicals against drugable targets of SARS-CoV-2 virus.

Protein	PubChem ID	Compound Name	Docking Score Kcal/mole
M^{pro}			
	14889736	10-Hydroxyaloin A	-8.57
	158096	7-Hydroxyaloin B	-8.37
	10648253	10-Hydroxyaloin B 6'-catate	-8.35
	12305761	Barbaloin	-7.54
	6857486	CHEBI:35671	-7.45
	–	AA	-7.38
	160190	Aloesin	-6.97
	10207	Aloe-Emodin	-6.95
	100450	MS 3	-6.93
	5464178	1,3,6,8-Tetranitro-4,5-Dihydroxy-2-Hydroxymethylanthraquinones	-6.81
	5317657	UNII-73899319HU	-6.62
	5317653	CHEMBL518845	-6.6
	442866	Mycosporine	-6.41
Spike Protein			
	100450	MS 3	-9.75
	–	AA	-8.57
	12305761	Barbaloin	-8.03
	158096	7-Hydroxyaloin B	-7.86
	5904246	SCHEMBL4210152	-7.71
	14889736	10-Hydroxyaloin A	-7.46
	5317657	10-Hydroxyaloin A	-7.46
	6857486	CHEBI:35671	-7.25
	160190	Aloesin	-7.22
	11850	Galactitol	-7.1
	5317653	CHEMBL518845	-6.91
RdRP			
	158096	7-Hydroxyaloin B	-7.07
	6857486	CHEBI:35671	-6.96
	5317657	UNII-73899319HU	-6.77
	10648253	10-Hydroxyaloin B 6'-catate	-6.66
	14889736	10-Hydroxyaloin A	-6.59
	–	AA	-6.46

Docking Score with cutoff <–6.0 kcal/mole is considered. MS 3-3-hydroxy-4,5-bis(hydroxymethyl)-2-(3-methylbut-2-en-1-yl)phenyl 2,4-dihydroxy-6-methylbenzoate; AA-(10R)-2,8-dihydroxy-6-(hydroxymethyl)-1-methoxy-10-[(2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-9,10-dihydroanthracen-9-one.

TABLE 2 | Docking score of *Azadirachta indica* phytochemicals against drugable targets of SARS-CoV-2 virus.

Protein	PubChem ID	Compound Name	Docking Score (Kcal/mole)
M^{pro}			
	10813969	Isoquercetin	-9.73
	97343-95-8	VEPAOL	-9.43
	29803-85-8	Nimbidiol	-6.8
	11334829	Nimbidiol	-6.62
	21725519	Vilasinin	-6.55
	13875774	Nimbocinolide	-6.44
	5280863	Kaempferol	-6.35
	102090424	Melianin B	-6.33
	185704	Salannic (nimbidic) Acid	-6.27
	102146586	Azadirachtanin	-6.19
	23256847	Azadirachtol	-6.18
	6442906	Nimocinolide	-6.1
	105404-75-9	Margosinolide	-6.03
Spike			
	10813969	Isoquercetin	-8.22
	97343-95-8	VEPAOL	-7.71
	5280863	Kaempferol	-6.24
RdRp			
	10813969	Isoquercetin	-6.86
	184310	Isonimocinolide	-6.29

Docking Score with cutoff <–6.0 kcal/mole is considered.

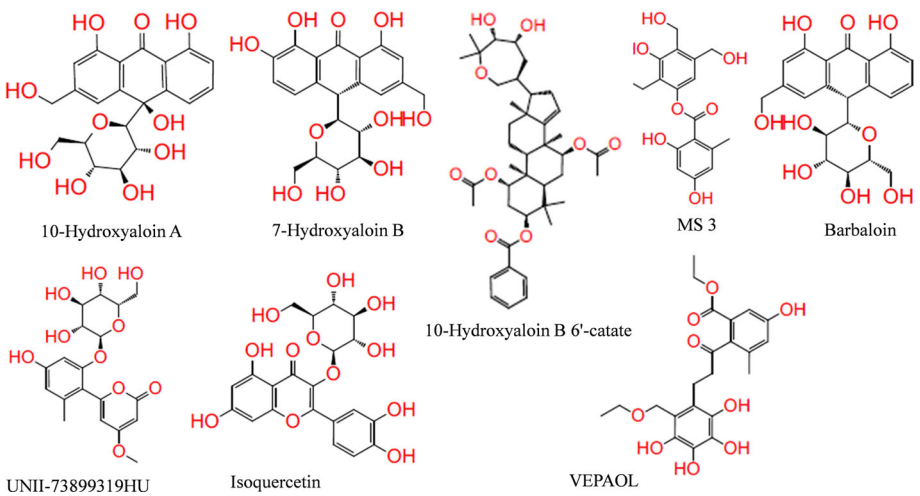

FIGURE 1 | Structure of lead phytochemicals present in *Aloe vera* and *Azadirachta indica* having potential to bind at the active sites/protein binding sites of the SARS-CoV-2 drugable proteins.

Figures 3B–D. Asp422 residue of Spike protein S1 domain was involved in hydrogen bond formation with the VE607. Besides, several amino acid residues Arg346, Thr345, Phe347, Arg509, Ser494, Lys417, Tyr421, Pro491, Leu492, Tyr351, Gln493, Ile418, Val350, Tyr495, Ile402, Ser349, Ala348, Phe497, and Asp442 were involved in hydrophobic interaction with the protein-binding domain (**Table 3**). MS 3 formed hydrogen with residues Ser494, and Gln493. Leu492, Pro491, Tyr421, Asn422,

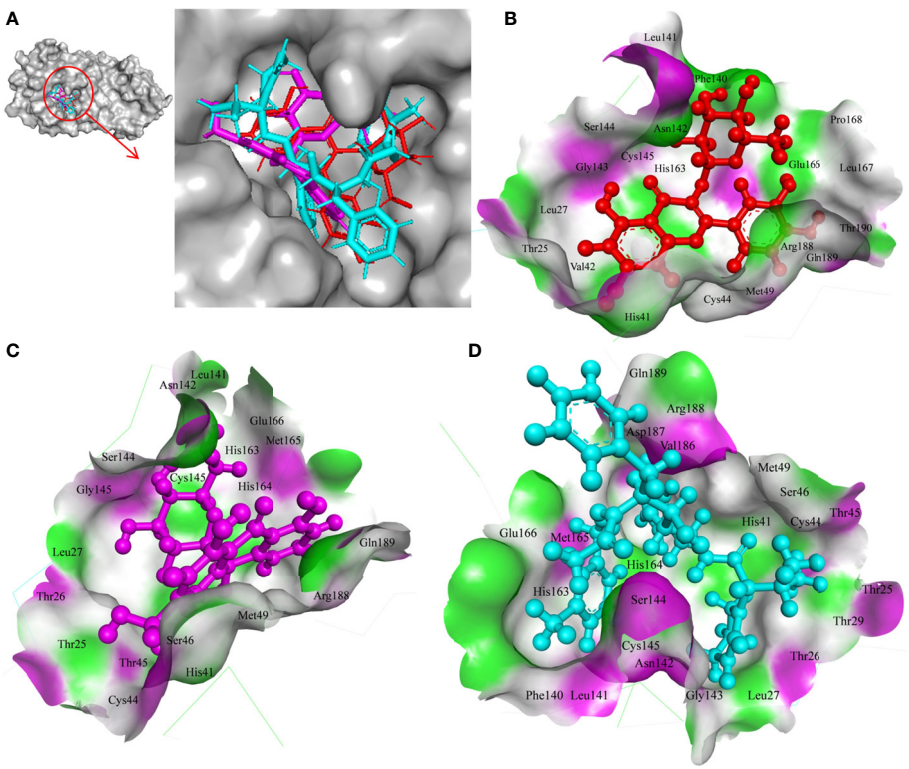

FIGURE 2 | Interaction of lead phytochemicals and standard inhibitor at the M^{pro} active site. **(A)** Binding pose of lopinavir (Cyan), Isoquercetin (Red), and 10-Hydroxyaloin A (Pink). **(B)** Interaction of M^{pro} protein with isoquercetin shown with the hydrogen bond surface of receptor. **(C)** Interaction of M^{pro} protein with 10-Hydroxyaloin A shown with the hydrogen bond surface of receptor. **(D)** Interaction of M^{pro} protein with lopinavir shown with the hydrogen bond surface of receptor.

TABLE 3 | Docking score and type of interaction of standard inhibitor and top three *Azadirachta indica* and *Aloe barbadensis* phytochemicals against SARS-CoV-2 M^{pro}, spike, and RdRp proteins.

Target	PMID of ligand	Ligand name	Hydrogen-forming residues	Residues with hydrophobic interaction
M^{pro}				
	Standard			
	92727	Lopinavir	**Asn142,** Ser46, Thr45, Cys44, Thr25, Thr24, Leu27, Gly143, Thr26, Met49, Arg188, His41, Asp187, Val186, His164, Met165, Ser144, Phe140, Leu141, Cys145, His163, Glu166, Gln189,	
	Aloe			
	14889736	10-Hydroxyaloin A	**His41, Leu141, Gly143, Cys145,** Leu27, Arg188, Thr26, Glu166, Ser144, His164, Asn142, His163, Thr25, Thr45, Cys44, Ser46, Met49, Gln189, Met165	
	158096	7-Hydroxyaloin B	**Leu141, His41, Gln189, Glu166, Thr190,** Met49, Asn142, His163, Pro168, Met165, Arg188,	
	10648253	10-Hydroxyaloin B 6'-catate	**Gly143, His164, Cys145, Phe140,** Ser46, Thr45, His41, Thr25, Cys44, Met49, Gln189, Met165, Leu141, Glu166, His163	
	Neem			
	10813969	Isoquercetin	**Glu166, Asn146, His41,** Gly143, Leu27, Thr25, Cys44, Val42, Met49, Cys145, Met165, Arg188, Gln189, Thr190, Pro168, Leu167, Leu141, Ser144, His163, Phe140	
	97343-95-8	VEPAOL	**Thr26, Asn142, Gly143,** Ser46, Gln189, Met49, His41, Leu27, His164, Met165, Glu166, His163, Ser144, Leu141, Phe140, HSl172, Cys145, Thr24, Thr25	
	29803-85-8	Nimbidiol	**Gly143, Thr26,** Met49, Gln189, Ser46, Asn142, Thr24, Thr25, Leu27, Ser144, Cys145, His164, His41, Met165, Glu166	
Spike				
	Standard			
	VE607	–	**Asp422,** Arg346, Thr345, Phe347, Arg509, Ser494, Lys417, Tyr421, Pro491, Leu492, Tyr351, Gln493, Ile418, Val350, Tyr495, Ile402, Ser349, Ala348, Phe497, Asp442	
	Aloe			
	100450	MS 3	**Ser494, Gln493,** Leu492, Pro491, Tyr421, Asn422, Lys417, Ile418, Tyr495, Val350, Gly496, Ser349	
	–	**BB**	**Phe497, Asp442, Phe347,** Tyr495, Ser349, Val401, Ser443	
	12305761	Barbaloin	**Tyr495, Ser494, Val401,** Gln493, Val350, Ser349	
	158096	7-Hydroxyaloin B	**Phe347, Asp442,** Arg346, Val401, Tyr495, Val350, Ser494, Ser349, Phe497	
	Neem			
	10813969	Isoquercitin	**Phe347, Val350, Asp442, Phe497,** Arg509, Arg346, Ala348, Val401, Ser349, Gln493, Tyr495, Tyr351, Ser494, Gln498, Pro499, Ser443	
	97343-95-8	Vepaol	**Val350, Asn422, Pro491, Tyr351,** Gln493, Leu492, Lys417, Ile418, Tyr495, Ser349	
RdRp				
	Standard			
	37542	Ribavirin	**Trp617, Asp618, Lys798, Glu811,** Tyr619, Ser814, Phe812, Asp761, Asp760, Ala762, Gly616, Trp800, Cys799,	
	Aloe			
	158096	7-Hydroxyaloin B	**Asp618, Tyr619, Lys621, Asp760, Lys798, Glu811,** Asp761, Asp623, Cys622, Pro620, Lys551, Arg553, Ser814	
	6857486	CHEBI:35671	**Asp760, Asp761, Ala762, His810, Glu811,** Asp618, Tyr619, Lys798, Cys799, Trp800, Gly616, Trp617, Val763, Phe812, Ser759, Ser814	
	5317657	UNII-73899319HU	**Arg553, Ser759, Asp760, Ser814, Lys798,** Lys545, Glu811, Phe812, Trp800, Cys799, Trp617, Tyr619, Trp617, Cys813, Asp618, Asp761, Leu758,	
	Neem			
	10813969	Isoquercetin	**Trp617, Asp618, Tyr619, Asp760, Lys798,** Pro620, Lys621, Cys622, Cys799, Trp800, Gly616, Ala762, Asp761, Glu811, Ser814, Ser759	
	184310	Isonimocinolide	**Tyr619, Lys621, Asp761, Ser814,** Asp618, Lys798, Asp623, Cys622, Pro620, Leu758, Asp760, Ser759, Cys813, Ala762, Phe812, Glu811, Trp617, Gly616, Trp800	

BB*- (10R)-2,8-dihydroxy-6-(hydroxymethyl)-1-methoxy-10-[(2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-9,10-dihydroanthracen-9-one.*
The amino acids shown in bold were involved in hydrogen bond formation with their respective protein.

Lys417, Ile418, Tyr495, Val350, Gly496, and Ser349 amino acid residues of the S1 domain of Spike protein interacted with MS 3 by hydrophobic interaction (**Table 3**). Isoquercetin showed hydrogen bonding with Phe347, Val350, Asp442, and Phe497. Furthermore, it interacted with Arg509, Arg346, Ala348, Val401, Ser349, Gln493, Tyr495, Tyr351, Ser494, Gln498, Pro499, Ser443 amino acid residues by hydrophobic interaction. The top two lead molecules from *Azadirachta indica* and top three *Aloe vera* (MS 3, **BB**; Barbaloin, Isoquercetin, and VEPAOL) showed H-bond interaction with Ser494, Gln493, Phe497, Asp442, Phe347 Tyr495, Val401, Val350, Val350, Asn422, Pro491, Tyr351 amino acid residues. Moreover, the active phytochemicals showed hydrophobic interaction at the test protein active site (**Figure 3** and **Table 3**).

The 7-Hydroxyaloin B, CHEBI:35671, and UNII-73899319HU *A. vera* compounds showed -7.07, -6.96, and -6.77 kcal/mole docking score against SARS-CoV-2 RdRp protein. The top RdRp protein inhibitors (above the cut-off value) present in *A. indica* (Isoquercetin and Isonimocinolide) showed -6.86 and -6.29 kcal/mole binding efficacy at the active site of the protein. The standard inhibitor ribinavir showed -6.13 kcal/mole binding efficacy. The binding pose of the lead RdRp protein inhibitors (7-Hydroxyaloin B and Isoquercetin) and standard compound is depicted in **Figure 4A**. Amino acids involved in the binding with

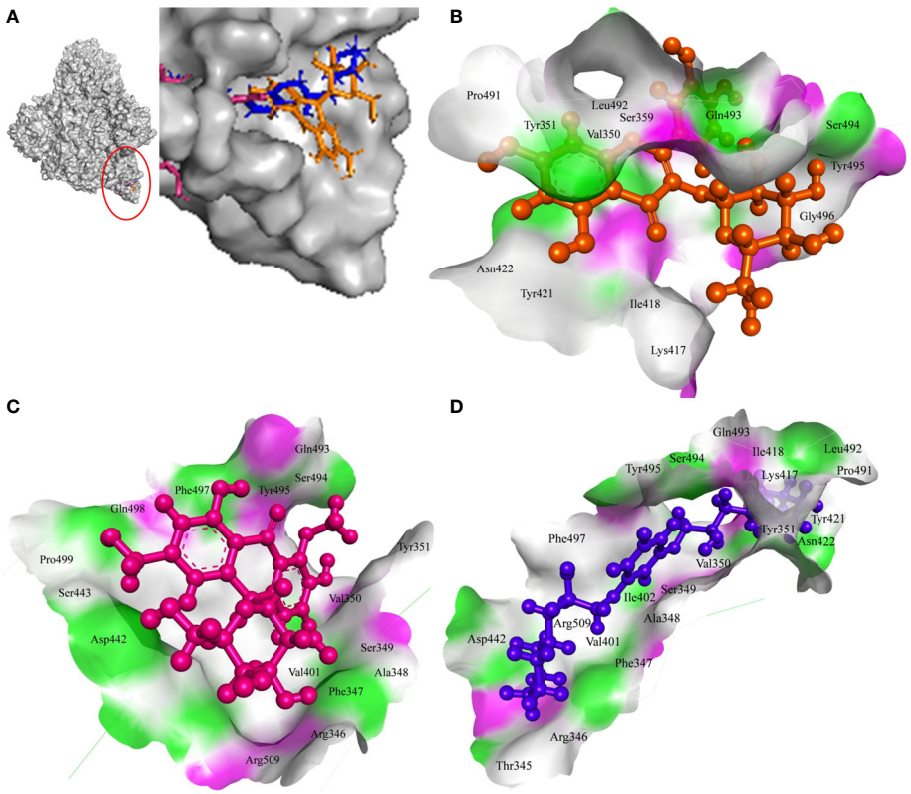

FIGURE 3 | Interaction of lead phytochemicals and standard inhibitor with RdRp protein of SARS-CoV-2. **(A)** Binding pose of VE607 (Blue), Isoquercetin (Orange), and 10-Hydroxyaloin A (Pink) at the active site of the RdRp. **(B)** Interaction of RdRp active site with isoquercetin shown with the hydrogen bond surface of receptor. **(C)** Interaction of RdRp active site with 10-Hydroxyaloin A shown with the hydrogen bond surface of receptor. **(D)** Interaction of RdRp active site with VE607 shown with the hydrogen bond surface of receptor.

7-Hydroxyaloin B, Isoquercetin, and standard inhibitor are depicted in **Figures 4B–D**. Trp617, Asp618, Lys798, and Glu811 residues of RdRp active site were involved in hydrogen bond formation with ribinavir. Besides, Tyr619, Ser814, Phe812, Asp761, Asp760, Ala762, Gly616, Trp800, and Cys799 amino acid residues showed hydrophobic interaction at the RdRp active site (**Table 3**). The 7-Hydroxyaloin B formed hydrogen with residues Asp618, Tyr619, Lys621, Asp760, Lys798, and Glu811. Furthermore 7-Hydroxyaloin B interacted with residues Asp761, Asp623, Cys622, Pro620, Lys551, Arg553, and Ser814 hydrophobically (**Table 3**). Isoquercetin formed hydrogen bonding with Trp617, Asp618, Tyr619, Asp760, and Lys798 amino acid residues. Furthermore, isoquercetin interacted with residues Pro620, Lys621, Cys622, Cys799, Trp800, Gly616, Ala762, Asp761, Glu811, Ser814, and Ser759 hydrophobically. The top two lead molecules from *Azadirachta indica* and top three from *Aloe vera* (7-Hydroxyaloin B, CHEBI:35671, and UNII-73899319HU, Isoquercetin, and Isonimocinolide) showed H-bond interaction with Asp618, Tyr619, Lys621, Asp760, Lys798, Glu811, Asp761, Ala762, His810, Arg553, Ser759, Ser814, and Trp617 amino acid residues of the RdRp protein. Moreover, the active phytochemicals showed hydrophobic interaction at the test protein active site (**Figure 4** and **Table 3**).

Molecular Dynamics Simulation

On the basis of docking results, we selected M^{pro} protein to perform molecular dynamics simulation study. The simulation was carried out on M^{pro} unbound, *A. indica*–, and *A. vera*–bound systems to study the dynamic behavior of the targeted protein. Simultaneously, the M^{pro} bound with its experimentally validated inhibitor was also performed to compare the results. Quality check parameters for the simulated system (temperature, pressure, potential/kinetic energy) were evaluated to check the validity of the performed simulations. Results showed that the quality check parameters were stable throughout the simulation period (**Supplementary Figures 1A–H**). MD simulation results for M^{pro}-10-Hydroxyaloin A (MHA) complex, M^{pro}-Isoquercetin (MIQ) complex, the unbound M^{pro} protein and M^{pro}-Lopinavir (MLP) complex are shown in **Figures 5A, B**. The systems were stabilized and showed no significant alterations in density, temperature, volume, kinetic/potential/total energies, and pressure in unbound and lead ligand/standard inhibitor–bound protein/protein complex during the 20 ns MD simulation period (data not shown). Root mean square deviation (RMSD) of the MHA, MIQ, and MLP complexes did not show any significant deviation in comparison to unbound protein (**Figures 5A, B**). RMSD of 10-Hydroxyaloin A and

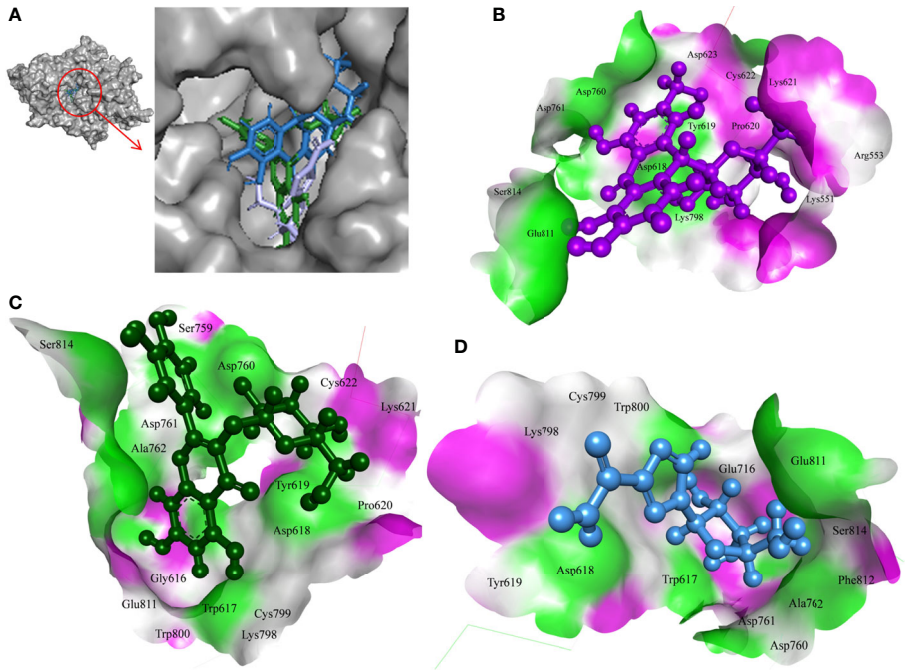

FIGURE 4 | Interaction of lead phytochemicals and standard inhibitor with RdRp protein of SARS-CoV-2. **(A)** Binding pose of ribinavir (Cyan), Isoquercetin (Violet), and 10-Hydroxyaloin A (Green). **(B)** Interaction of Spike-protein RBD domain with isoquercetin shown with the hydrogen bond surface of receptor. **(C)** Interaction of Spike-protein RBD domain with 10-Hydroxyaloin A shown with the hydrogen bond surface of receptor. **(D)** Interaction of Spike-protein RBD domain with ribinavir shown with the hydrogen bond surface of receptor.

Isoquercetin-bound M^{pro} complexes were comparable to known inhibitor-bound complexes. Root-mean-square fluctuation (RMSF) was assessed to analyze the impact of lead phytochemicals binding on the flexible portion of the targeted protein. Significant reduction in the RMSF values was observed in Isoquercetin-bound M^{pro} complex. The 10-Hydroxyaloin A and lopinavir-bound complexes did not show significant fluctuations in comparison to unbound protein (**Figures 5C, D**).

Radius of gyration (Rg) and Solvent accessible surface area (SASA) analyses for M^{pro}-10-Hydroxyaloin A (MHA) complex,

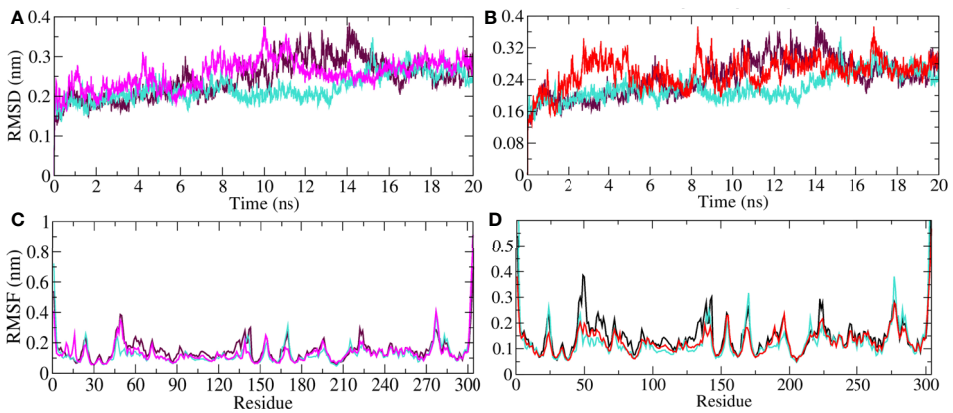

FIGURE 5 | MD simulation trajectory plot of SARS-CoV-2 main protease in unbound and standard inhibitor ligand/natural lead compound bound complex. **(A)** The RMSD of the M^{pro} protein and lopinavir/10-Hydroxyaloin A lead compound complex during 20 ns MD simulation. **(B)** The RMSD of the M^{pro} and lopinavir/Isoquercetin lead compound complex during 20 ns MD simulation. **(C)** The RMSF values of M^{pro} protein and lopinavir/10-Hydroxyaloin A lead compound complex during 20 ns MD simulation. **(D)** The RMSF values of M^{pro} protein and lopinavir/Isoquercetin lead compound complex during 20 ns MD simulation Unbound protein, black color; *Aloe vera*–bound complex, pink; Neem-bound complex, red; and Lopinavir-bound complex is shown in light-blue color.

M^{pro}-Isoquercetin (MIQ) complex, M^{pro}-Lopinavir (MLP) complex, and the unbound M^{pro} protein were compared, and the results are shown in **Figures 6A–D**. Result showed that 10-Hydroxyaloin A reduced the Rg value during the 20 ns simulation period in comparison to free protein and lopinavir-bound M^{pro} protein complex (**Figure 6A**). Isoquercetin showed similar Rg value pattern to lopinavir-bound M^{pro} complex (**Figure 6B**). Solvent accessible surface area analysis of the MD simulation trajectory revealed that 10-Hydroxyaloin A binding did not affect the SASA value significantly in comparison to MLP and pro systems (**Figure 6C**). It should be noted that the binding of Isoquercetin significantly reduced the SASA in comparison to lopinavir-bound and non-bound M^{pro} complexes during the entire 100 ns of MD simulation (**Figure 6D**).

Hydrogen bond formation between the protein (ligand bound and unbound state) and the surrounding water molecules, within the protein (ligand bound and unbound state), as well as between the protein and lead compound/standard inhibitor was assessed, and the results are shown in **Figures 7A–G**. About 570 intermolecular hydrogen bonds were formed between unbound protein and water molecules. 10-Hydroxyaloin A binding with the M^{pro} protein significantly reduced the protein-water molecule H-bonding (**Figure 7A**). Isoquercetin showed lesser effect on H-bond formation between the test protein and surrounding water molecules (**Figure 7B**). The results were comparable with the standard inhibitor–bound protein complex (**Figures 7A, B**). Binding of 10-Hydroxyaloin A phytochemical to M^{pro} active site significantly increased (250) the intraprotein hydrogen bonding in comparison to unbound and lopinavir-bound M^{pro} protein (**Figure 7C**). Isoquercetin did not affect the interprotein H-bond formation in comparison to unbound and inhibitor-bound M^{pro} complex (**Figure 7D**). The H-bond formation between the protein and ligand (lead phytochemicals/standard inhibitor) showed that 10-Hydroxyaloin A and Isoquercetin binding favored H-bond formation in comparison to lopinavir binding at the active site of the M^{pro} protein during the MD simulation period (**Figures 7F, G**).

Principal component analysis (PCA) of MD simulation trajectories was analyzed to show the collective motion of the active site–bound 10-Hydroxyaloin A and Isoquercetin-bound M^{pro} protein complexes. The results were compared with the unbound and standard inhibitor–bound M^{pro} protein/protein complex. The results of PCA analysis of the test system are depicted in **Figures 8A–F**. Results showed that binding of lead phytochemicals significantly decreased the collective motion of the M^{pro} protein in comparison to M^{pro} unbound and MLP systems.

Changes in secondary structure content (SSC) in the ligand-bound complex were studied for the change in structural behavior of the test protein in the presence of ligand at the active site. The SSC was studied in MHA, MLP, MIQ, and Mpro systems; and the results are depicted in **Figures 9A–D**. Results showed that the lopinavir binding did not affect significantly the various secondary structures in the Mpro protein (**Figures 9A, B**). A little fluctuation in the β-sheet of the M^{pro} protein was observed in 10-Hydroxyaloin A and Isoquercetin-bound M^{pro} protein complex (**Figures 9C, D**). To visualize the energy minima landscape of M^{pro}-10-Hydroxyaloin A (MHA) complex, M^{pro}-Isoquercetin (MIQ) complex, M^{pro}-Lopinavir (MLP) complex, and the unbound M^{pro} protein, the FEL against PC1 (Rg) and PC2 (RMSD) was studied. The concise minimal energy area (blue color) obtained for the M^{pro}-10-Hydroxyaloin A (MHA) and M^{pro}-Isoquercetin (MIQ) complexes in comparison to lopinavir-bound and unbound protein is shown in **Figures 9Ei–iv**. The DCCM analysis was performed to find the effect of ligand binding on the correlated and anti-correlated motions of the target protein. It is evident from the DCCM analysis that the binding of 10-Hydroxyaloin A results in the significant decrease in both correlated (cyan color) and anti-

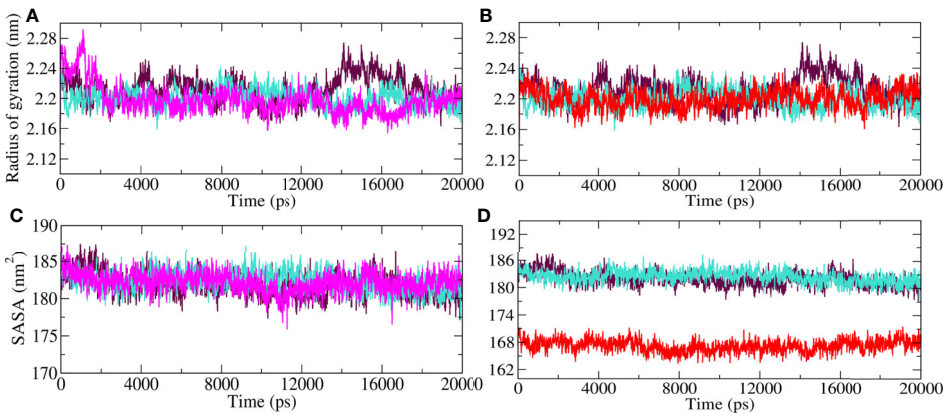

FIGURE 6 | Radius of gyration (Rg) and solvent accessible surface (SASA) region of SARS-CoV-2 main protease in unbound and standard inhibitor ligand/natural lead compound–bound complex. **(A)** The Rg of the M^{pro} protein and lopinavir/10-Hydroxyaloin A lead–bound complex during 20 ns MD simulation. **(B)** The Rg of the solvated M^{pro} protein and lopinavir/Isoquercetin lead–bound complex during 20 ns MD simulation. **(C)** The SASA values of M^{pro} protein and lopinavir/10-Hydroxyaloin A lead–bound complex during 20 ns MD simulation. **(D)** The SASA values of M^{pro} protein and lopinavir/Isoquercetin lead–bound complex during 20 ns MD simulation. Unbound protein, black color; *Aloe vera*–bound complex, pink; Neem-bound complex, red; and Lopinavir-bound complex is shown in light-blue color.

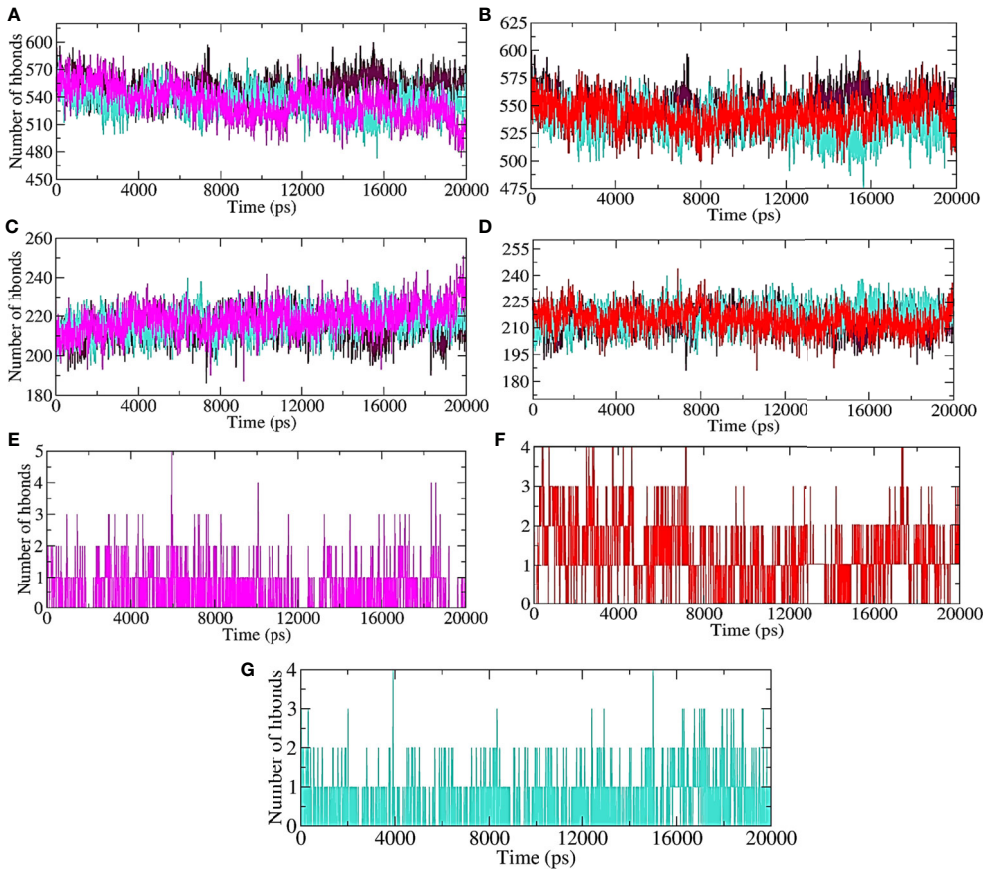

FIGURE 7 | Plot of hydrogen bond formation during the MD simulation period of M^{pro} protein in ligand-bound and unbound conformation. Hydrogen bond formation between water and test protein in unbound, lopinavir-bound, **(A)** *Aloe vera* lead compound 10-Hydroxyaloin A–bound, and **(B)** Neem lead compound Isoquercetin-bound complexes. Intraprotein hydrogen bond formation in unbound, lopinavir–bound, **(C)** *Aloe vera* lead compound 10-Hydroxyaloin A–bound, and **(D)** Neem lead compound Isoquercetin-bound complexes. Hydrogen bond formation between M^{pro} complexed with **(E)** *Aloe vera* lead compound 10-Hydroxyaloin A, **(F)** Neem lead compound Isoquercetin, and **(G)** Lopinavir. Unbound protein, black color; *Aloe vera*–bound complex, pink; Neem-bound complex, red; and Lopinavir-bound complex is shown in light-blue color.

correlated motions (pink color) of the M^{pro} protein in comparison to the unbound and lopinavir-bound M^{pro} protein (**Supplementary Figures 2A–C**). Binding of isoquercetin did not show significant effect on correlated and anti-correlated motions of target protein in comparison to the unbound and lopinavir-bound protein (**Supplementary Figure 2D**).

Further, we performed the MM-PBSA analysis of the last 5 ns (15–20 ns) of the lead phytochemical/standard inhibitor–bound M^{pro} complex trajectories (obtained from 20 ns MD simulations) to calculate the thermodynamics parameters of the complex such as binding free/van der Waals/electrostatic/polar solvation energies ($\Delta E_{binding}$; E_{vdw}; E_{elec}; ΔE_{polar} respectively) and SASA (**Figures 10A–D** and **Table 4**). The binding energy of *A. indica* and *A. vera* lead phytochemical–bound M^{pro} complexes was stable during the analysis of the simulation trajectory (**Figures 10A, B**). The MM-PBSA data analysis also allows us to calculate the contribution of amino acid residues in the studied parameters (total binding energy). The total binding free energy was decomposed into the per amino acid residue contribution energy. The results for the amino acid residue contribution in the binding of lead phytochemicals are shown in **Figures 10B, C**. The M^{pro}-10-Hydroxyaloin A binding analysis showed that the amino acid residues Met49, His41, Asp48, Cys145, Met165, Pro52, Leu50, and Leu27 played significant roles in the complex formation (**Figure 10A**). Similarly, M^{pro}-Isoquercetin binding involved the significant contribution of leu27, His41, Met49, Asp142, Gly143, Leu167, Asp187, and Glu189 amino acid residues (**Figure 10B**).

The boiled egg diagram for the lead phytochemicals (MS 3, 10-Hydroxyaloin A, 7-Hydroxyaloin B, and Isoquercetin) was prepared to study their blood–brain barrier crossing potential. All the phytochemicals showed satisfactory results (**Figure 11A**). The bioavailability radars for the lead phytochemicals against the Spike-protein, M^{pro}, and RdRp drugable targets of SARS-CoV-2 were studied, and the results are shown in **Figure 11**. The radar plot shows the important drug-likeness properties such as lipophilicity, molecular weight, polarity, insolubility, insaturation, and rotatable bond flexibility of the test compounds. The bioavailability radars of the lead compounds were in the range

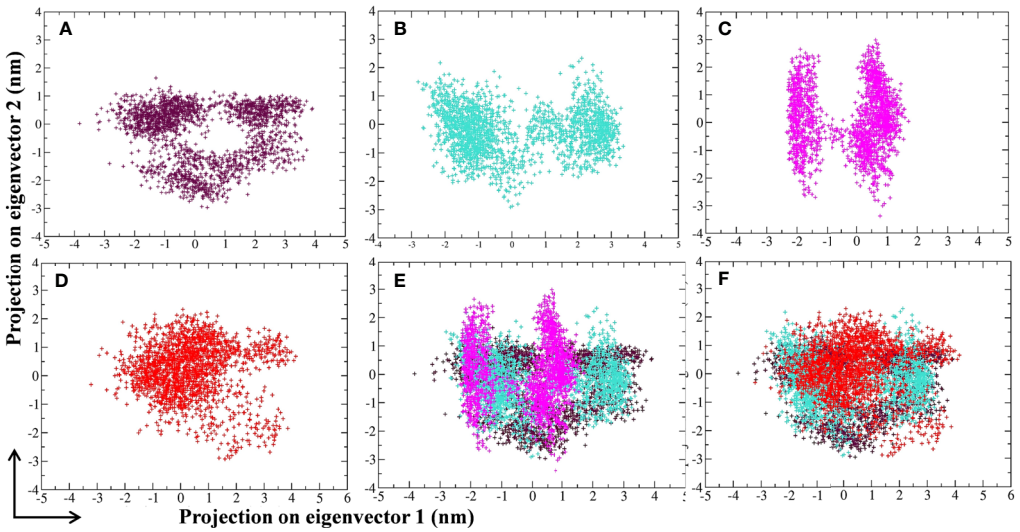

FIGURE 8 | Projection of SARS-CoV-2 M^{pro} protein atoms in phase space along the first two principal eigenvectors. **(A)** Unbound M^{pro} protein. **(B)** Lopinavir-bound M^{pro} complex, **(C)** *Aloe vera* lead compound 10-Hydroxyaloin A–bound M^{pro} complex, **(D)** Neem lead compound Isoquercetin-bound M^{pro} complex, **(E)** Unbound M^{pro} and lopinavir/*Aloe vera* lead compound–bound complex, **(F)** Unbound M^{pro} and lopinavir/Neem lead compound–bound complex. Unbound protein, purple color; *Aloe vera*–bound complex, pink; Neem-bound complex, red; and Lopinavir-bound complex is shown in light-blue color.

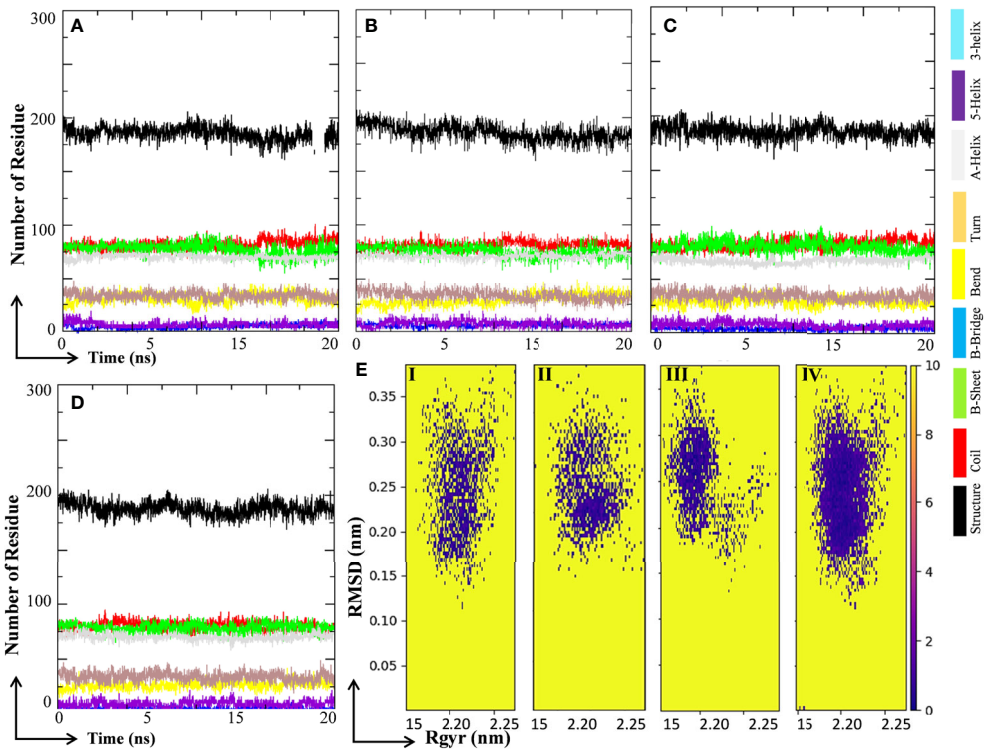

FIGURE 9 | Secondary structure change and protein-ligand energy landscape of SARS-CoV-2 Main protease unbound and ligand-bound conformation. The secondary structure changes during the 20 ns MD simulation in **(A)** Unbound M^{pro} protein, **(B)** Lopinavir-bound M^{pro} complex, **(C)** *Aloe vera* lead compound 10-Hydroxyaloin A–bound M^{pro} complex, and **(D)** Neem lead compound Isoquercetin-bound M^{pro} complex. The free energy landscape of the **(Ei)** Unbound M^{pro} protein, **(Eii)** Lopinavir-bound M^{pro} complex, **(Eiii)** *Aloe vera* lead compound–bound M^{pro} complex, and **(Eiv)** Neem lead compound–bound M^{pro} complex.

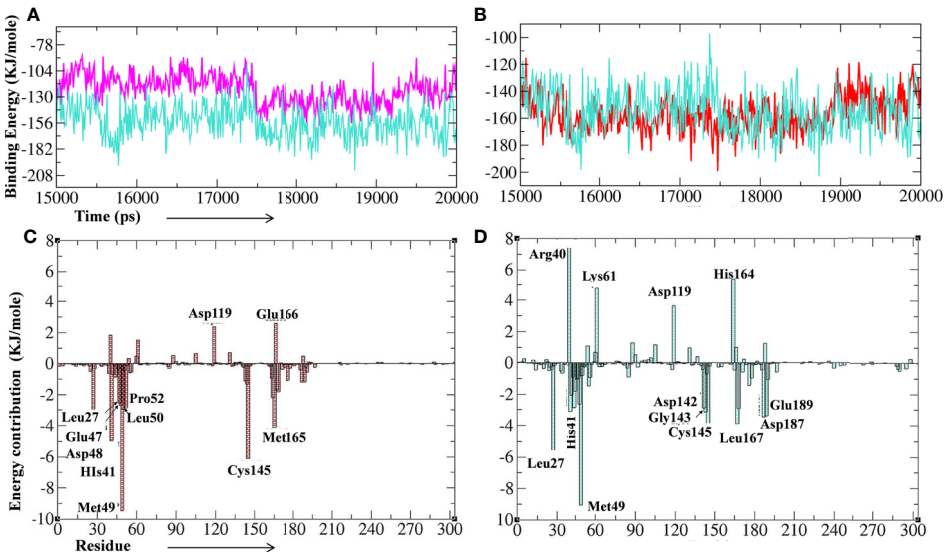

FIGURE 10 | Binding energy of the lead phytochemicals and contribution energy of amino acid residues in the g_mmpbsa analysis. **(A)** Binding energy plot of M^{pro}-lopinavir (light-blue color) and 10-Hydroxyaloin A (pink color) complex. **(B)** Binding energy plot of M^{pro}-lopinavir (light-blue color) and Isoquercetin (red color) complex. **(C)** Residue contribution plot of M^{pro}-10-Hydroxyaloin A complex. **(D)** Residue contribution plot of M^{pro}- Isoquercetin complex.

and satisfactory. The 10-Hydroxyaloin A, 7-Hydroxyaloin B, and Isoquercetin showed little deviation from the required flexibility region of the radar (**Figures 11C–E**). Further, the various physiochemical properties of MS 3, 10-Hydroxyaloin A, 7-Hydroxyaloin B, and Isoquercetin are summarized in **Table 5**.

DISCUSSION

In the present study we found the potential interaction of phytochemicals with the drugable targets of SARS-CoV-2-mediated infection in human. For this we targeted the three key steps of the viral pathogenesis, *viz.*, viral entry into the host cell (Spike-protein), conversion of viral precursor to functional proteins (M^{pro}), and viral genome replication (RdRp). Interaction of Spike-protein RBD with the ACE-2 receptor (human host protein) is a critical step in viral entry into the host cell (Senapati et al., 2020). Recently Shang et al. (2020) reported that residues 455, 482–486, 493, 494, and 501 are critical SARS-CoV-2 S1 domain amino acids involved in interaction with human ACE-2 protein (Shang et al., 2020). Our research group reported inhibitory potential of phytochemicals against SARS-CoV-2 drugable proteins. The *in silico* inhibitory potential of *Curcuma longa* and *Withania somnifera* phytochemicals against M^{pro} protein of SARS-CoV-2 has been reported recently (Gupta et al., 2020; Kushwaha et al., 2021c). The identified lead phytochemicals showed significantly increased binding potential at the M^{pro} active site in comparison to standard inhibitors in molecular docking and molecular dynamics simulation study (Gupta et al., 2020; Kushwaha et al., 2021c). In the present study, we found that lead *A. indica* and *A. vera* phytochemicals interacted with two of the critical amino acids (Gln493 and Ser494) (**Table 3**). Isoquercetin formed hydrogen bonding, while MS 3 showed hydrophobic interaction with the Gln493 and Ser494 amino acids, which are critical for viral Spike-protein and human host ACE-2 binding receptor. MS 3 showed H-bonding with the Asn422 amino acid residue, which is reported to be involved in ACE-2 binding of Spike-protein (Gordon et al., 2020). Lead phytochemicals (binding efficacy below <−6.0 kcal/mole) also showed hydrophobic and hydrogen bond interaction with the key amino acids involved in Spike-protein RBD domain and ACE-2 protein-protein interaction (Asp442, Ser494, and Gln493). The results indicate that Isoquercetin and MS 3 have potential to disrupt spike

TABLE 4 | Binding free energy for Main protease complexes with standard inhibitor and lead phytochemical–bound complexes.

BE type	BE values MLP	BE values MHA	BE values MIQ
$\Delta E_{binding}$ (kj/mol)	-154.850 +/− 16.491	-122.513 +/− 14.118	-156.650 +/− 12.970
SASA (kj/mol)	-25.582 +/− 1.701	-15.250 +/− 1.268	-19.524 +/− 1.316
$\Delta E_{polar\ solvation}$ (kj/mol)	145.474 +/− 18.030	49.261+/− 8.881	71.794 +/− 13.762
$\Delta E_{Electrostatic}$ (kj/mol)	-29.830 +/− 9.759	-7.357+/− 7.080	-5.438+/− 7.347
$\Delta E_{Van\ der\ Waal}$ (kj/mol)	-244.912 +/− 16.819	-149.166+/− 12.217	-203.481 +/− 14.122

BE, Binding Energy; MLP, M^{pro}-Lopinavir complex; MHA, M^{pro}-10-Hydroxyaloin A complex; MIQ, M^{pro}- Isoquercetin complex.

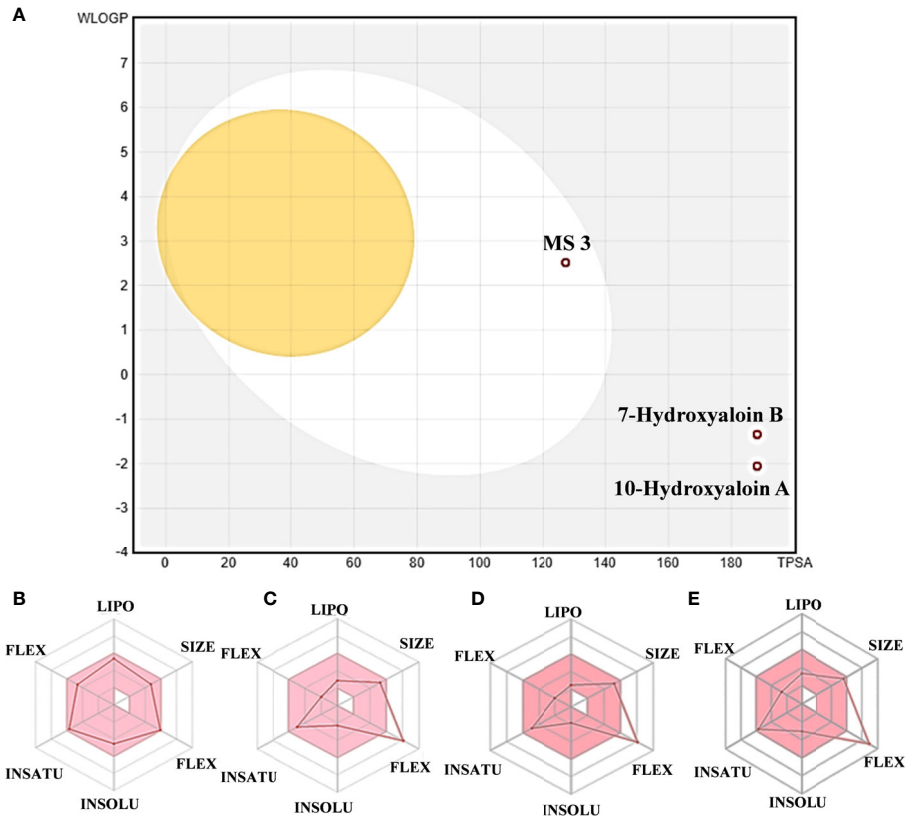

FIGURE 11 | Boiled egg diagram and bioavailability radar map of lead phytochemicals. **(A)** Boiled egg model of MS 3, 10-Hydroxyaloin A, and 7-Hydroxyaloin B phytochemicals. The isoquercetin molecule value was out of range of the boiled egg plot. Bioavailability radar map of **(B)** MS 3, **(C)** 10-Hydroxyaloin A, **(D)** 7-Hydroxyaloin B, and **(E)** Isoquercetin. LIPO, lipophilicity; SIZE, molecular weight; POLAR, polarity; INSOLU, insolubility; INSATU, insaturation; FLEX, rotatable bond flexibility.

glycoprotein-ACE-2 protein-protein interaction by binding at SARS-CoV-2 RBD. Thus, the lead molecules might inhibit viral entry into the cell. It has been shown that RdRp inhibitors (such as remdesivir) potentially block the RNA synthesis and thereby delay the chain termination process in SARS-CoV-2 RNA synthesis (Gordon et al., 2020). SARS-CoV-2 RdRp inhibitors are being studied in clinical trials in various countries of the world. Binding of *A. indica* and *A. vera* lead compounds at RdRp active site showed interaction with similar amino acids (Asp618, Tyr619, Asp760, Lys798, Glu811, and Asp761), indicating the SARS-CoV-2 RdRp inhibition potential in the test medicinal plants (**Table 3**). The SARS-CoV-2 RNA–mediated translation generated polyproteins that later on cleaved at some specific sites by Main protease enzyme to produce functional proteins.

TABLE 5 | Physiochemical properties of the lead phytochemicals.

Molecule	MS 3	7-Hydroxyaloin B	10-Hydroxyaloin A	Isoquercetin
Formula	$C_{21}H_{24}O_7$	$C_{21}H_{22}O_{10}$	$C_{21}H_{22}O_{10}$	$C_{21}H_{20}O_{12}$
MW(g/mol)	388.41	434.4	434.39	464.38
HA	28	31	31	33
AHA	12	12	12	16
FCsp3	0.29	0.38	0.38	0.29
HBA	7	10	10	12
HBD	5	8	8	8
MR	104.85	103.98	103.01	110.16
TPSA	127.45	188.14	188.14	210.51
ESOL-S (mg/ml)	1.08	2.08	6	4.23
ESOL-C	MS	S	VS	S

MW, Molecular weight; HA, Heavy atoms; AHA, Aromatic heavy atoms; FCsp3, Fraction Csp3; HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donors; MR, Molar refractivity; TPSA, The polar surface area; ESOL-S, ESOL-Solubility; ESOL-C, ESOL Class; S, Soluble; PS, Poorly soluble; MS, Moderately soluble; GIA, GI absorption; BBB-P, BBB permeant; Pgp-S, Pgp substrate; BS, Bioavailability score.

This step aids in the virulence of SARS-CoV-2. Literature reports that a Cystine-Histidine dyad is essential for the protease activity of M^{pro}. Besides, the alanine, glycine, glutamate, serine, and leucine residue also play important roles in the cleavage catalysis process (Gupta et al., 2020). In the present study, the 10-Hydroxyaloin A and Isoquercetin showed hydrogen bond interaction with the Cys and His residues, indicating the Cys-His dyad disruption potential of the lead phytochemicals. The dyad disruption might lead to the decreased M^{Pro} activity, which in turn inhibits the production of functional protein. Thus, the 10-Hydroxyaloin A and Isoquercetin might be involved in the SARS-CoV-2 virulence mitigation.

RMSD is a parameter that computes the distance between protein atoms. The average distance between the atoms in unbound and ligand/standard inhibitor–bound targeted protein allows us to assess the comparative conformation and stability of the protein (Gupta et al., 2020). The present study indicates that the binding of 10-Hydroxyaloin A and Isoquercetin did not affect the conformational stability of the M^{Pro} protein (**Figures 5A, B**). The result indicates the stable M^{pro}-10-Hydroxyaloin A (MHA) and M^{pro}-Isoquercetin (MNB) complex formation. RMSF is an important parameter to assess the fluctuation of protein atoms across the time duration from a reference position. This allows us to study the comparative fluctuations in the portion of target protein (residue) before and after the ligand binding. In this study, binding of 10-Hydroxyaloin A and Isoquercetin showed the stabilization of the 40–45 and 140–165 amino acid residues during the simulation time (**Figures 5C, D**). The key amino acids required for the catalytic activity of the test protein fall in these areas. Thus, it might be inferred that the lead phytochemicals tightly bind with the key amino acids and stabilize the active site of the protein. In the previous studies in our laboratory, we found that phytochemical binding mediated lesser fluctuations in target protein amino acid residues (Gupta et al., 2020; Kushwaha et al., 2021c). Radius of gyration (Rg) provides valuable information about the folding of regular secondary structure of the targeted protein into the tertiary or functional structure before and after binding of the ligand/inhibitor molecule. In comparison to unbound protein, the 10-Hydroxyaloin A and Isoquercetin binding mediated decreased Rg value during the simulation period, indicating the compact and stabilized folding in the ligand-bound complexes (**Figures 6A, B**). The binding of a ligand at the active site of a protein surrounding a solvent is a solvent-substitution process. Thus, calculations of Solvent accessibility surface area (SASA) of ligand-bound and unbound protein give important information about the potential ligand binding. Isoquercetin binding mediated significantly decreased SASA value of the complex (in comparison to standard inhibitor–bound and non-bound protein), indicating the stabilized protein structure/active site throughout the simulation period (**Figures 6C, D**). Although the various types of interactions among ligand and targeted protein are involved in the complex stabilization process, hydrogen bond formation plays a significant role in this process. The greater the number of hydrogen bond incidence during the ligand-protein complex formation, the greater the stability of the complex. In the present study, the increased hydrogen formation potential (in comparison to standard inhibitor) of the 10-Hydroxyaloin A and Isoquercetin phytochemicals during the simulation period indicates the potential stability of the protein complex (**Figures 7E–G**). The greater number of hydrogen bond formation in the test compound interaction with the M^{pro} protein corroborates the more negative docking score of the lead compounds in comparison to standard inhibitor. The PCA and free energy landscape results (compact structure and increased centric energy respectively) indicate the compactness of the M^{pro} protein structure after binding the lead molecules at the active site of the protein (**Figures 8**, **9**). The analysis of the various secondary structures of the test protein in the presence of 10-Hydroxyaloin A and Isoquercetin indicated the involvement of β-sheet and 5'-helix in the interaction (**Figure 9**). The DCCM analysis indicated that the 10-Hydroxyaloin A inhibited the amino acid motion in the test protein, but isoquercetin revealed similar pattern of amino acid motions in standard inhibitor–bound protein. Over all, the analysis of the MD simulation trajectory revealed the stable and energetically favorable complex formation in the presence of lead phytochemicals. The MM-PBSA analysis showed the contribution of amino acids in the ligand-protein binding (**Figure 10**). It should be noted that the His41 and Cys145 amino acid residues involved in catalytic dyad were contributing to the interaction of lead phytochemicals and the M^{pro} protein. The results substantiate the potential binding and active site inhibition potential in the lead molecules. Over all, the boiled egg diagram, the bioavailability radar, and the physiochemical properties of the *A. indica* and *A. vera* lead phytochemicals showed the drug-like potential that could be utilized for anti-SARS-CoV-2 drug discovery (**Figure 11**).

CONCLUSION

Plant-derived compounds possess single and/or multitargeted therapeutic potential against various diseases including viral disease. Thus, the computer-based identification of phytochemicals present in the medicinal plants is the need of time to identify potential inhibitors of SARS-CoV-2 drugable targets. The natural compounds possess less toxicity and associated side-effects, which make them a suitable candidate for drug discovery. In the present molecular docking study, we found that the phytochemicals present in *A. vera* and *A. indica* medicinal plants possess significant binding potential at the active site/protein-protein interaction sites of the SARS-COV-2 drugable targets (Spike-protein, RdRp, and M^{pro} proteins). Further, the molecular dynamic (MD) simulation and MMPBSA calculations revealed that 10-Hydroxyaloin A and Isoquercetin phytochemicals present in the *A. vera* and *A. indica*, respectively, stabilize the structure and energy of the M^{pro}-ligand complex. More importantly, the lead phytochemicals showed disruption of His-Cys dyad at the active site of the M^{pro} protein required for its catalytic activity.

In MMPBSA analysis, we found that the His and Cys residues contributed significantly in the binding free energy of the complex. Overall, we conclude that the *A. vera* and *A. indica* Indian medicinal plants could be taken as source of lead anti-SARS-CoV-2 agents for future drug discovery.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SK designed and conceptualized the study. PK, AS, TB, and AY contributed in the data generation. SK wrote the manuscript. The figures and tables were developed by KP, MS, and AS. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.730288/full#supplementary-material

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REVIEW
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A Comprehensive Overview on COVID-19: Future Perspectives

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The outbreak of COVID-19 has proven to be an unprecedented disaster for the whole world. The virus has inflicted billion of lives across the globe in all aspects—physically, psychologically, as well as socially. Compared to the previous strains of β-CoV genera-MERS and SARS, SARS-CoV-2 has significantly higher transmissibility and worst post-recovery implications. A frequent mutation in the initial SARS-CoV-2 strain has been a major cause of mortalities (approx. 3 million deaths) and uncontrolled virulence (approx. 1 billion positive cases). As far as clinical manifestations are concerned, this particular virus has exhibited deleterious impacts on systems other than the respiratory system (primary target organ), such as the brain, hematological system, liver, kidneys, endocrine system, etc. with no promising curatives to date. Lack of emergency treatments and shortage of life-saving drugs has promoted the repurposing of existing therapeutics along with the emergence of vaccines with the combined efforts of scientists and industrial experts in this short span. This review summarizes every detail on COVID-19 and emphasizes undermining the future approaches to minimize its prevalence to the remaining lives.

Keywords: COVID-19, clinical manifestations, epidemiology, future prospects, pathogenesis, treatments

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INTRODUCTION

The influence of viruses and viral infections on human history has been broadly described by the **social history of viruses** ever since the modifications in human behavior during the Neolithic period around 12,000 years ago (Baranowski et al., 2001; Fuchs et al., 2019). This was the period when humans began to expand their agricultural communities and an exponential increase in the spread of viruses leading to becoming endemic was observed the most. With the rapid globalization and anthropogenic activities with time, pathogenic transmission has escalated across the globe and resulted in viral pandemics (Fuchs et al., 2019). It was the mid-19th century that was remarkably known for pathogenic viral outbreaks and their multiplex associations with humans and animal species. This further leads to cross-species transmission, posing a high threat to human health and well-being (Ye et al., 2020).

Abbreviations: ACE, Angiotensin-converting enzyme; β-CoV, Betacoronavirus; BatCov, Bat coronavirus (HKU3); CoV, Coronavirus; COVID-19, Coronavirus disease 2019; FDA, Food and Drug Administration; GMT, Greenwich Mean Time; HCoV, Human coronavirus; hACE, Human Angiotensin-converting enzyme; HIV, Human Immunodeficiency Virus; ILs, Interleukins; IFN, Interferon; WHO, World Health Organization; MERS, Middle East respiratory syndrome; SARS, Severe Acute Respiratory Syndrome; TMPRSS2, Transmembrane protease, serine 2.

Later at the beginning of the 21st century, it was observed that due to the rapid globalization and human activities, pathogenic transmission across continents has escalated and resulted in several pandemics, especially viral pandemics (Ye et al., 2020). The pandemic caused by old diseases, namely plague, cholera, and yellow fever in addition to some emerging diseases such as Ebola, Zika, severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), and COVID-19, makes it the most ferocious century in human history (Ong et al., 2020). As per the reports, these viral pandemics have caused significant mortalities and majorly affected the international economy over the last three decades (Ong et al., 2020). For example, the Ebola viral disease (EVD) identified in 1976 in Central Africa for the first time and its outbreak in 2014–2016 has resulted in more than 40% mortality in West Africa (Control and Prevention, 2014). SARS-CoV infection was first identified in 2003 and has known to be originated from bats and transmitted to humans *via* palm civets (host) in Guangdong Province, China; there were 8422 reported cases including the mortality rate of 11% in 26 countries (of the International, 2020). Similarly, MERS-CoV also originated in bats, transmitted through dromedary camels as an intermediate host reported in 2494 cases with 858 deaths (mortality rate 34%) in 27 countries (Omrani et al., 2015).

Now the biggest threat that the world is facing today is the outbreak of novel coronavirus (COVID-19) that originated in Wuhan, Hubei Province, China, in December 2019 and rapidly spread over the rest of the world in a short time (Boni et al., 2020). It can best be characterized by pneumonia-like symptoms that may further extend up to major hypoxia and several cardiovascular complications (Boni et al., 2020). Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses belonging to the Coronaviridae family and a leading cause of acute respiratory, hepatic, and neurological diseases with variable severities in vertebrates (Wang et al., 2020). They are referred to be the common human pathogens with the tendencies of fleeting recombination and mutation (Wang et al., 2020). It is due to the presence of crown-like spikes on the periphery of these viruses, popularly called coronaviruses (Boni et al., 2020; Wang et al., 2020). These coronaviruses are segregated into four distinct genera based on phylogenetic clustering, namely alpha coronavirus (αCoV), beta coronavirus (βCoV), gamma coronavirus (γCoV), and delta coronavirus (δCoV) (Huang et al., 2020). Among these, α and βCoVs (mainly found in bats and rodents) are known to infect humans; however, γ and δCoVs (found in birds) are known to infect mainly aves and mammals including pigs (Paim et al., 2019). In addition to affecting a vast majority of humans by crossing the inter-species barrier, βCoVs (i.e., SARS-CoV and MERS-CoV) have been marked with the highest mortality rates among all the classes of coronaviruses (Huang et al., 2020). Structurally they are composed of four major proteins: (a) the spike (S) protein, (b) the nucleocapsid (N) protein, (c) the membrane (M) protein, and (d) the envelope (E) protein, playing a pivotal role in mediating the attachment of the virus to the host receptor, its subsequent fusion, and accelerating virus assembly within the host system (Samidurai and Das, 2020). Until 2003, barely two human CoV (HCoV) strains, HcoV-OC43 and HcoV-229E, were recognized, but from 2003 to 2021, the world has experienced havoc and an exponential increase in mortality rates due to the emergence of 5 other deadly strains of coronaviruses: HcoV-NL63, HcoV-HKU1, severe acute respiratory syndrome corononavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, which may cause fatal respiratory infections in humans (Ivanov and Ziebuhr, 2004; Ye et al., 2020). Due to the rapid spread of the disease and its manifestations (i.e., enhanced mortality rate) caused by the newest corona strain viz. SARS-CoV-2 originated from Wuhan, China, in December 2019 has raised the concerns of researchers and clinicians concerns across the world (Long et al., 2020). As a result, the World Health Organization (WHO) in February 2020 has named it coronavirus disease 2019, abbreviated as COVID-19, and on March 11, 2020, the situation was declared a pandemic (Long et al., 2020).

The statistical data on COVID-19 has reported around 4,995,996 confirmed cases of SARS-CoV-2 infection along with 327,821 deaths in 216 countries, and the number is increasing exponentially daily (Nižetić, 2020; Sharma et al., 2020). China, which is an epicenter of SARS-COV-2, has reported 84,520 confirmed cases with 4645 deaths, the United States of America (USA) has 1,528,186 confirmed cases including 92,000 deaths, and India has a maximum number of confirmed cases at 22,362,920 and 242,000 deaths (Sharma et al., 2020). To control COVID-19's superspread event and its impact on global health care infrastructure, the WHO has substantiated on early diagnosis, prevention, social distancing, proper sanitization, and complete lockdown-like strategies. Additionally, the guidelines approved by various national and international authorities about the dos and don'ts have been made available to national and international platforms (Saadat et al., 2020). Apart from this, rigorous efforts being made by our researchers and medical practitioners to disseminate accurate details mimetic to etiology, pathogenesis, clinical course, and protective measures for the widespread COVID-19 disease across the communities, one of the fastest and reliable sources is real-time counts infected cases worldwide (Nižetić, 2020).

In corroboration with the recent editorial in *Lancet* prioritizing the spread of reliable, adequate, and independently scrutinized data and information related to COVID-19 disease among the general audience, the present review abridges all the scientific findings related to the COVID-19 outbreak in one place and thereby minimizes the effort of readers in going through the enormous studies available online.

EMERGENCE AND EVOLUTION OF SARS-CoV-2

Since the outbreak of COVID-19 disease in December 2019 in Wuhan, China, the interest of epidemiologists has piqued in assessing the rationale behind the eruption of SARS-CoV-2 in humans, including the involvement of animal reservoir,

endemic circulation, co-infection, recombination events within RNA segments, and its time of divergence from animal species (Decaro et al., 2021).

In December 2019, when the cases of pneumonia were epidemiologically related to the open-air seafood market in Wuhan, China, the local authorities in China provisioned an epidemiological alert and issued a complete lockdown for a couple of weeks (Decaro et al., 2021). After rigorous research and clinical implications, in January 2020, the scientists at Wuhan obtained a complete genome sequence from the infected people and obtained around 80% sequence similarity with SARS-CoV, confirming pneumonia to be a SARS-induced condition (Zhou et al., 2020). Initially, this novel human pathogen was placed in the Sarbecovirus subgenus of the Coronaviridae family, the family in which SARS falls (Boni et al., 2020). The virus is responsible for more than 8,200 cases from 2002–2003. Later, by mid-January 2020, the virus did super-spread within China, and by mid-March 2020 was labeled as pandemic status (Cucinotta and Vanelli, 2020). This, in turn, enhanced the concern of the medical fraternity to prevent its spread and, at the same time, the researchers across the world were busy identifying the strain affecting millions of lives (Cucinotta and Vanelli, 2020). With the subsequent studies and ongoing reports the virus was named SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV) study group and also named hCoV-19 by Wu et al. (Wu et al., 2020a).

For the first time in a consortium on virological.org led by Zhang on January 10, 2020 (GMT), the advent of the first genome sequence of SARS-CoV-2, Wuhan-Hu-1 assisted the researchers to understand the ancestry of this novel coronavirus (SARS-CoV-2) (Boni et al., 2020; Wu et al., 2020b). Later the data from the bioinformatics analysis has evidenced the homology between SARS-CoV-2 and other members of the coronavirus family, especially with the betacoronavirus 2B (Boni et al., 2020). Thus further studies have been undertaken on SARS-CoV-2 by considering it to be a new member of betacoronavirus 2B lineage infecting humans (Boni et al., 2020). Upon aligning the full-length genome sequence of SARS-CoV-2 and obtainable genomes of beta coronaviruses, scientists observed around 96% of sequence identity within the genomes of SARS-CoV-2 and SARS-like BatCov and RaTG13 coronaviruses. This indicates the bat origin of SARS-CoV-2 or, in other words, SARS-CoV-2 has been naturally evolved from bats (Boni et al., 2020; Naqvi et al., 2020; Xiao et al., 2020; Zhou et al., 2020).

Concurrently, studies have also suggested Malayan pangolins (*Manis javanica*) to be the possible host in the emergence of SARS-CoV-2 infection in humans (Xiao et al., 2020). Pangolins, the scaly ant-eaters belonging to the mammalian order Pholidota, are among the illegally trafficked mammalian species used for food and medicine purposes (Lam et al., 2020). Due to the extensive manhandling of these pangolins, researchers have decided to conduct a study on frozen tissue samples (blood, lungs, and intestine) obtained from 18 Malayan pangolins during an anti-smuggling task by Guangxi Customs officers (Lam et al., 2020). In particular, data from high throughput RNA sequencing has confirmed the Malayan pangolins to be the intermediate host of coronaviruses to humans, and later the readouts from sequence similarity search have demonstrated nearly 85.5–93% identity in the sequences of pangolin coronavirus genome and SARS-CoV-2 (Lam et al., 2020). Hence, the local authorities in China have decided to remove pangolins from wet markets to avoid further zoonotic transmissions (Boni et al., 2020; Lam et al., 2020).

PORTAL OF SARS-CoV-2 ENTRY IN THE HOST CELL

The respiratory tract is considered to be the prominent portal for the ingression of viruses into the mammalian system, due to its direct contact with the external environment (Matrosovich et al., 2004). Therefore, the principal symptoms and complications of SARS-CoV-2 are observed in the respiratory tract at its primary stage (Belser et al., 2013). The viral particles encapsulated in the droplets or aerosols are released from a COVID positive individual when inhaled by a healthy and uninfected person, and the SARS-CoV-2 adheres to the specific cell-surface receptor for the viral protein. In due course it enters into the endosomes and, finally, the viral and lysosomal membranes fusion occurs (Peiris et al., 2003; Belser et al., 2013).

The process of SARS-CoV and SARS-CoV-2 coronaviruses entrance into the host is facilitated by the host cells Transmembrane protease serine 2 (TMPRSS2) and lysosomal proteases (especially cathepsins) through two independent mechanisms: proteolytic cleavage of ACE2 receptor which stimulates viral uptake and cleavage of coronavirus spike glycoproteins which turns on the glycoprotein for host cell entry (Zhang et al., 2021). The host cell entry mechanism of these coronaviruses has been extensively studied and found to be almost similar in the case of both SARS-CoV and SARS-CoV-2 (Zou et al., 2020). The virus entry into the cell is mediated by the spike proteins anchored onto the virus surfaces. The spike protein on a mature virus consists of three receptor-binding S1 heads existing on top of trimeric membrane fusion S2 stalk. Once the virus is inhaled by the healthy individual and enters into the respiratory tract, it is the S1 subunit of spike protein with receptor-binding domain (RBD) which first recognizes the human angiotensin-converting enzyme-2 (hACE-2) as its receptor (Zheng, 2020; Zou et al., 2020). In general, hACE-2 is a membrane-bound protein expressing in several human cells, namely respiratory tract (abundant in the lower respiratory tract), vascular endothelium, cardiovascular tissue, renal tissue, and intestinal epithelia (Wang et al., 2020). After the recognition of hACE-2 by S1, the proteolytic activation of SARS spike protein at S1/S2 boundary is triggered by the activity of cell surface protease TMPRSS2 and lysosomal proteases (cathepsins) (Wang et al., 2020). Their activity causes the dissociation of S1 from S2, and the segregated S2 molecule further undergoes dramatic conformational changes. This in turn activates the glycoprotein for host cell entry, causing ingression or release of viral RNA into the host cytoplasm, followed by a translation of new viral

proteins and affecting nearby cells in the vicinity (Walls et al., 2020; Wang et al., 2020).

The cellular entry mechanism for SARS-CoV and SARS-CoV-2 are reported to be almost similar, with a difference in receptor (hACE-2) recognition and binding potential of RBD units in S1 glycoproteins on the surface of SARS-CoV and SARS-CoV-2. The binding affinity of SARS-CoV-2 with hACE-2 is known to be comparatively higher than that of SARS-CoV. Apart from this, the presence of an extra proprotein convertase (PPC) motif in the spike protein of SARS-CoV-2 also distinguishes it from SARS-CoV (Berry et al., 2004; Wang et al., 2020).

EPIDEMIOLOGICAL TRAITS OF SARS-CoV-2

As per the literature, bats and pangolins are reported to be the primary and intermediate reservoirs for the SARS-CoV-2 strain of coronavirus infecting humans (Zheng, 2020). Apart from this, the animals residing in proximity with humans, especially cats, ferrets, and even golden hamsters, are at high risk of SARS-CoV-2 transmissions (Sharun et al., 2021). Initially, it was reported that the expected roots of transmission for SARS-CoV-2 are mainly droplets and fomites exchange between a nCoV infected and an uninfected, healthy individual (Jayaweera et al., 2020). Later a study has also stipulated the emergence of virus from the surrounding environment (air-borne), potentially affecting an uninfected person upon inhaling the aerosols emitted by an infected person while exhaling, sneezing, shouting, coughing, etc. (Gralton et al., 2011; Liu et al., 2017). The authors have also quoted that SARS-CoV-2 could remain stable in the aerosols for 3 h (van Doremalen et al., 2020). This report on the airborne transmission of respiratory viruses has been considered as the dominant mode of spread, as it was even difficult to demonstrate on ground levels compared to those of droplets and ferrets mediated transmission (van Doremalen et al., 2020).

As per the recent reports, this new human pathogen (SARS-CoV-2) can continue to stabilize in the digestive tract for a longer duration than in the respiratory tract (Xu et al., 2020). Studies on humans have noted the presence of viral RNAs in the excreta of infected people for more than 33 days after they have been detected as COVID negative (Arslan et al., 2020; Wu et al., 2020d). This particular finding substantiates another unprecedented mode of viral transmission, i.e., fecal-oral route of viral distribution in the environment (Wu et al., 2020d). The viral transmission through the fecal-oral route was further confirmed when children with COVID-19 positive tests have reported negative results in nasopharyngeal swabs while their rectal swabs indicated a consistently positive result for COVID-19 infection (Yeo et al., 2020; Wu et al., 2020d). Noteworthy, the discharge of COVID-19 patients' fecal matter may increase the potential risk for wastewater treatment plants (WWTPs), as the virus could embed into the fecal matter and settle in WWTPs (Arslan et al., 2020). Studies have indicated the presence of SARS-CoV-2 in sewage samples in seven cities of Netherlands and Schipol airport at Amsterdam between February and March 2020 (Arslan et al., 2020).

Initially, it was known that SARS-CoV-2 cannot be distributed through intra-uterine vertical transmission from a pregnant woman to an infant, but later the studies have changed the notion by demonstrating the possibility of vertical transmission of the virus in a newborn with few neurological disorganizations whose mother tested positive with SARS-CoV-2 during the last trimester of her pregnancy (Chen et al., 2020; Wang et al., 2020). Likewise, a case was reported wherein anti-SARS-CoV-2 IgM antibodies and IL-6 levels were noted to be comparatively higher than the normal neonates indicating the likelihood of transplacental transmigration of virus from COVID-19 positive mother to the neonate (Dong et al., 2020). There is an availability of a relatively good amount of data stating the perinatal transmission of SARS-CoV-2 in pregnant women, but the chances are quite low as compared to SARS-CoV-1 and MERS (Fan et al., 2020; Parazzini et al., 2020; Wang et al., 2020).

Among all the common and major spread routes for the SARS-CoV-2 virus is direct contact, i.e., person to person contact, public gatherings, and/or crowding at one place as compared to the fecal-oral transmissions, vertical transmissions, and aerosol-mediated spread of virus among the community (Ghinai et al., 2020b; Ghinai et al., 2020a). This viral transmittance is not only limited to human-to-human transmissions but also the pattern of animal to human, human to animal, and animal to animal transmissions being frequently observed in recent days. The best-known instances for animal to human transmission of the primary and intermediate reservoir of SARS-CoV-2 for humans is bats and Malayan pangolins as discussed above. However, transmissions from humans to animals was confirmed when a study indicated the resemblance in viral genetic sequences of SARS-CoV-2 diagnosed in two dogs with that of human SARS-CoV-2 virus (Sit et al., 2020). Hence it was discovered later that animals like tigers, cats, and dogs are on the higher edge of getting infected with the SARS-CoV-2 virus when residing in contact with an infected person for longer (Singla et al., 2020). As the virus can be transmitted from one infected person to another, a similar trend has been observed in the case of animals when for the first time a SARS-CoV-2 positive cat has affected the naïve cat with the same virus and an adult SARS-CoV-2 positive ferret affected a naïve ferret *via* close contact (Wang et al., 2020). See **Figure 1**.

VARIANTS OF CORONAVIRUS

There is a general tendency of viruses including COVID-19 causing SARS-CoV-2 to evolve and gradually change over time (Abdool Karim and de Oliveira, 2021). During the replication process, these viruses every-so-often undergo changes in the genetic code termed as "mutation," giving rise to a new strain of virus called "variant" (Abdool Karim and de Oliveira, 2021). Mutation in viruses is neither new nor unprecedented, it is a commonly occurring phenomenon in almost all viruses having RNA as a genetic material (Manrubia and Lázaro, 2006). It is

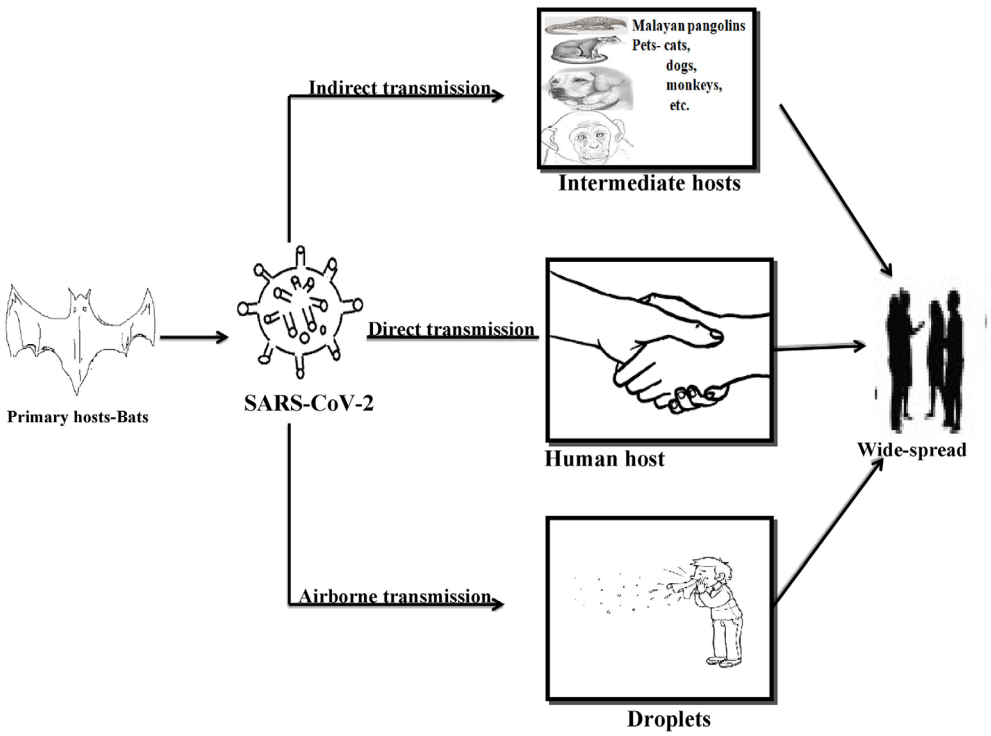

FIGURE 1 | The potential hosts and possible routes of SARS-CoV-2 dissemination.

mostly the geographic separation events that may result in genetically different variants (Manrubia and Lázaro, 2006).

The data from high throughput sequencing analysis has confirmed around 20 mutation events in the genome of SARS-CoV-2 collected in October 2020 over the first strain sequenced in January 2020 (Wuhan-Hu-1) (Fang et al., 2021). The virus has been reported evolving at a rate of $\sim 1.1 \times 10^{-3}$ substitutions per site per year, corresponding to one substitution every 11 days approximately (Martin et al., 2021). This contrasts with the mutations in the HIV occurring at a rate of $\sim 4 \times 10^{-3}$ substitutions per site per year (Andrews and Rowland-Jones, 2017). Based on the aforementioned details, the US government interagency established a variant classification scheme that classifies SARS-CoV-2 variants into three distinct groups:

(A). Variants of Interest (VOI): It can best be defined as an isolate of SARS-CoV-2 with genotypic and/or phenotypic changes compared to the reference genome. It is a variant with discrete genetic markers associated with inducing alterations in receptor binding, minimized neutralization by antibodies generated against previous exposure of viruses, affecting diagnostics and treatment strategies (Covid, 2021). The threshold for defining a VOI is quite low, to support surveillance efforts.

To date, eight different VOIs for nCoV-2 have been reported in the literature. These variants are an outcome of a common mutation, i.e., D614G, first documented in the United States of America (USA) during the initial phase of the pandemic (Control and Prevention, 2021). The variant with D614G mutation in SARS-CoV-2 spike glycoprotein curtails S1 shedding and enhances viral infectivity compared to the viruses without this mutation or with different mutations (Control and Prevention, 2021). Following is the list of VOIs known till date:

(i). B.1.526: This variant of SARS-CoV-2 was first identified in November 2020 and reported to spread at an alarming rate in New York. DNA sequencing analysis has confirmed the presence of B.1.526 sequence in approximately 27% of the total populatiobn of New York City (Annavajhala et al., 2021). The variant arose due to **E484K** and **S477N** mutations in the receptor-binding domain upraising complications associated with resistance to vaccine-elicited and therapeutics (Annavajhala et al., 2021).

(ii). B.1.526.1: The variant for the first time was identified in New York City (October 2020). It is a sub lineage of B1.526.1 with **T95I** and **D253G** spike mutation in nCoV-2 original strain (Wang et al., 2021). Like the parent strain (B.1.526), this variant also imparts potential resistance against monoclonal antibodies and reduction in neutralization by post-vaccination sera (Wang et al., 2021).

(iii). B.1.525: This variant was identified in December 2020 for the first time in Nigeria mainly and the sequence analysis studies later have confirmed its appearance in UK and France as well. It is also called the Nigerian strain of SARS-CoV-2. The variant is thought to be an outcome of E484K with H69–V70 deletion and Q677H mutation in the S1 domain of viral spike protein. The B1.525 mutant strains are attributed to

increased transmissibility, virulence, and immune escape (Ozer et al., 2021).

(iv). P.2: The whole-genome sequencing studies have reported their occurrences in Brazil mainly and in some regions of Manaus since April 2020. This variant is a sublineage of **B.1.128** lineage with **E484K** point mutation in the receptor-binding domain of SARS-CoV-2 S1 glycoprotein. Presence of E484K mutation in the virus-induced reduced neutralization by polyclonal antibodies in convalescent sera (Naveca et al., 2021b; Nonaka et al., 2021; Resende et al., 2021).

(v). B.1.617: It is the most prominent mutation in India now, which was detected for the first time in Maharashtra, India, in February 2021. It is often called a double mutant of novel coronavirus due to two prominent mutations: **E484Q** and **L452R** (Challen et al., 2021). The presence of this variant has triggered the transmittance and drug or vaccine resistance capacity of SARS-CoV-2 in infected people. Later on, UK detected three different but genetically resembling variants of COVID-19 that emerged in Indian: **B.1.617.1, B1.617.2,** and **B.1.617.3** found to be adversely affecting the U.K., U.S., and Israel health sector (Challen et al., 2021; Ferreira et al., 2021).

(B). Variants of concern (VOC) or emerging variants: As per the document issued by WHO on February 25, 2021, outlining the description of VOCs and VOIs, VOC can be expounded as a VOI with a noticeable increase in spread, virulence, and demonstrable impacts on diagnosis/treatment/vaccines (Harper et al., 2021). The mounting data on the initial variant of concerns have identified some of them, and the research is still underway to identify the presence of other unknown VOCs:

(i). B.1.1.7: It was first identified as VOC in December 2020 by COVID-19 Genomics (COG)-U.K. consortium, i.e., COG-UK. B.1.1.7 was recognized as the most frequently spreading variant across the UK during the nationwide lockdown; however, other strains usually demonstrate a significant reduction in their transmission by lockdown or social distancing (Frampton et al., 2021). Thus with a rigorous evaluation of retroactive data, the researcher has confirmed the existence of the variant in circulation since September 2020. The variant is also known as 20I/501Y.V1. Studies on B.1.1.7 stipulate that it is one of the well-versed and highly sequenced VOCs with the highest transmissibility (at a rate of between 40% to 70%), infectivity (30% to 50% higher than other strains), and demonstrable mortality rates (61% to 67%) due to mutation in the Y501 region of S1 protein of the virus (Galloway et al., 2021).

(ii). P.1: This particular variant has been detected in Japan by their surveillance system in 4 travelers who had returned from Brazil (Naveca et al., 2021a). The variant was noted to be emerged due to N501Y mutation associated with higher binding affinity to hACE receptor, **E484** mutation linked to drug/monoclonal antibodies/vaccine resistance, and **K417N/T** mutation responsible for imparting higher receptor binding affinity to virus in combination with N501Y mutation in the spike protein of the virus (Naveca et al., 2021a).

(iii). B.1.1351: The variant is popularly known as "20H/501Y.V2" or "**South African** variant." It was first identified in Nelson Mandela Bay, South Africa, in October 2020, after frontline clinicians were notified about the increased frequency of cases to the Network for Genomic Surveillance in South Africa (NGS-SA), which in turn promoted genomic investigations and analysis (Ellis, 2021). The variant is known to have multiple mutations in spike protein, especially the **K417N, E484K**, and **N501Y** mutations. These mutations make it a variant of concern as it exhibits enhanced transmissibility and resistance to vaccines (Ellis, 2021).

(iv). Cluster 5: Danish public health authorities first identified this variant on mink farms in Denmark and Netherlands on November 5, 2020 (Larsen and Paludan, 2020). After this, Denmark decided to halt all farmed mink in Denmark (Larsen and Paludan, 2020). The emergence of the Cluster 5 variant is due to the notable Y453 F mutation in the SARS-CoV-2 S1 domain, imparting resistance against neutralizing antibodies (Larsen and Paludan, 2020).

(C). Variants of high consequences: The presence of such variants ensures a considerable reduction in the effectiveness of preventive measures or medical countermeasures (MCMs) as compared to the variants in circulation. The relieving part is that currently there is no trace of the presence of such threatening variants reported or that has come forward from any region of the world (Control and Prevention, 2021).

It is very important to keep an eye on the circulation of these variants and to work efficiently on their preventive measures and vaccine suppression strategies. Moreover, it is equally important to keep track of any further mutation in the nCoV-2 genome by genomic surveillances and sequencing methodologies.

CLINICAL MANIFESTATIONS

Patients with COVID-19 may have an extensive range of clinical manifestations. The clinical attributes of COVID-19 may vary from patient to patient ranging from asymptomatic to acute respiratory distress syndrome (ARDS) (Nepal et al., 2020). In general, the disease manifestations of nCoV-2 are dominated by a respiratory condition known as interstitial pneumonia (Nepal et al., 2020). A person infected with SARS-CoV-2 will initially experience fever, sore throat, dry cough, headache, fatigue, restlessness, myalgia, anosmia, and dysgeusia (Nepal et al., 2020). Later it may progress to mild to moderate pneumonia followed by hypoxia, and if left undiagnosed and untreated, then it may further lead to severe complications such as acute respiratory disease syndrome (ARDS) and systemic inflammatory response syndrome (SIRS), and multiorgan failure (MOF) and/or shock (Nepal et al., 2020).

Regardless of respiratory symptoms, unrestricted SARS-CoV-2 infection may stimulate a severe immune reaction called a "Cytokine storm," in which a body dissipates too many cytokines in the blood very quickly and uncontrollably (Zhai et al., 2020). As a result, the production of neutrophils, proinflammatory cytokines (IL-1β, IL-6, TNF-α, etc.), and chemokines (Ccl1, CXCl10, Ccl3, etc.) exceeds the levels of anti-inflammatory cytokines in the body, which in turn leads to multiorgan

damages (Fu et al., 2021). The majority of patients together with the asymptomatic ones are reported to exhibit diffused bilateral pneumonia surrounded with ground-glassy opacity either progressing or coexisting with consolidation (Cui et al., 2020). Histological examinations have also evidenced that the lower respiratory tract holds a higher overall viral load than the upper respiratory tract (Wölfel et al., 2020). Besides this, pathological findings in the infected lungs have also clearly indicated the appearance of proteinaceous exudates in lung tissues as well as in BALF, development of pulmonary edema, bilateral diffuse alveolar damage (DAD), interstitial thickening, infiltration of T cells or inflammatory monocytes, etc. compared to a healthy lung (Wölfel et al., 2020). Moreover, COVID-19 patients have also been marked with relatively low levels of lymphocytic T cells ($CD4^+$ and $CD8^+$) and natural killer (NK) cells, i.e., overall low levels of lymphocyte counts in the blood profile (Varchetta et al., 2021). Two of the most prominent reasons for low lymphocytes are hypokalemia (low potassium levels) and hypophosphatemia (low sodium levels), induced due to the impact of SARS-CoV-2 on patients ACE-Angiotensin-II (ACE-Ang-II) that prevents the degradation of intact Ang-II within the system. As a result, aldosterone production triggers promoting to frequent vomiting, diarrhea, and urination. This in turn affects lymphocyte production in the infected person (Kordzadeh-Kermani et al., 2020). In addition to this, people with co-morbidities like diabetes, hypertension, hypothyroidism, chronic lung diseases (COPD, ALI, etc.), any malignancies, even obesity are at high risk of severe COVID-19 infection (Chen et al., 2020). In conformation with retrospective studies during the first wave of COVID-19 (i.e., unmutated strain), aged people (>50 years) were known to be at high risk, but the variant of SARS-CoV-2 as a leading cause for the second wave has affected youngsters mainly as compared to children and the aged once (Ioannidis et al., 2021). Hence aging cannot be precisely considered a factor for COVID-19 infection (Ioannidis et al., 2021).

Besides the involvement of the respiratory tract, the involvement of other vital organs has also been reported in the literature during infection either directly or indirectly (Catapano et al., 2021). According to the literature emergence of interstitial pneumonia in COVID-19 patients is an additive effect of respiratory complications and GI tract symptoms (Su et al., 2020). The presence of dense hACE-2 receptors on the epithelial cells of the GI tract promotes the viral ingression into the GI tract and causes GI associated abnormalities, namely vomiting, diarrhea, nausea, abdominal pain, etc. (Su et al., 2020). Data from the recent retrospective studies on COVID-19 has indicated that around 10% of the mortalities occurred because of low cardiac reserves, especially coronary artery disease and heart failure (Guzik et al., 2020). The laboratory findings suggest the spike in hs-Troponin-I levels and significant abnormalities in electrocardiogram (ECG) are the leading factors of nCoV-2 associated cardiac injuries, namely acute coronary syndrome (ACS), myocarditis, arrhythmias, venous thromboembolic episodes, and pericarditis (Guzik et al., 2020). Several case reports and series of surveys on hospitalized COVID-19 patients so far evidenced the acute kidney injury (AKI) to be a pivotal reason for COVID-19 related deaths (Lee et al., 2021). Similar to the lungs, kidneys are also the potential site of action for SARS-CoV-2 viruses due to the presence of enriched ACE-2 receptors (Lee et al., 2021). The clarity came after pathological examinations which demonstrated the dramatically higher counts of D-dimer, hematuria, proteinuria, serum creatinine, and microalbumin in the blood and urine samples of SARS-CoV-2 affected patients (Kordzadeh-Kermani et al., 2020). However, the effect of nCoV-2 infection on mammalian hematologic mechanism came into existence after autopsy reports obtained from six COVID-19 patients unveiled the SARS-CoV-2- mediated deterioration of spleen and lymph nodes, implicating the abnormal hematopoiesis, coagulopathy, and clear sign of thrombocytopenia to be the major cause of death (Connors and Levy, 2020; Fox et al., 2020; Qu et al., 2020).

According to Cai and Huang et al. (April 2020), more than 40% of COVID-19 patients have exhibited abnormal liver functioning and liver injuries due to increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Cai et al., 2020). Recently there have been some insights pointing towards the higher affinity of SARS-CoV-2 towards hACE-2 receptors present on cholangiocytes, leading to cholangiocytes deregulation followed by the induction of systemic inflammatory response, a major factor responsible for liver injury in the majority of cases (Kordzadeh-Kermani et al., 2020). Besides this elevated alkaline phosphatases, higher Gamma-glutamyltransferase (γ-GT) and lactate dehydrogenase (LDH) levels in the hospitalized COVID-19 patients have confirmed the impact of COVID-19 on the patient's liver (Cai et al., 2020; Kordzadeh-Kermani et al., 2020). The list of organs and vital parameters getting affected by COVID-19 hasn't been terminated here. The breakthrough occurred when the findings from Hamburg, Germany, confirmed the deleterious effects of SARS-CoV-2 on the mammalian endocrine system, by putting forward a report stating around 68% of the severe COVID-19 cases have presented critically low levels of testosterone and dihydrotestosterone along with elevated levels of estradiol in the males mimetic to higher IL-6 counts. Females with COVID-19 disease demonstrated higher testosterone levels correlated with IL-6 increase (Schroeder et al., 2020). Researchers from the retrospective cohort have already declared the direct impact of nCoV-2 on Leydig cells, a leading cause of testosterone secretions during stress or infection (Zou et al., 2020).

Later, as the knowledge on COVID-19 and its clinical features continued to expand, the observational studies came forward with shreds of evidence on neurological symptoms in the patients infected with COVID-19 (Kordzadeh-Kermani et al., 2020). The clinical implications of COVID-19 on the neurological system have been evidenced for the first time in a clinical report from Wuhan, China, when a 62-year-old severe COVID-19 patient admitted to a local hospital was shown to develop intracerebral hemorrhage which later progressed to intracranial hemorrhage, and the patient died finally (Li et al., 2020b). In corroboration to this was a clinical investigation of a 79-year-old male without any medical background of

hypertension and a 54-year-old woman with a medical history of hypertension suffering from COVID-19 and admitted to a local hospital in Iran, presented with fever, dry cough, and acute loss of consciousness in initial stages of infection. With the progression in severity of infection, the CT brain examinations unveiled an immense intracerebral hemorrhage in the right hemisphere along with intraventricular and subarachnoid hemorrhage in the former case and bilateral sub-acute basal ganglia hemorrhage in the latter case (Nepal et al., 2020). Subsequently, many other studies and case reports have confirmed the invasion of SARS-CoV-2 through the hACE-2 receptors on nasal and oral cavities leading to impaired functioning of sensory neurons, exhibiting neuromuscular symptoms, confirming the involvement of all three nervous systems (CNS, ANS, and PNS) in COVID-19 mediated mortalities (Mao et al., 2020). The nCoV-2 induced complications in CNS are confirmed by the commonly observed symptoms like headache, dizziness, ataxia, epilepsy, and impaired consciousness in COVID-19 patients (Mao et al., 2020). Intense nerve pain, skeletal muscle injury, cranial polyneuritis, neurosensory hearing loss, dysautonomia, neuro-ophthalmological disorders, Guillain-Barré syndrome, and similar signs represent PNS manifestation in COVID-19 infected patients (Andalib et al., 2021). However, the effect of COVID-19 on ANS is linked to cytokine storm as a response to viral ingression within the system. This exceedingly higher level of proinflammatory cytokines due to activation of the sympathetic system leads to vagal stimulation in order to produce anti-inflammatory cytokines to counter the higher levels of proinflammatory cytokines. As a result, symptoms like orthostasis hypotension, postural orthostatic tachycardia condition, and vasovagal syncope have been observed, which confirms the abnormal ANS (Dani et al., 2021).

Under mild COVID-19 conditions, the home-quarantined patients have also experienced erythema, papules, rashes, abnormal scaling patterns, the appearance of chicken-pox like vesicles followed by itching, and burning symptoms on the dermal tissues infrequently (Tsankov and Darlenski, 2020). Of note, references have also indicated the ocular manifestations in the patients with SARS-CoV-2 characterized with lacrimal infection, epiphora, chemosis, and conjunctival hyperemia (Recalcati, 2020). COVID-19 patients have shown interestingly higher levels of LDH, leukocytosis, CRP, and prolactin, which have been considered to be pivotal factors for ocular manifestations of COVID-19 (Wu et al., 2020c) (**Figure 2**).

POST-COVID-19 COMPLICATIONS

The majority of COVID-19 patients recover within a week or two after infection; on the other hand, some of them have been noticed to experience moderate to severe post-COVID conditions (Silva Andrade et al., 2021). The data from COVID-19 hospitals from different nations suggest multiple health issues extending from a week to a month, even in people who did not have symptoms during COVID-19

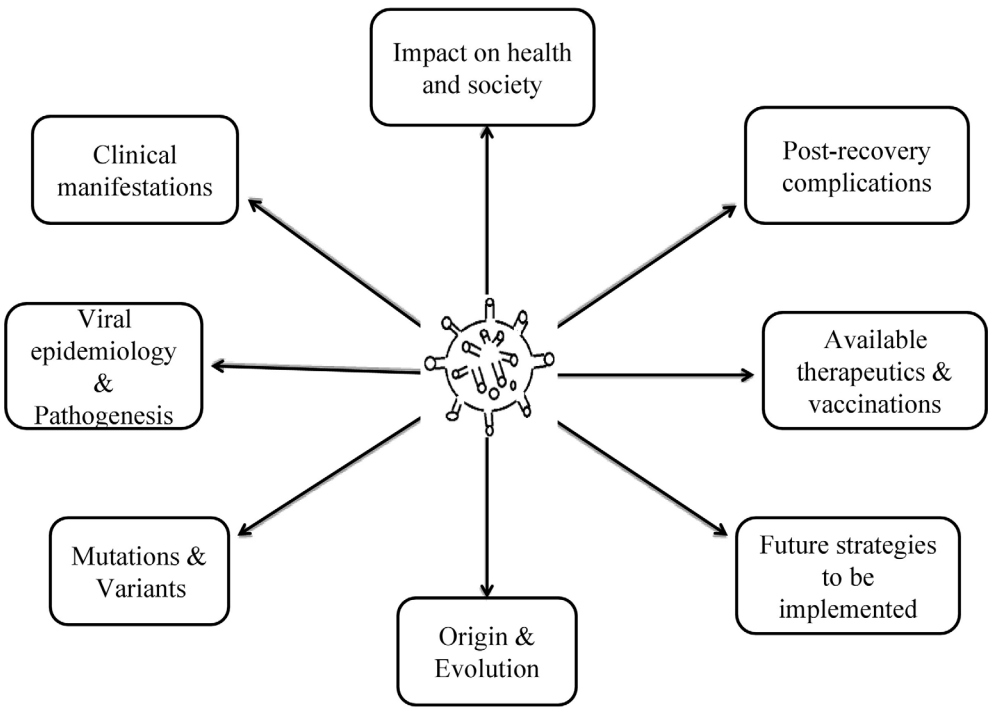

FIGURE 2 | Systematic overview of the complete article.

infection (Silva Andrade et al., 2021). Predominantly it is the fatigue, muscle ache, headache, chest pain, cough, reduced performance, anxiety, lack of concentration, and depression-like symptoms being experienced in the patients with the "Long COVID-19" condition (Silva Andrade et al., 2021).

After diving deep into the health status of patients diagnosed with negative COVID-19, symptoms such as acute disseminated encephalomyelitis, Guillain-barre syndrome (GBS), acute necrotizing hemorrhagic encephalopathy (ANHE), acute neuropathy, etc. arising due to deregulated immune response, cranial involvement, and impaired central and peripheral nervous system are seen nowadays as a post-COVID condition and have become a matter of concern Montalvan et al., 2020; (Sedaghat and Karimi, 2020; Shahmirzaei and Moghadasi, 2021). Apart from these multiorgan effects, especially autoimmune conditions and multisystem inflammatory syndrome (MIS), i.e., a clinical condition in which edema occurs in different regions of the body due to elevated proinflammatory cytokines production have also been reported by the patients after a week of recovering from COVID-19 (Ramos-Casals et al., 2021). Further progress in scientific and clinical investigations, pieces of evidence related to the post-COVID multiorgan condition with an extensive spectrum of manifestations of the disease, is brought into the knowledge. Examples of these multiorgan systems include rapid hair loss, feces with viral load for longer times, persistent palpitation, dyspnea, bone demineralization, uncontrolled diabetes, restrictive pulmonary physiology, elevated D-dimer, and COVID-19-associated nephropathy (COVAN) condition (a foremost pattern of renal injury in the majority of the African population) (Mokhtari et al., 2020; Velez et al., 2020).

Our local health sectors and doctors are required to provide maximum attention to the COVID-19 patients who were on ventilators and hospitalized for longer durations as they are the ones who have consistently been reported to present the most complicated manifestations of the disease such as severe weakness, post-intensive care syndrome (PICS), post-traumatic stress disorder (PTSD), and the most deadly "mucormycosis" infection (Garg et al., 2021; Smith and Rahman, 2020).

Mucormycosis is one of the devastating but rare fungal infections caused by exposure to a group of mucor moulds named mucormycetes. These mucormycetes, members of Mucorales order, are the cluster of fungi existing throughout the environment predominantly in soil rich in decaying organic matter such as animal drugs, composite piles, dead leaves, etc. (Lehrer et al., 1980). These fungi are more common in soil than in the air; similarly, they are noted to be more active in summers than winters or springs (Richardson and Rautemaa-Richardson, 2020). Among these mucormycetes, it is the *Rhizopus* and *Mucor* species that are commonly known to cause mucormycosis (Richardson and Rautemaa-Richardson, 2020). As per the literature, the majority of us are encountered with microscopic fungal spores on a regular basis, as it is almost impossible to 100% circumvent the contact with mucormycetes. Although it is not really harmful to most people, for those with a weak immune system and recovering from some critical pathologies and still breathing in mucormycetes spores, then the possibility of infection in the sinuses, brain, lungs, and to other body parts may occur (Spellberg et al., 2005). This particular fungal infection is noted to be life-threatening in severely immunocompromised individuals or patients with diabetes mellitus (Spellberg et al., 2005).

Depending on its clinical features and anatomical localization, mucormycosis is broadly classified into six distinct classes: (1) Rhinocerebral or rhino-orbitocerebral mucormycosis, (2) pulmonary, (3) cutaneous, (4) gastrointestinal, (5) disseminated, and (6) uncommon presentations (Spellberg et al., 2005). People infected with mucormycosis exhibit the presence of substantial angioinvasion followed by blood vessel thrombosis and tissue necrosis (Ibrahim et al., 2012). As a result, penetration through endothelial cells lining blood vessels and their deterioration is observed commonly (Ibrahim et al., 2012). Based on a retrospective cohort study on the mucormycosis, involvement of some predisposition conditions has been noted and reported in the present review; rhinocerebral, pulmonary, and disseminated muccormycosis are commonly known to affect those with uncontrolled diabetes mellitus (specifically in those with ketoacidosis), extensive burns, iron overload, solid malignancies, treatment with glucocorticosteroids, or patients with neutropenia (Skiada et al., 2020). GI mucormycosis may arise due to malnutrition (Skiada et al., 2020). However, cutaneous/subcutaneous mucormycosis may affect the patients who underwent prolonged hospitalization and have been in touch with catheters and ventilators (Castrejón-Pérez et al., 2017).

The massive upsurge or according to local news "Tsunami of black fungus" has been observed in India in the wake of a spike in COVID-19 cases. Patients infected with this black fungus are presenting with nasal congestion, headache, and facial swelling (Serris et al., 2019). In the worst scenario, fever, cough, and dyspnea are also reported when the infection reaches the lungs (Serris et al., 2019). As per the reports, more than 10,000 patients have been known to be infected with black fungus (mucormycosis) in different parts of India (Bhuyan, 2021; Moona and Islam, 2021). Statisticians have claimed that steroids useful in curtailing the mortality rate in COVID-19 patients are potential factors for mucormycosis infection (Mehta and Pandey, 2020). At the same time, the shortage of oxygen tanks and delivery devices due to fulfilling the demands of exceedingly high COVID-19 cases in India has compelled the local authorities to gather oxygen cylinders without keeping an eye on their sources, resulting in the use of outdated oxygen delivery devices and cylinders contaminated with black fungus colonies delivered to the local hospital authorities (Carter and Notter, 2021; Feinmann, 2021). Other possible risk factors could be steam inhalation abuse, genetic pre-disposition, higher use of antibiotics, poor oral-nasal hygiene, repeated use of the same mask, etc. (Spellberg et al., 2012).

There are not much data available on the treatment and preventive measures for black fungus so far; however, experts are only left with either advising the use of Amphotericin B and posaconazole, or isavuconazole, or mostly the employment of surgical procedures to remove infected or dead tissue (Ochi et al., 1988).

IMPACTS OF COVID-19

COVID-19 has frequently affected day-to-day life and decelerated the global economy by freezing world trade and moments in multiple ways (Chakraborty and Maity, 2020). Despite these, the biggest threat that the world is facing today is to slow down the mortality rates due to the rapid spread of SARS-CoV-2 and its associated manifestations. The complications of modern medicine and research have been aggravated by the emerging variants of nCoV-2, influencing the responses of the drugs and vaccines designed so far (Munzig, 2019). There are reports available that evidence the long-term psychological impact of COVID-19 on people even after being cured of it (Saladino et al., 2020).

The havoc caused by the COVID-19 pandemic has pushed the world into prolonged exposure to stress due to a sense of helplessness, lack of freedom, and separation from our dear ones (Saladino et al., 2020). As an aftermath, psychological disturbances, depression, anxiety, and inability to tackle negative emotions leading to suicidal attempts have become a subject of concern (Saladino et al., 2020). The group which is majorly affected due to this is school-going children, college students, earning youngsters, and the health professionals (Saladino et al., 2020). As per a recent survey on 1143 parents and children aged between 3–18 years in Italy and Spain, parents experienced drastic emotional and behavioral changes in their children during lockdown (Orgilés et al., 2020). Parents reported that their children have difficulty in concentrating, few of them have expressed consistent irritability, complaining of boredom, sensing uneasiness, and loneliness throughout the quarantine (Orgilés et al., 2020). On contrary, the observations in the parents have exhibited some worrying responses, including: most of the parents have undergone depression due to loss of their wages and earning resources, in some cases relationships among the couples have also been compromised, and staying disconnected for longer (Orgilés et al., 2020). In corroboration to this, the data collected from a small survey in China during the initial stages of quarantine due to COVID-19 also demonstrated its worsening impact on the socio-behavioral and psychological tendencies of college-going students and earning individuals especially (Li et al., 2020a).

Apart from this, literature has also emphasized the mental state of healthcare workers (HCWs) and health professionals, who have equally been affected and registered with a high level of stress due to soaring COVID-19 cases (Garcia-Castrillo et al., 2020; Saladino et al., 2020). Ever since the emergence of SARS-CoV-2, this particular segment of people is engrossed with direct contact of the COVID-19 patients and loaded with enormous responsibilities, making them suffer from a high level of psycho-physical stress (Mohindra et al., 2020). This causes them to enter into secondary traumatic stress disorders, emotional and physical exhaustion, and sometimes a sense of helplessness is observed when sufficient resources and treatments lack to save lives (Mohindra et al., 2020; Saladino et al., 2020).

Therefore, to combat these alterations in the socio-psychological behavior of the majority of the population across the globe, World Health Organization (WHO) and Centers for Disease Control and Prevention (CDCP) have advocated specific conventions on the correct usage of health protection to curtail the distress and anxiety in the communities getting affected during the pandemic (Saladino et al., 2020). Besides this, local governments have also decided to avail psychotherapists to provide psychological support online and help the youngsters and HCWs to deal with their challenges (Saladino et al., 2020).

TREATMENTS AVAILABLE

The global threat that prevailed due to this emerging SARS-CoV-2 virus has been a challenge to the health sector and medicine due to lack of information on specific anti-viral therapies or pharmacological entities to prevent nCoV-2 infection. As emergency medicine, doctors are relying on oxygen therapy, extracorporeal membrane oxygenation (ECMO), glucocorticoid supplementation (Dexamethasone), a common antibiotic, and antifungal treatments (van Paassen et al., 2020). Refer to **Table 1** (Han et al., 2020; Madjid et al., 2020; Zheng, 2020; Ardestani and Azizi, 2021; Malgotra and Sharma, 2021; Shamsi et al., 2021).

The increasing number of cases and deaths due to COVID-19 has raised the concerns of local governments in all the nations across the globe. This has further created immense pressure on researchers and clinicians to expand trials on unknown or new drug moieties. According to the undergoing clinical trials the Food and Drug Administration (FDA) has recommended and allotted **"emergency use authorization (EAU)"** certification only to the three types of vaccines:

1. **mRNA-based vaccines:** exhibit an encouraging alternative to conventional vaccine approaches with their expeditious development capacities, low manufacturing investments, high potency, and secure administration (Verbeke et al., 2021). mRNA vaccines carry material from the COVID-19 causing virus that instructs our cells to synthesize a harmless protein that is unusual to the virus. Once the copies of such proteins are prepared by the cells, the genetic material from the vaccine is destroyed. Immediately after this body recognizes no more need for those proteins, so it starts generating relevant T- and B- lymphocytes to store the memory of how to combat the encounter of the body with the same virus for the next time (Verbeke et al., 2021).
2. **Subunit vaccines:** Protein subunit vaccines also called "A cellular vaccines" are considered to be the safest vaccine types over other categories. Instead of injecting a whole pathogen to trigger an immune response, they include purified, harmless protein fragments of the viral or bacterial pathogen selected especially for stimulating immune cells. After having sufficient copies of the desirable protein, our body cells then promotes the production of T- and B-lymphocytes for the memory to protect the body from future attack of the same virus (Kaur and Gupta, 2020).
3. **Viral vector-based vaccines:** Viral vector vaccines employ live viruses to convey DNA into host cells for synthesizing antigenic proteins that can further be tailored to trigger the range of immune responses, especially the production of antibodies, cytotoxic T lymphocytes, T-helper cells, etc.

TABLE 1 | The list of potential therapeutics against clinical manifestations of COVID-19 in use and their side effects.

S.No.	Drugs	Primary manufacturer (Year of approval)	Primary target condition	Route of administration	Mode of action	Recommended dose	Complications/ Limitations
1.	Ribavirin (Avigan)	International Chemical and Nuclear Cooperation (ICN)/ Valeant pharmaceuticals (1970)	Pediatric respiratory syncytial virus (RSV) infection	Intravenous (I.V.)	Inhibits both RNA and DNA viruses, prevents mRNA capping, and has anti-MERS-CoV activity as well.	Adults: 500 mg/ time, twice or thrice a day for not more than 10 days.	Anxiety, insomnia, possibility of hemolytic anemia, etc.
2.	Remdesivir (RDV)	Gilead Sciences, Inc. (2009)	Hepatitis C virus (HCV) and RSV	Intravenous (I.V.)	Halts viral genome replication by inhibiting RNA-dependent RNA polymerases (RdRp) activity.	Adults:100 mg once daily for 5–10 days.	Black fungus, altered blood pressure, nausea, etc.
3.	Lopinavir-Ritonavir	Lopinavir by Abott laboratories (1997) and Ritonavir by Abb Vie Inc. (1996)	Human immunodeficiency virus (HIV)	Oral	Inhibits cytochromes (CYP3A4 and CYP2D6) and P- glycoprotein. Prevents Gag-Pol polyprotein breakdown and thereby increases plasma concentrations of anti-viral drugs.	Adults: 400 mg/ 100 mg each time, for not more than 10 days.	Muscle cramps, vomiting, acidity, insomnia, etc.
4.	Chloroquine	Discoverer and primary manufacturer- Bayer laboratories (1934)	Malaria	Oral	Interferes with endocytic pathway, seizes sialic acid receptors, resists pH mediated spike (S) protein cleavage at hACE-2 binding site and prevents cytokine storm.	Adults: 500 mg/ d, orally, 5–7 days, in addition to standard care.	Loss of appetite, weakness, skin rashes, itching, etc.
5.	Darunavir-Cobicistat	Darunavir- Tibotec pharma. In association with Johnson & Johnson (marketed in 2006) and Cobicistat- Janssen pharmaceuticals (2012)	Human immunodeficiency virus (HIV)	Oral	Selectively blocks the cleavage of viral-encoded Gag-Pol polyprotein in virally infected cells, thereby inhibiting viral replication	Adults: 400 mg and 100 mg, respectively, twice a day for 14 days, in addition to standard care.	High blood pressure, frequent urination, clay colored stool, possibility of jaundice, etc.
6.	Favipiravir	Glenmark pharmaceuticals (2014)	Human influenza A and B infections	Oral	Halts viral genome replication by inhibiting RNA-dependent RNA polymerases (RdRp) activity.	Adults: 1800 mg bid on day 1, followed by 800 mg bid on days 2–14.	Diarrhea, elevated uric acid, reduced leukocytes, etc.
7.	Abidol	USSR Research Center for Medical Chemistry (2010-2011)	Human influenza A and B infections	Oral	Prevents the fusion of viral lipid membranes with host cell surface receptors, and thereby inhibits replication.	Adults: 200 mg, twice for 5 d.	Constipation, nausea, dizziness, vomiting, etc.
8.	Oseltamivir (Tamiflu)	Gilead Sciences (2015)	H1N1 flu and its subtypes	Oral	Selectively inhibits neuraminidase enzyme and thereby preventing viral entry to host cells, viral release from infected cells, and further spread in the body.	Adults: 75 mg, twice for 10 d.	Insomnia, eye redness, nose bleeding, dizziness, and diarrhea mainly.
9.	ASC09F	Ascletis Pharmaceuticals Co., Ltd (under trials)	HIV protease inhibitor	Oral	Inhibits viral proteases and in turn blocks viral replication.	Adults: 400 mg, twice for 14 d.	Hepatotoxicity, retinal damage, nephrotoxicity, and cardiotoxicity.
10.	2-Deoxy-D-glucose	Institute of Nuclear Medicine & Allied Sciences (INMAS), Defence Research and Development Organization (DRDO)- Dr. Reddy's Laboratory (2021)	Tumor	Oral	It accumulates selectively in the virus-infected cells and prevents viral replication by suppressing glycolysis by competitively inhibiting hexokinase 2 (HK2) as minimizing ATP synthesis for viral synthesis within the host cell.	Adults: 2.34 g, in dissolved form (water), for 10 d.	Still under trials, cannot be recommended to people with respiratory distressed conditions, allergic, or high diabetes.

(Crommelin et al., 2021). In other words, such vaccines generally embody live attenuated viruses which can genetically be engineered to carry DNA encoding antigens (protein) from a different organism (Crommelin et al., 2021). Therefore, with the combined efforts of research and development (R&D) sectors at present, we have some efficient anti-viral vaccines from different pharmaceuticals to guard us against the pandemic. Refer to **Table 2** (Food and Drug Administration, 2019; Chagla, 2021; Kashte et al., 2021; Kumar et al., 2021).

Almost a year after the start of the COVID-19 pandemic, the U.S. Food and Drug Administration authorized the emergency use of the first nCoV-2 vaccine (Quinn et al., 2020). Raising vaccines and conducting vaccination drives has been realized to be the foremost requisite of public health intervention. Indeed, it is the

TABLE 2 | Update on FDA authorized COVID-19 vaccines for emergency use.

S.No.	Vaccines (Code names)	Manufacturer	Type of the vaccine	Efficacy rate	Doses/ Shots	Side effects	FDA authorization/Approval
1.	BNT162b2	Pfizer- BioNTech Ltd., USA	mRNA-based vaccine	94–95%	2 shots (21 days apart)	Site of vaccination-pain, erythema, swelling. General side-effects: tiredness, muscle pain, headache, nausea, and mild fever for few days.	Yes (Emergency Use Authorization (EUA))
2.	mRNA-1273	Moderna TX, Inc., USA	mRNA-based vaccine	94–95%	2 shots (28 days apart)	At the place of vaccine: pain, redness, swelling of the lymph node. General side-effects: severe allergic reaction, fatigue, muscle pain, headache, vomiting, and mild fever for few days.	Yes (Emergency Use Authorization (EUA))
3.	JNJ-78436735	Johnson & Johnson's-Janssen pharmaceuticals, USA	Viral vector-based vaccine	75%	1 shot	At the place of vaccine: pain, redness, swelling of the lymph node. General side-effects: fatigue, muscle pain, headache, vomiting, and mild fever for few days.	Yes (Emergency Use Authorization (EUA))
4.	AZD1222 (Covishield- India And Vaxzevria-Europe)	Oxford-AstraZeneca, USA and Serum Institute, India	Viral vector-based vaccine	64%	2 shots (28 days apart)	At the place of vaccine: pain, redness, swelling of the lymph node. General side-effects: vomiting, diarrhea, dizziness, etc. insomnia for few days to weeks.	Yes (Emergency Use Authorization (EUA))
5.	NVX-CoV2373	Novavax and Coalition for Epidemic Preparedness Innovation (CEPI)	Subunit and viral vector-based vaccine	89%	2 shots (28 days apart)	At the place of vaccine: pain, redness, swelling of the lymph node. General side-effects: vomiting, diarrhea, dizziness, etc. insomnia for few days to weeks.	Yes (Emergency Use Authorization (EUA))
6.	Sputnik V	Gamaleya Research Institute, Russia	Viral vector-based vaccine	92%	1 shot	Site of vaccination: pain, erythema, swelling. General side-effects: tiredness, muscle pain, headache, nausea, and mild fever for few days.	Yes (Emergency Use Authorization (EUA))
7.	BBV152/ Covaxin	Bharat Biotech, India	Inactivated vaccine	81%	2 shots (28 days apart)	At the place of vaccine: pain, redness, swelling of the lymph node, itching. General side-effects: mild fever, vomiting, diarrhea, malaise, dizziness, etc. insomnia for few days to weeks.	Pending

only way to stay protected during the pandemic. Again, this incredible and prolific outcome of researchers' great input has been found to be associated with a limitation, known as "vaccine hesitancy" (Sallam, 2021). According to the World Health Organization (WHO), vaccine hesitancy is a retarded acceptance or rejection of vaccine despite the ongoing vaccination drives; it has been labeled as among the top-10 global threats of 2019 (Recio-Román et al., 2021). A significant proportion of the U.S. population has experienced a pattern of hesitancy against this first COVID-19 vaccine (Coustasse et al., 2021). This skeptical attitude of individuals against the non-authorized SARS-CoV-2 vaccine has been more aggravated by the previous experience from the approved flu A and B vaccine reported with minimal acceptance and in turn introduced an anonymous distrust among the community (Razai et al., 2021). Additionally the political influence and fake media announcements have also played a considerable role in manipulating the notions of the general public on available COVID-19 vaccine (Mills et al., 2020; Razai et al., 2021). As per a small survey conducted on 10-item vaccine hesitancy scale developed by WHO SAGE working group in Canada, China, Ethiopia, and Guatemala, maximum hesitancy towards any novel vaccine is expressed by parents of the middle age group (Wagner et al., 2021). Around 19% of the total parents within any country have expressed their distrust against un-authorized or non-FDA approved vaccines, making the vaccination drive challenging (Wagner et al., 2021).

Indeed the rationale behind vaccine hesitancy has been categorized into three categories: (1) lack of trust on the efficacy and safety profile of the vaccine, (2) missing complacency, i.e., confidence on not perceiving vaccine preventable diseases (VPDs) in the future, and (3) inconvenience due to managing authorities responsible for vaccination in terms of accessibility and unavailability of vaccine, unorganized vaccination pattern, inequitable distribution of vaccine, appeal of immunization services such as time, place, language, and cultural contexts (Razai et al., 2021).

Therefore, to overcome this COVID-19 induced catastrophic moral failure of the world, first we need to untidily work on pushing the equitable production and equitable distribution of vaccine to every single individual across the globe at our earliest convenience. Second, we need to keep on track on boosting our testing and tracing, oxygen supplies, and therapeutic and public health measures approaches.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

As the COVID-19 pandemic continues to unfurl, the complications of the general public and health sector crisis are presumed to continue. A subsequent increase in cases and flaring death cases due to the outbreak of this disease created

unprecedented havoc initially in most afflicted nations such as China, Italy, the United States, and Iran, and eventually to the entire world. Earlier the WHO and local governments of all the countries decided to impose global lockdown to minimize social gatherings and in-person contact with the thought of preventing SARS-CoV-2 distribution. Later it was reported that the virus can spread even through the aerosols in the atmosphere and can be transmitted from pets to humans and vice versa. This has further compounded an inevitable stress on researchers and doctors and poses a serious threat to public health.

Henceforth, the need for enormous research and meta-analysis was realized and the significant findings from those studies to justify the reason for viral spread, possible preventive measures, and future approaches to be adopted made available to the general audience in the form of distinguished articles in pieces. Therefore, to save the precious time and effort of our readers, the authors in the present review have strived to gather, compile, and place every possible detail on COVID-19 disease from different sources and available literature in one place. By diving deep into the details shared in retrospective cohort studies and considering the present scenario, the current review has intensively discussed the origin of COVID-19, its pathogenesis, epidemiology, possible routes of SARS-CoV-2 invasion and zoonotic dissemination, virus variants, its implications on mental as well as on physical health, post-COVID-19 side-effects, and details on pros and cons of available curatives (therapeutics and vaccines) across the globe.

Depending on the rate of mortalities and the catastrophe caused by nCoV-2 infection, the production and administration of vaccines should be prioritized by limiting the usage of corticosteroids to COVID-19 patients to prevent the ferocious side effects, especially mucormycosis infection. We should also speed our trials on ongoing therapeutics with a special emphasis on some minimally studied nanoparticles, bio-flavonoids, nano-nutraceuticals, etc., with reported antiviral attributes to obtain certain novel and efficient pharmacological moieties to combat COVID-19 and post-COVID-19 clinical manifestations with minimal or no side effects.

AUTHOR CONTRIBUTIONS

RR contributed to the study design and concept. RR, AT, NK and NG were involved in the study organization. RR and AT wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Genomic Variations in the Structural Proteins of SARS-CoV-2 and Their Deleterious Impact on Pathogenesis: A Comparative Genomics Approach

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A continual rise in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection causing coronavirus disease (COVID-19) has become a global threat. The main problem comes when SARS-CoV-2 gets mutated with the rising infection and becomes more lethal for humankind than ever. Mutations in the structural proteins of SARS-CoV-2, i.e., the spike surface glycoprotein (S), envelope (E), membrane (M) and nucleocapsid (N), and replication machinery enzymes, i.e., main protease (M^{pro}) and RNA-dependent RNA polymerase (RdRp) creating more complexities towards pathogenesis and the available COVID-19 therapeutic strategies. This study analyzes how a minimal variation in these enzymes, especially in S protein at the genomic/proteomic level, affects pathogenesis. The structural variations are discussed in light of the failure of small molecule development in COVID-19 therapeutic strategies. We have performed in-depth sequence- and structure-based analyses of these proteins to get deeper insights into the mechanism of pathogenesis, structure-function relationships, and development of modern therapeutic approaches. Structural and functional consequences of the selected mutations on these proteins and their association with SARS-CoV-2 virulency and human health are discussed in detail in the light of our comparative genomics analysis.

Keywords: severe acute respiratory syndrome coronavirus-2, coronavirus disease 2019, single amino acid substitutions, SARS-CoV-2 mutations, SARS-CoV-2 pathogenesis

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a seventh strain and the third member of the coronavirus family, has rapidly spread all across the globe since 2019 and has been a leading cause of death worldwide (Seo et al., 2020). The urgency and health crisis forced the World Health Organization (WHO) to enforce a state of health emergency and declare it a pandemic

(Jebril, 2020). People with existing comorbidity and those belonging to the elderly were more prone to this infection earlier. Still, now many young individuals are losing the battle to Coronavirus disease 2019 (COVID-19) (Ruan, 2020; Brookman et al., 2021). Such changes in the patterns of SARS-CoV-2 infection as compared to the previous strains of coronaviruses and among different variants of SARS-CoV-2 has been attributed to the mutations of the virus in the Spike (S) protein, a part of the structural component which allows it to enter into the host cells (Wan et al., 2020). Currently, the total number of confirmed cases across the world as of now stands at > 219 million, whereas 4.5 million people have died (assessed on 11th September 2021) (Organization, 2020). Many countries have witnessed this expeditious spread of infection in the form of different waves occurring after a certain time interval (Asrani et al., 2021c; Boroujeni et al., 2021; Iftimie et al., 2021). Many scientists have warned against the upcoming peaks of the current waves and the arrival of new waves, which are yet to come in several countries in the future (Lai and Cheong, 2020). The incubation period of this virus is said to be between 10-14 days. Therefore, immediate diagnosis post-viral entry is not possible, putting undue pressure on the healthcare infrastructure and medical facilities. Diagnosis is mostly performed through reverse transcription real-time – polymerase chain reaction (rRT-PCR) approach, but it takes time to provide results (Singh et al., 2020a; Singh et al., 2020b; Vo et al., 2020; Asrani et al., 2021a). Rapid antigen test is also being used in certain parts; however, rRT-PCR is a more accurate procedure to be followed for the diagnosis (Asrani et al., 2021b). The serious complications of this virus have led to the complete lockdown in major parts of the world, leading to physical and psychological effects on their citizens (Ju et al., 2021; Kim et al., 2021; Van Vo et al., 2021).

SARS-CoV-2 mediates its entry into the host *via* the S protein of the virus, which interacts with the ACE2 receptors on the host cells (Lan et al., 2020). In this positive sense, a single-stranded RNA virus escapes the host's innate and adaptive immune response, causing overproduction of cytokines leading to the formation of cytokine storm (Song et al., 2020). Patients in serious conditions have shown an alleviated expression of IL-2, IL-7, IL-10, IP10, MIP1A, MCP1, G-CSF and TNFα cytokines (Huang C. et al., 2020). The death is mainly observed to be caused by pneumonia affecting the patient's respiratory system (Xu et al., 2020). Along with acute respiratory distress syndrome, COVID-19 causes the manifestation of acute heart injuries, heart failures, inflammation leading to sepsis and multi-organ dysfunction in individuals in chronic cases (Wang D. et al., 2020). The virus was initially thought to spread through droplets of infected individuals *via* sneezing or coughing; however, recent reports claim their airborne transmission (Zou et al., 2020; Tang et al., 2021).

The virus possesses four structural proteins- spike (S) protein that helps in attachment of the virus to the host cells ACE-2 receptors (Kandeel et al., 2018); membrane (M) protein typically involved in the formation of viral membrane for enclosing the mature virus particles (Neuman et al., 2011); nucleocapsid (N) protein involved in the formation of a viral protein coat, i.e., N which surrounds the genetic material of the virus (Risco et al., 1996); and envelope (E) protein which is involved in the formation of the envelope that assembles the virion particles (Ruch and Machamer, 2012). The following gene arrangement has been observed in SARS-CoV-2 structural analysis: 5' untranslated region (UTR) [non-structural genes (ORF 1a/ ORF1b replicase gene), structural genes (S, M, E, and N) and accessory genes (ORF 3, ORF 6, ORF 7a, ORF 7b, ORF 8, ORF 9b)] 3' UTR (Song et al., 2019; Asrani et al., 2020). Replicase genes account for the synthesis of non-structural proteins (NSPs). Sixteen NSPs assist in the replication and packaging of the virus (Naqvi et al., 2020). Accessory proteins usually differ among the different strains of Coronaviruses (Li et al., 2020). SARS-CoV-2 shares more than 80% genomic similarity to the previous SARS-CoV strain that caused an outbreak in 2003 (Asrani et al., 2020; Malik, 2020). Thus, it is known to exhibit a similar replication process as observed in the previous cases.

Now, different mutant strains of this virus have been identified from different parts of the world, such as B.1.1.7 variant of SARS-CoV-2 was originally acknowledged in United Kingdom (UK), B.1.351 variant from South Africa, B.1.1.28 variant from Brazil, B.1.36 variant, N440K and E484Q mutations from India however; all these variants have now been identified and cultured following their spread to different parts of the world (Tang J. W. et al., 2020; Islam et al., 2021; Planas et al., 2021). Apart from these single-site mutations, few variants have been reported to have double and triple mutations. B.1.617, a double mutant variant that originated from a combination of previously identified Coronavirus variants L452R and E484Q, has been found to cause major deaths in certain parts of India (Cherian et al., 2021). A triple mutant (B.1.618) strain was recently found to cause major outbreaks and deaths in the Bengal region in India, leading to the worst COVID-19 outbreak (Huh et al., 2021).

Since the mutation rate of SARS-CoV-2 is very high, it is important to identify the major sites in its genome that show potential in mutating further and posing a risk to humankind (Chen J. et al., 2020). It is also necessary to identify the mutation types that have occurred predominately to understand the selection pressure on this novel coronavirus strain (Presti et al., 2020). In this article, we have performed mutational analysis on different proteins specific to SARS-CoV-2. We have explored the structural and functional consequences of the selected mutations on the protein structures and their interaction with respective binding partners. The association between SARS-CoV-2 virulence properties and its effect on human health has also been discussed subsequently so that different mutations that may happen in the future and their implications on humanity could be assessed.

The presence of the SARS-CoV-2 S protein assists in attaching the virus to the host cell membrane (Letko et al., 2020; Walls et al., 2020). It belongs to transmembrane (TM) glycoprotein class I and is trimeric in structure (Hoffmann et al., 2020). The activation of S protein occurs by TM protease serine 2, which is present on the host cell membrane. Post-viral entry,

the release of viral RNA, translation of polyproteins and assembly of replicase-transcriptase complex for replication and transcription of viral genome occurs. This results in the synthesis of structural proteins assembled, packed and released from the host cells (Fehr and Perlman, 2015). S protein plays an important role in recognizing the ACE2 receptor, attaching virion to the host cell, and their subsequent coronavirus entry induced pathogenesis (Lan et al., 2020; Wang Q. et al., 2020). The S proteins are common to many coronaviruses and other members of the influenza family, including HIV, Ebola virus, influenza virus, paramyxovirus etc. (Hoffmann et al., 2020; Huang Y. et al., 2020).

The size and shape of the virus are maintained by the action of the most abundant structural protein in a virus referred to as M proteins (Mahtarin et al., 2020). These are membrane glycoproteins that are conserved among the β-coronaviruses (Bianchi et al., 2020). They have embedded inside the lipid bilayer and consist of an amino-terminal (NH_2) domain at the extracellular region and a cytoplasmic domain (COOH) within the viral cell (Mousavizadeh and Ghasemi, 2020). M proteins have 222 amino acid residues in length, and they exhibit a conserved sequence suggesting a common structure of these proteins among different variants (Tang T. et al., 2020). Although higher conservation in the M protein sequence was observed among BAT-CoV, SARS-CoV and SARS-CoV-2 through multiple sequence alignment (MSA) studies, despite this, great variability was observed within the sequence of MERS-CoV suggesting their divergence from the traits shown by other members of coronaviruses (Naqvi et al., 2020).

M proteins usually interact with other (structural, non-structural, and accessory) proteins of the virus to mediate several functions. One of the main features of M protein is that it helps assemble structural proteins (S, E, and N) required for virus budding (Neuman et al., 2011; Schoeman and Fielding, 2019). These interactions between M, N, and E proteins help form virus-like particles (VLP), their intracellular trafficking, and subsequent release from the host cells (Siu et al., 2008). The stabilization of the viral RNA-N protein complex is maintained by the interaction of M with N structural proteins (Astuti, 2020). Similarly, they also interact with S protein and help in their incorporation into the virion. Their interaction is also observed during viral attachment to the host cells and in the regulation of entry processes (Naskalska et al., 2019). M proteins show self-association behavior, and their protein-protein interactions account for their ability in processing, modification and trafficking of structural proteins for assembling virus particles before release (Li et al., 2021).

Experiments involving the deletion of E protein from a highly pathogenic strain of SARS-CoV showed attenuated properties, which could be a basis of an effective vaccine against the virus (Dediego et al., 2008; Netland et al., 2010; Fett et al., 2013); however, reversions into the virulent form were reported when similar experiments were performed in cell cultures and *in-vivo* (Jimenez-Guardeño et al., 2015). Soon after, stable vaccine candidates in mice were identified by introducing deletion mutations in the C-terminal region without interrupting the PDZ binding motif (PDM) (Jimenez-Guardeño et al., 2015). Therefore, E protein serves as an excellent candidate for vaccine development in comparison to the other structural proteins (Mandala et al., 2020).

Among all structural proteins, N protein is a potent immunogen whose expression increases during infection (Shang et al., 2005; Liu et al., 2006). Most of the serological assays for the coronavirus diagnosis rely on identifying N proteins during the diagnostic procedures (Ahmed et al., 2020). More N protein-specific antibodies were detected in the serum of patients infected with SARS-CoV (Tan et al., 2004). These antibodies were more persistent and highly sensitive than other structural proteins within serum (Shi et al., 2003). Post-viral infection, these proteins bind to the viral RNA genome and play a major role in forming a ribonucleoprotein core and assist in their replication, assembly, and subsequent release from the cells to infect the new host (Ji et al., 2020). In complex with genomic RNA of the virus, N proteins provide stability and improve viral transcription and assembly (Mcbride et al., 2014). In addition to this, they also assert their role in mediating the viral life cycle (Chang et al., 2014).

To get deeper insights into the mechanism of pathogenesis, we have performed extensive sequence and structure analysis of structural and enzymatic proteins of SARS-CoV-2. The emergence of new variants and their harmful impact on human health concern healthcare experts and drug/vaccine development. In such context, our findings establish gene to disease relationships and provide the molecular basis of pathogenesis.

MATERIALS AND METHODS

Mutational and Structural Data

The mutational data for the SARS-CoV-2 proteins, i.e., S, E, N, and M^{pro} was fetched out from the NCBI Virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/scov2_snp). The structural coordinates of all four proteins were taken from the Protein Data Bank (PDB). The sequence information was taken from the UniProt database.

Mutational Analysis

To study the impact of the reported mutations on the S, E, N, and M^{pro} proteins structure, we have performed a structure-based analysis using various bioinformatics tools, such as DynaMut2 (Rodrigues et al., 2021), mCSM (Pires et al., 2014), CUPSAT (Ham, 2020), MAESTROweb (Laimer et al., 2016), SDM (Ju et al., 2021). MAESTROweb, mCSM, CUPSAT. SDM provides Gibbs free energy values ($\Delta\Delta G$); The change in free energy during the unfolding of a kinetically stable protein is described by this $\Delta\Delta G$ value. Sometimes the mutation in proteins differentiates the free energy landscape between the mutant and the native protein. This variance in the free energy landscape is why the mutation affects the stability of a protein. DynaMut2 is based on vibrational entropy (VE); VE describes how a protein

residue in an energy landscape is likely to be occupied based on average configurational entropy. A decrease in VE would increase the rigidity of a protein. If a mutation is shown destabilizing by four out of the five tools, we have considered that as a destabilizing mutation. A detailed protocol of structure-based mutational analyses can be found in our previous reports (Amir et al., 2019; Mohammad et al., 2020; Choudhury et al., 2021; Habib et al., 2021; Umair et al., 2021).

RESULTS

Stabilizing and Destabilizing Mutations in SARS-CoV-2 Proteins

Different sets of reported mutations in the SARS-CoV-2 proteins were extracted from the NCBI Virus database. For S protein, 229 mutations were analyzed, where 123 mutations were found to be destabilizing (**Figure 1**). In contrast, 6 mutations were destabilizing out of 18 mutations in E protein (**Figure 2**). At the same time, out of 57 mutations in M^{pro}, 36 mutations have a destabilizing effect (**Figure 3**). Here, 25 mutations are present in domain I and 11 mutations in domain II. While two mutations lie in the loop region, and 19 mutations are in the C-terminal domain III. The analysis of 162 N protein mutations showed 85 mutations as destabilizing (**Figure 4**). The analysis revealed that the SARS-CoV-2 structural proteins, i.e., S, E, and N, and replication machinery enzyme, i.e., M^{pro} have several mutations found in the concerning variants (**Table 1**).

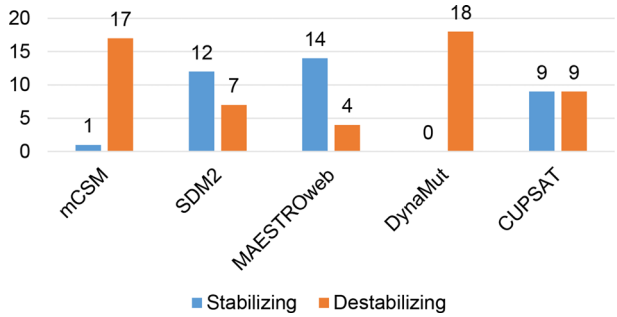

FIGURE 2 | Distribution of stabilizing and destabilizing mutations in SARS-CoV-2 Envelope protein predicted by structure-based tools.

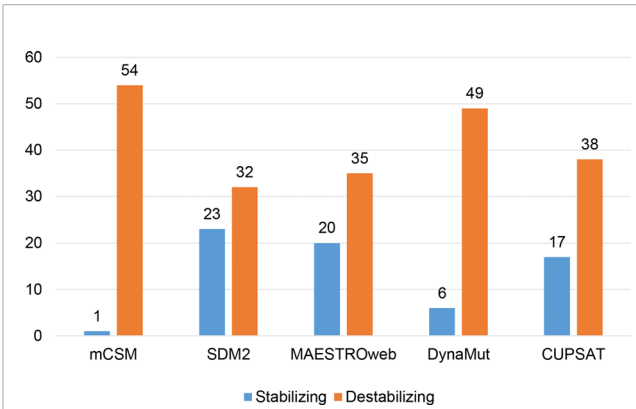

FIGURE 3 | Distribution of stabilizing and destabilizing mutations in SARS-CoV-2 Main Protease predicted by structure-based tools.

DISCUSSION

The SARS-CoV-2 proteins have several mutations found in the different variants emerging day by day. These mutations have various adverse impacts on the structure functions of the SARS-CoV-2 proteins making COVID-19 complex to administrate. Here we have discussed such mutations and their roles in the SARS-CoV-2 virulence.

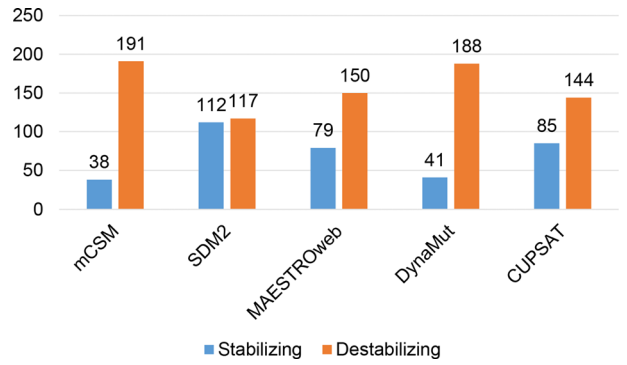

FIGURE 1 | Distribution of stabilizing and destabilizing mutations in SARS-CoV-2 Spike protein predicted by structure-based tools.

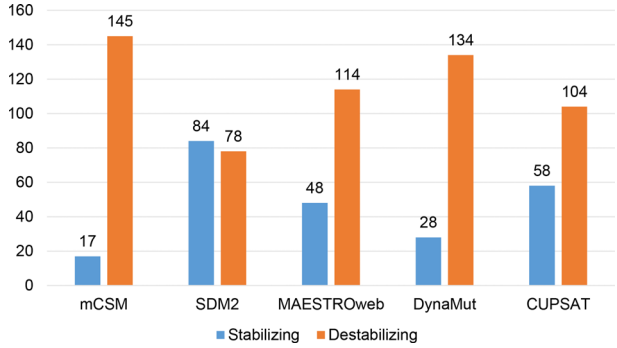

FIGURE 4 | Distribution of stabilizing and destabilizing mutations in SARS-CoV-2 Nucleocapsid protein predicted by structure-based tools.

TABLE 1 | Selected mutations predicted to be destabilizing and stabilizing through different structure-based approaches.

Protein	Destabilizing Mutations	Stabilizing Mutations
Spike Protein	L5F, Q14H, T20I, T22N, P26S, P26L, P26T, T29I, T29P, N30K, S31T, F32L, R34P, R34S, L54F, V62L, V70I, S71F, G75V, T76I, D80Y, T95I, D111N, L141F, G142S, G142D, Y144N, Y145H, W152C, R158T, L176F, M177I, L189F, L212F, D215N, D215E, L216I, L216F, P217A, P217H, G219C, S221L, W258L, G261D, A262S, A262T, F318Y, F318I, V320F, V320I, V367F, V382L, I402V, R403K, E406D, K417N, K417E, L452R, L455F, R466G, R466I, G485R, Q498H, P499T, N501Y, N501T, L518Q, A520S, A520E, P521T, A522V, A522S, A522G, E583D, D614G, V622F, A626V, T632N, S640F, E654K, Q675H, S691F, M731I, T732A, A771S, Q779H, I788M, I794F, I794M, S810P, L822F, I834M, A845D, A845S, A846S, E868D, A879S, I882T, I882V, S884A, A899S, A930V, S939T, S940T, A942S, L945F, L1004F, Y1047N, S1055A, V1068F, A1070S, K1073N, A1078T, A1078S, V1104L, P1112L, V1122L, G1124V, N1125S, V1137I, Y1138H, P1140A, L1145F	P9L, S12F, S13I, L18F, R21I, A27V, G35C, G35R, H49Y, S50L, A67V, H69Y, D88H, S98F, D138Y, H146Y, N149Y, M153I, S155I, Q173K, E180V, G181R, V213L, R214L, R214S, D215V, D215Y, Q218L, A222V, H245Y, S247R, D253G, S254F, S255F, T307I, Q321R, Q321H, K417M, N439K, G446V, S477N, S477I, Q493K, S494P, S514C, L517P, L518I, H519Y, H519Q, H519N, K558N, Q613H, N641K, A653V, H655Y, Q677H, Q677P, P681H, P681L, R682W, R682L, A688V, S698L, A701V, S704L, T716I, T719I, G769V, V772I, T778I, Q779R, P793T, D796Y, D796H, S810L, P812S, P812L, S813T, D839E, D839Y, L841I, L841R, A845T, R847T, T859I, L894F, S929N, S929R, G932S, K933Q, D936Y, D936E, S939F, S943T, T1027I, A1078V, D1084E, D1084Y, S1097L, H1101Y, T1117I, V1133I, D1139V, L1141M, Q1142E, E1144Q
Envelope Protein	L37H, V49L, L51F, F56V, R69K, V75L	T9I, T30I, S55T, S55F, S68F, R69I, R69G, P71S, P71L, D72G, L73F, L74M
MPro	F8L, G15S, L30F, D34G, V35A, I43V, T45I, L50F, K61R, G71S, L75F, R76S, V86L, K88R, L89F, A94V, P96L, P108S, P108L, P132S, F134L, F134Y, V157L, L167F, P168S, N180K, V202F, V204A, L227F, M235L, M235I, E270A, L282F, L286F, P293S, R298K,	S10A, S10Y, L32F, V35I, S46A, L67F, K90R, A94S, T135I, N180T, A191V, T196M, A234V, S254F, A266V, S267A, R279C, A285T, L286I
Nucleocapsid	N11S, P13L, R14C, P20S, T24I, G25D, S26N, E31G, R32H, G34W, A35V, K65R, P67S, V72I, P80Q, D81Y, I94V, D103Y, A119S, G120A, I131V, L139F, D144Y, T148S, P151L, A152T, A152S, A156S, I157T, P168S, P168A, G178V, S180I, S183Y, S188P, R191L, N192D, S194L, P199S, R203K, R203S, G204R, T205I, T205N, P207L, R209S, R209K, G212S, G212V, N213K, N213D, G215D, D216E, A217T, Q229H, M234V, S235F, G238C, A251V, T265I, E290D, I292T, A308S, S327L, S327W, T334K, T334P, N345K, N345H, D348H, Q349K, A359S, T366A, E367G, P368S, A376T, E378Q, T379A, A381P, P383L, Q384H, T391I, L407F, Q409K, Q409H	Q9H, S21T, D22G, S33N, S37P, N48I, D63E, D63Y, S79G, S79T, P80T, D103E, D128E, D128Y, T135I, A152D, A173V, A182S, R185C, S187L, S188L, S190I, N192S, S193N, S193I, S194T, R195I, S197L, P199L, S202C, S202N, S202T, G204V, S206F, A208G, A208S, R209I, M210I, M210K, A211V, N213S, A220V, L230F, M234I, G243C, T247I, A267E, A267P, V270L, T271I, Q289H, H300Y, A305V, T325I, T329M, T334I, P344S, Q349H, T362I, T362K, P364L, T366I, D371Y, D377Y, T379I, P383S, Q389L, Q390P, Q390H, T393I, A398V, L400M, D401Y, D402Y, K405R, S413R, A414S

Spike Protein Mutations

S protein consists of 1273 amino acids and is approximately 180-200 kDa (Hoffmann et al., 2020). Several polysaccharide molecules are coated onto the surface of S protein to help escape the host's immune response (Watanabe et al., 2020). Like other typical proteins, S protein also has an extracellular N-terminal domain (NTD), transmembrane domain and a cytoplasmic C-terminal domain (CTD) (Hwang and Yu, 2021). It has a signal peptide ranging from 1 to 13 amino acid (aa) residues followed by two different subunits, namely S1 (14-685 aa residues) and S2 (686-1273 aa residues), each one of which plays an essential role in adherence of SARS-CoV-2 to the host cells for their entry (Xia et al., 2020). In the native state, S protein is inactive. The presence of TMPRSS2 on the target cell membrane and other cellular proteases cleaves the S protein into its S1 and S2 subunits required for the fusion of viral-host membrane after the viral invasion (Bertram et al., 2013; Hoffmann et al., 2020).

S1 subunit further consists of an N-terminal region and a receptor-binding domain (RBD) (Xia et al., 2020). It initiates the viral infection cycle by binding of trimeric S protein *via* RBD of S1 subunit to ACE2 receptors on the host cell (Wrapp et al., 2020; Yan et al., 2020). This interaction induces a conformational change that directs them to form endosomes to trigger viral fusion with the host cell under the influence of low pH (Shang et al., 2020). Understanding such conformational changes provides a base for the development of drugs that disrupt the entry mechanisms (Walls et al., 2020; Shamsi et al., 2021). The atomic-level studies by cryo-electron microscopy have revealed different open and closed conformations of the RBD. These domains account for the variability of SARS-CoV-2 (Walls et al., 2020; Wrapp et al., 2020). The amino acid composition of the RBD reflects the evolutionary relatedness of SARS-CoV-2 with other members of the Coronavirus family, and it is the least conserved unit. However, the RBD of MERS-CoV was distinct, indicating the divergence from the previous strains (Andersen et al., 2020). The intermediate hosts of SARS-CoV-2 can be identified by analyzing binding affinities and the RBD domain of the S1 subunit with the ACE2 receptors (Chen Y. et al., 2020; Huang Y. et al., 2020). Mutations in the S1 region are associated with changed antigenicity, and thus, it accounts for some strains to be more infectious than others. The affinity of the receptor binding region of S1 subunit with ACE-2 receptors might change because of mutations, but their interaction is preserved among humans, cats and swine (Chan et al., 2020; Wrapp et al., 2020; Yan et al., 2020).

S2, another subunit of S protein, mediates the fusion of the virus to the host cell membrane. It consists of a fusion peptide (FP), heptapeptide repeat sequence 1 (HR1), HR2, TM domain and a cytoplasm domain at the last (Xia et al., 2020). Various large-scale structural rearrangements allow the virus to fuse with the host cell membrane (Watanabe et al., 2020). The main basis of viral-host fusion lies in the cleavage of S1 and S2 subunits, releasing the viral genome into the host cell (Tortorici et al., 2019; Rabaan et al., 2020). Multiple furin cleavage sites have been found in SARS-CoV-2, which are susceptible to attack by furin-like proteases (Millet and Whittaker, 2015; Hasan et al., 2020). These sites were absent from previously known SARS-CoV and, thus, might have increased the infectivity of SARS-CoV-2

(Coutard et al., 2020; Rabaan et al., 2020). Besides, furin-like proteases TMPRSS2 and trypsin have been found to exhibit a profound role in activating the membrane fusion domain (Heurich et al., 2014; Limburg et al., 2019; Ou et al., 2020). The fusion peptide, rich in hydrophobic residues like glycine and alanine, anchors to the host cell membrane and disrupts their lipid bilayer (Millet and Whittaker, 2018). The exposure of FP following the cleavage of S protein into two subunits and the two HR domains of the S2 subunit is important for mediating the viral fusion (Kawase et al., 2019). Certain receptor (FP) and ligand (on host cell) interactions trigger a conformational change which shortens the distance between the two membranes for fusion (Harrison, 2015). As a result, the HR1 domain becomes closer to the host cell, whereas the proximity of the HR2 domain increases with the viral membrane. The folding of HR1 and HR2 together creates a six-helical bundle structure aligned in an antiparallel form to the fusion core. In this way, viral and host cell membrane pulls each other close for bringing out the necessary fusion (Eckert and Kim, 2001). The potential mutations in the SARS-CoV-2 S protein found in different variants are discussed here:

N501Y: The N501Y mutation was initially found in the B.1.1.7 UK variant (Leung et al., 2021), and later it appeared in South African and Brazilian variants, which were B.1.128.1 and B.1.351, respectively (Weisblum et al., 2020). It was also detected in the Theta (P.3 or GR/1092K.V1) variant in Japan and the Philippines in February 2021. This amino acid substitution is located in the RBD of the S protein. It was found that the N501Y substitution increases the affinity of RBD to bind to the ACE2 receptor and thus enhances the transmission rate (Starr et al., 2020; Makowski et al., 2021). Our study also shows that the N501Y mutation destabilizes the SARS-CoV-2 S protein (**Table 1**).

K417N: This mutation is first reported in the B.1.351 variant (Beta variant or GH501Y.V2) in South Africa (Tegally et al., 2021). Later it was also detected in the P.1 variant (GR/501Y.V3, Gamma variant) in Brazil in December 2020 (Faria NR, 2021). The K417N substitution is also present in the RBD. It was found that this substitution significantly increases the binding affinity of SARS-CoV-2 RBD to the ACE2 receptor. Also, the mutation causes a huge decrease in the binding affinity STE90-C11 antibody to RBD (Fratev, 2020).

E484K: The E484K mutation was first reported in the South African variant B.1.351 variant (GH501Y.V2, Beta variant) (Tegally et al., 2021) and later in the P.1 variant (Gamma variant or GR/501Y.V3) (Faria NR, 2021). This mutation was also found in the Zeta (P.2), Eta (B.1.525) and Iota (B.1.526) variants (Huh et al., 2021). This mutation significantly alters the electrostatic complementarity of antibody binding to the RBD (Andreano et al., 2020). The impact of E484K mutation in the RBD was also seen in the binding of serum polyclonal neutralizing antibodies to SARS-CoV-2 (Jangra et al., 2021).

L18F: This mutation occurred in the South Africa B.1.351 variant (Beta variant or GH501Y.V2) [5]. Later it was also found in the P.1 variant (Gamma variant or GR/501Y.V3) and Zeta (P.2) variants in Brazil. This substitution has been found to affect the binding of neutralizing antibodies negatively [16]. Studies have shown that mutants with L18F substitution are highly sequenced variants, escaping S2L28-mediated neutralization (Mccallum et al., 2021).

A570D: The A570D substitution was first identified in the B.1.1.7 UK variant (Alpha or GRY GR/501Y.V1) in December 2020. Along with D614G, this mutation induces significant conformational destabilization (Socher et al., 2021).

P681H: This mutation was detected first in the UK B.1.1.7 UK variant. Later it was identified in P.3 (Theta or GR/1092K.V1) variant found in Japan and the Philippines in February 2021. The P681H mutation is present at a proteolytic cleavage site for furin or furin-like proteases at the junction of the fusion domain and S protein RBD (Jaimes et al., 2020). It has been shown that P681H increases cleavability at the S1/S2 junction but does not surely indicate increased membrane fusion and infectivity (Lubinski et al., 2021).

S982A: The S982A substitution was first identified in the B.1.1.7 UK variant (Alpha or GRY GR/501Y.V1). The S982A mutation of B.1.1.7 lineage is present on the S2 subunit of the S protein. This substitution in UK variant B.1.1.7 does not have intermolecular hydrogen bonding potential between S protein subunits (Ostrov, 2021).

D1118H: This mutation was detected first in the B.1.1.7 UK variant. The D1118H substitution is also present in the S2 subunit of the S protein (Chrysostomou et al., 2021). It has been suggested that this mutation can alter the stability and dynamics of trimer assembly (Zhao et al., 2021).

A701V: The A701V substitution was first detected in the B.1.351 variant (Beta variant or GH501Y.V2) in South Africa and later found in the Iota (B.1.526) (Annavajhala et al., 2021) variant in New York. This non-synonymous substitution is located in the cleavage site of the neighboring promoter of the S2 subunit (West et al., 2021).

D614G: The D614G is the most widespread mutation of the S protein; it has been found to increase the infectivity of the SARS-CoV-2 virus (Korber et al., 2020). This non-synonymous substitution was identified in the South Africa B.1.351 variant (Beta variant or GH501Y.V2), B.1.617.2 variant (Delta variant), Epsilon (B.1.427 and B.1.429) variants, Zeta (P.2), Kappa (B.1.617.1), Eta (B.1.525) and Iota (B.1.526) variants. The D614G mutation increases infectivity and enhances the replication of the SARS-CoV-2 virus in the upper respiratory tract (Plante et al., 2021). This substitution is away from the RBD. It decreases the binding affinity of S protein to the ACE2 receptor and introduces a conformational change in the S1 subunit of the S protein (Yurkovetskiy et al., 2020). D614G is present at the SD2 domain, enhancing the furin cleavage at the S1/S2 domain junction (Gobeil et al., 2021).

R158G: The R158G substitution was first found in the B.1.617.2 variant (Delta variant) in December 2020 in India. The mutation is absent from the RBD of the S protein (Baral et al., 2021).

L452R: This mutation was reported in the B.1.617.2 variant (Delta variant) and Kappa (B.1.617.1) variant in India as well as in the Epsilon (B.1.427 and B.1.429) variants in the US (Huh et al., 2021). This mutation is present in the RBD of SARS-COV-

2 S protein and was found to reduce neutralizing activities in many monoclonal antibodies (Baral et al., 2021).

T478K: This mutation was first identified in the B.1.617.2 variant (Delta variant) in India. The mutation is also present in the RBD of the S protein (Baral et al., 2021).

P681R: This mutation was first detected in the B.1.617.2 variant (Delta variant) in December 2020 in India and later in the Kappa (B.1.617.1) variant. The mutation is absent from the RBD of the S protein.

Envelope Protein Mutations

The E protein with an 8-12 kDa size is one of the smallest structural proteins in SARS-CoV-2 (Schoeman and Fielding, 2019). The sequence of E protein is highly conserved among different members of Coronaviruses. The major function of E protein involves activation of host inflammasome, viral progeny budding and release from the host cells (Nieto-Torres et al., 2015; Schoeman and Fielding, 2019). Like other structural proteins, it also possesses three distinct domains- the extracellular domain at N-terminus consisting of hydrophilic (7-12 amino acid) residues followed by a transmembrane domain of 25 hydrophobic amino acid residues and a cytosolic or C-terminus domain-containing hydrophilic amino acid sequences (Corse and Machamer, 2000; Torres et al., 2007; Surya et al., 2018). The characteristic feature of E protein is viroporin, a pentameric ion channel with no or low selectivity of ions formed from the oligomerization of the transmembrane domain (Verdiá-Báguena et al., 2012; Nieto-Torres et al., 2014). Viroporins are small M proteins that get incorporated into the host membrane and assists in the maturation and release of the viral particles (Nieva et al., 2012). Therefore, these pentameric structures with an ion-conducting pore mediate the host-pathogen interactions (Torres et al., 2006; Parthasarathy et al., 2008; Pervushin et al., 2009). Besides regulating the assembly and release of virions, they have been found to possess a significant role in the pathogenesis of the virus (Nieto-Torres et al., 2014), where pathogenesis is directly proportional to the ion channeling (IC) activity (Chellasamy et al., 2020). For example, no effect on the replication of the virus was observed after E gene knockdown, but reduced edema accumulation was witnessed. This might be because the loss of ion channeling activity of E protein resulted in the correct localization of Na^+/K^+ ATPase, which is probably involved in decreased edema accumulation and an increase in edema resolution. Often, the accumulation of edema is one of the reasons contributing to ARDS. Also, studies on animal models infected with IC activity lacking viruses exhibited reduced levels of IL-1β, which further reduced the production of TNF and IL-6 in the lung airways. Therefore, it was estimated that IC activity of E protein is essential in the development and progression of cytokine storm leading to permanent lung damage and ARDS in the later stages (Nieto-Torres et al., 2014).

In addition to this, the selective cation ion channel formed from viroporin is localized towards the ERGIC membrane (Wilson et al., 2004; Verdiá-Báguena et al., 2012). The C-terminal region within the E protein contains a β motif with a conserved proline amino acid residue that is important for localization into the ER-Golgi complex (Li et al., 2014; Chellasamy et al., 2020). A small part of E protein inside the host cell membrane is transferred to the virion when a virus replicates. In contrast, the larger section of this protein remains at the location of intracellular trafficking within the mammalian cells, i.e., ER-Golgi network and the ERGIC (Nieto-Torres et al., 2011; Venkatagopalan et al., 2015). Such localization of E proteins assists in viral structural assembling and budding from the host cell (Nieto-Torres et al., 2011).

In all the variants of SARS-COV-2 except the Beta (B.1.351) variant, there are no reported mutations in the E protein. The Beta variant was first discovered in the Eastern Cape province of South Africa in October 2020 (Tegally et al., 2021). India has reported more than 200 cases of the Beta variant from the time of its discovery. The potential mutation in the SARS-CoV-2 E protein found in the Beta (B.1.351) variant are discussed here:

P71L: It is the amino acid substitution found in the E protein of the Beta variant. Statistically, the mutation P71L was associated with disease severity and death rate. The mutation was present in deceased patients' datasets and virus isolates of patients from high case-fatality-ratio countries (Rizwan et al., 2021).

M^{pro} Mutations

The SARS-CoV-2 M^{pro} has several mutations reportedly found in different variants are discussed here:

T45I: This mutation in the domain I region of SARS-CoV-2 M^{pro} is reported in variants B.1.1.7, B.1.351, P.1, B1.617, B.1.429$^+$ B.1.427 (Philot et al., 2021). It presented a polar to non-polar substitution due to which there is a reduction in its hydrogen bonding potential. SDM predicted the free energy change as stabilizing w.r.t. WT SARS-CoV-2 M^{pro}. Also, Dynamut2 indicates no substantial change in the flexibility of the protein compared to the WT SARS-CoV-2 M^{pro}.

K90R: This mutant lies in the domain-I region; it has shown relevant modifications in its potential energy concerning the WT SARS-CoV-2 M^{pro} (Philot et al., 2021). SDM has indicated this substitution as destabilizing, and the Dynamut2 score suggests the substitution will increase the flexibility of the protein. K90R mutant is reported in the variants B.1.1.7, B.1.351, P.1, B1.617, which are mainly are our variant of concern. Furthermore, a lower energy configuration and a more extensive dimeric interface have resulted from the mutant K90R (Philot et al., 2021).

P99L: It is reported in the B.1.1.7 variant only. The Dynamut2 score suggests the mutant will induce stability to the structure, whereas SDM shows destabilizing; a higher probability of the formation of dimeric interfaces was reported than WT SARS-CoV-2 M^{pro} (Sheik Amamuddy et al., 2020; Philot et al., 2021).

P108S: This mutant is reported only in the B.1.1.7 variant. The substitution showed a significant variation in potential energy (Philot et al., 2021); nevertheless, the SDM and Dynamut2 scores suggest no substantial change in the structure.

T135I: This substitution brought polar to non-polar replacement. It is reported in variant B.1.1.7 and P.1. SDM has predicted this as stabilizing, whereas there may be a potential increase in the protein flexibility as per Dynamut2 w.r.t. WT SARS-CoV-2 M^{pro}.

N151D: This substitution is reported in variants B.1.1.7, B.1.351, P.1, B1.617. Compared to WT SARS-CoV-2 M^{pro}, this mutant shows behavior that may induce catalysis and create distinct dimeric interfaces (Philot et al., 2021). In addition, a significant variation in potential energy was also detected (Sheik Amamuddy et al., 2020). SDM predicts destabilizing, whereas, according to Dynamut2, there will be no change in the structure.

A234V: The substitution A234V in the Domain III region is reported in variants B.1.1.7, B.1.351, P.1, and B.1.617. This mutant is associated with the protein's mobility. However, it might also affect the flexibility since it lies in the highly flexible region of the protein (Sheik Amamuddy et al., 2020).

A266V: This mutation was found in B.1.1.7, B.1.351, P.1, and B.1.617 varients. The mutation occurred in a highly flexible region involved in protein mobility and might affect protein flexibility (Sheik Amamuddy et al., 2020). The variation might induce rigidity as per the Dynamut2 result and is also destabilizing according to SDM.

R279C: This mutant is also related to protein's mobility and lies in the Domain III region. It is reported in variants B.1.1.7, B.1.351, P.1, and B.1.617. The substitution R279C increases potential energy more than WT SARS-CoV-2 M^{pro} (Philot et al., 2021). As per SDM, the mutation may destabilize the protein. However, there may be an increase in protein's flexibility, as per Dynamut2, which might benefit the protein.

Nucleocapsid Mutations

N proteins play a major role in packaging the viral genome into a ribonucleoprotein complex known as a capsid. This packaging ensures the proper replication and self-assembly of the virus (Chang et al., 2014). N protein contains N-terminal (NTD) and C-terminal domains (CTD) connected by a linkage region (LKR) having serine/arginine-rich (SR-rich) domain within their structural sequence (Huang et al., 2004; Hurst et al., 2009). The presence of these positive amino acid residues favors the binding of the viral genome to both the domains, i.e., NTD and CTD (Chen et al., 2007; Saikatendu et al., 2007). LKR is mostly associated with the oligomerization process (He et al., 2004a; Chang et al., 2013). This binding occurs through a long stretch of RNA binding domain in N protein consisting of approximately 140 amino acid residues (Fehr and Perlman, 2015). Structural analysis of the N protein of SARS-CoV-2 has revealed a disordered region in high content that is not in the bound state to the genomic DNA (Zeng et al., 2020). The linker region is also disordered (Chang et al., 2006; Yu et al., 2006), suggesting its ability to bind to several other partners to maintain appropriate N protein conformation (He et al., 2004a; He et al., 2004b; Luo et al., 2005). Likewise, other structural proteins, N protein of SARS-CoV-2 is also conserved among coronavirus family and shares~90% sequence similarity with SARS-CoV N protein sequence (Naqvi et al., 2020). The potential mutations in the SARS-CoV-2 N protein found in different variants are discussed here:

R203K: Mutation R203K was observed in the B.1.1.7 variant or the alpha variant and the P.1 variant or the gamma variant, initially found in the United Kingdom and Japan/Brazil. It is found in the Ser/Arg-rick linker region (LKR), one of the protein's most phosphorylated regions. It is one of the most found mutants in N protein. Variant R203L and G204R were aroused by homologous recombination in the SARS-CoV-2 genome (Leary et al., 2021).

G204R: The mutation G204R was also observed in the B.1.1.7 variant (Alpha variant) and the P.1 variant (Gamma variant), initially found in the United Kingdom and Japan/Brazil. It is also located in the LKR region. G204R, along with R203K, is one of the most found mutations in the N protein. Their presence was associated with the increase in N protein and sub-genomic RNA expression from the other ORFs (Leary et al., 2021). N protein shows the high protein intrinsic disorder, and 203/204 residue sites showed increasing entropy and their neighborhoods aligned with areas of the high disorder (Tomaszewski et al., 2020).

T205I: The mutation T205I was first observed in the B.1.351 variant or the beta variant of the Coronavirus, which was first observed in South Africa in October 2020. It is also found in the LKR region. T205I mutant was a common mutation at around 43% [35] since it is highly phosphorylated. Hence, the mutation disrupts the activation of N protein and thus interferes with the virus life cycle.

S235F: The mutation S235F was observed in the B.1.1.7 variant, I.e., the alpha variant, first observed in the United Kingdom in November 2020. It is also found in the LKR region. This mutation was seen to alter the corresponding epitopes, which can cause changes in the specificity of certain antibodies and alter the vaccine-induced protection against the disease.

Altogether, studies suggest that mutations affect pathogenesis by changing the phenotype of a protein, disrupting its stability, structure, macromolecular binding, ablation of posttranslational modification sites, etc. (Jubb et al., 2017; Joo and Liu, 2021; Padhi et al., 2021a). Some mutations increase/decrease the binding affinity of the protein towards its receptor (Lee et al., 2020). The increased binding affinity in viral proteins results in a higher infection probability (Lee et al., 2020). The N501Y mutation, located in the receptor-binding domain of the S protein, increases its binding affinity towards the ACE2 receptor (Shi et al., 2021). Various mutations in the S protein reportedly affect vaccine development, efficacy, and neutralization. The D614G mutation of S protein enhances the viral replication rate and is the most prevalent mutation, predominantly reported in B.1.617.2, B.1.427, B.1.429, P.2, B.1.617.1., B.1.525, and B.1.526 variants. It was also found that the D614G mutation may decrease binding affinity and could also change the predicted MHC binding (Akkiz, 2021; Daniloski et al., 2021). The spike protein mutations, N501Y, E484K, P681H, and K417N, found in variant B.1.1.7, B.1.351, and the B.1.128.1 could decrease the virus's ability to attach to antibodies (Mahase, 2021). Hence, it becomes a necessity to consider the variant during the development of vaccines. However, according to some recent findings, despite the N501Y and P681H mutants in B.1.1.7, vaccine efficacy would not be affected (Seo et al., 2020; Mahase, 2021; Shen et al., 2021). The Spike mutations N501Y (B.1.1.7, B.1.351 and the B.1.128.), L18F, K417T (B.1.351), E484K (B.1.1.7, B.1.351, B.1.128.1, B.1.525 and B.1.526), and D614G (B.1.617.2, B.1.427, B.1.429, P.2, B.1.617.1., B.1.525, and B.1.526) are reported in most concerning variants and has significant

inferences for evading antibody-mediated immunity (Altmann et al., 2021; Gupta, 2021; Mohammadi et al., 2021).

The T190I mutation in M^{pro} has brought a polar to non-polar substitution near the active site cavity, which might have a significant role in enzymatic activity, particularly when coupled with mutations in neighboring areas (Sheik Amamuddy et al., 2020). Another mutation adjacent to T190I is A191V; both residues belong to the most flexible regions, as substrate recognition sites often require structural flexibility to recognize the binding sites precisely (Sheik Amamuddy et al., 2020). Such mutations would alter the native conformation and activity of the SARS-CoV-2 M^{pro} and RdRp, which might affect the binding of therapeutic molecules (Padhi et al., 2021b; Padhi and Tripathi, 2021).

CONCLUSION

SARS-CoV-2 is more potent than the previous strains of coronaviruses; this reflects their enormous ability to mutate into new strains. When a virus enters a host cell, it uses its machinery to replicate and synthesize viral particles. Mutations lead to the evolution of the viral genome, allowing them to better adapt and survive in the human host for their active reproduction. Such mutations are achieved by modifications in the epitopes of viral genes, making them more infective, transmissible and helps in escaping the immune responses of the host. Most of the structural proteins of SARS-CoV-2 conserved among coronavirus family and shares ~ 90% sequence similarity. However, a slight change in sequence causes a great impact on the structure and pathogenesis of SARS-CoV-2. Despite major mutations do not affect vaccine efficacy, however, sometimes become more pathogenic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Conceptualization, TM, FA, and MIH. Methodology, AC, IH, DY, TM, and YM. Software, AC and MU. Validation, TM and MIH. Formal analysis, AC, AS, and MU. Investigation, MA, AS, and TM. Resources; data curation AC, and IH. Writing—original draft preparation, TM, PA, DY, AS, and AC. Writing—review and editing, TM, FA, and MH. Visualization, FA, AC, and IH. Supervision, MIH. Project administration, DY and MIH. Funding acquisition, FA and MIH. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.765039/full#supplementary-material

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REVIEW
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Host Response to SARS-CoV2 and Emerging Variants in Pre-Existing Liver and Gastrointestinal Diseases

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Background: Novel coronavirus SARS-CoV2 is evolving continuously with emergence of several variants of increasing transmission capabilities and pandemic potential. Generation of variants occurs through accumulation of mutations due to the RNA nature of viral genome, which is further enhanced by variable selection pressures of this ongoing pandemic. COVID-19 presentations of SARS-CoV2 are mainly pulmonary manifestations with or without mild gastrointestinal (GI) and hepatic symptoms. However, the virus has evolved beyond pulmonary manifestations to multisystem disorder due to systemic inflammation and cytokine storm. Definitive cause of acute or late onset of inflammation, infection in various organs, and host response to emerging variants lacks clarity and needs elucidation. Several studies have reported underlying diseases including diabetes, hypertension, obesity, cardio- and cerebrovascular disorders, and immunocompromised conditions as significant risk factors for severe form of COVID-19. Pre-existing liver and GI diseases are also highly predominant in the population, which can alter COVID-19 outcome due to altered immune status and host response. We aim to review the emerging variants of SARS-CoV2 and host response in patients with pre-existing liver and GI diseases.

Methods: In this review, we have elucidated the emergence and characteristic features of new SARS-CoV2 variants, mechanisms of infection and host immune response, GI and hepatic manifestation with radiologic features of COVID-19, and outcomes in pre-existing liver and GI diseases.

Key Findings: Emerging variants of concern (VOC) have shown increased transmissibility and virulence with severe COVID-19 presentation and mortality. There is a drastic swift of variants from the first wave to the next wave of infections with predominated major VOC including alpha (B.1.1.7, UK), beta (B.1.351, South Africa), gamma (B.1.1.28.1, Brazil), and delta (B1.1.617, India) variants. The mutations in the spike protein of VOC are implicated for increased receptor binding (N501Y, P681R) and immune escape (L452R, E484K/Q, T478K/R) to host response. Pre-existing liver and GI diseases not only have altered tissue expression and distribution of viral entry ACE2 receptor but also host protease TMPRSS2, which is required for both spike protein binding and cleavage to

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initiate infection. Altered immune status due to pre-existing conditions results in delayed virus clearance or prolonged viremia. Even though GI and hepatic manifestations of SARS-CoV2 are less severe, the detection of virus in patient's stool indicates GI tropism, replication, and shedding from the GI tract. COVID-19-induced liver injury, acute hepatic decompensation, and incidences of acute-on-chronic liver failure may change the disease outcomes.

Conclusions: The changes in the spike protein of emerging variants, immunomodulation by viral proteins, and altered expression of host viral entry receptor in pre-existing diseases are the key determinants of host response to SARS-CoV2 and its disease outcome.

Keywords: SARS-CoV2, COVID-19, variants of concern, emerging variants, gastrointestinal disease, liver, manifestations

INTRODUCTION

Novel coronavirus with the earliest reported pneumonia-like symptoms originated in the Wuhan City of China in December 2019, hence was designated as 2019-nCoV (Phelan et al., 2020), and the resulting outbreak was termed as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). Subsequently, the International Committee on Taxonomy of Viruses (ICTV) renamed the virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) after its genome sequencing and phylogenetic analysis, which revealed 79.5% genome identity with SARS-CoV. From December 2019 till July 29, 2021, WHO has reported 195,886,929 confirmed cases of COVID-19, with 4,189,148 reported deaths worldwide. Despite its apparent correlation of origin, COVID-19 is distinct from symptoms associated with other coronaviruses such as SARS-CoV, 2003, and the Middle East respiratory syndrome by (MERS-CoV). Clinical presentation of COVID-19 varies from asymptomatic to severe such as acute respiratory distress syndrome (ARDS), multisystem inflammatory syndrome (MIS), fever, cough, dyspnea, fatigue, and pneumonia (Guan et al., 2020). In addition, loss of taste and smell, myalgia, conjunctivitis, and skin rash have also been associated with atypical and early presentation of COVID-19. Severe presentation of COVID-19 requires immediate medical attention, whereas asymptomatic presentations go unnoticed, resulting in virus dissemination and spread. SARS-CoV2 infection also presents with gastrointestinal (GI) manifestations such as diarrhea, nausea, vomiting, and abdominal discomfort. Despite the low severity ascribed to GI symptoms, the detection of virus in patient's stool is an indication of virus replication, shedding, and GI tropism.

With emerging variants of SARS-CoV2 contributing towards enhanced infectivity and transmission as well as reports of multiple organ involvement, COVID-19 has transformed from being a respiratory disease to multisystemic disorder. Since the onset of this pandemic, association of severity of COVID-19 with old age and pre-existing comorbidities in patients has been observed. Various studies have reported that underlying diseases such as diabetes mellitus, hypertension, cardio- and cerebrovascular disorders, obesity, coronary heart disease, HIV infection, cancer, and other immunocompromised conditions are significant risk factors for developing a severe form of COVID-19, which may turn fatal (Fathi et al., 2021; Ng et al., 2021). In addition to the respiratory and GI system, involvement of the hepatobiliary system either as a pre-existing condition or consequence of treatment may also exacerbate disease progression. The contribution of indirect hepatic injury owing to cytokine storm and systemic inflammation towards COVID-19 severity currently lacks clarity (Hunt et al., 2020). Current studies suggest a relatively mild disease discourse for patients presenting with GI symptoms, but the effect of pre-existing GI and liver diseases on the prognosis of COVID-19 or resulting long COVID disease remains largely inconclusive. An in-depth analysis of case studies for a longer duration is required to accurately suggest the contribution of underlying GI and liver diseases towards altered disease outcomes. We have reviewed emerging variants of SARS-CoV2 and host response in patients with pre-existing gastrointestinal and liver diseases. Pre-existing comorbidities, including hepatic and gastrointestinal diseases, may have a role in severe presentations altering the disease outcome.

SARS-CoV2 INFECTION

SARS-CoV2 is an enveloped positive-stranded RNA virus that belongs to the genus *Betacoronavirus* of the family Coronaviridae. The viral genome is 29,870 nucleotides long (Ref. NC_045512), which encodes five typical open reading frames (ORF) known as ORF1ab polyprotein (7096 amino acid, aa), Spike (S) glycoprotein (ORF2, 1273-aa), Envelope (E) protein (ORF4, 75-aa), Membrane (M) protein (ORF5, 222-aa), and Nucleocapsid (N) protein (ORF9, 419-aa) and six accessory protein-coding ORFs known as ORF3a, ORF6, ORF7a-b, ORF8, and ORF10 (Wu et al., 2020; Yadav et al., 2020). The major non-structural polyprotein ORF1ab encodes viral replicase complexes, including non-structural proteins (NSP 1-16), mainly processed by viral proteases.

SARS-CoV2 infection begins with the entry of the virus through binding of spike protein to cell entry receptor ACE2 (angiotensin-converting enzyme 2) and its cleavage by

transmembrane protease serine subtype 2 (TMPRSS2) (Lu et al., 2020). Viral entry is followed by uncoating of the genomic RNA, translation of the first open reading frame (ORF1ab), and its processing by viral proteases into several non-structural proteins (nsp 1-16), including viral replicase RdRp (RNA-dependent RNA polymerase). Further, viral genomic RNA replicates through viral RdRp, and synthesis of viral structural proteins (S, N, M, and E) occur, necessary for viral assembly and release for subsequent infection.

HOST IMMUNE RESPONSE AND IMMUNOMODULATION BY SARS-CoV2

Host innate and adaptive immune response to the viral infection determines the disease's clinical course and viral clearance. The innate immune system is the first line of defense against viral infections. A diverse set of immune cells such as macrophages, dendritic cells (DCs), neutrophils, natural killer (NK) cells, and innate lymphoid cells (ILCs) sense viral infection through recognition of viral PAMPs/DAMPs (Pathogen/Damage Associated Molecular Patterns) using pattern recognition receptors (PRRs). The PAMPs majorly include viral nucleic acids (ss/ds RNA), proteins, lipids, and other viral components. The PRRs are mainly Toll-like receptors (TLRs), the NOD-Like receptors (NLRs), the RIG-I-Like receptors (RLRs), the C-type lectin receptors (CLRs), and inflammasomes. This PAMP-PRR interaction activate multiple innate immune signaling pathways such as TLR/TRIF/MyD88/IRF3/7- IFN, RLR/RIG-1/MDA5/NFkB- IFN/IL, NLR/NLRP/AIM2/Inflammasome/IL for the production of interferon and cytokines/chemokines (Seth et al., 2006). Activation and priming of innate and adaptive immune responses result in pathogen clearance and recovery. But the viruses can evade host immune responses, rendering the host susceptible to infection. Evasion of host immune response is an early occurrence initiated with viral entry, translation of first ORFs, and subsequent immunomodulation by viral proteins (Chan and Gack, 2016).

During the initial phases of infection, the virus evades the host's innate immune system through immunomodulation by viral proteins, which leads to productive infection and unchecked viremia. SARS-CoV2 non-structural proteins such as nsp1, PLpro, 3CLpro, nsp10, and nsp16 may suppress host antiviral response, mainly type I IFN response, facilitating virus replication. Later, host may recognize viral structural proteins to mediate inflammatory response (**Figure 1**). Both T and B cell epitopes are extensively mapped to the structural proteins including S, N, M, and E protein of SARS-CoV2 (Liu et al., 2017). However, the role of all viral proteins for immunomodulation are not yet clear. For SARS-CoV2 infection, the initial immunosuppressive phase is characterized by lymphopenia, T cell exhaustion, and inadequate adaptive immune response (Diao et al., 2020). Host recognition of more viral proteins mounts severe inflammatory response at a later stage as characterized by a cytokine storm with neutrophil, monocyte/macrophage infiltration, and activation (Jacques and Apedaile, 2020). Generally, during viral infections, lymphocytes inhibit overactive innate immune responses, but lymphopenia during SARS-CoV2 infection may result in an increased release of

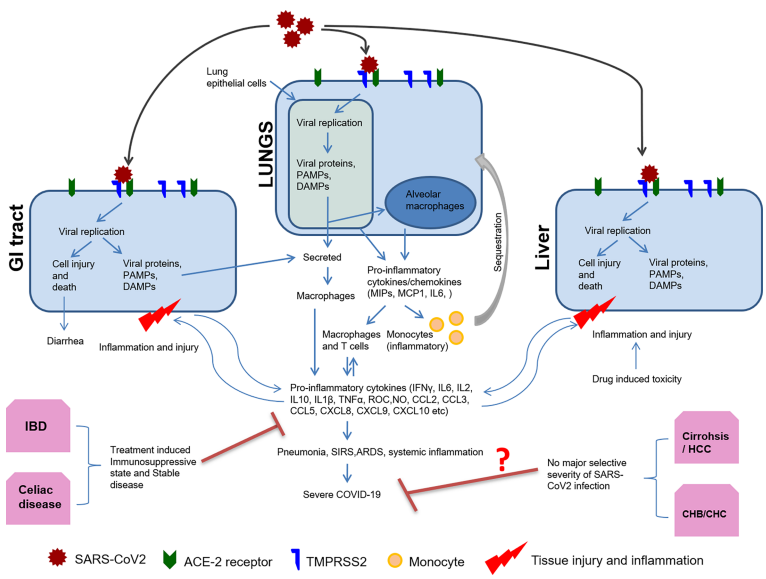

FIGURE 1 | Host immune response to SARS-CoV2 and organ-specific immunomodulation. Organ specific expression of viral entry receptor ACE2 and host protease TMPRSS2 determine SARS-CoV2 tropism and replication. Host cell mounts immune response by sensing viral replication and its intermediates through PAMPS or DAMPS. Subsequently, there is antigen presentation, the release of pro-inflammatory cytokines/chemokines, and activation of the adaptive immune response. Monocytes are also sequestered to the site of inflammation by chemotaxis and differentiate into macrophages forming the pro-inflammatory milieu. This results in pneumonia, ARDS, SIRS, and systemic inflammation. Pre-existing disease conditions including liver and GI tract may affect COVID-19 outcome as summarized in this figure.

cytokines such as interleukin (IL)-6, IL-10, IL-2, and IFN (Musa, 2020). This aggravated inflammatory response is an accentuated immune response, like SIRS, and implicated in multisystem aggravation contributing towards the severity of COVID-19 in patients (**Figure 1**).

GASTROINTESTINAL MANIFESTATIONS OF COVID-19

COVID-19 was primarily considered a respiratory disease, but it has now evolved into a multisystem disorder with transmission *via* inhalation and oral ingestion (Roberts et al., 2020). As discussed earlier, COVID-19 disease affects various organs with no quantifiable way of predicting the affected systems with any certainty. SARS-CoV2 infection presenting itself as GI symptoms such as anosmia, diarrhea, vomiting, and abdominal pain, with or without respiratory symptoms, has widely been reported in infected patients (Smyk et al., 2020). A recent study with a large cohort of 1,099 COVID-19 patients showed that diarrhea occurred in 3.8% and vomiting in 5.0% of the cases (Wong et al., 2020). The prevalence of GI manifestations in COVID-19 patients could be as high as 50%, as approximately 50% of patients with COVID-19 have detectable virus in their stool till 27.9 days of infection (Ouali et al., 2020). The frequency of GI symptoms significantly varies depending on the geographical area, studied population, and time of assessment of the COVID-19 patients (Papa et al., 2020; Redd et al., 2020). About 17% of COVID-19 pneumonia patients experienced pancreatic injury defined by any abnormality in amylase or lipase as reported in a recent study (Patel et al., 2020). The cause of injury may be attributed to the direct cytopathic effects of SARS-CoV2 or indirect systemic inflammatory and immune-mediated cellular responses, resulting in organ damage or secondary enzyme abnormalities (Patel et al., 2020). The pancreatic injury could also occur due to circulating microthrombi causing an ischemic injury in the pancreas (Eketunde et al., 2020). GI tropism of SARS-CoV2 is attributed to the presence of ACE2 entry receptor throughout the GI tract with the greatest functional role in the small bowel and colon. Increased ACE2 expression has been observed in the enterocytes of the proximal to the distal region of the small intestine, making it more vulnerable to infection (Smyk et al., 2020). Another key player is the host protease that cleaves viral spike protein into S1 and S2 subunits that are required for attachment of the virion to both the ACE2 receptor and the cell membrane. The distribution of these proteases varies across the GI tract and probably contributes to the lack of correlation between the expression levels of ACE2 receptors and the degree of infection (**Figure 2**). Viral nucleocapsid protein has also been detected in the cytoplasm of gastric, duodenal, and rectal epithelium, indicating viral replication at these sites (Wong et al., 2020). Viral RNA also has been found in esophageal, gastric, duodenal, and rectal biopsies, but only in the patients with severe disease, suggesting that the presence of SARS-CoV2 in GI tissue is associated with a more severe disease course (Almeida and Chehter, 2020). Conversely, several studies associate presentation of GI symptoms with milder form of disease (Nobel et al., 2020; Papa et al., 2020). The current lack of clarity regarding whether GI symptoms lead to a severe or mild form of COVID-19 requires further studies and needs observation of large cohorts of patients for more assertive conclusions.

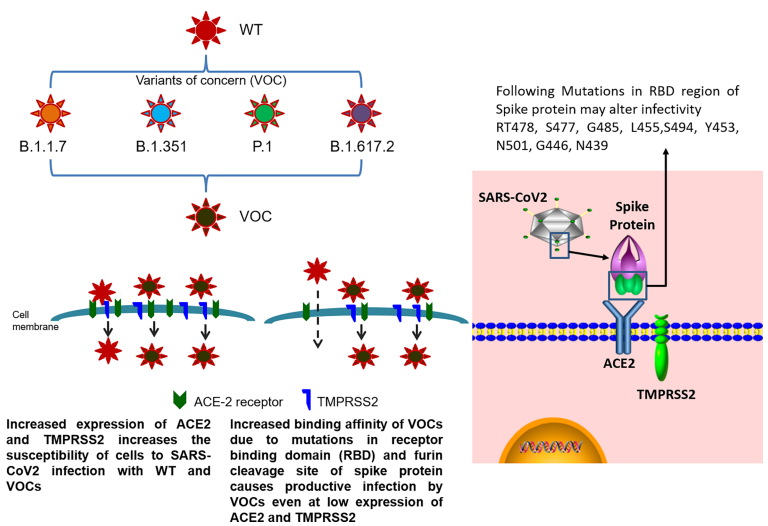

FIGURE 2 | Infective potential of wild-type SARS-CoV2 and its VOC. SARS-CoV2 infection occurs by the receptor-mediated entry of the virus into the host cells using ACE2 receptor in the presence of TMPRSS2 protease. Higher expression of ACE2 receptor and TMPRSS2 on the cell membrane of host cells causes internalization of both wild-type SARS-CoV2 and its VOC. In cells with low levels of ACE2 and TMPRSS2 expression, the mutations present in the receptor binding domain (RBD) of the spike protein (RT478, S477, G485, L455, S494, Y453, N501, G446, and N439) of VOC allows for efficient binding of the variant to the host receptors even at low expression levels as compared to the wild-type virus, contributing towards the enhanced transmissibility of VOC.

HEPATIC MANIFESTATIONS OF COVID-19

Hepatic manifestations of COVID-19 have been observed as a marked rise of transaminases in the blood indicative of liver injury. Several studies have reported abnormal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels with an elevation of serum bilirubin (Guan et al., 2020). Initially, a study from Wuhan, China, reported liver manifestations in 43 out of 99 patients (Chen et al., 2020). Later, a study involving a larger (n=1,099) cohort of patients from China has shown elevated serum levels of ALT, AST, and total bilirubin in 22, 21, and 10% of the cases, respectively. Comparison of non-severe *vs* severe cases has shown higher levels (ALT 19.8 *vs* 28.1%, AST 18.2 *vs* 39.4%, and bilirubin 9.9 *vs* 13.3%) in severe COVID-19 patients (Guan et al., 2020). Hepatic dysfunction in these severe COVID-19 patients is also associated with activation of coagulative and fibrinolytic pathways, reduced platelet counts, and increase in ferritin levels, neutrophil counts, and neutrophil-to-lymphocyte ratios (Wang et al., 2020). Hepatic dysfunction either due to replication of virus in liver or by other mechanisms is lacking of histological correlation. COVID-19 patients' post-mortem liver biopsies have shown microvesicular steatosis, hepatocyte degeneration, lobular focal necrosis, and neutrophil infiltration, but the mechanisms of liver injury in these patients remain uncertain (Lu et al., 2020). The mechanisms of hepatic injury in COVID-19 patients can be virus-induced cytopathic effects during its replication in hepatocytes, systemic inflammation-mediated immune injury through cytokine storm, pneumonia-associated hypoxia, or drug-induced hepatotoxicity (Xu et al., 2020). In addition, viral replication in liver may be more extensive as hepatic distribution of ACE-2 receptor is not limited to hepatocytes, but also expressed in the cholangiocytes and in the endothelial layer of small blood vessels of liver. Additionally, expression of ACE-2 receptor is much higher in cholangiocytes (59.7%) than hepatocytes (2.6%), which suggests that viral entry and replication in cholangiocytes may also dysregulate the liver function (Chai et al., 2020). Cholangiocytes are known to play important roles in liver regeneration, and immune response (Banales et al., 2019), and damage to these cells may cause liver injury during SARS-CoV2 infection.

RADIOLOGIC FEATURES OF GI AND HEPATOBILIARY SYSTEM IN COVID-19

Imaging manifestations of COVID-19 in organs other than the respiratory system is attributed to the viral entry receptor expression, local organ specific virus replication, and its cytotoxic effects. Typical imaging manifestations of COVID-19 pneumonia observed in CT scan are peripheral, multifocal consolidative and ground-glass opacities as observed in high-resolution CT (HRCT) scan (**Figures 3A**, **C**). In contrast, manifestations in other organs are mainly attributed to derangement of the renin-angiotensin system due to death of cell expressing ACE2 elevating angiotensin-II and reduced angiotensin 1–7, which further potentiates endothelial inflammation, thrombosis, and hypertension (Roberts et al., 2020). Abdominal imaging in COVID-19 patients is generally performed to evaluate the cause of abdominal pain. Radiologic findings on abdominopelvic CT are small bowel thickening, solid organ infarctions, and vascular thrombosis (Goldberg-Stein et al., 2020). The proposed mechanisms of bowel wall thickening could be SARS-CoV2 binding to bowel epithelial cells, leading to mucosal inflammation and prothrombotic state (Ahmad et al., 2021). This binding can decrease ACE2 receptor expression, resulting in dysregulation of B0AT1 (broad neutral amino acid transporter, SLC6A19), which influences, COVID-19-associated diarrhea (Barbosa da Luz et al., 2020). Other potential mechanisms are altered microbiota homeostasis, enterocyte damage, release of virulent proteins or toxins, and hypercoagulable state (Suryana et al., 2021). Bowel-wall thickening can occur non-specifically and also due to neoplastic, inflammatory, infectious, or ischemic conditions (Macari and Balthazar, 2001). Other causes of abdominal pain in COVID-19 patients are presented with epiploic appendagitis due to inflammation and venous thrombosis (Bashari et al., 2020). Other related manifestations such as bowel wall hyper-enhancement, pneumatosis, portal venous gas- and fluid-filled colon have been described in literature (Bhayana et al., 2020).

Almost 40% of COVID-19 patients have abnormal liver function tests (LFT) on admission, and this LFT abnormalities are associated with high fever, increased C-reactive protein, and longer hospital stay (Fan et al., 2020). Liver dysfunction mostly correlates well with imaging. Patient with altered LFT's may show fatty infiltration in the liver as evidenced in **Figure 3B** (Fan et al., 2020). Several COVID-19 patients with abdominal pain also have shown imaging findings similar to acute pancreatitis (**Figure 4**) (Saeed et al., 2020). In an ultrasonography-based study, gallbladder wall thickening, presence of pericholecystic fluid and sludge were reported in 3, 3, and 60% of COVID-19 patients, respectively (Bhayana et al., 2020). Definite cholecystitis with gallbladder distension and wall edema was also reported in 25% COVID-19 (Goldberg-Stein et al., 2020). Gallbladder wall edema in COVID-19 patients may be a reflection of direct hepatocellular damage due to the virus or by a reactive inflammatory response such as systemic inflammatory response syndrome or Kawasaki-like multisystem inflammatory syndrome (Ahmad et al., 2021). CECT abdomen and CT angiography can show gall bladder wall thickening and edema (**Figure 5A**), small (**Figure 5B**) and large (**Figure 5C**) bowel wall thickening, vascular thrombosis and solid organ infarctions, pneumatosis, portal venous gas- and fluid-filled colon (Bhayana et al., 2020). Imaging plays a pivotal role in diagnosing various complications of GI and hepatobiliary system and may play a significant role in prognosis of patients.

EMERGING VARIANTS OF SARS-CoV2

Full-length genome sequencing and phylogenetic analysis during this pandemic period have given insight into the genetic diversity of SARS-CoV2. High rates of mutation and emerging variants

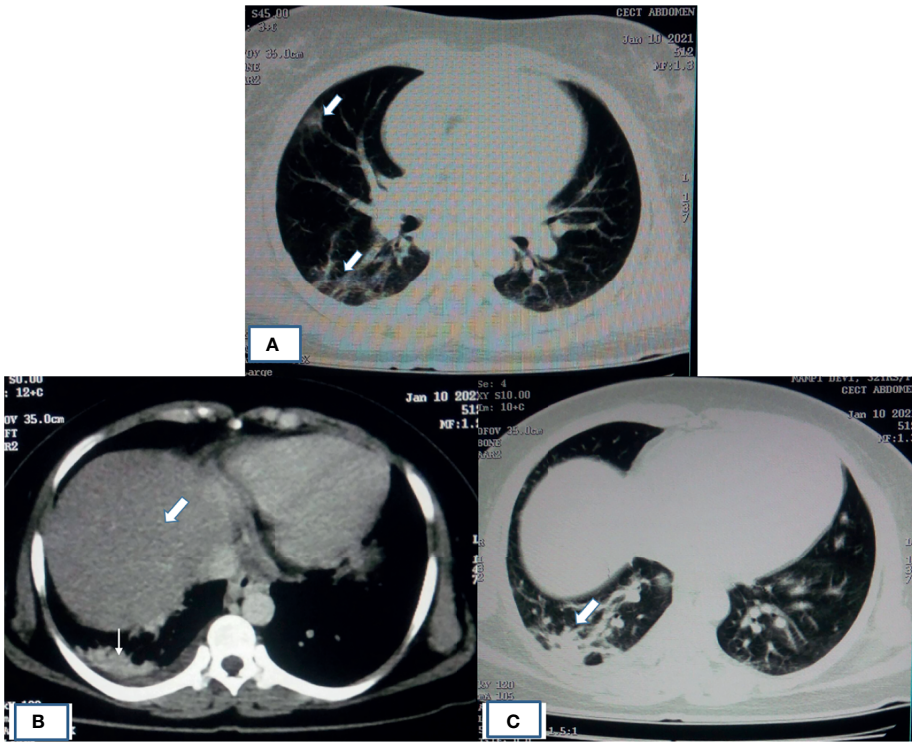

FIGURE 3 | HRCT of patient showing typical imaging manifestations of COVID-19 pneumonia and fatty infiltration of liver. **(A)** HRCT of chest showing pneumonia in the form of peripheral, multifocal consolidative and ground-glass opacities as indicated by thick white arrow. **(B)** Mediastinal window image of same COVID-19 patient with altered LFT had shown fatty infiltration of liver (thick white arrow) with opacity in the right lung base (thin white arrow). **(C)** Corresponding lung window image showing ground-glass haziness of COVID-19 pneumonia (thick white arrow).

are attributed to the varying selection pressure during its global spread in the current pandemic. Several variants of this pandemic virus are emerging rapidly with altered infectivity and enhanced transmission abilities. These SARS-CoV2 variants are classified into two major lineages (A, B) with several sublineages (1, 2, 3, and 5) as per the dynamic lineage or PANGO (Phylogenetic Assignment of Named Global Outbreak) lineage classification method (Rambaut et al., 2020;

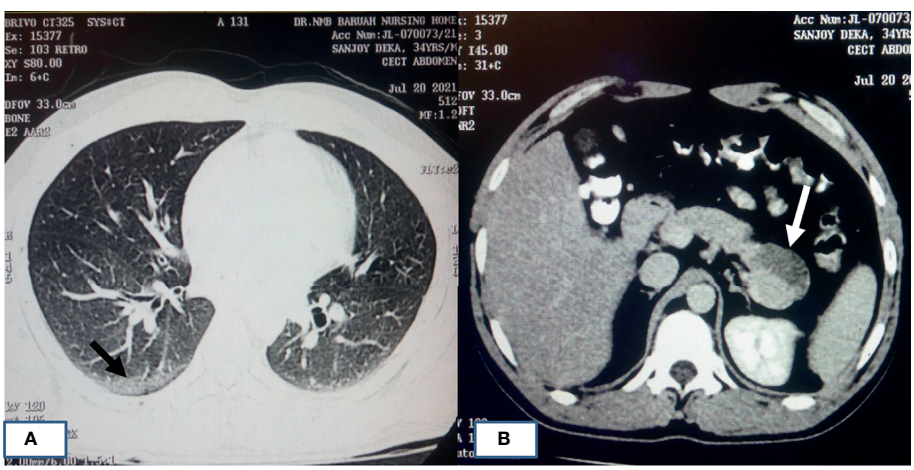

FIGURE 4 | HRCT chest of patients with COVID-19 pneumonia and acute pancreatitis. **(A)** A 34-year-old male who had cough and painful abdomen showing typical imaging manifestations of COVID-19 pneumonia in the peripheral basal segment of right lower lobe (black arrow). **(B)** CECT abdomen image of same patient showing hypodensity in the tail of pancreas (white arrow) suggestive of acute necrotizing pancreatitis.

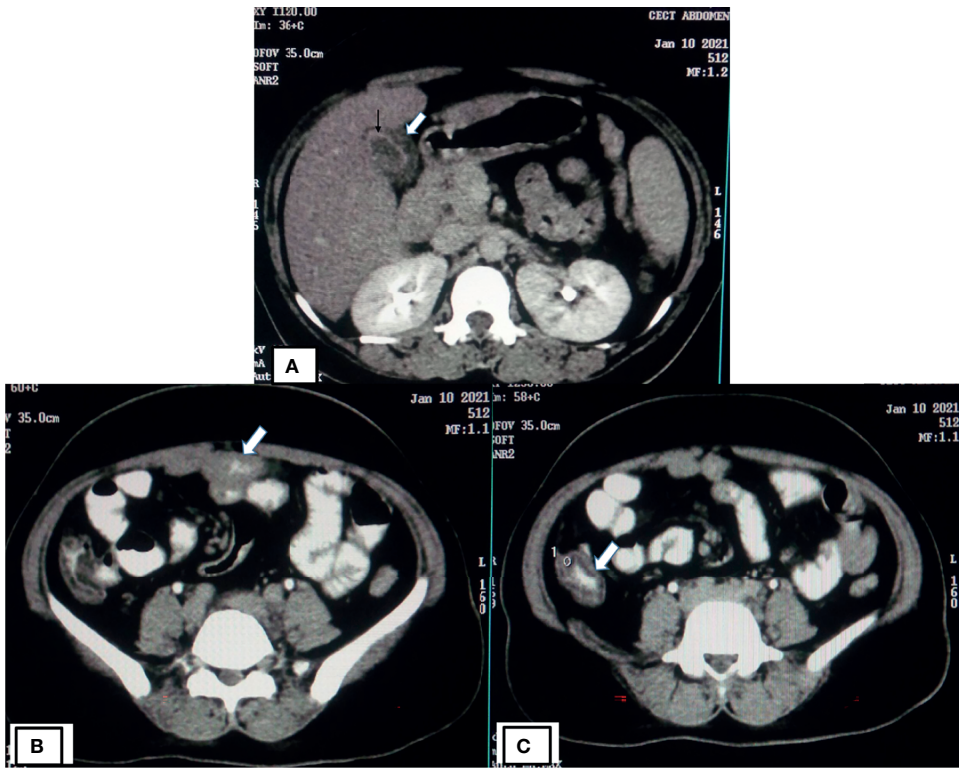

FIGURE 5 | CECT of COVID-19 patients showing gall bladder, small and large bowel wall thickening. **(A)** CECT abdomen of COVID-19 patients (same patient as shown in **Figure 3**) showing gall bladder wall thickening (thick white arrow) likely due to edema and mucosal enhancement (black arrow). CECT abdomen of same patient showing small bowel (thick white arrow) wall thickening **(B)** and large bowel (thick white arrow) wall thickening **(C)**.

Rambaut et al., 2021). Lineage-A variants mostly harbor two unique mutations (8782 C>T and 28144 T>C), which are absent in Lineage-B variants. These variants of different lineages and sublineages also lead to varying manifestations after their respective infections. Association of Lineage-A variants is mostly with self-limiting upper respiratory infections, whereas Lineage-B variants are associated with severe lower respiratory tract infections, ARDS, and extrapulmonary manifestations (Brufsky, 2020). Several synonymous and non-synonymous mutations get accumulated in the entire genome, leading to the emergence of viral variants. Recently, emerging variants of concern (VOC) were identified by comparing their common ancestor with relevant amino acid substitutions at different ORFs. The GISAID (Global Initiative on Sharing All Influenza Data) introduced another nomenclature system for major clades, developed by Sebastian Maurer-Stroh et al., based on marker mutations (alphabet for non-synonymous and number for synonymous substitutions). The hCOV-19 Metadata (2,564,149 sequence submission, as of August 1, 2021) in GISAID is now classified into 10 clades (S, L, V, G, O, GH, GR, GV, GK, and GRY) using specific combinations of genetic markers. The early splits of phylogenetic clades were S (marker C8782T, T28144C and NS8-L84S) and L (C241, C3037, A23403, C8782, G11083, G26144, T28144). Further, L clade evolved to V (G11083T, G26144T, NSP6-L37F + NS3-G251V) and G (C241T, C3037T, A23403G, and S-D614G); and later G clade evolved into GH (C241T, C3037T, A23403G, G25563T includes S-D614G + NS3-Q57H), GR (C241T, C3037T, A23403G, G28882A includes S-D614G + N-G204R), GV (C241T, C3037T, A23403G, C22227T includes S-D614G + S-A222V), and GK. Recently, GR into GRY (C241T,C3037T, 21765-21770del, 21991-21993del, A23063T, A23403G, G28882A includes S-H69del, S-V70del, S-Y144del, S-N501Y + S-D614G + N-G204R) along with GK. The sequences that do not fall under above clades were assigned O clades (GISAID, 2021). The Nextstrain clades naming is the third nomenclature system, which follows Year-Letter nomenclature to label clades that persist for at least several months and have significant geographic spread (20% frequency—globally at any time point). These clades are also defined by signature mutations and differ in at least two positions from their parent clade. The circulating clades for the year 2019 were 19A and 19B, for the year 2020 were clade 20A-20J, and till now, the 2021 clades are emerged to 21A-21H (Bedford et al., 2021). Recently, WHO using letters of the Greek alphabet to name major VOC as Alpha, Beta, Gamma, Delta, and the variants of interest (VOI) as Kappa, Epsilon, Eta, Theta, Iota, and Lambda. In the year 2020, Alpha variant (Pango Lin, B.1.1.7/GSIAID, GRY/Nextstrain, 20I clade) emerged from UK, Beta variant (Pango Lin, B.1.351/GSIAID, GH/501Y.V2/Nextstrain, 20H clade) emerged from South Africa, and Gamma variant (Pango Lin, P.1/GSIAID,

GR/501Y.V3/Nextstrain, 20J clade) emerged from Brazil, whereas Delta variant (Pango Lin, B.1.617.2, AY.1/2/3/GSIAID, G/478K.V1/Nextstrain, 21A clade) recently emerged from India in the year 2021 (WHO, 2021).

The emerging variants with D614G aa substitution in the spike protein are now dominant and circulating globally. The variants with D614G substitution do not cause severe illness but alter its infectivity, competitive fitness, and transmission as evident from laboratory studies (Hou et al., 2020). Alpha variant emerged from the UK (20I/501Y.V1) during September 2020 and has shown increased transmissibility with minimal impact on the disease severity and is now reported in several countries. This variant of B.1.1.7 lineage harbors receptor-binding domain (RBD) N501Y mutation and other mutations including 69/70 deletion, spike P681H, and ORF8 stop codon (Q27stop) mutation (Leung et al., 2021).The beta variant (20H/501Y.V2) of B.1.351 lineage harbors spike N501Y, E484K, and K417N/T mutations without 69/70 deletion and is predicted to have emerged in South Africa during October 2020 with potential of global spread (Tegally et al., 2020). It is important to note that the E484K mutation, termed as "escape mutation," which partially aids the virus to evade host immunity, is acquired either through natural infection or vaccines. These three mutations have also been observed in gamma variant (P.1/20J/501Y.V3), which is responsible for the drastic rise in COVID-19 infections and related deaths in Manaus, Brazil, during December 2020 to early 2021. This P.1 variant has 17 unique mutations, 12 of which are present in the spike protein region where the abovementioned triplet mutations cause increased binding affinity of spike protein to ACE2 receptor, probably contributing towards its enhanced infectivity and transmissibility (Faria et al., 2021). Despite these commonalities, P.1 variant is significantly less resistant to natural infection or vaccine-induced antibody response as compared to the beta variant (B.1.351), indicating viral neutralization is not restricted to only the RBD region of virus (Dejnirattisai et al., 2021). Similar to E484K, another probable escape mutation, E484Q, has been observed in the delta variant (B.1.617, G/452R.V3) currently dominant in India and detected in more than 50 other nations. Additionally, this variant has P681R and L452R mutations in the RBD region (Cherian et al., 2021; Ferreira et al., 2021), with L452R also present in B.1.427/B.1.429 variants found in California, USA, which have been associated with increased transmissibility of virus (Deng et al., 2021). As of May 2021, three sublineages have been detected for this variant (B.1.617.1/2/3) with B.1.617.2 being the most dominant, having a unique T478K mutation absent in sublineages 1 and 3, serving as a possible route of immune evasion (Di Giacomo et al., 2021). The delta variant, B.1.617.2, has been implicated in the devastating surge of infections in India from February 2021 to May 2021. In addition to the SARS-CoV2 variants discussed above, numerous other variants are currently in circulation and will emerge due to natural selection of virus resulting in accumulation of favorable mutations for the continued survival of virus (**Figure 2**). In-depth description of all these variants remains out of the scope of this review.

EFFECT OF SARS-CoV2 ON PRE-EXISTING LIVER AND GI DISEASES

COVID-19 patients with pre-existing morbidities such as diabetes, hypertension, coronary artery disease, cancer, and pre-existing liver disease are at risk of a severe disease and death (Boettler et al., 2020; Fix et al., 2020). Pre-existing liver diseases of major concern in COVID-19 are history of chronic viral hepatitis, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), autoimmune hepatitis, hepatic compensated and decompensated cirrhosis. The impact of pre-existing liver disease due to different etiology is not completely known. The risk of SARS-CoV2 infection in advanced chronic liver disease (CLD) may be higher due to cirrhosis-induced immunodeficiency, but conversely their immunosuppressive state may provide protection from cytokine storms, modulating disease outcome of multiorgan failure in COVID-19 (Albillos et al., 2014). The effects of SARS-CoV2 infection on different pre-existing liver and GI diseases are mentioned below and summarized in the **Table 1**.

Chronic Hepatitis B

Chronic hepatitis B (CHB) is one of the most prevalent pre-existing liver diseases that affect the disease outcome of COVID-19. Dysregulation of host innate and adaptive immune response during CHB may hinder SARS-CoV2 clearance. The median time for SARS-CoV2 clearance in CHB group was reported to be longer (21 days) than in the non-HBV group (14 days) (Liu et al., 2020a). COVID-19 patients with chronic HBV infection are more prone to liver injury and poor prognosis with increased mortality and incidence of complications such as acute-on-chronic liver failure (ACLF), acute cardiac injury, and shock (Zou et al., 2020). Additionally, the clinical presentation of COVID-19 patients with pre-existing CHB are leukopenia, erythropenia, and thrombocytopenia, along with moderate liver injury and inflammation; however, the HBV co-infection with SARS-CoV2 did not significantly affect the outcome of COVID-19 (Liu et al., 2020b). Hepatitis B virus reactivation is also a major concern in COVID-19 patients with chronic HBV infection (Liu et al., 2020a). This is mostly associated with COVID-19 management due to immune suppressive corticosteroid therapy or biological therapies such as IL-6 receptor antagonists in patients with current or past HBV exposure (Rodriguez-Tajes et al., 2020). Such patients require HBV DNA load monitoring and treatment with antivirals for HBV such as Entecavir or Tenofovir to reduce viral load and hepatitis B flares (Mehta et al., 2020).

Chronic Hepatitis C

A recent study of chronic HCV-infected veteran cohort found similar SARS-CoV2 infection rate in patient groups of varying degrees of liver fibrosis (Butt and Yan, 2020). It was also observed that virus clearance and COVID-19 recovery were delayed with late antibody response in chronic hepatitis C (CHC) patients co-infected with human immunodeficiency virus and following liver transplantation (Muller et al., 2020; Zhou et al., 2020). Another

TABLE 1 | Effects of SARS-CoV2 infection on pre-existing hepatic and gastrointestinal conditions.

	Pre-existing condition	Effects of SARS-CoV2 infection	Clinical manifestations	References
Hepatic	CHB	Delayed SARS-CoV2 clearance, liver injury, incidence of ACLF, acute cardiac injury, shock	Leukopenia, erythropenia, thrombocytopenia, moderate liver injury, inflammation, HBV reactivation	(Liu et al., 2020a; Liu et al., 2020b; Mehta et al., 2020; Rodriguez-Tajes et al., 2020; Zou et al., 2020)
	CHC	Delayed SARS-CoV2 clearance, delayed recovery, late antibody response in CHC-HIV co-infection, higher cases of mortality in cirrhotic patients of metabolic origin	No significant alteration from general COVID-19 symptoms	(Butt and Yan, 2020; Muller et al., 2020; Zhou et al., 2020; Mangia et al., 2020)
	NAFLD	Increased risk of COVID-19 severity, liver damage, increased expression of ACE2 and furin receptors	Hepatic injury and failure, hepatic encephalopathy, GI bleeding.	(Ji et al., 2020; Mahamid et al., 2020; Sachdeva et al., 2020; Meijnikman et al., 2020; Mushtaq et al., 2020; Biquard et al., 2020; Singh et al., 2021)
	Cirrhosis	Increased mortality, higher rate of acute hepatic decompensation and ACLF, progressive liver injury	Hepatic decompensation, liver injury, and liver failure	(Rosenblatt and Verna, 2020; Marjot et al., 2020; Sarin et al., 2020; Shalimar et al., 2020; Bajaj et al., 2020)
	HCC	Major risk factor, increased mortality	Exacerbation of hepatic injury and severity	(Jothimani et al., 2020; Kim et al., 2020; Chai et al., 2020)
	Liver transplant	Higher risk of severity in long-term (>10 yrs) patients on immunosuppressive therapy and advanced age (>65 yrs) and comorbidities	GI symptoms (nausea, vomiting, abdominal pain diarrhea), higher ICU admissions, invasive ventilation procedures	(Fix et al., 2020; Huang et al., 2020; Webb et al., 2020; Manzia et al., 2021; Theodore et al., 2021)
Gastrointestinal	IBD	Higher mortality risk associated with advanced age (>60 yrs), comorbidities, active colonic disease, corticosteroid therapy, combination of anti-TNF and corticosteroid therapy	No significant alteration from general COVID-19 symptoms	(Hunt et al., 2020; Bezzio et al., 2020; Fiorino et al., 2020; Singh et al., 2020; Lukin et al., 2020)
	Celiac disease	No major association as risk factor for COVID-19 or its severity	No significant alteration from general COVID-19 symptoms	(Zingone et al., 2020; Gokden et al., 2020; Lebwohl et al., 2021)

CHB, chronic hepatitis B; CHC, chronic hepatitis C; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; IBD, irritable bowel disease; ACLF, acute-on-chronic liver failure; HIV, human immunodeficiency virus; TNF, tumor necrosis factor; GI, gastrointestinal.

study also found lower-risk mortality in cirrhotic CHC and DAA treated or cured CHC patients, whereas case fatality rate was higher in cirrhotic patients of metabolic origin (Mangia et al., 2020).

NAFLD

Non-alcoholic fatty liver disease (NAFLD) is considered a greater risk for COVID-19 progression and liver damage, which is further complicated by hepatic failure, hepatic encephalopathy, and GI bleeding as reported in the initial studies during pandemic (Ji et al., 2020). Another retrospective case-control study of 71 COVID-19 patients with or without fatty liver showed a significant severe COVID-19 presentation in NAFLD as compared to non-NAFLD subjects (36.3 *vs* 10.2%, OR 3.57, 95% CI 1.22–14.48) (Mahamid et al., 2020). Pooled data analysis of NAFLD in COVID-19 patients had shown an association with a higher risk of symptomatic, severe, and progressive COVID-19. The odds ratio of pooled cases after adjusting the obesity factor was found significant for this risk (OR=2.358, 95% CI: 1.902–2.923) (Sachdeva et al., 2020). Increased expression of SARS-CoV2 entry receptor ACE-2, furin, and protease TMPRSS2 was observed in liver tissue of NAFLD patients (Meijnikman et al., 2020). Meta-analysis of array-based liver gene expression data found a positive correlation of NAFLD with the expression of ACE-2 and furin but not with the expression of TMPRSS2 (Singh et al., 2021). Other studies debated that NAFLD could predict liver injury but not mortality or disease severity of COVID-19 presentation or progression (Mushtaq et al., 2020). Another study concluded that increased ACE-2 and TMPRSS2 gene expression in liver tissue of NAFLD patients was not necessary for increased liver uptake of SARS-CoV2 (Biquard et al., 2020). Association of NAFLD with severe presentation of COVID-19 is due to the fact that patients with NAFLD tend to be obese, be diabetic, and have metabolic syndrome, which have also been associated with severe form of COVID-19.

Cirrhosis

Pre-existing cirrhotic patients are at increased risk of SARS-CoV2 infection due to their immune dysfunction status, frequent hospitalization, and associated comorbidities such as hypertension, diabetes, and obesity (Rosenblatt and Verna, 2020). Cirrhosis is not necessarily associated with diabetes, hypertension, and obesity, unless it is due to NASH/NAFLD etiology. Outcome of SARS-CoV2 infection was studied in a large number of cirrhotic patients (n=386) by comparing with non-cirrhotic patients (n=359) in and international registry study from UK hospital network (Marjot et al., 2020). Rate of mortality in cirrhotic patients has shown to be increased according to the severity of cirrhosis (Child-Pugh class A, 19%; B, 35%; and C, 51%). Higher rate of acute hepatic decompensation and acute-on-chronic liver failure occurred in

patients with cirrhosis following SARS-CoV2 infection. Poor outcome to SARS-CoV2 infection in patients with cirrhosis was observed in the APCOLIS study (Sarin et al., 2020), as well as one study from our center (Shalimar et al., 2020). In the APCOLIS study, a higher rate of progressive liver injury (57%) and mortality (43%) was observed in decompensated cirrhotic patients following SARS-CoV2 infection. About 20% of cirrhotic patients presented with either acute-on-chronic liver failure or acute decompensation following infection. Similarly, from our center, high mortality (42.3%) was observed in cirrhotic patients superimposed with COVID-19 than historical cirrhotic control (23.1%) (Shalimar et al., 2020). Another multicenter study observed similar mortality risk in patients compared to superimposed cirrhosis and COVID-19, cirrhosis alone, or COVID-19 alone (Bajaj et al., 2020).

Hepatocellular Carcinoma

Most patients with hepatocellular carcinoma (HCC) have underlying chronic liver disease, and therefore, they fall under this high-risk category and are likely to have worse outcome (Jothimani et al., 2020). A multicenter study from USA had observed HCC as an independent risk factor for higher overall mortality with a hazard ratio (HR) of 3.31 (95% CI,1.53–7.16) (Kim et al., 2020). SARS-CoV2 entry receptor ACE-2 gene expression in cancer and its impact on overall survival (OS) and disease-free survival (DFS) were recently studied (Chai et al., 2020). Higher ACE-2 expression predicted better outcome of HCC with disease-free survival, indicating a dual role of ACE-2 function predicting outcome.

LIVER TRANSPLANT AND THE RISK OF COVID-19 SEVERITY

SARS-CoV2 pandemic has brought with it new considerations in the cases of transplant procedures. In case of transplant patients, their immunocompromised condition may have a two-pronged effect of SARS-CoV2 infection, such as increased viremia and decreased chances of systemic inflammation. Increased risk of developing a severe pulmonary disease in liver transplant (LT) patients following SARS-CoV2 infection was not observed compared to general COVID-19 patients (Bhoori et al., 2020; D'Antiga, 2020). However, the risk of severity and mortality increased up to 23% in LT patients who were on long-term (>10 years) maintenance immunosuppressive therapy (Fix et al., 2020; Huang et al., 2020) and those with comorbidities such as >65 years age, diabetes, hypertension, and obesity (Webb et al., 2020). LT success in COVID-19 patients is high when both donor and recipients have neutralizing SARS-CoV2 antibodies prior to LT (Manzia et al., 2021). It was also observed that a large proportion of LT patients with COVID-19 were presented with GI symptoms such as nausea, vomiting, abdominal pain, and diarrhea compared to non-LT COVID-19 patients. These patients also required higher rate of ICU admissions (28 *vs* 8%) and invasive ventilation procedures (20 *vs* 5%) (Theodore et al., 2021). It should be noted that the level and nature of immunosuppression in LT patients may hinder the development of anti-SARS-CoV2 immunity (Theodore et al., 2021). Current knowledge regarding the safety of LT procedures on COVID-19 patients and the expected outcome of patients infected with SARS-CoV2 post-LT remains ambiguous. To draw clear conclusions, long-time follow-up studies are required in large cohorts of LT patients.

GI DISEASES

Irritable Bowel Disease

IBD, being the chronic inflammation of GI tract, presents an acute challenge during the time of COVID-19 pandemic. The widespread use of immune-modulators and biological agents for complete remission and mucosal healing in Crohn's disease and ulcerative colitis may contribute towards the progression of SARS-CoV2 infections. Various international organizations involved in research and care for IBD patients have reached the consensus of continuation of immune-active therapies with strict disinfection and social distancing measures to ablate the risk of relapse in future. International registry SECURE-IBD, to monitor outcomes of IBD patients worldwide, has reported a low fatality rate for the 6,262 registered patients from 33 countries. It did implicate age, comorbidities, steroid and mesalamine therapy with ICU admissions and death. Highest death rate of 12–25% was observed in patients >60 years of age. Mortality also increased in patients having more than one comorbidities (15–33%) and taking oral/parenteral steroids (13%), Budesonide (7%), mesalamine/sulfasalazine (6%), azathioprine and methotrexate monotherapy (6%). Conversely, anti-TNF or anti-interleukin 12/23 monotherapy only had 1–2% risk of adverse outcomes. This risk significantly increased in case of combination therapy of anti-TNF with other corticosteroids (Hunt et al., 2020). Various other studies with regards to IBD patients have reported on the prevalence of COVID-19, risk assessment, severity of disease, and mortality (Bezzio et al., 2020; Fiorino et al., 2020); and a general consensus suggests no significant rise in severity or mortality due to IBD. But advanced age, comorbidities, active colonic disease (Singh et al., 2020), and use of corticosteroid therapy (Lukin et al., 2020) are contributing risk factors. Additionally, the effect of IBD medications on the expression of ACE-2 and TMPRSS2 in ileum and colon and their contribution towards increased susceptibility to local or systemic disease are being explored (Bangma et al., 2020). Studies have shown increased ACE-2 and TMPRSS2 expression in active colon disease and ileal inflammation, respectively (Nowak et al., 2020). Currently, no association has been established with increased ACE-2 expression and risk of SARS-CoV2 infection in IBD patients.

Celiac Disease and COVID-19

Celiac disease (CeD) is an autoimmune multiorgan disease affecting the small bowel of patients in a genetically predisposed manner. It is characterized by hyper-responsiveness of innate and adaptive immune system to gluten. Studies have established

increased susceptibility of CeD patients to invasive pneumococcal disease (Simons et al., 2017) and viral infections such as influenza (Marild et al., 2010) and varicella zoster (Ludvigsson et al., 2017). In the current scenario, studies involving patient-response-based data collection and analysis have been done to determine the risk associated with CeD towards susceptibility and severity of COVID-19. A large CeD subset (n= 40,963) from nationwide histology-based population of patients with GI diseases in Sweden, ESPRESSO, was identified, and no significant increase in COVID-19-based hospitalizations, severity, or mortality was observed as compared to the control group (n= 183,892) (Lebwohl et al., 2021). Another study from Italy, involving 171 CeD patients on gluten-free diet, also suggests no major association of CeD with increased risk of SARS-CoV2 infection or severity (Zingone et al., 2020). Similarly, a questionnaire-based study conducted with 101 CeD patients in Turkey also suggests no association of CeD with COVID-19 susceptibility in patients on gluten-free diet as compared to non-CeD population (Gokden et al., 2020). Current data suggest no association of CeD with COVID-19 risk, but majority of these studies are limited in their scope due to the design of studies being unable to identify asymptomatic SARS-CoV2 infections and no long-term analysis of associated debilitations.

EMERGING VARIANTS AND DISEASE OUTCOME IN PATIENTS WITH PRE-EXISTING LIVER-GI DISEASES

The effects of emerging variants on the clinical outcome can be studied by comparing outcomes of COVID-19 patients in the first wave *versus* the second wave of SARS-CoV2 infections in India. Second wave surge was defined after March, 15, 2021, where emerging delta variant was widespread in India (Kar et al., 2021). Recently, our study compared the outcome of patients with clinical presentations of cirrhosis and COVID-19 patients admitted during the first (Shalimar et al., 2020) and second wave of the pandemic in India. It was observed that the clinical presentation with severe COVID-19 infection was more frequent in the second wave than the first wave of infections (51.5 *vs.* 25.0%; p=0.006). However, both the subgroups had similar duration of hospital stay and mortality.

HOST GENE EXPRESSIONS IN LIVER AND GI TISSUE OF SARS-CoV2-INFECTED PATIENTS

Several studies have shown transcriptome analysis using tissue samples of COVID-19 patient or cell line infected with SARS-CoV2 for understanding host immune response and expression of viral entry receptor. Single-cell RNA sequencing analysis (accession no: GSE164547) of cells in human respiratory epithelium had shown variable expression of host entry molecules (ACE2, TMPRSS2, and FURIN) in different cell types, including ciliated, goblet, club, alveolar type 1 (AT1), and AT2 cells. Abundant expression of ACE2, TMPRSS2, and FURIN was observed on the apical side of ciliated cells, which support massive replication of SARS-CoV2 during early stage of infection (Ahn et al., 2021). Transcriptomic analysis (GSE148697) of SARS-CoV2-infected lung organoids enriched with permissive AT2 cells has shown upregulation of chemokines and cytokines (CXCL2, CCL2, CXCL3, IL1A, BCRC3, AADAC, and ATPB4) without upregulation of type I/III interferon signaling (Han et al., 2020). However, differential expression study in postmortem lung tissue of COVID-19 patients *vs* healthy controls (GSE147507) had shown increased expression of Type I IFN genes (IFNA4, IFNA6, IFNA10) and significant enrichment of the common Type I and Type II Interferon stimulating genes including IFNA2, IFNB1, and IFNG genes. Increased expression of antiviral innate immune response genes (IFIH1, DDX58, EIF2AK2, OAS2) and decreased expression of its negative regulators (IRF2BP1, SKIV2L) were also observed (Daamen et al., 2021). Transcriptome (GSE161881) analysis of SARS-CoV2-infected Vero E6 cells at 8 h post-infection has shown upregulations of antiviral genes (IFIT1, ZC3HAV1), NF-κB targets (CCL5, CXCL10), and ER stress response (DDIT3, PPP1R15A, GADD45B) genes (Zhang et al., 2021).

Few transcriptomic studies in extrapulmonary organ have been reported for SARS-CoV2 infection. Differential expression of genes (DEGs) in liver tissues of severe COVID-19 (GSE150316) and non-Covid 19 (GSE112356) patients have shown differential upregulation of host genes implicated in tissue remodeling (DNAJB1/hsp40, IGF2, EGFR, and HDGF), liver inflammation, and fibrosis such as metalloproteinases (MMP-3, 16,17, TIMP-1, 2, 4), collagens (COL6A3, 18A1-AS1, 20A1, 24A1, EC12, 13A1, 22A1, COLGALT2), and VCAM1. These genes are also upregulated in liver fibrosis, cirrhosis, and NAFLD. Downregulated genes such ACAD11, CIDEB, GNMT, GPAM, and cytochrome P450 (CYP450) family members are mostly implicated in metabolic pathways, mitochondrial function, and xenobiotic/drug metabolism (Hammoudeh et al., 2021). Expression profile observed in COVID-19 samples (GSE147507) has similar DEGs as seen with other diseases and comorbidities such as liver cirrhosis, influenza, and colonic neoplasms. These DEGs are also positively regulated in respiratory diseases, arthritis, psoriasis, glioblastoma, Crohn's disease, ulcerative colitis, colorectal cancer, skin diseases, pneumonia, keratosis, lupus erythematosus, esophageal cancer (Nain et al., 2021). Single-cell transcriptome analyses of colon mucosae of normal, colorectal adenoma (CRA), and colorectal cancer (CRC) patients have shown increased expression of SARS-CoV2 entry-related genes such as ACE2, CD147 (Basigin), TMPRSS2, Cathepsin B (CATB), Cathepsin L (CATL), and Furin. Expression of these entry receptor genes is found positively correlated with genes related to inflammatory cytokine-mediated signaling pathways and virus infection. Expression of these genes may be attributed to the tissue tropism of SARS-CoV2 on intestinal cells (Chen et al., 2020).

COVID-19 VACCINE AND DILEMMA OF VACCINATION WILLINGNESS, EMERGING VARIANTS, AND PRE-EXISTING DISEASES

SARS-CoV2 vaccines received fast-track emergency use authorization due to catastrophic consequences of ongoing COVID-19 pandemic and emergence of variants. These vaccines include mRNA vaccine expressing full-length spike protein (BNT162b2, Pfizer-BioNTech and mRNA-1273, Moderna), adenoviral vectored vaccine (ChAdOx1 nCoV-19, AstraZeneca; Covishield, Serum Institute of India; JNJ-78436735 or Ad26COVS1, Johnson & Johnson; and Sputnik V or Gam-COVID-Vac, Gamaleya National Research Centre, Russia), and whole inactivated virion (Covaxin or BBV152, Bharat Biotech; BBIBP-CorV, Sinopharm; and CoronaVac, Sinovac Biotech). The vaccine safety and efficacy data till date suggest complete safety and varying levels of efficacy, which may be further enhanced by additional booster dose or combination of different vaccines. There is also hesitancy in the general population to receive vaccine. Several surveys have reported variations in the global vaccine hesitancy average rate, which was 21% in April 2020, 36% in July 2020, and later declined to 16% in October 2020. These variations are mostly attributed to socio-demographic determinants including age, gender, ethnicity, education, risk perception of infection, and vaccine safety and efficacy (Guidry et al., 2020; Joshi et al., 2021). A recent survey (VACUNEII project) on SARS-CoV2 vaccine acceptance among gastroenterologists (n=144) and inflammatory bowel disease (IBD, n=1302) patients reported willingness of 43% of patients to receive the vaccine and 43% are not sure, whereas 95% of the physicians recommended vaccine for IBD patients (Ferreiro-Iglesias et al., 2021). Another survey on celiac disease patients (n=103) has shown 30% vaccine hesitancy including refusal due to fear of adverse events or distrust on the fast vaccine production (Costantino et al., 2021). Adverse effects have been reported in very few cases including vaccination-induced autoimmune hepatitis (AIH) (Bril et al., 2021; Rocco et al., 2021) and hepatotoxicity or drug-induced liver Injury in a patient with known history of IBD (Mann and Sekhon, 2021). Cytokine release syndrome as an adverse effect was observed after vaccination with BNT162b2 in a patient with colorectal cancer who is on longstanding anti-PD-1 monotherapy (Au et al., 2021).

The paradigm of COVID-19 vaccination in context to pre-existing liver and GI diseases does not fall under the linearity of vaccine efficacy due to the immunosuppressive state of patients either caused by the disease (cirrhosis, HCC) or the treatment (liver transplant, IBD). A recent prospective study analyzed for immune response at 4 weeks after the 2nd dose of mRNA vaccines or after the single dose of Johnson & Johnson vaccine in LT recipients (n=62) and in chronic liver disease (CLD) patients with (n=79) and without (n=92) cirrhosis. Of these, 61.3% of LT recipients and 24% of CLD patients had shown poor (undetectable <0.4 U/ml or suboptimal <250 U/ml) antibody response (Thuluvath et al., 2021). A multicenter study from China in NAFLD patients (n=381) had shown detectable antibody responses in 95.5% of patients at 14 days post-vaccination of the second dose of the alum-adjuvanted inactivated COVID-19 vaccine (Wang et al., 2021). Current vaccines indicated for protection against SARS-CoV2 have shown reduced efficacy to emerging variants such as delta as compared to the earliest strain (alpha) of SARS-CoV2 (Lopez Bernal et al., 2021).

DRUG, TARGET, AND FUTURE PROSPECTIVE OF TREATMENT FOR SEVERE COVID-19 WITH GASTROINTESTINAL MANIFESTATIONS

As discussed earlier, mild to moderate GI symptoms of nausea, vomiting, diarrhea, anorexia, and abdominal pain have been observed in COVID-19 patients. The certainty of association of these symptoms with severe progression of COVID-19 remains to be established, but COVID-19 cases presented with gastrointestinal symptoms are more likely to be severe and complicated by acute respiratory distress (ARDS), liver damage, and poor prognosis. Two separate studies (n= 141 and n=1,314) have shown that almost 74 to 86% critically ill COVID-19 patients, owing to the vigorous treatment and prolonged hospitalization, are more susceptible to develop severe gastrointestinal complications (Sun et al., 2020; Kaafarani et al., 2020). These include acute cholecystitis, acute pancreatitis, ileus and feeding intolerance, acute colonic pseudo-obstruction, hemorrhagic and ischemic colitis, and mesenteric ischemia. The cause of these severe GI manifestations may be attributed to systemic inflammation affecting extrapulmonary organ systems, side effects of drugs/antibiotics, or dysbiosis of gut microbiome or dysregulation affecting gut-liver and gut-lung axis due to SARS-CoV2 infection (Ye et al., 2020).

As first line of treatment, several repurposed drugs were explored by *in silico* studies for treatment of moderate to severe COVID-19. These drugs are broad-spectrum antivirals (remdesivir, lopinavir, ritonavir, arbidol, baloxavir marboxil, favipiravir), antiparasitic drugs (chloroquine, hydroxychloroquine, and ivermectin), immunomodulators (dexamethasone, tocilizumab, and interferon), antibiotics (azithromycin and doxycycline), and antitumor therapeutic (2-Deoxy-d-glucose, 2-DG) (Andersen et al., 2020; Mesri and Lampidis, 2021). Besides antivirals, immunomodulators are aimed to block the cytokine storm in severe COVID-19, and neutralizing antibody cocktails have been employed to prevent subsequent virus entry (Rai et al., 2021). Out of the abovementioned drugs, remdesivir and dexamethasone have shown promising clinical benefits in randomized controlled trials. The drug remdesivir is an inhibitor of RNA polymerase essential for replication of SARS-CoV2 (Pan et al., 2020). The double-blind, randomized, placebo-controlled trial of intravenous remdesivir in severe COVID-19 patients (n=1,062, remdesivir, n=541 and placebo, n= 521) found remdesivir superior to placebo in shortening the time to recovery (Beigel et al., 2020). In a real-life

study of severe COVID-19 patients (n=52) receiving non-invasive ventilation, global clinical improvement was observed in 43% of patients at 12 days, 71% at 20 days, and 53% patients had de-escalation of oxygen support post-treatment with remdesivir (Simioli et al., 2021). In a controlled open-label trial of dexamethasone (n= 2,104 patients *vs* usual care n= 4,321), reduction of mortality by 3.5% was observed in patients on oxygen and reduction of mortality by 11.7% in those requiring mechanical ventilation (Horby et al., 2020).

Currently there is no effective targeted therapy for SARS-CoV2 infection. Novel drug candidates should be designed to overcome this lacuna by targeting viral proteins as well as their interacting host proteins to arrest viral infection and to promote its clearance. For this, computer-aided drug design (CADD) is an effective way of identifying lead drug molecules. The CADD uses crystallographic structure of target protein/receptor for docking of lead molecule/ligand with absolute precision and specificity. This will not only help in drug development but also help in repurposing of the available drugs for new targets. Virtual screening of small inhibitory molecules through *in silico* docking studies and its evaluation by *in vitro* assays is an effective way for quick drug discovery during virus breakthrough or pandemic. Molecular docking and molecular dynamic (MD) simulation studies have been performed by using HCV and HIV protease inhibitors as ligands on M3CLpro (protease) of SARS-CoV2 virus (Manandhar et al., 2021). Major SARS-CoV2 target proteins are the spike or S-protein; two proteases, main protease (Mpro) and papain-like protease (PLpro) enzymes, helicase and RNA-dependent RNA polymerase (RdRp) (Cavasotto and Di Filippo, 2020). Host viral entry receptor ACE2 and host protease TMPRSS2 also have been used as targets (Wu et al., 2020). Availability of crystallographic structure of these target proteins may lead to discovery of new drugs with high specificity.

CONCLUSION

The SARS-CoV2 infection has taken the world by storm. The pandemic is yet to reach a plateau, with the incidences rising and newer populations getting infected. However, physicians, researchers, and scientists have left no stone unturned to understand the pathogenesis of this multisystem-afflicting disease, find effective therapies, and contain the pandemic. While the disease primarily affects the respiratory system, the liver injury does pose problems in the management of COVID-19 patients. Both direct virus-mediated cytopathic effects and indirect immune-mediated, drug-induced, or hypoxic states are probably responsible for causing and perpetuating the liver injury. However, a word of caution: Transaminitis in patients with COVID-19 should not be overly investigated. Only in patients with suspicion of a cholestatic pattern of injury, investigations like ultrasonography and magnetic resonance cholangiopancreatography may be performed. Besides, although it may sound slightly premature, in view of the recent case report, clinicians should also, in this era of COVID-19 infection, keep in mind that acute non-icteric hepatitis may be the virus's initial presentation prior to the development of respiratory symptoms (Wander et al., 2020). As of now, no major association of risk has been ascribed to either GI symptoms or pre-existing GI diseases towards COVID-19 susceptibility. But detection of SARS-CoV2 genome in fecal matter of COVID-19 patients with GI symptoms should be treated with caution as a probable feco-oral transmission route. Recent literature suggests a major role of age and comorbidities in severe outcome of COVID-19 as seen in case of IBD patients. Role of immune-modulatory medications used for treatment of pre-existing conditions also plays a decisive role in determining the trajectory of COVID-19 progression. It should be evaluated on an individual basis until a common consensus supported by exhaustive data has been reached. As new evidence trickles in and more facts come to light, we will be in a better position to understand and tackle liver injury in COVID-19. Intensive monitoring and individually tailored approach are the need of the hour to treat patients with severe liver injury or patients with pre-existing liver diseases.

AUTHOR CONTRIBUTIONS

BN: Review concept, draft writing. GL: Draft writing, image preparation. SK: Literature review, draft writing. CD: Radiological feature writing and data. AS: Proof correction, draft writing. S: Proof correction, draft writing. All authors contributed to the article and approved the submitted version.

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Association of ABO and Rh Blood Group in Susceptibility, Severity, and Mortality of Coronavirus Disease 2019: A Hospital-Based Study From Delhi, India

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Background: ABO and Rh blood group systems are associated with many diseases including cancerous, infectious, non-infectious, bacterial and viral diseases. Studies have shown association of blood groups A and O with higher and lower odds for coronavirus disease 2019 positivity, respectively.

Methods: This is a single-center, retrospective study conducted at Sir Ganga Ram Hospital, Delhi. We investigated the association of ABO and Rh blood groups with susceptibility to coronavirus disease 2019 infection, severity of disease, recovery period, and mortality of patients. Patients were enrolled from April 8, 2020 to October 4, 2020. A total of 2,586 real-time PCR (RT-PCR)-confirmed coronavirus disease 2019 (COVID-19) patients were recruited. Data was analyzed using chi-square test, odds ratio, and Mann–Whitney test to determine the association of blood groups.

Results: In the 2,586 COVID-19-infected patients, the frequencies of A, B, O, and AB were 29.93%, 41.80%, 21.19%, and 7.98%, respectively. Of the patients, 98.07% were Rh positive. Blood group A (odds ratio, 1.53; CI, 1.40–1.66; $p < 0.001$) and B (odds ratio, 1.15; CI, 1.06–1.24; $p < 0.001$) is observed to be significantly associated with COVID-19 susceptibility, whereas blood group O (odds ratio, 0.65; CI, 0.59–0.71; $p < 0.001$) and AB (odds ratio, 0.66; CI, 0.59–0.71; $p < 0.001$) have low risk of COVID-19 infection.

Conclusion: A, B, and Rh+ are found to be more susceptible to COVID-19 infection, whereas blood groups O, AB, and Rh– are at a lower risk of COVID-19 infection. No association was found between blood groups and susceptibility to severity of disease and mortality.

Keywords: ABO and Rh blood groups, susceptibility, severity, mortality, coronavirus disease (COVID-19) outbreak

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged from Wuhan, China in December 2019 has posed a great threat to global public health. It causes coronavirus disease 2019 (COVID-19) (Long et al., 2020; Wang et al., 2021). COVID-19 affects people in different ways. It has a wide range of symptoms like fever, dry cough, shortness of breath, muscle pain, fatigue, sore throat, ageusia, and anosmia. However, a large proportion of infected patients remain asymptomatic (Lovato et al., 2020; Rodriguez-Morales et al., 2020). COVID-19 has an incubation period of 1–14 days, but typically, it takes 3–7 days to present symptoms. There are many cases where the COVID-19 infection has taken more than 14 days to present any symptoms (Fan et al., 2020; Huang et al., 2020).

The knowledge of blood group types and their association with COVID-19 may help in disease management and treatment. Out of 34 blood group types that the International Society of Blood Transfusion (ISBT) recognizes, ABO and Rh blood types are the most investigated, studied, and clinically applied. The antigenic structure present on the surface of erythrocytes determines the ABO blood group and also whether there is antigenic structure present or not, determines the Rh system (Sayli, 2020).

Previous studies have found an association between rheumatological diseases, cancers, cardiovascular diseases, infectious and non-infectious diseases, and bacterial and viral diseases, and ABO blood group. Previous studies have shown susceptibility of ABO blood groups to viruses such as Middle East Respiratory Syndrome (MERS), hepatitis B, human immunodeficiency virus, norawalk virus, rotavirus, dengue virus, and SARS coronavirus (Mehta, 2009; Degarege et al., 2012; Chen et al., 2016; Batool et al., 2017; Murugananthan et al., 2018; Wolpin et al., 2010).

Recent studies from China and other parts of the world have reported that there is an association of ABO and Rh blood group with SARS-CoV-2 infection. Blood group type A has high odds of getting infected, while the rate of infection and severity seems less among the blood group O. Rh (D) positive blood group is also associated with increased COVID-19 infection and mortality (Boudin et al., 2020; Dzik et al., 2020; Fan et al., 2020; Goker et al., 2020; Noor et al., 2020; Zietz et al., 2020; Zhao et al., 2021). The underlying mechanism is still unknown and needs to be investigated. Several theories have been proposed to elaborate the mechanism of this association. Genetically encoded blood group antigens might be a predisposing factor for SARS-CoV-2 infection. The human ABO blood group is located on chromosome 9 (9q34.1-34.2) (Amundadottir et al., 2009; Wiggins et al., 2009; Vasan et al., 2016). The ABO blood groups are associated with several bacteria, parasites, and virus infections and also have shown major role as a receptor and coreceptor. ABO blood groups represent a polymorphic trait that has histo-blood group antigens (HBGAs), which are present on the outer surface of red blood cells (RBCs). The expression of HBGAs can decrease or increase the susceptibility of disease (Singh et al., 2016; Liu et al., 2018).

Severe acute respiratory syndrome coronavirus 2 is a novel virus, and it is indeterminate whether blood groups have any impact on COVID-19 susceptibility or progression. Therefore, we investigated the association of ABO and Rh blood group with COVID-19 susceptibility, prognosis, recovery time, and mortality in this study. An overview of the study is depicted in **Figure 1**.

MATERIALS AND METHODS

Study Design

This is a single-center, retrospective study that was carried out at Sir Ganga Ram Hospital, Delhi. The Ethical Committee of Sir Ganga Ram Hospital reviewed and approved the study.

We investigated the association of ABO and Rh blood groups with susceptibility to coronavirus disease 2019 infection, severity of disease, length of stay, and mortality of patients. Association was analyzed with ABO blood group and Rh blood group system. This study includes real-time PCR (RT-PCR)-confirmed COVID-19 patients who were admitted to the hospital.

Case and Control Selection

A total of 2,586 patients with confirmed COVID-19 positivity, tested through real-time PCR for coronavirus disease 2019, were recruited for this study as a case group. All the patients were admitted to Sir Ganga Ram Hospital, Delhi, from April 8, 2020 to October 4, 2020, and follow-up was taken up till their last date of admission as either discharged or deceased.

The control group data were extracted from a review study on blood group distribution in India (Patidar and Dhiman, 2021). The blood group distribution data of Delhi was selected, which was extracted from five independent studies, totalling to 79,325 people (Agarwal et al., 2013; Ahuja et al., 2015; Arora et al., 2015; Garg et al., 2015; Kaur et al., 2016).

Data Analysis

Statistical Package for Social Sciences (SPSS) Version 18 and MedCalc (statistical software) were used for the analysis. Chi-square test was used to analyze the distribution of ABO and Rh blood groups. Odds ratio (OR) test was applied to study the odds of ABO and Rh blood groups. Odds ratios were reported with 95% confidence intervals (CIs). Mann–Whitney test was used to explore the relationship between recovery period (length of stay at hospital) and blood group. All the tests were applied in a one-vs.-all manner.

RESULTS

Susceptibility of COVID-19 Infection

As shown in **Table 1**, the control group is comprised of 79,325 individuals having a high frequency of blood group B, followed by O, A, and AB reported. A total of 2,586 COVID-19-infected patients showed different order of frequency, where blood group B, followed by A, O, and AB were reported as highest to lowest.

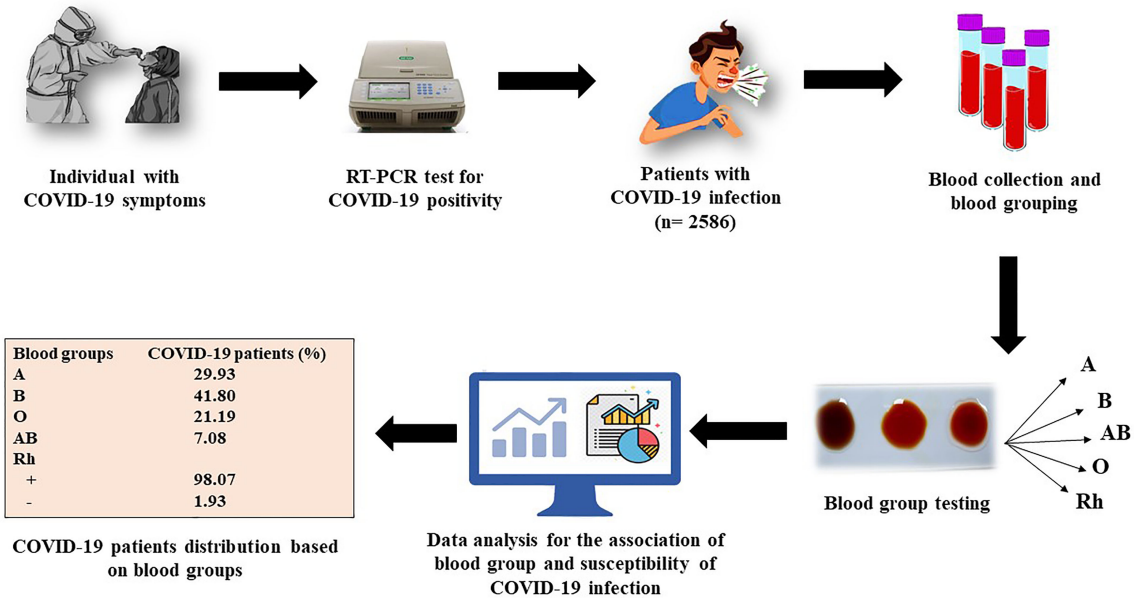

FIGURE 1 | A schematic overview of the study.

ABO blood group distribution was statistically different between the two groups ($p < 0.001$). The Rh blood group of the control population was Rh (D) + (n = 73,479, 92.63%) and Rh (D)– (n = 5,846, 7.37%) vs. Rh (D) + (n = 2,536, 98.07%) and Rh (D)– (n = 50, 1.93%) in the COVID-19-infected individuals. Chi-square analysis showed that blood groups A and B were associated with high risk of infections, while blood groups O and AB were associated with a decreased risk of infection. Similarly, Rh+ was found to be associated with increased risk of COVID-19 infection.

Association Analysis by Sex

The COVID-19-infected patients were divided into two groups by sex as shown in **Supplementary Table S1**. Out of 2,586, the male group comprised 1,800 patients. ABO blood group distribution among the two groups showed no difference except for the blood group B. Male patients of blood group B are more prone to COVID-19 than the female patients with blood group B. The association is compared in one-vs.-all blood group manner. Similarly, in comparing Rh blood group, no significant difference was found among the two groups. Chi-square analysis showed that the sex of the patients and ABO and Rh blood groups were not associated with susceptibility of COVID infection

Association Analysis by Age

Chi-square test was used to compare the blood groups and age groups of the COVID-19 patients. COVID-19-infected patients were divided into two groups as ≤60 and >60 years as shown in **Supplementary Table S2**. Blood group AB was observed to be more susceptible to infection in patients with age group ≤60 years. No other association was observed among other ABO or Rh blood groups.

Severity of COVID-19 Infection

The severity of COVID-19 is measured based on the admission to intensive care unit (ICU). The association of ABO and Rh blood groups with susceptibility to severity of COVID-19 was analyzed by blood group distributions of COVID-19-infected individuals that required ICU admission (COVID+ ICU+) and

TABLE 1 | Distribution of ABO and Rh blood groups among controls group and COVID-19-infected patients.

Blood group	COVID-19 patients n = 2,586	Control group n = 79,325	*p*-value	OR (95% CI)
A	774 (29.93)	17,340 (21.86)	<0.001	1.53 (1.40–1.66)
B	1,081 (41.80)	30,532 (38.49)	0.001	1.15 (1.06–1.24)
O	548 (21.19)	23,298 (29.37)	<0.001	0.65 (0.59–0.71)
AB	183 (7.08)	8,155 (10.28)	<0.001	0.66 (0.57–0.77)
Rh			<0.001	4.04 (3.05–5.35)
+	2,536 (98.07)	73,479 (92.63)		
–	50 (1.93)	5,846 (7.37)		

those who did not require ICU admission (COVID+ ICU−). The distribution of the two groups is shown in **Table 2**. Chi-square analysis of the blood groups applied on one-vs.-all showed no significant difference. There was no association observed between ABO and Rh blood groups with susceptibility to a severe infection.

Analysis of Recovery Duration

As shown in **Table 3**, the median (IQR) hospital length of stay (LOS), i.e., the recovery duration, was found to be significantly increased among the patients with blood group O, whereas in patients with blood group A, the median (IQR) LOS was observed to be significantly low when analyzed in manner one-vs.-all using Mann–Whitney test. There was no difference observed in case of patients with blood groups B and AB. Comparing the Rh blood groups, the median (IQR) LOS of patients with Rh (D)+ was significantly lower than that of patients with Rh (D)−.

Analysis of Risk of Mortality

To test the association of ABO and Rh blood groups with deceased COVID-19 patients, blood groups distribution was compared between deceased and recovered COVID-19-infected patients. There was no significant difference and association found with the ABO and Rh blood groups, as shown in **Table 4**.

LIMITATIONS

The major limitation of this study is that it does not consider underlying factors like comorbidities of the patients. Furthermore, the duration of the study was wide, and the treatment guidelines are dynamic and change over time as per the revised national guidelines, which might have affected the progression of disease.

DISCUSSION

This is a single-center, retrospective study. The patients visiting to the hospital with COVID-19 symptoms were tested for SARS-CoV-2 positivity *via* RT-PCR test. Blood sample was collected from the COVID-19-positive admitted patients for blood group testing and other pathological tests. Then, the medical history and records were extracted from the hospital database as shown in **Figure 1**.

ABO and Rh blood group types are the most widely used blood groups in clinical studies. The ABO blood group system basically contains two antigens, namely, A and B. The antigen coding gene is located on chromosome 9q34.1 and 9q34.2. It consists of three alleles (A, B, and O) and is four phenotypes (A, B, O, and AB) (Amundadottir et al., 2009; Vasan et al., 2016).

HBGAs are complex molecules present on the surface of RBC membranes. The involvement of complex molecules in modifying the progression of disease is through the action of natural antibodies (Neil et al., 2005; Storry and Olsson, 2009; Ewald and Sumner, 2018). The association between ABO blood groups and diseases have been widely explored for several diseases including viral diseases.

The current study was conducted to observe the association of ABO and Rh blood groups on susceptibility of coronavirus disease 2019 infection, disease severity, recovery time (LOS), and mortality. The study also compares the susceptibility of infection with sex, age, and blood group types. We seek to analyze the association of ABO and Rh blood groups for risk of coronavirus disease 2019 infection among the population in Delhi, India.

A total of 2,586 patients were recruited for the study. In coronavirus disease 2019-infected patients, it was observed that blood groups A and B were more disposed to SARS-CoV-2, whereas blood group O and AB had a significantly lower risk of infection. On comparing the distribution of ABO and Rh blood groups of COVID-19-infected patients with the general population of Delhi, there was a significant increase in infected individuals with blood groups A and B. A significant decrease in the number of COVID-19 patients with blood groups O and AB was also observed.

In a study by Li et al. on 265 SARS-CoV-2 patients, it was observed that blood group A was significantly higher in infected individuals than in the healthy control group population, while blood group O in COVID-19-infected patients was significantly less. Fan et al. from a study on 105 infected patients reported that an individual with blood group A is associated with a high risk of COVID-19 infection, whereas other blood groups had no association (Fan et al., 2020). A study by Zhao et al. on 2,173 COVID-19-infected patients reported that blood group A was higher in COVID-19-infected patients, and blood group O was associated with low risk of infection (Zhao et al., 2021). Similar association of blood group A with increased risk and O with low risk was reported by Solmaz and Araç, who also reported almost a significant increase in risk of infection in patients of blood group AB (Solmaz and Araç, 2021).

TABLE 2 | Distribution of ABO and Rh blood groups between COVID+ ICU+ and COVID+ ICU−.

Blood group	COVID+ ICU+ n = 779	COVID+ICU− n = 1,807	*p*-value	OR (95% CI)
A	228 (29.27)	546 (30.22)	0.629	0.96 (0.80–1.15)
B	329 (42.23)	752 (41.62)	0.770	1.03 (0.87–1.22)
O	174 (22.34)	374 (20.70)	0.349	1.10 (0.90–1.35)
AB	48 (6.16)	135 (7.46)	0.234	0.81 (0.58–1.43)
Rh			0.546	0.83 (0.46–1.51)
+	762 (97.82)	1,774 (98.17)		
−	17 (2.18)	33 (1.83)		

TABLE 3 | Comparison of ABO and Rh blood groups distribution and recovery period (LOS) of patients with COVID-19 infection.

Blood group	Median LOS	IQR	*p*-value
A	9	6–12	<0.001
B	9	7–13	0.858
O	10	7–15	<0.001
AB	10	7–13	0.290
Rh			<0.001
+	9	7–13	
–	11.5	8.25–16	

Our study also reported similar findings with the association of A and O blood groups. Furthermore, our study adds to the previous results and found that blood group B is also associated with increased risk of coronavirus disease 2019, whereas AB is associated with lower risk of coronavirus disease 2019.

Comparing the Rh (D)+ and Rh (D)– COVID-19-infected individuals with the healthy group, Rh (D)+ individuals are significantly at greater risk of infection when compared to Rh (D) negative individuals.

Esref et al. found a significant difference in Rh blood group system, whereas in a study by Solmaz and Araç on 1,667 patients in the Diyarbakir community of Turkey, no significant difference was found (Esref et al., 2020; Solmaz and Araç, 2021). In our study, comparing the Rh (D)-positive and Rh (D)-negative COVID-19-infected individuals with the control group, a strong association was observed in Rh (D)-positive individuals having a significantly more susceptibility to COVID-19 infection than the Rh (D)-negative individuals.

The current study compared ABO and Rh blood groups distribution and found no association of sex and age groups with the susceptibility of COVID-19 infection, the exception being blood group B, where male patients in the blood group are more disposed to the COVID-19 infection as compared to female patients of same blood group.

ICU-admitted COVID-19 patients were compared to non-ICU-admitted patients to study the association of blood groups and susceptibility to severity of disease. In a study reporting 2,334 COVID-19 patients, Almadhi et al. also found no association between blood groups and severity of disease. In the current study, the Rh blood group was also investigated for association and found to have no association with susceptibility to severe infection (Almadhi et al., 2021).

The recovery period (length of stay at hospital) and blood groups were tested for association. The recovery period of COVID-19-infected individuals was found to be significantly less in patients with blood group A than non-A blood group, whereas patients with blood group O are observed to have significantly greater recovery period than non-O blood group. Rh (D) positivity is also associated with significantly decreased number of days for recovery. Mahmud et al. compared the relation of recovery period in blood group A to non-A blood group. It was found that COVID-19 positivity at 14th day of infection is significantly higher in blood group A. To the best of our knowledge, there are not many studies in the aspect of association of recovery period and blood groups (Mahmud et al., 2021).

In a meta-analysis of 10 studies by Liu et al., it was found that 5 out of 10 studies have analyzed the association between ABO and mortality due to COVID-19 (Liu et al., 2020). It reported that blood group A was associated with significantly increased risk of mortality when compared to non-A blood group. Muniz-Diaz et al. found that blood group A has significantly high risk of mortality as compared to non-A blood groups, whereas blood group O is associated with significantly low risk of mortality (Muniz-Diaz et al., 2021). Our study contrasts with these studies, as we found that ABO and Rh blood groups are not associated with mortality in COVID-19 patients. Similar to the current results, Solmaz and Araç also found that blood groups have no association with mortality of the patients (Solmaz and Araç, 2021).

CONCLUSION

Our study has found that blood groups A, B, and Rh+ are more disposed to COVID-19 infection, whereas blood groups O, AB, and Rh– are significantly of lower risk of COVID-19 infection. ABO and Rh blood groups show no impact on the progression of disease and are not associated with susceptibility to severity of disease or mortality. We also found that blood groups A and Rh+ types are associated with a decrease in recovery period, whereas

TABLE 4 | Distribution of ABO and Rh blood groups between deceased and recovered COVID-19-infected patients.

Blood group	Deceased n = 317	Recovered n = 2,269	*p*-value	OR (95% CI)
A	81 (25.55)	693 (30.54)	0.069	0.09 (0.05–0.17)
B	136 (42.90)	945 (41.65)	0.672	1.05 (0.83–1.34)
O	80 (25.24)	468 (20.63)	0.60	1.30 (0.99–1.71)
AB	20 (6.31)	163 (7.18)	0.569	0.87 (0.54–1.41)
Rh			0.211	0.63 (0.30–1.31)
+	308 (97.16)	2,228 (98.19)		
–	9 (2.84)	41 (1.81)		

blood groups O and Rh− are associated with increase in recovery period.

However, the ABO and/or Rh blood groups may not be responsible for this association, as these may indicate an unexplored underlying factor like comorbidity. Therefore, larger, multicenter, and prospective studies are needed to ascertain the relationship of between blood groups and SARS-CoV-2.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institute ethics committee, Sir Ganga Ram Hospital, Delhi. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

RR and NK wrote the manuscript and performed data analysis. VR and RR had full access to data in the study and take responsibility of the data integrity. RR and NK take responsibility of the accuracy of data analysis. RR edited the manuscript, and VR is responsible for data collection. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.767771/full#supplementary-material

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Studies on Growth Characteristics and Cross-Neutralization of Wild-Type and Delta SARS-CoV-2 From Hisar (India)

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly evolved to generate several antigenic variants. These variants have raised concerns whether pre-existing immunity to vaccination or prior infection would be able to protect against the newly emerging SARS-CoV-2 variants or not. We isolated SARS-CoV-2 from the coronavirus disease 2019 (COVID-19)-confirmed patients in the beginning of the first (April/May 2020) and second (April/May 2021) waves of COVID-19 in India (Hisar, Haryana). Upon complete nucleotide sequencing, the viruses were found to be genetically related with wild-type (WT) and Delta variants of SARS-CoV-2, respectively. The Delta variant of SARS-CoV-2 produced a rapid cytopathic effect (24–36 h as compared to 48–72 h in WT) and had bigger plaque size but a shorter life cycle (~6 h as compared to the ~8 h in WT). Furthermore, the Delta variant achieved peak viral titers within 24 h as compared to the 48 h in WT. These evidence suggested that the Delta variant replicates significantly faster than the WT SARS-CoV-2. The virus neutralization experiments indicated that antibodies elicited by vaccination are more efficacious in neutralizing the WT virus but significantly less potent against the Delta variant. Our findings have implications in devising suitable vaccination, diagnostic and therapeutic strategies, besides providing insights into understanding virus replication and transmission.

Keywords: SARS-CoV-2, wild type, delta, antibody, cross-neutralization

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in 2019 in Wuhan, China, is responsible for the current pandemic of coronavirus disease 2019 (COVID-19) (Cao et al., 2020) and has resulted in more than 241.8 million infections and 4,919,755 deaths as of October 22, 2021 (WHO, 2021). Currently, there is no specific antiviral drug to treat COVID-19. However, vaccine is available, and currently, the world's 7.9 billion population is being vaccinated to generate herd immunity. The original SARS-CoV-2, first detected in Wuhan, has undergone extensive mutations and has resulted in the generation of several antigenic variants. The major antigenic

variants include Alpha, Beta, Gamma, and Delta and are believed to have originated in UK (December 2020), South Africa (September 2020), Brazil (November 2020), and India (May 2021), respectively (Duong, 2021). Currently, SARS-CoV-2 Delta variant (B.1.617.2) is predominantly circulating in India and several other parts of the world (Noh et al., 2021; Sanyaolu et al., 2021; Winger and Caspari, 2021). It has been classified as a variant of concern and is believed to be 60% more transmissible than the Alpha variant (B.1.1.7) (Duong, 2021). New variants of interest are Eta, Iota, Kappa, Lambda, and Mu, and many more are likely to emerge (Areo et al., 2021; Janik et al., 2021; Parums, 2021). The emergence and widespread prevalence of new SARS-CoV-2 variants can reduce the effectiveness of the current vaccines and pose a significant threat to combat the pandemic (Becker et al., 2021; Duong, 2021; Ferraz et al., 2021; Sharun et al., 2021). Besides vaccine efficacy, genetic/antigenic variations may also affect the capability of the diagnostic tests, which are based on WT SARS-CoV-2. Besides, there is a significant gap in our understanding about the life cycle of the wild-type (WT) and Delta strains of SARS-CoV-2. This study provides a comparative insight on the growth characteristics and cross-neutralization of WT and Delta strains of SARS-CoV-2.

MATERIALS AND METHODS

Cells

Vero cells were available at the National Centre for Veterinary Type Cultures (NCVTC), Hisar, and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with antibiotics and 10% fetal calf serum.

Nasopharyngeal Swabs and Serum Samples

Nasopharyngeal swabs were received from the Civil Hospital Hisar, Haryana (India). Serum samples from the vaccinated and/or COVID-19-positive individuals were also received from the Civil Hospital, Hisar. Depending on the exposure to SARS-CoV-2, serum samples were categorized into the following types, namely, infected during the first wave (W1), infected during the second wave (W2), uninfected but vaccinated (V), infected during the first wave and subsequently vaccinated (W1V), and infected during the second wave and later vaccinated (W2V). Paired samples were also collected from an individual who succumbed to COVID-19 during both the first and second waves (W1W2).

Ethics Statement

Samples were collected from patients by the authorized District Medical Officer Hisar, India. A due consent was taken from the patients before collection of the serum samples.

Virus Isolation

SARS-CoV-2 was propagated in Vero cells in the Biosafety Level 3 (BSL-3) laboratory of ICAR-National Research Centre on Equines (ICAR-RCE), Hisar. The work was approved by the Institute Biosafety Committee and Review Committee on Genetic Manipulation (RCGM), Department of Biotechnology, Government of India (No. BT/BS/17/436/2011-PID).

Virus isolation was attempted from the nasopharyngeal swabs received for COVID-19 testing at our facility at ICAR-NRCE Hisar. Nasopharyngeal swabs that were positive for SARS-CoV-2 genome with a cycle threshold (cT) value of <20.0 in quantitative real-time PCR (qRT-PCR) were considered for virus isolation in Vero cells. Samples that produced cytopathic effect (CPE) within three successive blind passages in Vero cells were authenticated and accessioned. Samples that did not produce CPE up to the third blind passage were discontinued. The first attempt of virus isolation was made in April 2020, which was the beginning of COVID-19 pandemic in Haryana (India). Similarly, virus isolation was also attempted from nasopharyngeal swabs (n = 11) that were collected following the onset of the second wave in India (April/May 2021).

Identification

The viral RNA was extracted using QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany). This was followed by amplification of SARS-CoV-2 RdRp and E gene using LabGun COVID-19 Assay (LabGenomics Co., Ltd., Suwon-si, Republic of Korea) using CFX96 PCR Detection Systems (BioRad, USA).

Complete Genome Sequencing of the Viral Isolates

Viral RNA was extracted by TRIzol Reagent (Invitrogen, CA, USA). Complementary DNA (cDNA) was synthesized as per the protocol described by the manufacturer (Fermentas, Hanover, USA) and sent to Clevergene Biocorp Pvt Ltd. (Bengaluru, India) for complete genome sequencing. Briefly, first-strand cDNA reactions were converted to double-stranded DNA (dsDNA). The double-stranded cDNA fragments obtained were cleaned up by using 1.8× of AMPure XP beads (Catalog no. A63881, Beckman Coulter). The purified cDNA was run on the tape to check the fragment size of cDNA. The library concentration was determined in a Qubit.3 Fluorometer (Catalog no. Q33216, Life Technologies) using the Qubit dsDNA High Sensitivity Assay Kit (Catalog no. Q32854, Thermo Fisher Scientific). The library quality assessment was done using Agilent D5000 Screen Tape System (Catalog no. 5067-5588, Agilent) in a 4150 Tape Station System (Catalog no. G2992AA, Agilent).

The sequence data were generated using Illumina HiSeq. Data quality was checked using FastQC (Ewels et al., 2016) software. Raw sequence reads were processed to remove adapter sequences and low-quality bases using fastp (Chen et al., 2018). To make consensus sequences, quality trimmed were aligned to respective genomes transcriptome using bwa-mem. The mean depth was calculated using mosdepth (Pedersen and Quinlan, 2018) and sambamba (Tarasov et al., 2015). From the aligned bam files, variants were called with –ploidy 1 option, and consensus genome sequences were built using the reference genome using bcftools from the samtools package (Li et al., 2009). SnpEff v 4.1 was used to annotate the variants (Cingolani et al., 2012) with respect to their reference sequence.

The complete nucleotide sequences of WT and Delta strains reported in this study were compared with the original SARS-CoV-2, reported for the first time in late 2019 in Wuhan, China (GenBank Accession Number MN996528.1), and with a reference Delta strain (GenBank Accession Number OK091006.1), and the mutations were identified by using an online server (https://www.gisaid.org).

Plaque Assay

SARS-CoV-2 plaque assay was performed as described previously (Kumar et al., 2018; Kumar et al., 2019). Briefly, the confluent monolayers of Vero cells were infected with 10-fold serial dilutions of SARS-CoV-2 for 1 h at 37°C, after which the infecting medium was replaced with an agar overlay containing equal volume of 2× L-15 medium and 1% agar. Upon development of plaques, the agar overlay was removed, and the plaques were stained by 1% crystal violet.

Virus Neutralization Assay

The virus neutralization assay was carried out as per the previously described method with some modifications (Kumar et al., 2020; Kumar et al., 2021). Serum samples were initially heated at 56°C for 30 min to inactivate the complement. Vero cells were grown in 96-well tissue culture plates at ~90 confluency. Twofold serum dilutions (in 50 µl volume) were made in phosphate-buffered saline (PBS) and incubated with equal volume of SARS-CoV-2 (10^4 PFU/ml) for 1 h. Thereafter, a virus–antibody mixture was used to infect Vero cells. The cells were observed daily for the appearance of the CPE. Final reading was taken at 48 h postinfection (hpi) (SARS-CoV-2 Delta) or at 72 hpi (SARS-CoV-2 WT) for the determination of antibody titers.

One-Step Growth Curve

Confluent monolayers of Vero cells, in triplicates, were infected with SARS-CoV-2 at multiplicity of infection (MOI) of 5 and thereafter washed with PBS, and fresh MEM was added. Infectious progeny virus particles released in the cell culture supernatant at various time points were quantified by plaque assay.

Kinetics of SARS-CoV-2 Genome Synthesis (qRT-PCR)

Confluent monolayers of Vero cells, in triplicates, were infected with SARS-CoV-2 at MOI of 5, followed by washing with PBS and addition of fresh MEM. Cells were scrapped at indicated time points and subjected to RNA extraction and quantitation of viral RNA by qRT-PCR as describe above. cT values were normalized with β-actin [primers describe elsewhere (Khandelwal et al., 2020)] housekeeping control gene, and relative fold change in RNA copy numbers was calculated by ΔΔCt method (Holmes et al., 2010).

RESULTS

Complete Genome Sequencing (Genotyping)

During the first wave of COVID-19, 11 nasopharyngeal swabs from COVID-19-confirmed patients were subjected to complete genome sequencing. Their sequences are available in GenBank with Accession Numbers, namely, MW555317, MW555320, MW555325, MW555334, MW555576, MW555595, MW555597, MW555280, MW927136, MW555786, and MW555598. Upon comparison of the nucleotide/amino acid sequences, rather than any variants of concern (observed later during the pandemic), these viruses were found to be more closely related with the original SARS-CoV-2 reported during the early stage of pandemic in China. Hereinafter, we refer them as wild-type (WT) SARS-CoV-2 strain(s), although only one of them was used for the detailed investigation in this study. Six amino acid mutations (**Table 1**) were observed in the WT as compared to the Wuhan SARS-CoV-2.

Likewise, three virus isolates from the second wave (April/May 2021) were also subjected to complete genome sequencing (sequence will be provided on request). On BLAST search, these sequences were found to be closely related with the Delta variants of SARS-CoV-2 and possess all the Delta-specific signature mutations (**Table 1**). One of the Delta strains (employed for detailed investigations in this study) that was compared with the reference Delta strain showed six amino acid mutations (**Table 1**).

Virus Isolation

The first COVID-19-positive case in Haryana was reported from the Gurugram district on March 17, 2021. By April 2020, it had spread to almost the entire state. Our laboratory started testing for COVID-19 from April 12, 2020. Some of the samples that were received in April/May 2020 and had a cT value of <20 were subjected to virus isolation. In April/May 2020, out of the 11 nasopharyngeal swabs subjected to virus isolation, only 4 produced CPE up to the third blind passage, 3 of which were further authenticated and deposited with accession numbers of VTCCAVA 294 (SARS-CoV-2/India/2020/tc/Hisar/4907), VTCCAVA295 (SARS-CoV-2/India/2020/tc/Hisar/2710), and VTCCAVA296 (SARS-CoV-2/India/2020/tc/Hisar/1469) at the National Repository of Animal Microbes (NCVTC, Hisar, Haryana; www.ncvtc.org.in). The virus with accession number VTCCAVA295 was used as a prototype of WT SARS-CoV-2 for various biological assays described in this study.

On the onset of the second wave of COVID-19 (April/May 2021) in India, we again attempted virus isolation from the samples received from the Hisar district of Haryana. Out of the 11 nasopharyngeal swabs, 1 swab sample produced CPE even on the first blind passage, whereas 2 produced CPE during the second blind passage. These three virus isolates were further authenticated and deposited at the repository described above with accession numbers, namely, VTCCAVA318 (SARS-CoV-2/India/2021/tc/Hisar/177124), VTCCAVA319 (SARS-CoV-2/India/2021/tc/Hisar/177405), and VTCCAVA320 (SARS-CoV-2/India/2021/tc/Hisar/177961). The virus with accession number VTCCAVA319 was later used as a prototype of Delta variant of SARS-CoV-2 for various biological assays described in this study.

At passage level 5 (Vero cells), whereas WT SARS-CoV-2 took 3–4 days in producing appreciable CPE in Vero cells, the Delta variant was able to produce significant cell death within 24–36 h, suggesting a higher replication rate of the Delta variant. Besides, the nature of the CPE was also strikingly different;

TABLE 1 | Mutational analyses of WT and Delta strains of SARS-CoV-2.

Sr. No.	Gene Name	WT*	Delta (Reference Strain)	Delta*
1.	NSP3	T1198K	A488S	A488S
			V932A	H795Y
			P1228L	P1228L
			P1469S	P1469S
2.	NSP4		V167L	V167L
			T492I	T492I
3.	NSP5			S123F
				V296I
4.	NSP6	L37F	T77A	T77A
		M83I		
5.	NSP12	A97V	P323L	P323L
			G671S	G671S
6.	NSP13		P77L	P77L
7.	NSP14		A394V	A394V
10.	Spike		T19R	T19R
			G142D	**G142D**
			L452R	**L452R**
			T478K	**T478K**
			D614G	**D614G**
			P681R	**P681R**
			D950N	**D950N**
				T95I
				P812R
11.	NS3		S26L	S26L
13.	NS7a		V82A	V82A
				T120I
14.	NS7b		T40I	T40I
15.	NS8	E106Q	F120L	
16.	M			I82T
17.	N	P13L	D63G	D63G
			R203M	R203M
			G215C	G215C
			D377Y	D377Y
				S79I

Comparisons were made with a reference strain (GenBank Accession Number MN996528.1, reported in the beginning of the COVID-19 epidemic in Wuhan, China). GenBank Accession Number OK091006.1-a Delta reference strain was also included in the study. Bold letters represent signature mutations of the Delta variant.
**SARS-CoV-2 strains belong to this study.*

whereas infection of WT virus resulted in cell rounding, detachment, degeneration, and occasionally small syncytia (**Figure 1A**), the Delta variant produced elongated and extremely large syncytia, besides inducing degeneration and detachment of the cells (**Figure 1A**). Most importantly, plaques produced by the Delta variant were much larger in size as compared to the WT SARS-CoV-2 (**Figure 1B**).

SARS-CoV-2 Life Cycle (One-Step Growth Curve)

In order to determine the length of the viral life cycle, Vero cells were infected with high MOI (MOI = 5), and the virus released in the infected cell culture supernatant at different times postinfection was quantified. There was no significant difference in the vial titers in the infected cell culture supernatant that were collected at 2 h postinfection (hpi) and 4 hpi in both WT (**Figure 2A**) and Delta (**Figure 2B**) strains. However a sudden increase in viral titers was observed at 8 and 6 hpi, respectively in WT (**Figure 2A**) and Delta (**Figure 2B**) strains. This increase in viral titers was presumably due to the appearance of infectious progeny virus particles in the infected cell culture supernatant and hence indicated the completion of viral life cycle at these time points. The higher viral titers in Delta as compared to the WT SARS-CoV-2-infected cells at 6 hpi (**Figure 2C**) suggested that Delta variant has a significantly shorter life cycle than the WT SARS-CoV-2. Although the peak viral titers (~10^7 pfu/ml) were almost similar in the supernatant collected from both WT (**Figure 2A**) and Delta (**Figure 2B**) strains, this was achieved significantly faster in Delta (~ 24 hpi) as compared to the WT strain (48 hpi) of SARS-CoV-2 (**Figure 2C**).

We also examined the kinetics of the SARS-CoV-2 RNA synthesis in cultured cells wherein Vero cells were infected with high MOI (MOI = 5), followed by quantifying the levels of SARS-CoV-2 RNA in the cell pellet at different times postinfection. A peak level of viral RNA was observed at ~9 h and ~6 h (**Figure 3**), respectively, in WT and Delta variant of SARS-CoV-2, which again suggested the faster replication (fitness) rate of the Delta variant.

Cross-Neutralization Between WT and Delta Variants of SARS-CoV-2

We performed a virus neutralization assay in order to determine whether the sera from the COVID-19 vaccine recipient

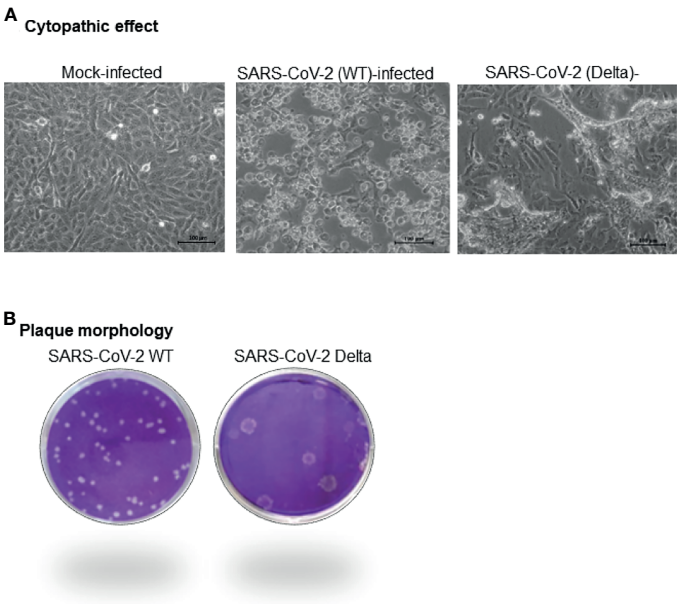

FIGURE 1 | Virus isolation. Nasopharyngeal swabs positive for COVID-19 with cT value of <20.0 in qRT-PCR were considered for virus isolation in Vero cells. The samples were filtered in a 0.45-µm syringe filter, and 500 µl of the filtrate was used to infect Vero cells. Samples that produced CPE within three successive passages in Vero cells were authenticated and accessioned. Characteristics of the CPE produced by WT and Delta **(A)** variant of SARS-CoV-2 at passage level 5 is shown. Plaque morphology of WT and Delta variant of SARS-CoV-2 is also shown **(B)**.

(Covishield/Covaxin used in India) and those who recovered naturally from the disease are able to neutralize the variants of concerns. There was a poor cross-reactivity between the sera derived from V/W1/W1V individuals with Delta variants; the antibody titers were in the range of 16–256 and 0–16 when neutralized with WT and Delta variant, respectively (**Table 2**). Likewise, sera derived from W2 individuals more effectively neutralized the Delta (titers range from 32 to 128) as compared to the WT SARS-CoV-2 (titers range from 0 to 32) (**Table 2**). The W2V individuals had higher antibody levels against both Delta and WT virus as expected. Exceptionally, two of the V-type sera (**Table 2**; SR134 and SR139) from diabetic patients were equally neutralized by both WT and Delta variants. Interestingly, one of the individuals (SR116A and SR116B, **Table 2**) developed clinical disease and tested positive for COVID-19 during both the first and second waves. Initially, 29 days following primary infection, the antibody titers in this individual were 32 and <8, respectively, against WT and Delta SARS-CoV-2, whereas at 18 days following secondary infection, the titers were 64 and 128, respectively.

DISCUSSION

Vaccinating the world's 7.9 billion population against COVID-19 is a huge challenge (Asawapaithulsert et al., 2021; Eichhorst, 2021; Perkins, 2021). As on September 2, 2021, only 40.1% of the world population has received at least one dose of the vaccine, while 38.92 million are now vaccinated each day. Only 1.8% of people in low-income countries have received at least one dose (Organization, 2021). Most of the vaccines being used worldwide are based on the SARS-CoV-2 strain(s) isolated in patients in December 2019 or early 2020. Since the virus rapidly undergoes mutations, a lot of antigenic variants have been reported. Currently, the major variants of concerns are Alpha, Beta, Gamma, and Delta (Lessells, 2021; Noh et al., 2021; Sander et al., 2021). New variants of interest are Eta, Iota, Kappa, Lambda, and Mu (Areo et al., 2021; Janik et al., 2021; Parums, 2021). New variants are believed to have more transmissibility and produce more lethal disease (Gravagnuolo et al., 2021). Most of these variants harbor mutations in the spike protein, thereby raising concerns whether pre-existing antibodies due to vaccination or infection (recovered individuals) would be able to protect against the newly emerging variants or not (Sharun et al., 2021). Besides vaccine efficacy, genetic/antigenic variations may also affect the capability of the diagnostic tests and therapeutic agents, most of which are based on WT SARS-CoV-2. Likewise, SARS-CoV-2 literature, particularly on the *in vitro* experiments that is mostly based on WT SARS-CoV-2, may lead to misleading conclusions if being extrapolated to understand the Delta virus biology. Despite several studies on virus isolation (Keyaerts et al., 2005), the precise natures of the kinetics of viral life cycle and nature of CPE produced by WT and Delta variants of SARS-CoV-2 are not well studied. Therefore, we compared the growth characteristics in terms of the kinetics of RNA synthesis, virus production (viral life cycle), plaque formation, and nature of CPE between WT and Delta variants of SARS-CoV-2.

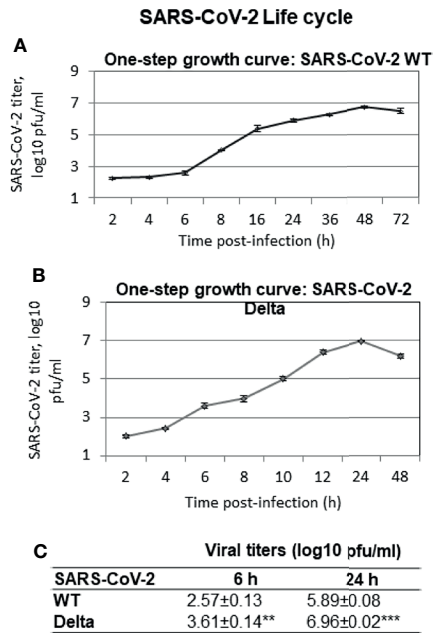

FIGURE 2 | SARS-CoV-2 life cycle. Confluent monolayers of Vero cells, in triplicates, were infected with SARS-CoV-2 at MOI of 5 and thereafter washed with PBS, and fresh MEM was added. Infectious progeny virus particles released in the infected cell culture supernatant at indicated time points were quantified by plaque assay. One-step growth curve of WT **(A)** and Delta variant **(B)** of SARS-CoV-2 is shown. Statistical comparisons of viral titers (WT versus Delta SARS-CoV-2) were performed at 6 hpi (when progeny virus particles start appearing in the infected cell culture supernatant) and at 24 hpi (when Delta virus is about to complete its life cycle) by two-tailed Student's t-test **(C)**. $^{**}p < 0.01$, $^{***}p < 0.001$.

Mutations in envelope proteins of RNA viruses have been shown to be associated with altered viral fitness, eventually affecting the viral plaque morphology (Mandary et al., 2019). In our study, we observed that the Delta variant of SARS-CoV-2 produces a rapid CPE within 24–36 h as compared to 48–72 h by WT. Besides, the Delta variant had bigger plaque size and a shorter life cycle (~ 6 h as compared to the ~8 h in WT). In SARS-CoV-2, D614G or P681R mutations (**Table 1**) were shown to enhance the replication fitness of Delta strain, although no alteration in plaque morphology was observed (Liu Y. et al., 2021; Plante et al., 2021), suggesting that plaque morphology and replication fitness may be determined by independent residues (Liu J. et al., 2021). In addition, the Delta variant rapidly synthesized viral RNA and achieved peak viral titers within 24 h as compared to the 48 h in WT. These observations on faster replication rate of the Delta variant reported in this study support the clinical finding, namely, high transmissibility of Delta variant (Gan et al., 2021; Xu et al., 2021).

Summarily, our neutralization experiments indicated that antibodies elicited by vaccination or infection with WT virus can more effectively neutralize WT SARS-CoV-2 (potential source of the available vaccine) but are significantly less potent against the Delta variant (**Table 2**), the strain that is currently circulating in India. Likewise, W2 sera were more strongly neutralized by Delta as compared to WT SARS-CoV-2 (**Table 2**). These findings on poor cross-neutralization are somewhat in agreement with few other recent findings (Hammerschmidt et al., 2021; Planas et al., 2021).

Exceptionally, two of the sera from vaccinated individuals (**Table 2**; SR134 and SR139), which had no prior history of COVID-19 infection but were diabetic, equally neutralized (antibody titer 128 against each) by both WT and Delta

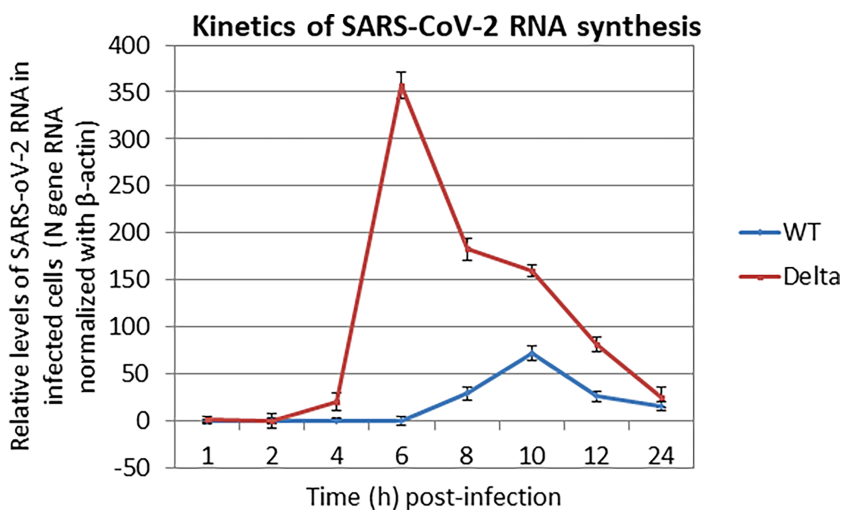

FIGURE 3 | Kinetics of SARS-CoV-2 RNA synthesis. Confluent monolayers of Vero cells, in triplicates were infected with SARS-CoV-2 at MOI of 5, followed by washing with PBS and addition of fresh MEM. Cells were scrapped at indicated time points and subjected for the quantitation of SARS-CoV-2 RNA (N gene) by qRT-PCR. cT values were normalized with β-actin housekeeping control gene, and relative % fold change in viral RNA copy numbers at various time points (as compared to 1 hpi) was calculated by ΔΔCt method.

TABLE 2 | Cross-neutralization of SARS-CoV-2 WT and Delta variant.

	Serum ID	Infection/Vaccination status	Sample History	Vaccine type	Ab titer (WT)	Ab titer (Delta)	Significance (T test)
History of exposure to WT	SR-6	W1	Day 15	NA	16	8	$p < 0.041$
SARS-CoV-2	SR-8	W1	Day 18	NA	32	<8	
	SR-9	W1	Day 10	NA	16	8	
	SR-13	W1	Day 14	NA	32	0	
	SR-14	W1	Day11	NA	32	8	
	SR-17	W1	Day 12	NA	16	8	
	SR-18	W1	Day 14	NA	32	0	
	SR-19	W1	Day 15	NA	32	16	
	SR-21	W1	Day 10	NA	<8	<8	
	SR-116A*	W1	Day 29	NA	32	<8	
	SR-119	W1V	Day 98 (2nd dose)	Covishield	256	16	
	SR-125	V	Day 40 (2nd dose)	Covishield	32	16	
	SR-127	W1	Day 105	NA	128	32	
	SR-132	V	Day 42 (2nd dose)	Covishield	16	16	
	SR-133	V	Day 22 (1st dose)	Covishield	16	8	
	SR-134	V	Day 48 (2nd dose)	Covishield	128	128	
	SR-135	V	day 19 (1st dose)	Covishield	16	0	
	SR-136	V	Day 30 (2nd dose)	Covaxin	16	<8	
	SR-137	V	Day 109 (2nd dose)	Covishield	32	8	
	SR-138	V	Day 28 (2nd dose)	Covishield	32	16	
	SR-139	V	Day 10 (2nd dose)	Covaxin	128	128	
	SR-140	V	Day 57 (2nd dose)	Covishield	16	8	
	SR-141	V	Day 62 (2nd dose)	Covishield	128	64	
	SR-142	V	Day 58 (2nd dose)	Covishield	16	<8	
	SR-143	V	Day 58 (2nd dose)	Covishield	32	16	
	SR-144	V	Day 84 (2nd dose)	Covishield	32	16	
History of exposure to Delta	SR-126	W2	Day 45	NA	32	128	$p < 0.035$
SARS-CoV-2	SR-128	W2	Day 51	NA	32	128	
	SR-129	W2	Day 22	NA	16	32	
	SR-130	W2	Day 56	NA	16	64	
	SR-131	W2	Day 37	NA	<8	32	
Exposure to both WT and Delta variant	SR-116B*	W1W2	Day 18 (2nd infection)	NA	64	128	NA
	SR-124	W2V	Day 34 (1st dose)	Covishield	64	64	

Pairwise statistical comparison of antibody titers in serum samples (WT versus Delta variant) was performed by two-tailed Student's t-test. For statistical analysis, antibody titer <8 was considered as 8.

W1, infection during the first wave; W2, infection during the second wave; V, uninfected but vaccinated; W1V, infected during the first wave and then vaccinated; W2V, infection during the second wave and later vaccinated; Ab, antibody; NA, not applicable.

**The individual infected during both the first and second waves (SR116A, sample collected at 29 days post-first wave infection; SR116B, sample collected at 18 days post-second wave infection).*

variants. One possibility is that these vaccinated individuals could have been exposed to the Delta virus but did not develop any clinical disease. Although we cannot make any firm conclusions based on mere two samples, immune response against SARS-CoV-2 in diabetic patients could certainly be a matter of further research (Heald et al., 2021a; Heald et al., 2021b; Zheng et al., 2021).

Interestingly, one of the individuals (**Table 2**, SR116A and SR116B) tested positive for COVID-19 during both the first and second waves (also developed clinical disease on each occasion). The antibody titers in this individual were 32 and <8, respectively, against WT and Delta SARS-CoV-2, following 1 month after primary infection, whereas after 18 days following secondary infection, the titers were 64 and 128, respectively. This single but rare sample has again raised concern about the protective efficacy of cross-neutralizing antibodies elicited by vaccination in providing protection against Delta or other newly emerging strains of SARS-CoV-2.

Potential limitations of our work include the low number of infected individuals analyzed and the lack of data on cellular immune responses. Future studies on large number of sera for longer time periods to characterize the role of humoral responses could elaborate on the efficacy of the existing vaccine (prepared from WT SARS-CoV-2) against the circulating variants.

In conclusion, our results demonstrate that Delta variant is poorly neutralized by antibodies elicited by previous infection with SARS-CoV-2 or by vaccination.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, MW555317.

ETHICS STATEMENT

Samples were collected from patients by the authorized District Medical Officer Hisar, India. A due consent was taken from the patients before collection of the serum samples.

AUTHOR CONTRIBUTIONS

Conceptualization: NKu and BT. Formal analysis: NKu and BT. Funding acquisition: NKu and BG. Methodology: NKu, NKh, RK, AV, HN, YC, PM, RT, SV, SKa, and SKh. Writing—first draft: NKu. Writing—review and editing: NKu, NKh, RK, AV, HN, YC, PM, RT, SV, SKa, BT, BG, and YP. All authors contributed to the article and approved the submitted version.

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REVIEW
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SARS-CoV-2: Emergence of New Variants and Effectiveness of Vaccines

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The emergence of SARS-CoV-2 variants may cause resistance at the immunity level against current vaccines. Some emergent new variants have increased transmissibility, infectivity, hospitalization, and mortality. Since the administration of the first SARS-CoV-2 vaccine to a human in March 2020, there is an ongoing global race against SARS-CoV-2 to control the current pandemic situation. Spike (S) glycoprotein of SARS-CoV-2 is the main target for current vaccine development, which can neutralize the infection. Companies and academic institutions have developed vaccines based on the S glycoprotein, as well as its antigenic domains and epitopes, which have been proven effective in generating neutralizing antibodies. The effectiveness of SARS-CoV-2 vaccines and other therapeutics developments are limited by the new emergent variants at the global level. We have discussed the emergent variants of SARS-CoV-2 on the efficacy of developed vaccines. Presently, most of the vaccines have been tremendously effective in severe diseases. However, there are still noteworthy challenges in certifying impartial vaccines; the stories of re-infections are generating more stressful conditions, and this needs further clinical evaluation.

Keywords: SARS-CoV-2, variant, vaccine, neutralization, infectivity

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1 INTRODUCTION

The emergence globally of multiple variants of concern (VOCs) may cause greater severity of infection and transmissibility (Abouelela et al., 2021). Neutralization effects reduced by antibodies are attained *via* naturally occurring infection or vaccination and decrease the effectiveness of vaccines or therapeutics options. Classification systems for genetic variants have been established by the CDC (Center for Disease Control and Prevention) and the WHO (World Health Organization) independently for distinguishing the emerging VOCs and variants of interest (VOIs). The VOCs are classified by the WHO as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) (Wang et al., 2021). All These strains have shown genetic modification in the S gene in comparison with the native Wuhan strain. The maximum number of mutations in S protein change the infection rate, severity, affinity with host receptor ACE2, and also the possibility to alter the effectiveness of neutralizing antibodies and vaccine efficacy. B.1.351 variants of SARS-CoV-2 have been identified in more than 40 countries at the global level, P.1 variants have been identified in 20 countries (Weisblum et al., 2020). Most of the vaccines are produced for the early strain circulating at the

global level. Therefore, some vaccines had reduced efficacy against the newly emerged SARS-CoV-2 variants. Nevertheless, The US FDA (Food and Drug Administration) stated that all FDA-approved vaccinations are still effective against circulating SARS-CoV-2 strains as of February 2021. Here, we will discuss SARS-CoV-2 New Variants and characteristic features and impact on the efficacy of different vaccines to understand their role in the transmissible and vaccine effectiveness.

2 PHYLOGENETIC ANALYSIS OF SARS-COV-2 GENOME

The Phylogenetic analysis of the SARS-CoV-2 genome with the related family members of SARS-CoV-2 from various organisms disclose that the genome of SARS-CoV-2 shows a high evolutionary association with Bat-SL-CoV. The phylogenetic tree is classified into three clades. Clade I consist of Bat-SL-CoV genomes and SARS-CoV with shared sequence identity ranging from more than 80% to 98%. Clade III consists of 11 complete genomes of a mixed form of coronavirus and MERS-CoV-2 genomes and shares the sequence identity from more than 75% to 85%. Clade II consists of 12 SARS-CoV-2 from India (CCMBOM9/2020/EPI ISL 495297), Korea (KCDC2059/2020/EPI ISL 481379), and two Bat-SL-CoV complete genomes and share sequence identity range from 85% to 99%, mainly the SARS-CoV-2 genomes isolated from human samples show a sequence identity range from 98% to 100%. In the analysis of the genome, there is no major divergence was observed in different countries as shown in **Figure 1**.

3.SARS-COV-2 VACCINE AND VARIANTS

3.1 SARS-CoV-2 Vaccine

The current pandemic situation is ongoing and a continuous threat to public health, and still, no anti- SARS-CoV-2 drugs or vaccine options have shown absolute health benefits (Singh et al., 2020a; Singh et al., 2020b). SARS-CoV-2 is very challenging due to age factors, gender differences, ecological factors, and its quick

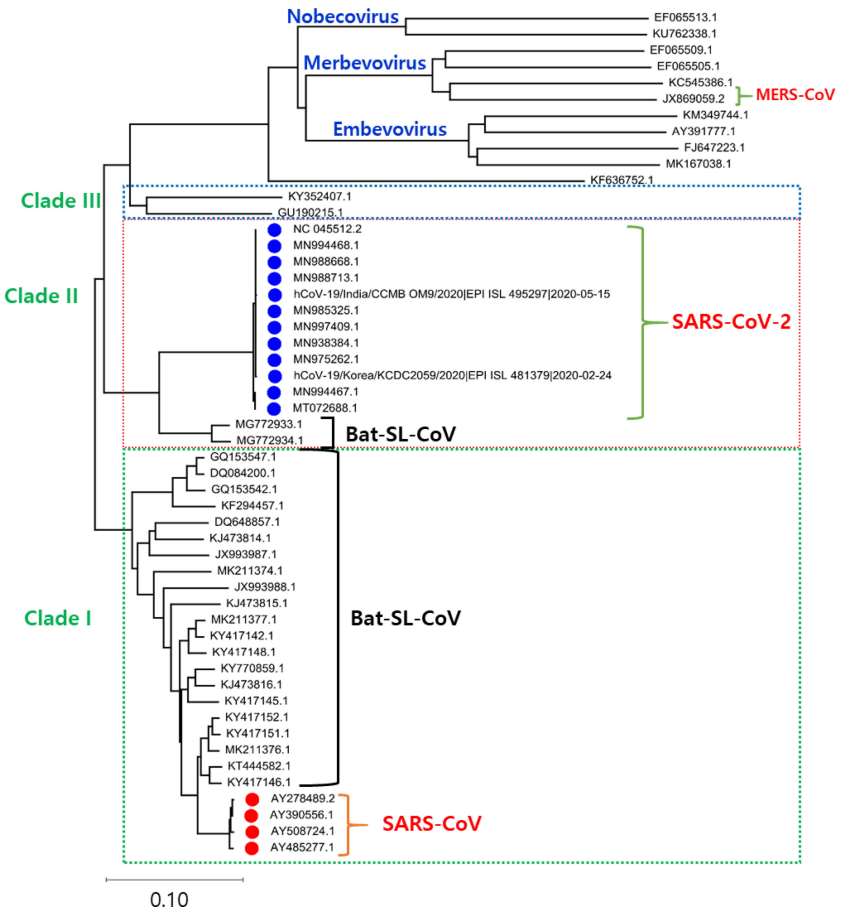

FIGURE 1 | The phylogenetic tree is generated using the latest complete genome sequences of different neighbours, MERS-CoV, Bat-SL-CoV, and SARS-CoV. The tree is divided into three clades according to the grouping of clusters. In Clade I: Bat-SL-CoV-2 and SARS-CoV were showing a close evolutionary relationship with each other. Clade II: A mixture of human and bat coronavirus including the MERS-CoV. Clade III: This clade represents all the SARS-CoV2 genomes isolated from humans, interestingly it is also observed that these genomes are showing a close evolutionary relationship with Bat-SL-CoV-2.

evolution (de Oliveira et al., 2021). The investigations from various fields to come up with effective treatment options and vaccine developments are shown in **Figure 2** (Marian et al., 2021). The challenge of finding a final targeted drug is still difficult and ongoing, 405 therapeutic drugs are under investigation in various clinical stages and 242 vaccines are under clinical 139 research (Park et al., 2021).

Effective vaccines are required against the infection of SARS-CoV-2 for lifelong immunity, and various types of vaccines are under clinical investigation (**Table 1**) such as

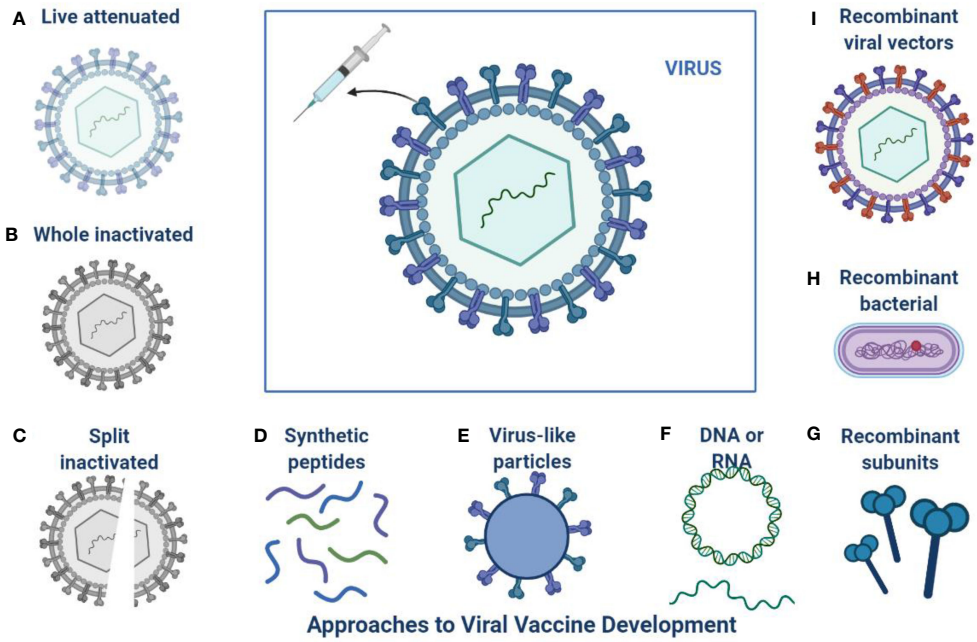

FIGURE 2 | Approaches to SARS-CoV-2 Vaccine development: This is an overview of different platforms for viral vaccine development including **(A)** Live attenuated **(B)** Whole inactivated **(C)** Split inactivated **(D)** Use of synthetic peptides **(E)** Virus like particles **(F)** DNA or RNA **(I)** Recombinant viral vectors **(H)** Recombinant bacterial **(G)** Recombinant subunits.

TABLE 1 | Efficacy of SARS-CoV-2 vaccine on Clinical trials.

SARS-CoV-2 variant	Key mutations	First detected	Transmissibility	Vaccine-mediated protection						
				Corona Vac (Sinovac)	NVX-CoV2373 (Novavax)	mRnA-1273 (moderna)	Ad26.CoV2-S (Johnson & Johnson)	AZD1222 (AstraZeneca–university of oxford)	BnT162b2 (Pfizer–BionTech)	References
Wuhan strain	Reference strain	China, December 2019	Original strain	50–90%	89%	94.1%	66%	55–81%	95%	Rahimi et al., 2021
B.1.617.2 (Delta)	L452R T478K D614G P681R	India, December 2020	97% increase	Not known	Not known	Neutralization titer 6.8-l	Reports of 60% effectiveness	92% effective against hospitalization	one dose of vaccine is 88% effective	Tregoning et al., 2021
Alpha, B.1.1.7	H69/V70 deleti	UK, September 2020	~50% increase	Unknown	86%	2.3–6.4 in titres of neutralizing antibodies	70%	75%	90%	Wang et al., 2021; Abu-Raddad et al., 2021
Beta, B.1.351	D614G K417N E484K N501Y	South Africa, September 2020	25% increase	Unknown	60%	Reduced levels of neutralizing antibodies	72% efficacy in the USA, and 57% in South Africa	10%	75%	Hoffmann et al., 2021
Gamma, P.1 (B.1.1.28.1)	E484K K417N/T N501Y D614G	Japan/ Brazil, December 2020	1.4–2.2 times more transmissible	51%	Unknown	Reduced levels of neutralizing antibodies	68%	Unknown	No evidence of reduced protection	Aleem et al., 2021

nucleic acids, lipid-coated, mRNA, peptides, live or attenuated vaccines, and adenovirus-based anti-SARS-CoV-2 (Kyriakidis et al., 2021; Lazarus et al., 2021). The efficacy of SARS-CoV-2 vaccine clinical trials is shown in **Table 1**. Approximately 17 vaccines are in Phase I and 23 vaccines are in Phase-I-II, while 20 have reached stage III for clinical evaluation, and 10 various types of vaccines are approved by different regulatory agencies for community use. CanSino Biologics has developed a Vector-based S protein vaccine and efficacy was evaluated in 603 volunteers and observed effective humoral immune response (Hou et al., 2021). A high level of antibodies production was analyzed after the booster dose. Inactivated and whole vaccines are under clinical examination and 320 Individuals have established effective neutralizing antibodies (Khoury et al., 2021).

3.2 SARS-CoV-2 Variants

SARS-CoV-2 is susceptible to genetic modification which in multiple variants has changed the potential transmission mechanism and transmission rate as shown in **Figure 3**. SARS-CoV-2 variants have been increased in rate of infection, hospitalization, and mortality (Chu et al., 2020; Tregoning et al., 2021). Multiple mutations have been observed in the spike protein and other areas of the genome as shown in **Figure 4** (Garcia-Beltran et al., 2021). The B.1.1.7 genomic variant of SARS-CoV-2 has a significant transmission advantage, the R0 (Reproduction Numbers) ranging from 0.4 (B.1.1.7) to 0.7 (non-B.1.1.7) variants. B.1.1.7 variants have been identified with a mutation in the region of the viral spike protein in the RBD (receptor-binding domain) at the global level (Wang et al., 2021; Abu-Raddad et al., 2021). Genomic sequencing of SARS-CoV-2 viral samples is essential to control the pandemic and it helps in the identification of emergent genetic variants of SARS-CoV-2 (Konings et al., 2021). The efficacy of any SARS-CoV-2 vaccine may change among the notable variants reported during this pandemic, are as shown in **Table 2**.

3.2.1 D614G Variant

Spike D614G variant became prevalent at the global level within a few months after observation of original strain, D614G Variant high affinity with human ACE2 receptor in comparison to the parental strain. The D614G mutation was able to increase replication capacity and susceptibility in both human and animal models and high disease severity was observed in a patient with the D614G variant (Korber et al., 2020; Plante et al., 2021). A high level of neutralizing antibodies was analyzed in animal models with the D614G variant, and compared with the parental strain (Chen LL et al., 2021; Ozono et al., 2021). Vaccine and therapeutics agents are less effective against D614G (Korber et al., 2020). TheD614G in the Furin binding is prominent common mutations described in nearly all the new variants (Ozono et al., 2021).

3.2.2 Alpha (B.1.1.7) Variant

In Dec 2020, B.1.1.7 (Alpha) of SARS-CoV-2 was reported in the United Kingdom (Oude Munnink et al., 2021). Seventeen mutations were observed in the viral genome, of which eight mutations were in the spike (S) protein such as Δ69-70 deletion, Δ144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H. Another important mutation N501Y has shown an increased severity of the infection and high binding affinity of the spike protein to ACE 2 receptors, enhancing the viral attachment and subsequent entry into host cells (Cascella et al., 2021). This variant was observed in the UK for the first time in September 2020, and in December 2020 in the USA (Galloway et al., 2021). The mortality rate was observed to be high in B.1.1.7 variant

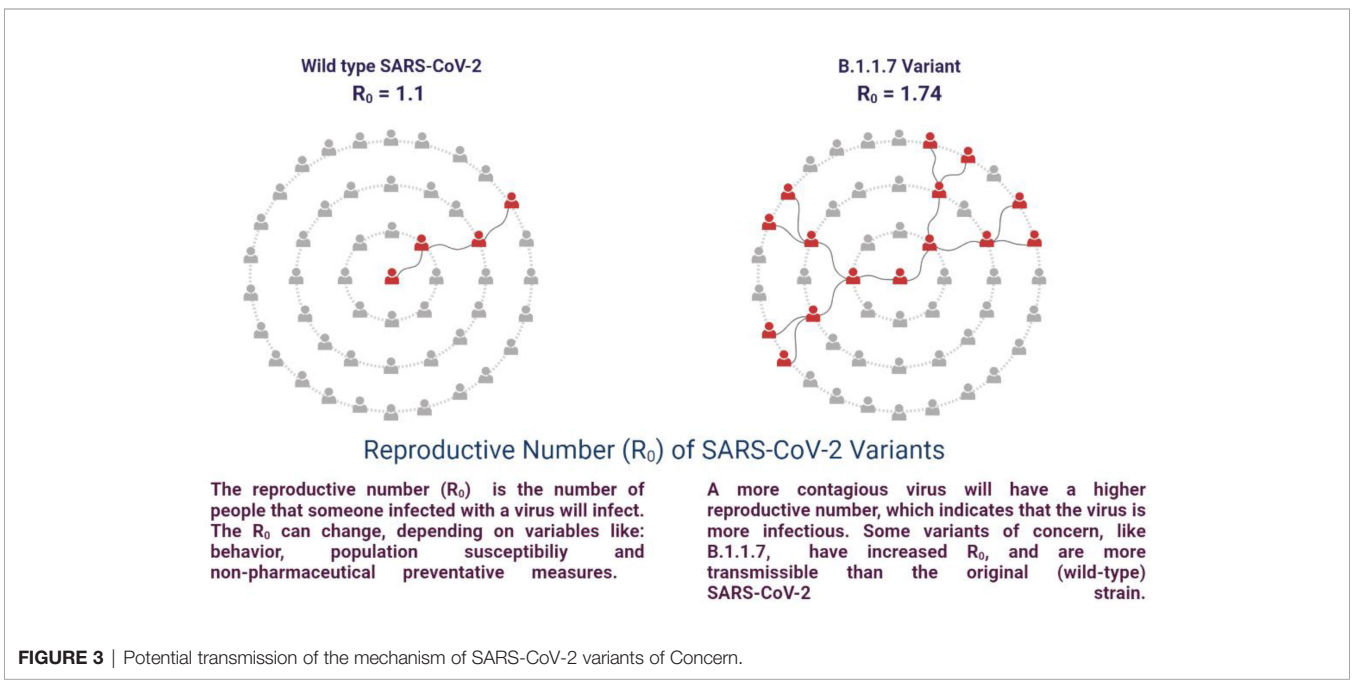

FIGURE 3 | Potential transmission of the mechanism of SARS-CoV-2 variants of Concern.

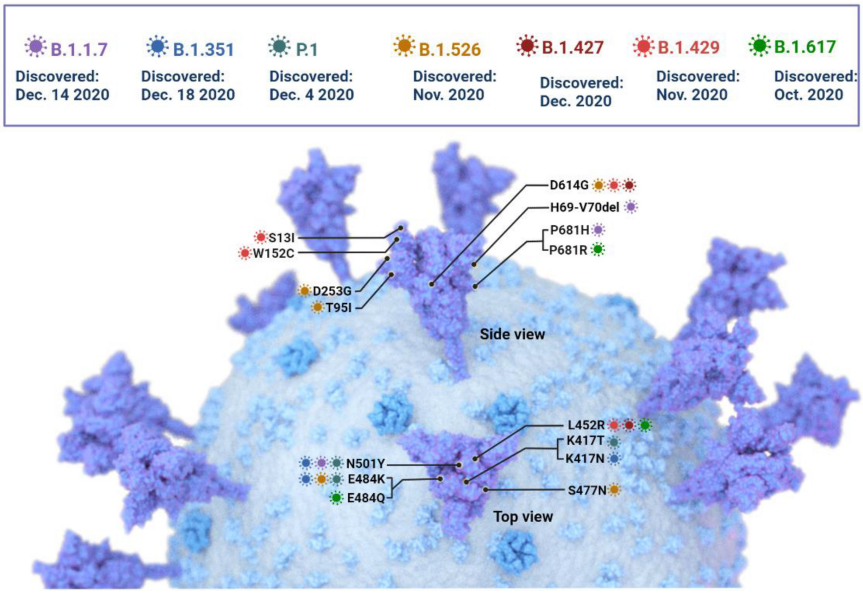

FIGURE 4 | The SARS -CoV-2 Variants of concern: Key mutations in the spike protein are shown, but mutations in other areas of the genome have been identified and are currently under investigation.

infected patients and the adjusted hazard ratio was analyzed as 1.67, 95% CI 1.34-2.09 (Cascella et al., 2021). The B.1.1.7 dominant variant SARS-CoV-2 strain is circulating in various countries globally (Port et al., 2021). BNT162b2-immunized individuals with B.1.1.7 mutations was observed a substantial reduction in neutralization titters (Emary et al., 2021; Kernéis et al., 2021). Persons immunized with Ad26.COV2-S observed a neutralization effect against the B.1.1.7 variant *in-vitro*, but was less effective than against the reference strain (Emary et al., 2021). NVX-CoV2373 SARS-CoV-2 vaccine was investigated and observed more effective (86%) against B.1.1.7 variant in comparison to the original strain (96%). The phase III clinical trial was evaluated with 15,000 participants (18 and 84 years of age) in the UK (Galloway et al., 2021; Shinde et al., 2021). The efficacy of AZD1222 was observed 70% in patients with B.1.1.7. In the case of non-B.1.1.7 Lineages, 77% efficacy was observed (Lopez Bernal et al., 2021). Various amino acid modifications have been observed in the spike protein of B.1.1.7. including P681H, N501Y, 69/70, E484K, and ORF8 mutations (Collier et al., 2021; Kernéis et al., 2021; Seghatchian, 2021). Another mutation F888L in the spike protein was also identified in the Nigerian variant along with the E484K (Port et al., 2021; Ashoor et al., 2021). This mutation has been reported to alter the biological efficiency of SARS-CoV-2 by performing the hydrolysis by TMPRSS2 and augmenting viral invasion (Ashoor et al., 2021; Liu C et al., 2021). It is also probable that this major variation supports the viruses' misleading of the immune response of the host (Kernéis et al., 2021; Liu J et al., 2021; Quinonez et al., 2021). All the above investigations were carried out with their limitations in respect to methodology, sample size, and immune response (Kernéis et al., 2021).

3.2.3 Lineage B.1.351 (Beta)

The B.1.351 variants were reported in South Africa in December 2020 (Shinde et al., 2021). B.1.351 variants carry E484K mutations and cause more severe symptoms than the other variants (Hoffmann et al., 2021). Emergent mutant variants from the UK and South Africa are more infectious, but effective against developed vaccines (Voysey et al., 2021). The 501.V2 variants was the first time observed in South Africa, it carries K417N and E484K mutations. The 501.V2 are more transmissible and cause severe in comparison to the parental strain (Wang et al., 2021). E484K mutation also plays an important role in immune mechanism, host receptor affinity, and infectivity (Shastri et al., 2021). Initial findings have indicated that the Oxford–AstraZeneca vaccine has shown considerable reduction in effectiveness against these variants and was reviewed by the WHO (Lopez Bernal et al., 2021). Novavax can protect up to a moderate level, while the Pfizer–BioNTech and Johnson & Johnson vaccines also have reduced the efficacy against the β-lineage, although for the other vaccines the data is not yet available (Heath et al., 2021; Sadoff et al., 2021; Lopez Bernal et al., 2021). It has also been identified that the most extensively distributed vaccines may have reduced antibody neutralization against Beta variants of SARS-CoV-2, which is relevant to how vaccines can stop the disease by reducing asymptomatic infection (Grint et al., 2021). Sera from patients immunized with the Moderna and Pfizer-BioNTech vaccines had less activity against Beta (B.1.351) (Bates et al., 2021). On 1 April 2021, an investigation on a Pfizer/BioNTech South African vaccination analysis showed that the vaccine has been 100% effective against Beta variants (Seppälä et al., 2021). In January 2021 Ad26.COV2. S Vaccine developed by Johnson & Johnson

TABLE 2 | Efficacy of SARS-CoV-2 vaccine and effectiveness on variants.

Name of Vaccine with manufacturer	Type of Vaccine	Administration Of Clinical trial	Sample size of clinical trial	Efficacy	Endpoint Analysis	Admissibility	Phase III trial with follow up	Circulating genotypes	Disease severity	References
BnT162b2 (Pfizer–BioNTech)	mRNA	2 doses (21 days apart)	43,548	95%	Positive case tested by RT–PCR and Symptomatic COVID-19 and	>16 years old	Up to 24 months after second dose (NCT04368728)	B.1.351, P.1, B.1.427/ B.1.419, P.2 and B.1.526	95.3% effectivity was observed As per FDA-guideline	Lopez Bernal et al., 2021
mRnA-1273 (Moderna)	mRNA	28 days apart (2 doses)	30,420	94%	Symptomatic COVID-19	≥18 years (NCT04649151) and 6 months to 12 years (NCT04796896)	Up to 24 months after second dose (NCT04470427)	B.1.526 and B.1.427/ B.1.429	More than 95% Efficacy against severe disease	Baden et al., 2021
AZD1222 (AstraZeneca University of Oxford)	Viral vector	<6 weeks apart (2 doses) 2 doses (>12 weeks	17,178	55% 81% (Pooled efficacy 67%)	Symptomatic COVID-19 NAAT result	≥18 years Age, ≥40 years old and not pregnant in the UK	24 months (NCT04516746) 12 months after second dose (NCT04400838, NCT04536051 and NCT04516746)	B.1.1.7, B.1.351, P.1, B.1.427/ B.1.429, P.2, B.1.526 and C.37	More than 95% efficacy against hospitalization	Voysey et al., 2021; Emary et al., 2021
Ad26.CoV2-S (Johnson & Johnson)	Viral vector	1 dose	44,325	66%	Tested Positive cases	≥18 years old	25 months (NCT04505722) and 27 months (NCT04614948) after the first dose	B.1.351, P.1, B.1.427/ B.1.429, P.2, B.1.526 and C.37	85.4% efficacy against severe cases	Sadoff et al., 2021
Sputnik V (Gamaley)	Viral vector	21 days apart (2 doses)	19,866	92%	Tested Positive cases	≥18 years old	6 months after the first dose (NCT04656613 and NCT04642339)	No variants have been observed in the trial region up to June 2021.	No data available up to July	Logunov et al., 2021
Covaxin (Bharat Biotech)	Viral vector	28 days apart (2 doses)	25,800	25,800 78%	Symptomatic COVID-19 and positive RT–PCR test result at least 14 days after second dose	≥18 years old (2–18 years old: study ongoing)	12 months after second dose (NCT04641481); pediatric cohort followed up for 9 months (NCT04918797)	B.1.617.2 and B.1.617.1	More than 95% efficacy against hospitalization	Yadav et al., 2021
CoronaVac (Sinovac Biotech)	Inactivated virus	14 days, (2 doses)	2,300 (Chile) 13,000 (Turkey), 12,688 (Brazil)	Various investigations; Brazil 50.7% Chile (56.5%), Turkey (91%) Indonesia, (65%), and Brazil (78%)	Tested Positive cases	≥18 years old	12 months after the first dose	P.1 and P.2	51% efficacy SARS-CoV-2, More than 95% efficacy against hospitalization infection; from 14 days after vaccination	Tanriover et al., 2021
BBIBP-CorV (Sinopharm)	Inactivated virus	2 doses (21 days apart)	45,000	78%	Occurrence of COVID-19	≥18 years old	12 months after the first dose (NCT04510207)	No variants have been identified	79% efficacy against hospitalization	Xia et al., 2021
NVX-CoV2373 (Novavax)	Protein subunit	21 days apart (2 doses)	>15,000	89%	COVID-19 positive at least 7 days after second dose	≥18 years, study ongoing, NCT04611802)	24 months after the first dose (NCT04611802)	B.1.1.7, B.1.351, B.1.427/ B.1.429 and B.1.526	More than 95% efficacy against hospitalization infection	Heath et al., 2021

(Continued)

TABLE 2 | Continued

Name of Vaccine with manufacturer	Type of Vaccine	Administration Of Clinical trial	Sample size of clinical trial	Efficacy	Endpoint Analysis	Admissibility	Phase III trial with follow up	Circulating genotypes	Disease severity	References
EpiVacCorona (VECTOR)	Protein subunit	2 doses (21–28 days apart)	3,000	Data not available July 2021	Tested Positive cases, 6 months after the first dose	≥18 years old	9 months after the first dose (NCT04780035)	No variants have been identified	No data	Dorofte et al., 2021

has been tested in South Africa and reported 72% efficiency against SARS-CoV-2 infection (mild to severe) in the US and 57% in South Africa (Shinde et al., 2021; Voysey et al., 2021).

3.2.4 Cluster 5 Variant

The Cluster 5 Variant may decrease the strength of immune defenses, after a decrease in the viral neutralization sensitivity which is obtained by vaccination and the normal procedure of infection (Becker et al., 2021). In Denmark, ΔFVI-spike was categorized as Cluster 5 strains transmitted from mink worsened the situation and may further aggravate it. As of November 2020, active mink-mediated corona cases are confirmed (Lassaunière et al., 2021). As per WHO data, the cluster 5 variant resisted diminishing sensitivity to countering antibodies. Viral expansion in mink lakes leads to a recurring risk of human infection from mink, and the adaptability of this variant in mink is a huge health concern in the future (Hoffmann et al., 2021).

3.2.5 Lineage B.1.258Δ

This variant was identified in the Czech Republic and Slovakia in late 2020, within the clade B.1.258 (Brejová et al., 2021). It has been observed to escape the immune response and increase the severity of infection. This variant has been analyzed with an N439K mutation in the terminal regions of the spike glycoprotein while showing similar deletions 69-70 at the receptor-binding domain (RBD) (Gómez et al., 2021). H69/V70 deletions mutations have been modified. The antigenic peptides in the amino-terminal region are changed, subsequent in confrontation to neutralization by improving sera and vaccination (Jeong et al., 2021).

3.2.6 P.1 or 20 J/501Y.V3 Variants

A new variant has been observed in Lineage P.1 with 11 mutations in the spike protein. Since December 2020, approximately 42% of SARS-CoV-2 positive tested samples were analyzed with P.1 lineage infection (Hoffmann et al., 2021). These mutations are closely linked with antibody-mediated immune evasion high infection rate (+161%), the mortality rate was also observed to be high, up to 50%, making it 2.2 times more transmissible than the baseline virus (Sarkar et al., 2021). P.1 and P.1-like clades are more infective in younger people. P.1 or 20 J/501Y.V3 were classified as gamma mutations (K417T, E484K, and N501Y) in the RBD domain (Hirotsu and Omata, 2021). P.1 or 20 J/501Y.V3 variants with E484K substitution were reported in Brazil (Hirotsu and Omata, 2021). In November 2020 and January 2021, it has been observed that the Gamma variant is 1.4-2.2 times more infectious than baseline (Hitchings et al., 2021). People who have been completely vaccinated with Pfizer or Moderna have shown significant neutralization against Gamma variants (Planas et al., 2021). The data from various clinical trials carried out by the WHO, CoronaVac, and BBIBP-CorV shows effectiveness against Gamma variants (Barros-Martins et al., 2021). They also found that Oxford–AstraZeneca, and CoronaVac had preserved antibody neutralization against Gamma linage, and Pfizer–BioNTech and Moderna had minimal to moderate reduction

in neutralization, with no information for other vaccines so far (Barros-Martins et al., 2021).

3.2.7 Lineage B.1.617 and B.1.617.2

Three major substitution mutations were observed in Lineage B.1.617 and B.1.617.2, namely P681R, L452R, and E484Q (Liu J et al., 2021). Two substitution mutations were observed in the RBD domain, and one nearby the furin binding the site, which increases the mode of transmission (+64%), hospitalization (+85), and mortality, with natural immunity also affected. The chances of reinfection were decreased, but the efficacy of the vaccine was also compromised in Lineage B.1.617 and B.1.617.2 (Lopez Bernal et al., 2021). Various investigations were examined by the WHO and found that vaccines from Oxford–AstraZeneca, and Pfizer–BioNTech, are likely to sustain efficacy/effectiveness against delta's variants. (Liu J et al., 2021). Researchers from the field also investigated and found that the vaccines produced by Oxford–AstraZeneca have shown a reduced neutralization effect against the Delta virus (Pascarella et al., 2021). Spike protein mutations D111D, E484Q, G142D, and P681R are found in the delta variants of 15 mutations, which may escape antibody neutralization. Initial observations have shown that Emergent variants reduce the efficacy of the mRNA-based vaccine (Cherian et al., 2021). Researchers from the field also explored whether the E484K variant may compromise the efficiency of the current vaccine. More clinical investigation is required to finally reach a conclusion for therapeutic strategies against new variants.

3.2.8 Other Variants

Lineage B.1.168 was observed in West Bengal, India with two amino acid deletions, Tyr145 and His146, and E484K and D618G mutations (Cherian et al., 2021). These substitutions can escape convalescent plasma and multiple monoclonal antibodies (Bates et al., 2021). Other 1.5.9 variants are also reported in different countries (Singh et al., 2021). The B.1.429 or Epsilon lineage was reported in 50% of samples in Los Angeles, which exhibits different mutations in ORF1ab and spike protein (Shen et al., 2021). The variants B.1.429 and B.1.427 were classified as VOCs by the CDC (Shen et al., 2021). These variants with D1183Y and I4205V mutations in the ORF1ab and S13I, W152C, and L452R mutations in the spike protein are also known as CAL.20C, 20C/S:452R, CA VUI, or 21C. CAL.20C variants were identified in November 2020 in California, this variant was classified Epsilon (McCallum et al., 2021). The prevalence of the variant in sequenced samples from Northern California increased from 3% to 25% between November and December 2020 in total samples tested in California (Zhang et al., 2021). In January 2021, the prevalence of the variant 20G was observed in the United States. This variant was also seen in some SARS-CoV-2 cases in Europe, Asia, and Australia (McCallum et al., 2021). The frequency of this variant was decreased in February 2021, until April 2021 by when this Epsilon variant had disappeared from southern California and comprised just 3.2 percent of cases were observed in the United States, while 2/3 cases were observed from Alpha variants (Zhang et al., 2021). Theta variants (P.3.) were reported on February 18, 2021, with two mutations E484K and N501Y, by the Central Visayas Department of the health service of the Philippines (Oude Munnink et al., 2021). Theta variants (theta) were also identified in Japan, the United Kingdom, and Malaysia in July 2021. Theta variants disappeared by July 2021. Another, the R.1 variant was reported by Japan on the RBD of the spike protein with E484K mutation, and another W152L mutation was observed in the N-terminal Domain. These mutations also play an important role in immune evasions, and are reported by various countries at the global level (Chen RE et al., 2021). The Pfizer-BioNTech vaccine was shown to be 94% effective in preventing R.1 infected hospitalization and mortality. As Alpha and then Delta increase in Japan, R.1 illustrations are becoming increasingly rare (Kernéis et al., 2021). Linage B.1.620 was analyzed in Lithuania in March 2021, also known as the Lithuanian strain, it was found in Central Africa, North America, France, and Belgium. In an analysis of the original variant, this lineage revealed 23 substitutions, most of which are discrete mutations (Silva et al., 2021; Korber et al., 2020). Lineage B.1.618 was discovered for the first time in October of 2020, this variant contained E484K mutation with many other variations and showed substantial development in West Bengal, India, in April 2021 (Biswas et al., 2021). On 23 April 2021, The PANGOLIN analysis identified 135 sequences in India (Singh et al., 2021). Sixteen cases in the United Kingdom were identified under Lineage B.1.1.318 as a VUI (VUI-21FEB-04) (Seghatchian, 2021).

4 CONCLUSION

Researchers from the field are focused on the eradication of SARS-CoV-2 infection from serious health risks to humans. In this regard various clinical approaches and scientific methods are exponentially used against SARS-CoV-2 from virtual drug screening to the molecular mechanism, and from vaccine designing to SARS-CoV-2 platforms development, computational approaches are of great interest. They have enhanced the understanding of genomics designs, proteomics, structures determination, mutation solidity, function connection, and tracing. There are now enough investigations of the altering antigenicity of the SARS-CoV-2 spike protein and of the amino acid variations that can change antibody neutralization. Spike amino acid substitutions and deletions influence neutralizing antibodies efficacy in the global virus population. However, our knowledge about SARS-CoV-2 is very limited. No effective treatment option and anti- SARS-CoV-2 approaches are have reached their final design. Further clinical investigation is required to prevent infection and control the pandemic situation at the global level.

AUTHOR CONTRIBUTIONS

D.D.S. and D K Y. conceived and designed the project, D.D.S., A.P. and D K Y. collected data from the literature. D.D.S. analyzed the data and wrote the manuscript. All authors have read and approved the final version of the manuscript. Figure

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REVIEW
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Molecular Evolution of Severe Acute Respiratory Syndrome Coronavirus 2: Hazardous and More Hazardous Strains Behind the Coronavirus Disease 2019 Pandemic and Their Targeting by Drugs and Vaccines

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Within almost the last 2 years, the world has been shaken by the coronavirus disease 2019 (COVID-19) pandemic, which has affected the lives of all people. With nearly 4.92 million deaths by October 19, 2021, and serious health damages in millions of people, COVID-19 has been the most serious global challenge after the Second World War. Besides lost lives and long-term health problems, devastating impact on economics, education, and culture will probably leave a lasting impression on the future. Therefore, the actual extent of losses will become obvious only after years. Moreover, despite the availability of different vaccines and vaccination programs, it is still impossible to forecast what the next steps of the virus are or how near we are to the end of the pandemic. In this article, the route of molecular evolution of the coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is thoroughly compiled, highlighting the changes that the virus has undergone during the last 2 years and discussing the approaches that the medical community has undertaken in the fight against virus-induced damages.

Keywords: COVID pandemic, variants, molecular evolution, therapeutics, vaccination

INTRODUCTION

From the end of 2019, life has been greatly affected by the coronavirus disease 2019 (COVID-19) all over the world. Based on the data from Worldometers, this pandemic has afflicted more than 241.97 million human lives and has claimed nearly 4.92 million lives around the globe during the last 1.5 years (https://www.worldometers.info/coronavirus/; data from October 19, 2021). At that, the elderly people and

those with underlying cardiovascular, respiratory, and metabolic disorders have been found to be especially vulnerable by severe course of the disease, causing bilateral pneumonia, acute respiratory distress syndrome (ARDS), failure of multiple organs (including, but not limited to, the brain, heart, liver, and kidneys), or even mortality (Abdullahi et al., 2020; Li et al., 2021). In addition to the direct health damages, devastating impact on education, culture, economics, and general public welfare proceeding from the strict restrictions in social contacts established for the disease prevention cannot be underestimated (Sood et al., 2020).

COVID-19 is caused by an infection with the single-stranded RNA virus with positive polarity, i.e., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that transmits mainly *via* respiratory droplets, aerosols, and fomites (Abdullahi et al., 2020; Kawabata et al., 2020; Li et al., 2021; Mallah et al., 2021). Coronaviruses consist of enveloped virus particles with 80–120 nm of diameter; they have typically spherical or pleomorphic structure with spike-like projections of glycoproteins on surface, giving them a crown-like appearance under electron microscopy (Tuli et al., 2021). The initial reservoir of SARS-CoV-2 is hypothesized to be bats transmitting the virus particles to human beings (Tuli et al., 2021). Within the time of the pandemic course, SARS-CoV-2 virus has been in a continuous molecular evolution, displaying genetic diversity and mutations with varied degrees of transmission and virulence (Abdullahi et al., 2020; Deimel et al., 2021). Such mutations can help virus particles to escape the immune system and/or replicate more efficiently once it has entered the host organism, making the virus more infectious and pathogenic (Adedokun et al., 2021; Hossain et al., 2021). The impact of viral changes on the COVID-19 pandemic has been apparent in the disease outbreaks occurring disproportionately in different parts of the world (Abdullahi et al., 2020; Fraser, 2020; Vudathaneni et al., 2021). Therefore, the virus variants are designated by the geographical regions where the mutations have emerged, including the UK (B.1.1.7), Brazilian (B.1.1.248), and South African (1.351) strains, among others (Hossain et al., 2021). Furthermore, as mutations in the virus genome can change also the susceptibility of the virus to both clinically used drugs and vaccines, concerns have been arisen about the efficacy of current preventive and therapeutic interventions for stopping the pandemic (Chiam et al., 2021; Hossain et al., 2021; Matta et al., 2021; Robinson et al., 2021).

In this state-of-the-art review article, molecular characteristics of the currently emerged variants of SARS-CoV-2 are under discussion, analyzing their infectivity, morbidity, and mortality potential, as well as susceptibility to the current intervention measures applied for achieving control over the pandemic.

MOLECULAR EVOLUTION OF CORONAVIRUS DISEASE 2019 FROM ITS EMERGENCE TO THE CURRENT STATE

Mutations originate as a result of viral replication during circulation. Despite being an RNA virus, coronaviruses undergo fewer mutations because of their strong proofread mechanism. Moreover, the fate of mutations is determined by the natural selection, meaning that those favored with respect to viral better survival will increase in frequency, and those that reduce viral fitness tend to be eliminated from the population of circulating viruses. However, mutations can also happen due to chance events. Therefore, the interplay of natural selection and chance events leads to virus evolution.

The SARS-CoV-2 virus has been mutated over time, resulting in different genetic variations in the population of circulating viral strains over the course of the COVID-19 pandemic. The evolution of SARS-CoV-2 suggests strong purifying selection and modest divergence; one of the most closely related strain of SARS-CoV-2 is "RaTG13" found in a bat sample from Yunnan Province, China, in 2013. RaTG13 (horseshoe bat, *Rhinolophus affinis*) shows 96% similarity to SARS-CoV-215. Though RaTG13 is closely related to SARS-CoV-2, there is a significant level of variation in sequence similarity across the genomes of these two viruses, ranging between 93.1% and 99.6% (Zhou et al., 2020). However, comparisons with other coronavirus strains suggest complex recombination events during its evolution. Various recombinations were detected across the genome majorly in ORF1a and in the region marking the N-terminus of the S protein (Li et al., 2003; Li, 2016; Hoffmann et al., 2020; Wan et al., 2020). S protein binds to angiotensin-converting enzyme 2 (ACE2) receptors and mediates viral entry into the human cells. One such mutation, D614G, arises as a result of single-nucleotide polymorphism (SNP) and results in amino acid change from an aspartate [D] to a glycine [G] at residue 614, increasing the efficiency of viral entry into the human cells (Isabel et al., 2020; Korber et al., 2020).

The D614G mutation in the spike glycoprotein of SARS-CoV-2 was significantly detected for the first time in early March 2020 and has spread globally across multiple geographic regions over the next month (Korber et al., 2020). However, various sequencing studies have already identified the D614G mutation in viruses in China in late January, which dispersed globally. Similarly, the population genetics analysis of more than 25,000 sequences from the United Kingdom also found that viruses with 614G are more transmissible and affect larger phylogenetic clusters (Volz et al., 2021). Even parallel studies in animal models also indicate that 614G viruses are more transmissible. As a result of more favored mutation, this strain has now become a dominant global strain (Hou et al., 2020; Plante et al., 2021).

Apart from its evolution in humans, there is evidence of cross-specific transmission in other animals like mink, which can even lead to emergence of potentially dangerous recombinant SARS-CoV-2 strains. Outbreaks of SARS-CoV-2 on mink farms in the Netherlands and Denmark that started in late spring and early summer 2020 demonstrated human-to-mink, mink-to-mink, and mink-to-human transmissions (European Centre for Disease Prevention and Control, 2020, Oude Munnink et al., 2021). In early November 2020, 214 cases of mink-associated human COVID-19 were reported. These cases where Y453F mutation in the receptor binding domain of spike might be responsible for increased binding affinity for ACE2 in mink. Eleven patients from the Danish outbreak had a cluster 5 variant

having three additional mutations in spike (del69_70, I692V, and M1229I). An investigation of human serum samples in nine patients showed a significant reduction in neutralization activity against cluster 5 viruses (mean, 3.58-fold; range, 0–13.5). Therefore, continued evolution and adaptation of SARS-CoV-2 in an animal reservoir resulted in novel SARS-CoV-2 from mink to humans and other mammals.

Another lineage B.1.1.7 (also called 501Y.V1) was identified in southeastern England (Rambaut et al., 2021) and became one of the variants of the highest concern. This variant has already highly evolved, having 17 lineage-defining mutations even prior to its detection in early September. Seven of these mutations were in the spike proteins only that later formed the basis for the vaccine in the United Kingdom. This variant was found to be 56% more transmissible and was responsible for approximately 28% of cases of SARS-CoV-2 infection in England within 1 month (Davies et al., 2020). Unlike D614G, which could be because of chance events, B.1.1.7 (Alpha variant) strongly seems to have arisen as a result of natural selection. It came into existence after outcompeting already circulating widespread SARS-CoV variants.

Most of the mutations in B.1.1.7 lineage include mutations in the spike glycoprotein, N501Y in the receptor binding domain, deletion 69_70, and P681H in the furin cleavage site, which could probably influence ACE2 binding and viral replication. Specifically, the 501Y spike variants were predicted to have an increased affinity for human ACE2, and another variant, also with an N501Y mutation, was spreading fast in South Africa (Beta variant—B.a351, B.1.351.2, and B.1.353.3). Immunogenic effects of these mutations are currently not clear. Similarly, the Gamma variant (P.1) was emerged in the Amazon city of Manaus in December 2020 and has led to a surge in cases in Brazil (Buss et al., 2021).

Recently, the Delta variant (B.1.617.2, AY.1, and AY.2) having multiple mutations originated in India is of major concern (Centers for Disease Control and Prevention (CDC), 2021; Public Health England, 2021). This variant is the highest transmissible variant and hence favored by evolution. Therefore, different mutants originated in different geographical areas as a combinatorial result of selective advantage or chance mutation. Variants having mutations in spike to increase transmissibility could quickly outcompete and replace other circulating variants. Moreover, widespread infection among humans is now posing a huge threat to other mammals that usually interact with human populations and worsen the severity of disease by creating more dangerous recombinant SARS-CoV-2 strains. It would be important to consider the epidemiological, genetic, and functional studies of different variants and come up with a strong strategy to stop its transmission across the species.

GEOGRAPHICALLY EMERGED STRAINS AND THEIR STRUCTURAL DIFFERENCES

Accumulation of mutations within the genome is the primary driving force in viral evolution within an endemic setting (Dan et al., 2020; Baden et al., 2021). This inherent feature often leads to altered virulence, infectivity and transmissibility, and antigenic shifts to escape host immunity, which might compromise the efficacy of vaccines and antiviral drugs (Upadhyay et al., 2021; Yadav et al., 2021a). The SARS-CoV-2 as RNA virus lacks mismatch repair mechanism and replication accompanied by a high mutation rate (Domingo and Holland, 1997). Therefore, the mutations of the coronavirus are commonsensical and predictable, which leads to several rapidly spreading variants (**Table 1**). At present, emergence of fast-spreading three SARS-CoV-2 variants (B.1.1.7, B.1.351, and B.1.1.28.1) due to rapid mutations in ACE2 became dominant strains all around the world, causing concern on prevention and treatment of COVID-19 (Krammer, 2020; Callaway, 2021; Zhou and Wang, 2021). The morphological and physiological assessments of the P.1 or B.1.1.28.1 variant of SARS-CoV-2 from Brazil reflected less resistance to antibodies produced from natural infection or vaccination compared with other parallel variants B.1.351 from South Africa, and B.1.1.7 from the United Kingdom (Faria et al., 2021). It is noteworthy that P.1, B.1.1.7, and B.1.351 have accrued multiple mutations in the NTD (N-terminal domain) and can be neutralized by a monoclonal antibody, mAb 222 (Cerutti et al., 2021; Dejnirattisai et al., 2021). In addition, these mutated residues also have the potential to modulate vaccine-induced antibody responses (Supasa et al., 2021; Zhou et al., 2021). The three central variants by analyzing 160 sequences claimed that B-type viruses (with substitution, NS8_L84S) were common in East Asia, whereas A-type (ancestral lineage) and C-type (NS3_G251V variant) viruses were prevalent in Europe and North America (Forster et al., 2020). Along with other co-evolving mutations, NSP12_P323L and S_D614G probably provide variants with an evolutionary advantage over their ancestral types, allowing them to survive and circulate in this densely populated region (Becerra-Flores and Cardozo, 2020; Islam et al., 2021). Thus, the recent emergence of a number of variants of concern (VOCs) has led to design of new vaccines that will be able to protect against the emerging viral variants.

The comprehensive analysis of whole-genome sequences of 837 Indian SARS-CoV-2 strains revealed the occurrence of 33 different mutations, 18 of which were unique to India (Tang et al., 2020; Sarkar et al., 2021b). The second SARS-CoV-2 epidemic wave in India began around March 2021, and just weeks after, it became the dominant lineage by superseding the previous lineages (Kar et al., 2021; Salvatore et al., 2021). Almost all new cases of COVID-19 are the Delta variant (B.1.617.2) with augmented cases, but the rate of growth is slower than that of the Alpha variant (O'Dowd, 2021). The data showed the even at the higher risk of hospitalization for patients with the Delta variant compared with the Alpha variant (B.1.1.7), two doses of vaccine gave a high degree (90%) of protection (Shrotri et al., 2021; Stowe et al., 2021; Williams et al., 2021). The identification and spread of various dreading variants including B.1.1.7, B.1.351, and P.1 in India led to global VOCs (Alai et al., 2021). The Kappa and Delta variant lineages of SARS-CoV-2 were first detected in December 2020 in India (Cherian et al., 2021). Rapidly between January and February 2021, the Delta (B.1.617.2) variant became dominant in Maharashtra and was marked as a VOC in early May by the WHO (2021b). Therefore, it is imperative that currently known variants of COVID-19 and new variants should be carefully considered in the design of an effective vaccine.

TABLE 1 | Different variants of SARS-CoV-2 according to the WHO.

S. no.	Variant name	1st detected by	Month, year of detection	Key mutations in spike protein	Reference
1	614G	Bavaria, Germany	January, 2020	D614G	Brüssow, 2021; Plante et al., 2021
2	20C-US	United States	May, 2020	Q677; Q173	Pater et al., 2021
3	B.1.427/B.1.429 (also known as Epsilon variant)	United States	June, 2020	L452R; W152C; S13I; D614G	Tomkins-Tinch et al., 2021
4	B.1.1.7 (also known as 20I/501Y.V1 or VOC202012/01 or Alpha variant)	United Kingdom	September, 2020	H69/V70; Y144; N501Y; A570D; P681H	Leung et al., 2021; Sarkar et al., 2021a
5	CAL., 20C	Southern California	October, 2020	ORF1a: I4205V; ORF1b: D1183Y; S13I; W152C; L452R	Zhang et al., 2021
6	B.1.526 (also known as Iota variant)	United States	November, 2020	L5F; T95I; D253G; D614G; A701V; E484K or S477N	West et al., 2021b
7	B.1.525 (also known as Eta variant)	United Kingdom, Nigeria	December, 2020	H69-V70; Y144; Q52R; E484K; Q677H; D614G; F888L	Faria et al., 2021
8	B.1.351 (also known as 20H/501Y.V2 or Beta variant)	South Africa	December, 2020	L242/A243/L244; K417N; E484K; N501Y	Tegally et al., 2021; WHO, 2021a
9	B.1 descendant with 9 mutations	France	January, 2021	G142; D66H; Y144V; D215G; V483A; D614G; H655Y; G669S; Q949R; N1187D	West et al., 2021a
10	B.1.1.28.1 (also known as P.1 or Gamma variant)	Brazil/Japan	January, 2021	K417T, E484K; N501Y	Sabino et al., 2021; Chudik et al., 2021
11	B.1.1.28.3 (also known as P.3 or Theta variant)	Philippines	February, 2021	E484K; N501Y; P681H	Haseltine, 2021
12	B.1.1.28.2 (also known as P.2 or Zeta variant)	Brazil	April, 2021	L18F; T20N; P26S; F157L; E484K; D614G; S929I; V1176F	Faria et al., 2021
13	B.1.617.2 (also known as Delta variant)	London, United Kingdom, India	March–May, 2021	T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, D614G, P681R, D950N	Salvatore et al., 2021; Williams et al., 2021
14	B.1.617.1/B.1.617.3 (also known as Kappa variant)	Maharashtra India	February, 2021	G142D; E154K; L452R; E484Q; D614G; P681R; Q1071H	Cherian et al., 2021; WHO, 2021b

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Variability in Infectivity, Morbidity, and Mortality of Different Strains

The case fatality rate (CFR) in COVID-19 seems to be elevated than that of in seasonal influenza, whereas both diseases principally have an effect on older adults above 65 years of age with infirmity (Dan et al., 2020; Yadav et al., 2021b). The augmented fatality rate of COVID-19 could be because of variations in underlying comorbidities of patients, pathogenicity of the causative agent SARS-CoV-2, immunity of population, and responses of host to the infection (Jha et al., 2020; Upadhyay et al., 2020a; Upadhyay et al., 2020b). It has been reported that the COVID-19 patients were more frequently obese and suffered from diabetes, hypertension, and dyslipidemia than influenza patients; on the contrary, the influenza patients often had cardiac failure, chronic respiratory disease, cirrhosis, and anemia (Piroth et al., 2021). Patients admitted to care centers with new variant of SARS-CoV-2 more frequently experienced acute respiratory failure, pulmonary embolism, septic shock, or hemorrhagic stroke, but less frequently developed myocardial infarction or atrial fibrillation (Dan et al., 2020). In-hospital mortality was comparatively multifold higher in patients with COVID-19 than conventional influenza patients (16.9% *vs.* 5.8%, respectively), with a relative risk of death of 2.9 (West et al., 2021b). Quantitatively, there was less pediatric patients (<18 years) for COVID-19 than influenza among the patients admitted in the hospital, but a bigger proportion of patients younger than 5 years required intensive care unit (ICU) support to COVID-19 than influenza (Piroth et al., 2021). As per the report, in-hospital mortality of adolescents (11–17 years) was manyfold higher for COVID-19 than for influenza as well. Thus, the effect of the SARS-CoV-2 variant is tremendous for all sex and age groups of the human population but was supposed to be the most common challenging health risk factor to immunocompromised septuagenarians and octogenarians (**Figure 1**).

SUSCEPTIBILITY OF CORONAVIRUS DISEASE 2019 TO CLINICALLY USED DRUGS

Currently, the strategy to treat the COVID-19 infection comprises social distancing and vaccination. However, with the sharp rise in the cases and variable symptoms, various pharmacotherapies were explored for enhancing viral clearance and other symptomatic relief (Rahman and Idid, 2021). Until now, no specific drug for the treatment and management of COVID-19 has been developed. Hence, the focus has been shifted towards drug repurposing, which is time saving, is an accepted approach, and has an unmet need of time (Stasi et al., 2020). At present, many of the existing drugs have been repurposed and tested in preclinical and clinical trials (**Table 2**). However, with the advancement and better understanding of pathophysiology and clinical presentation

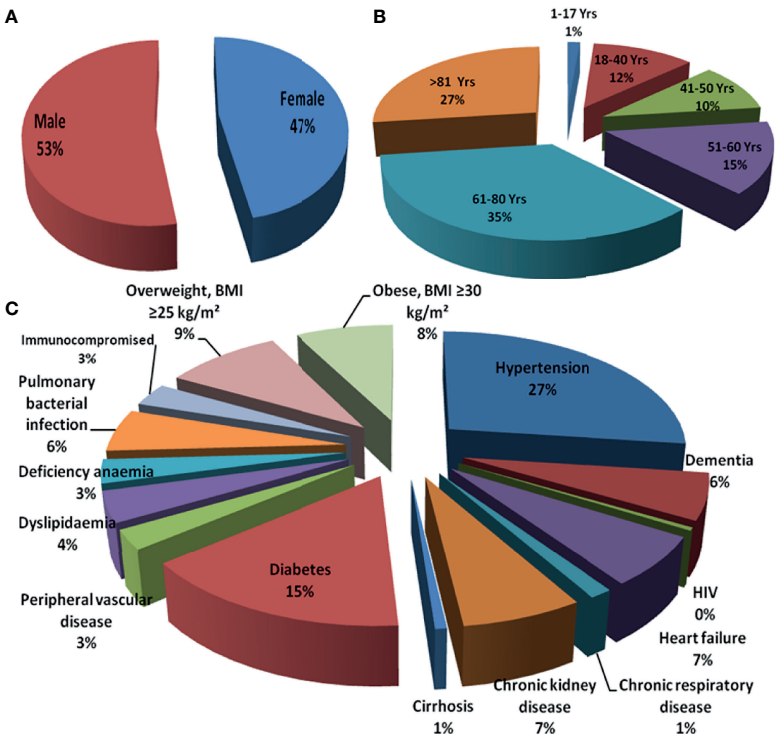

FIGURE 1 | Risk of infectivity and morbidity among COVID-19 patients: **(A)** sex based, **(B)** age based, and **(C)** comorbidities. COVID-19, coronavirus disease 2019.

among patients, it was noticed that the clinical efficacy of these drugs depends on timing of use, disease stage, and dose regimen (Iqubal et al., 2021a). Antiviral drugs are important when used during the early stage, as they inhibit viral entry and replication (Şimşek Yavuz and Ünal, 2020). Among antiviral drugs, remdesivir is one of the extensively used drugs. Initially, the *in vitro* study has shown antiviral potential against COVID-19. Later on, the US Food and Drug Administration (FDA) approved this drug to shorten the recovery time in adults and children (below the age of 12) (Young et al., 2021). However, the outcome of the WHO SOLIDARITY trial that involved 11,330 patients across 40 countries showed a non-significant effect on reducing mortality, duration of hospitalization, and need of a mechanical ventilator (Horby et al., 2020). Lopinavir/ritonavir is a combination therapy for HIV, and it was proposed to be an effective therapy for COVID-19 (Cao et al., 2020). Ivermectin is an approved antiparasitic drug (Caly et al., 2020). Initially, the *in vitro* study showed that ivermectin significantly inhibited the replication; but based on the outcome of a double-blinded randomized trial, no clinical efficacy of lopinavir/ritonavir and ivermectin among COVID-19-infected patients were found (López-Medina et al., 2021). These drugs are not in use now. Hydroxychloroquine and chloroquine were also claimed to be promising therapeutic modality against COVID-19 infection, but the outcome of the randomized trial showed a non-significant effect against symptomatic relief among COVID-19 patients (Mitjà et al., 2021).

Use of corticosteroids and immunotherapy is preferred during cytokine storms or at the hyperinflammatory stage, and inappropriate use of these drugs often results in fetal immunogenic reactions (Esmaeilzadeh and Elahi, 2021; Rabaan et al., 2021).

Based on various clinical findings, corticosteroids were reported to be effective against cytokine storm and hyperinflated lungs (Hassan et al., 2020; Shang et al., 2020). The outcome of the landmark RECOVERY trial that involved confirmed patients of COVID-19 showed that the use of dexamethasone resulted in reduced mortality and need of mechanical ventilators or oxygen supply (Hamilton et al., 2021). Based on this trial, dexamethasone was approved among critically ill patients, either alone or in combination with remdesivir (Vetter et al., 2020; Mehta et al., 2021). Interferon-β-1a, a cytokine, exhibits an immunogenic response against viral infection (Yuen et al., 2020). Previously, interferon-β-1a showed clinical ineffectiveness against ARDS but exhibited a positive response among the patients of COVID-19 (Bosi et al., 2020; Kali et al., 2021; Tortajada et al., 2021). Interferon-β-1a, when used during the early stage of infection, reduced the duration of hospitalization and mortality rate (Davoudi-Monfared et al., 2020). However, recent findings have shown that interferon-β-1a is ineffective against Alpha (B.1.1.7), Beta (B.1.351), Gamma (P1), and Delta (B.1.617.2) strains (Davoudi-Monfared et al., 2020). Currently, interferon-β-1a is not recommended for treating COVID-19 patients (Davoudi-Monfared et al., 2020). Similar to interferon-β-1a, anakinra (interleukin-1 antagonist) was found to be effective in reducing mortality during the initial investigation, but recent findings have shown its ineffectiveness against B.1.1.7; B.1.351, and P.1 variants and, hence, are not recommended to treat COVID-19-infected

TABLE 2 | Details of various repurposed drugs in COVID-19 infection.

Class of drugs	Drugs	Mechanism of action	References
Antivirals	Remdesivir	Inhibitor of RNA-dependent RNA polymerase and, hence, compete for viral ATP, which results in inhibition of viral replication	Young et al., 2021
	Lopinavir/ritonavir	Inhibitor of 3-chymotrypsin-like protease ($3CL^{pro}$) and inhibit viral replication	Cao et al., 2020
	Ivermectin	Blocker importin α/β receptor and, hence, inhibit the transmission of viral protein into the nucleus of host cell	Caly et al., 2020
	Ribavirin	Potent inhibitor of viral RNA synthesis	Iqubal et al., 2021b
	Favipiravir	Inhibitor of RNA-dependent RNA polymerase and, hence, compete for viral ATP, which results in inhibition of viral replication	Iqubal et al., 2021b
	Umifenovir	Affects the S protein activity and, hence, inhibit its fusion with the host cell	Iqubal et al., 2021b
Immunomodulators	**Corticosteroids** • Dexamethasone • Hydrocortisone • Methylprednisolone	Effectively mitigate the pro-inflammatory signaling pathways, stimulate the anti-inflammatory pathways, inhibit COX as well as NF-kB-mediated hyperinflammation, and, hence, reduce the cytokine storm	Hamilton et al., 2021
	IFN β-1a	Potentiate the interferon and assist in viral clearance	Davoudi-Monfared et al., 2020
	IL-6R-antagonists • Tocilizumab • Sarilumab	Inhibit IL-6-mediated hyperinflammation and cytokine storm	Michot et al., 2020; Gordon et al., 2021
	IL-1R antagonists Anakinra		
	TNF-α inhibitors Adalimumab	Inhibit TNF-α-mediated hyperinflammation and control cytokine storm	Iqubal et al., 2021a
	Bruton's tyrosine kinase inhibitors • Ibrutinib • Rilzabrutinib • Acalabrutinib	Potent inhibitor of TLR-4 activation and, therefore, mitigate the cytokine storm and inflammatory pathway	Roschewski et al., 2020
	JAK inhibitors • Baricitinib • Fedratinib	Inhibit JAK and activate STAT pathway, leading to inhibition of cytokine production and maturation. Additionally, these drugs inhibit the viral endocytosis *via* interacting with ACE2	Stebbing et al., 2020
	Calcineurin inhibitors * Cyclosporine * Tacrolimus	Reduced the production of T-lymphocytes *via* tumbling the expression of IL-2 receptor and production of IL-2. Inhibit the viral replication	Cavagna et al., 2020
Complement inhibitors	Eculizumab	Inhibit the production of inflammatory C5a and C5b-9	Laurence et al., 2020
Kinin–kallikrein pathway inhibitors	Lanadelumab	Inhibitor of kallikrein and hence offers relief from ARDS	Lipcsey et al., 2021
	Icatibant	Antagonist of bradykinin receptor type 2 and thus, inhibit hyperinflammation	
Serine protease inhibitors	**C1 esterase inhibitor** • Camostat mesylate • Nafamostat mesylate	Inhibit the coagulation and ARDS *via* interacting with FXIIa and kallikrein	Urwyler et al., 2020
Antimalarials	Hydroxychloroquine	Inhibit the viral entry, replication, cytokine production and coagulation	Mitjà et al., 2021
	Chloroquine		
Blood-derived products	Convalescent plasma	Maintain and stimulate the physiological defense against viral infection	Iqubal et al., 2021c
	Hyperimmune immunoglobulin		
	Bamlanivimab	Anti-spike neutralizing IgG1 monoclonal antibody that interferes with the function of viral spike proteins	Gottlieb et al., 2021
	REGN-COV2 • Casirivimab • Bamlanivimab • Imdevimab • Etesevimab • Sotrovimab	Cocktail of two anti-spike neutralizing antibodies that that interfere the function of viral spike proteins	Tardif et al., 2021
Miscellaneous	Colchicine	Reduce hyperinflammation	Tardif et al., 2021
	Vitamin D	Maintain the immune function (innate and adaptive immune system). Reduce oxidative stress, inflammation and scavenge free radicals.	Giannini et al., 2021

(Continued)

TABLE 2 | Continued

Class of drugs	Drugs	Mechanism of action	References
	Azithromycin	Assist in viral clearance and inhibit viral replication.	Oldenburg and Doan, 2020
	Sirolimus	Inhibit T-cell differentiation *via* inhibiting mTOR pathway and, hence, reduces cytokine storm and ARDS.	Omarjee et al., 2020
	Bevacizumab	Inhibition of IL-6 and hence reduces the severity of cytokine storm and ARDS	Pang et al., 2021

COVID-19, coronavirus disease 2019; ARDS, acute respiratory distress syndrome.

patients (Huet et al., 2020). Tocilizumab (IL-6 receptor antibody) and sarilumab as well as siltuximab (IL-receptor antagonist) are effective during hyperinflammatory state; and hence, they were explored for possible protective effects in COVID-19 infection (Michot et al., 2020). Some clinical trials, such as REMAP and RECOVERY, showed the benefit of using tocilizumab, sarilumab, and siltuximab, which reduced mortality and showed a better safety profile among infected patients (Michot et al., 2020; Gordon et al., 2021). Janus kinase (JAK) inhibitors (baricitinib, ruxolitinib, and tofacitinib) are well-known drugs approved for rheumatoid arthritis and other inflammatory conditions (Stebbing et al., 2020). Baricitinib is considered as one of the potential drug candidates against COVID-19 infection (Saber-Ayad et al., 2021). This drug acts by inhibiting viral endocytosis in the *in vitro* study and inhibits the altered hyperinflammatory signaling pathway (Richardson et al., 2020). In ACTT-2 trial, when baricitinib was used in combination with remdesivir, it showed superior clinical efficacy in reducing ARDS and mortality rate as compared with baricitinib alone (Kalil et al., 2021). Currently, the combination of baricitinib and remdesivir is approved by the US FDA for the treatment of COVID-19 infection (Kalil et al., 2021). Apart from JAK inhibitor, Bruton's tyrosine kinase inhibitors such as rilzabrutinib, ibrutinib, and acalabrutinib are currently approved by the US FDA for the treatment of hematological malignancy (**Table 2**) (Rezaei et al., 2020; Benner and Carson, 2021; Rada et al., 2021). These drugs act as an inhibitor macrophage activation, which is a rate-limiting step during cytokine storm (Roschewski et al., 2020). Therefore, these drugs are hypothesized to be a future therapeutic candidate against COVID-19 infection (**Figure 2**). More recently, anti-SARS-CoV-2-neutralizing antibodies such as casirivimab, bamlanivimab, imdevimab, etesevimab, and sotrovimab were approved by the US FDA for the treatment of non-hospitalized patients with a confirmed report of COVID-19 infection (Mahase, 2021; Verderese et al., 2021; Weinreich et al., 2021).

DIFFERENCES IN EFFICACY OF VACCINES ON PREVENTING INFECTION WITH CORONAVIRUS DISEASE 2019 STRAINS AND CONTROLLING NECESSITY FOR HOSPITALIZATION

COVID-19 vaccines play a critical role in helping the countries to overcome the challenging pandemic that they are currently

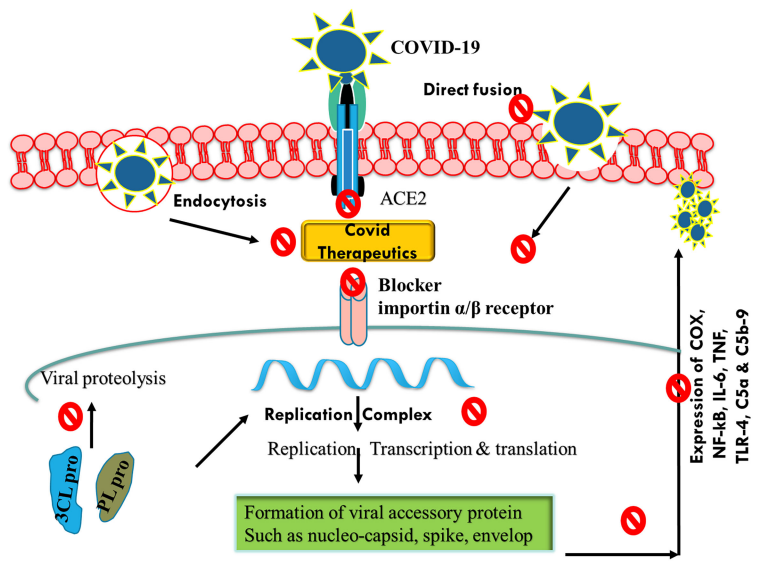

FIGURE 2 | Schematic representation of mechanisms of action of COVID-19 therapeutics by inhibiting endocytosis, ACE2 receptor, and viral replication. COVID-19, coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2.

grappling with. It is believed that the severity of the pandemic will gradually reduce as the herd immunity is achieved. However, there may be factors that make it difficult to achieve herd immunity such as receiving only one dose of the vaccine for which two doses are required, denial to get vaccinated, and shortage of the vaccines. Therefore, it is very important to mass vaccinate the population completely if we want to win the battle over the pandemic (Chen and Lu, 2021). A public–private partnership was initiated by the US government to speed up development, approval, and distribution of the COVID-19 vaccines (Corey et al., 2020). Most of the COVID-19 vaccines have spike glycoprotein of SARS-CoV-2 as their basis. The commonly used vaccines are as follows: BNT162b2 (Pfizer-BioNTech) (Polack et al., 2020), ChAdOx1 nCOV19 (Oxford-AstraZeneca) (Voysey et al., 2021), NVX-CoV2373 (Novavax) (Keech et al., 2020), mRNA-1273 (NIAID-Moderna) (Baden et al., 2021), and Ad26COV2S (Janssen) (Sadoff et al., 2021). There are several preprints, peer-reviewed publications, press releases, policy documents, and public regulatory documents that demonstrate the efficacy and safety of these vaccines (Keech et al., 2020; Polack et al., 2020; Baden et al., 2021; Voysey et al., 2021). A study was conducted to study the efficacy of BNT162b2 vaccine (Dagan et al., 2021) during the mass vaccination in Israel. The participants were followed up 7 days after the second dose, and it was found that the vaccine has an efficacy of 94% for symptomatic COVID-19 participants, 92% for people with severe COVID-19, 92% for people with documented infection, and 87% for the people admitted in the hospitals. It was also concluded that the effectiveness of the vaccine was lower in people who suffer from various coexisting medical conditions like hypertension and obesity than in healthy individuals. Similar results were found in England for adults aged 70 years and over, indicating that the BNT162b2 vaccine showed 85%–90% efficacy after the second dose (Lopez Bernal et al., 2021). The risk of being admitted to hospitals was reduced by 44% in the vaccinated people, whereas the risk of death was reduced by 51%. They also studied the efficacy of ChAdOx1-S vaccine and found out that a single dose was 60%–75% effective in people with symptomatic COVID-19 and that the risks of hospital admission were reduced up to 80% in the vaccinated people. Various vaccines are being manufactured and distributed across the globe (**Table 3**) to control the pandemic. **Figure 3**

TABLE 3 | Various vaccines available for COVID-19.

Vaccine	Manufacturer	Origin of vaccine	Dose(s) required	Efficacy against COVID-19
BNT162b2 or Comirnaty	Pfizer-BioNTech; Fosun Pharma	m-RNA-based vaccine	2 doses, 21 days apart	94% (Dagan et al., 2021)
ChAdOx1-S or AstraZeneca or Covishield (India)	Oxford-AstraZeneca	Adenovirus vector expressed in chimpanzee	2 doses, 28 days apart	60%–75% (Lopez Bernal et al., 2021)
NVX-CoV2373	Novavax	Spike protein expressed in baculovirus	2 doses, 21 days apart	95.6% (Mahase, 2021)
Gam-Covid-Vac or Sputnik V	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Spike protein expressed in adenovirus Ad5 and Ad26 vectors	2 doses, 21 days apart	92% (Roxby, 2020)
Moderna COVID-19 vaccine or mRNA-1273	Moderna, U.S. Biomedical Advanced Research and Development Authority (Dagan et al., 2021), National Institute of Allergy and Infectious Diseases (NIAID)	m-RNA vaccine expressing adenovirus type 26 (dose 1) and adenovirus type 5 (dose 2)	2 doses, 28 days apart	94.5% (Voysey et al., 2021)
Covaxin	Bharat Biotech, Indian Council of Medical Research (ICMR)	Inactivated virus vaccine	2 doses, 28 days apart	81% (Biotech, 2021)
BBIBP-CorV	Beijing Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	Inactivated virus vaccine	2 doses, 21 or 28 days apart	79% (Yan et al., 2021)
JNJ-78436735 or Ad26.COV2.S or Janssen COVID-19 vaccine	Janssen Biotech Inc.—Janssen Pharmaceutical Company of Johnson & Johnson	Spike protein expressed in adenovirus Ad26 vector	2 doses, 56 days apart	76.7%–85.4% for severe COVID-19 patients (Yan et al., 2021)
CoronaVac	Sinovac	Whole inactivated virus vaccines with alum as an adjuvant	2 doses, 14–28 days apart	50%–91% (Yan et al., 2021)
EpiVacCorona	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Subunit vaccine	2 doses, 21–28 days apart	100% (Phase I and Phase II trials) Merah et al. (2021)
Ad5-nCoV or Convidicea	CanSino Biologics	Spike protein expressed in adenovirus Ad5 vector	1 dose	90.98% (interim analysis) (Peshimam and Farooq, 2021)
ZF2001	Anhui Zhifei Longcom Biopharmaceutical, Institute of Microbiology of the Chinese Academy of Sciences	Recombinant vaccine	3 doses within 90 days	NA
Name not yet announced	Wuhan Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	Inactivated vaccine	NA	72.5% (interim analysis) (Yan et al., 2021)

COVID-19, coronavirus disease 2019.
NA, Not Applicable.

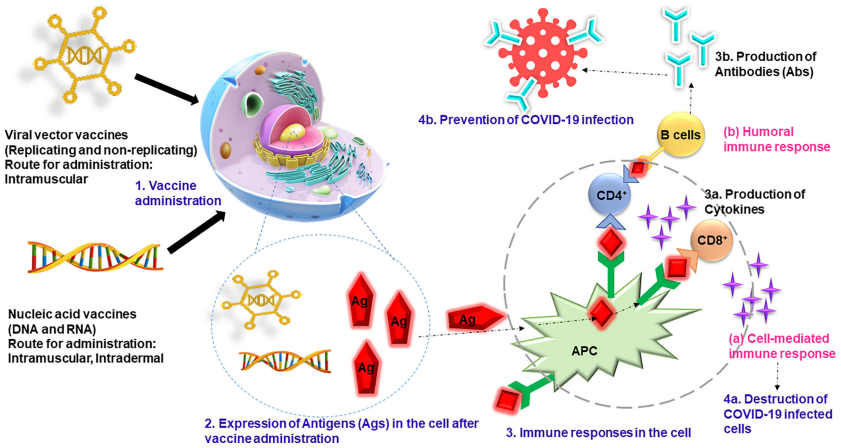

FIGURE 3 | The vaccines (viral vector and nucleic acid vaccines) are administered through intramuscular or intradermal routes, and antigen expression is initiated in the cells. The B cells and T cells generate the humoral immune response and cell-mediated immune response, respectively. The cell-mediated immune response produces the cytokines that kill the infected cells, and humoral cells produce antibodies that prevent the COVID-19 infection. COVID-19, coronavirus disease 2019.

summarizes the mechanisms of action of investigated anti-COVID-19 vaccines. These vaccines have helped in reducing the number of COVID-19 cases; however, the efficacy may vary in different studies. In the earlier phases of vaccination, it was found that the people receiving the vaccination were more prone to COVID-19 infection, which encouraged people to defer the vaccines. However, it was found that the infection occurred when people travelled to infected region or encountered COVID-19-positive patients, and the risk of infection was higher in the first 3 days of vaccination. This period was before the incubation of vaccine occurred, which rules out the odds of vaccination.

Most of these vaccines were manufactured against the original strain of SARS-CoV-2, and since then, the virus has mutated several times. It is crucial to develop a wide-spectrum vaccine that is effective against the various strains of SARS-CoV-2. In addition, for controlling the COVID-19, it is very important that the global population may be vaccinated completely. It is the duty of the officials to build trust among the public and encourage them to get vaccinated. The eradication of this disease is only possible when the herd immunity is achieved by vaccinating the people globally.

POSSIBILITIES TO FACILITATE OVERCOMING CORONAVIRUS DISEASE 2019 PANDEMIC

Considering the rapid molecular evolution of SARS-CoV-2 virus from its emergence to the present moment, continuous surveillance is required to identify novel mutations with potential ability to bypass current measures for controlling COVID-19. In the near future, readiness to react to such changes in virus genome is probably unavoidable. Rapid ongoing vaccination with continuously improved and updated vaccines or even vaccine cocktails is obviously the only human-controlled proactive way to impede the pandemic. Taking into consideration the fact that increased transmission can enhance the probability of further mutations (Matta et al., 2021), quick vaccination of the most active (younger) age groups seems to be the best strategy for preventing the appearance of novel hazardous mutations. On the other hand, the possibility of emergence of a mutant virus variant with high prevalence (high transmissibility) but low virulence cannot be avoided, overriding the spread of the current high-lethality strains and changing the fatal disease course to be much milder, thereby ensuring the "friendly" coexistence of virus and humankind in the future. Which of these scenarios will come true is just the question of time; still, it is clear that the lessons that this pandemic has taught to humankind are absolutely unique and tremendous.

CONCLUSIONS

Within already nearly the last 2 years, humankind of the 21st century has undergone unexpectedly complicated challenges related to the COVID-19 pandemic, from total social isolation to different mass-vaccination campaigns. However, despite biotechnological prosperity and ultrafast preparation of vaccines, we still cannot look to the future with peace of mind, as the virus is circulating among populations even after the use of current vaccines, and we have no means to forecast the virulence and lethality of potentially developing novel strains. Therefore, our location within this pandemic can be decided only retrospectively, and it remains to be hoped that after 5 years we will estimate today's position as the end of the pandemic.

AUTHOR CONTRIBUTIONS

HT performed the literature survey and data extraction. KS contributed in the introduction and conclusion. PA contributed in the molecular evolution. AI contributed in the therapeutic section. SU contributed in the geographic distribution section. JK contributed in the vaccination section. GK and DA contributed in final proofing and editing. All authors contributed to the article and approved the submitted version.

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Learning From Biological and Computational Machines: Importance of SARS-CoV-2 Genomic Surveillance, Mutations and Risk Stratification

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The global coronavirus disease 2019 (COVID-19) pandemic has demonstrated the range of disease severity and pathogen genomic diversity emanating from a singular virus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2). This diversity in disease manifestations and genomic mutations has challenged healthcare management and resource allocation during the pandemic, especially for countries such as India with a bigger population base. Here, we undertake a combinatorial approach toward scrutinizing the diagnostic and genomic diversity to extract meaningful information from the chaos of COVID-19 in the Indian context. Using methods of statistical correlation, machine learning (ML), and genomic sequencing on a clinically comprehensive patient dataset with corresponding with/without respiratory support samples, we highlight specific significant diagnostic parameters and ML models for assessing the risk of developing severe COVID-19. This information is further contextualized in the backdrop of SARS-CoV-2 genomic features in the cohort for pathogen genomic evolution monitoring. Analysis of the patient demographic features and symptoms revealed that age, breathlessness, and cough were significantly associated with severe disease; at the same time, we found no severe patient reporting absence of physical symptoms. Observing the trends in biochemical/biophysical diagnostic parameters, we noted that the respiratory rate, total leukocyte count (TLC), blood urea levels, and C-reactive protein (CRP) levels were directly correlated with the probability of developing severe disease. Out of five different ML algorithms tested to predict patient severity, the multi-layer perceptron-based model performed the best, with a receiver operating characteristic (ROC) score of 0.96 and an F1 score of 0.791. The SARS-CoV-2 genomic analysis highlighted a set of mutations with global frequency flips and future inculcation into variants of concern (VOCs)

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and variants of interest (VOIs), which can be further monitored and annotated for functional significance. In summary, our findings highlight the importance of SARS-CoV-2 genomic surveillance and statistical analysis of clinical data to develop a risk assessment ML model.

Keywords: COVID-19, SARS-CoV-2, genomic surveillance, risk stratification, machine learning, healthcare

INTRODUCTION

Since December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been observed to cause coronavirus disease 2019 (COVID-19). Thereafter, we have observed 254 million COVID-19 cases, 5.12 million deaths, 4 SARS-CoV-2 variants of concern (VOCs), 5 variants of interest (VOIs), and 11 other variants under monitoring. In India, we have witnessed 0.465 million deaths due to COVID-19 until mid-November 2021 (WHO Coronavirus (COVID-19) Dashboard). During the second surge of COVID-19 by the 21A (Delta) variant (Dhar et al., 2021; Mlcochova et al., 2021), India witnessed the effects of an overburdened healthcare infrastructure. Similarly, in many parts of the world, the COVID-19 pandemic has caused distress and resulted in mortalities that are not only direct consequences of the disease but also as associated consequences of an overburdened medical infrastructure (Xie et al., 2020; Usher, 2021; Singh, 2021).

Patient severity of COVID-19 ranges from being asymptomatic to symptomatic and a fraction resulting in mortality, with nearly 1.3% patients succumbing to the disease in India (Johns Hopkins Coronavirus Resource Center). Thereby, a majority of the patients exhibited varying levels of intermediate severity. These patients reported symptoms ranging from cough, fever, breathlessness to chest pain and loss of movement (Huang et al., 2020). Effective triage of these patients reaching a healthcare facility at the early phases of the disease when the symptoms are mild is crucial for effective healthcare management. This can help in improved resource allocation such as hospital beds, respiratory support, and targeted drugs. Furthermore, it can also help in administering drugs that are effective only at a particular severity stage (Beigel et al., 2020).

The novel coronavirus, similar to other coronaviruses, has been observed primarily to be spreading *via* fomites and direct human-to-human interactions. Transmission through the fecal-oral route and intrauterine vertical transfer have also been reported for SARS-CoV-2, as the pathogen has been demonstrated to stabilize in human digestive tract. Fecal matter and wastewater have been shown to contain active viral particles, which are shed even after the upper respiratory tract turns negative for viral RNA (Rana et al., 2021). This has led to initiatives of wastewater surveillance for SARS-CoV-2 to complement the naso/oropharyngeal sampling-based genomic surveillance. Similar to other RNA viruses, SARS-CoV-2 exists in the global population as a group of similar strains due to its rate of mutation acquisition. This ability to acquire mutations and thereby gain clinically and epidemiologically significant functions is a global concern during this pandemic (Barr and Fearns, 2016; Kanakan et al., 2020). To track this evolution of SARS-CoV-2 and flag the emergence of novel mutations, many countries have initiated integrative SARS-CoV-2 genome surveillance initiatives. This includes the COVID-19 Genomics Consortium in the UK (COVID-19 Genomics UK Consortium), SARS-CoV-2 Sequencing for Public Health Emergency Response, Epidemiology, and Surveillance (SPHERES) in the United States (SPHERES|CDC), and the Indian SARS-CoV-2 Genomics Consortium (INSACOG) (INSACOG|Department of Biotechnology). Such combined efforts of sequencing have facilitated SARS-CoV-2 genome sequences from their respective regions for functional insights (GISAID - Initiative). This aids in the early detection of potential gain-of-function mutations and tracking the selection of these mutations in emerging SARS-CoV-2 strains. Based on subsequent functional annotations of these mutations through the combination of *in silico* and *in vitro* experiments, SARS-CoV-2 strains have been classified according to the risks they pose to global health as VOCs and VOIs (Oude Munnink et al., 2021).

Pathological findings of COVID-19 have been implicated in the prediction of their severity capacity in many different studies using various approaches. The statistical significance of many such factors has been reported in different population cohorts. Studies have highlighted the statistical significance of the pathological findings of COVID-19 (Hu et al., 2020; Li et al., 2020; Yang et al., 2020; Zhou et al., 2020), including Indian cohorts (Gupta et al., 2020; Khan M. et al., 2020). Similarly, machine learning (ML)-based models have been built for COVID-19 using cohorts from North America (Cheng et al., 2020a; Khan A. I. et al., 2020), South Korea (Kim et al., 2020), and China (Wu et al., 2020; Karthikeyan et al., 2021). Due to the overwhelming host genetic, immunological, environmental, and healthcare factors, population-level differences in COVID-19 manifestations have been observed (Sorci et al., 2020; Lo et al., 2021; Maurya et al., 2021; Ong et al., 2021; Zhang et al., 2021), therefore necessitating the development of a severity prediction algorithm based on an Indian cohort, which is presented in this manuscript. At the same time, it is important to integrate different aspects of COVID-19 rather than only one at a time.

Using our dataset, inclusive of a broad range of biochemical test reports over multiple demographic and clinical observations of COVID-19 patients, we present an approach to narrow down the diverse clinical factors of COVID-19 into a few functionally important variables. We analyzed the early-onset symptoms presented by COVID-19 patients associated with the development of severe disease and further used statistical

methods to identify important biomarkers for severity progression of the disease. It is important to use and analyze clinical data as these can provide insightful information about the disease patterns, risk factors, and outcomes of treatment (Nass et al., 2009). We devised a ML pipeline to predict the outcomes of patients as severe or mild using the nested cross-validation (nested CV) algorithm (Bhargava et al., 2020; Hao et al., 2020; Liang et al., 2020; Gupta R. K. et al., 2021). We studied five computational learning models, namely, logistic regression, random forest, XGBoost, support vector machine (SVM), and multilayer perceptron. The predictive results of each model were compared and analyzed, and together, they can be implemented in clinical settings to predict COVID-19 severity in patients during the early stages of SARS-CoV-2 infection. Patient risk stratification and identification of the relative contributions of specific risk factors to overall risk are two of the widest applications of ML in healthcare (Wiens and Shenoy, 2018). ML/deep learning (DL) promote a data-driven approach to common yet important problems such as patient categorization and can greatly improve the management of patients in hospitals (Ching et al., 2018). They also have the potential to increase the efficiency and minimize the failure rates in drug discovery and development (Vamathevan et al., 2019).

Beyond contextualizing our study by providing the SARS-CoV-2 genomic constitution of our cohort, we further highlight the mutational diversity of SARS-CoV-2 in the background of its phylogenetic diversity in the cohort. Herein, we observe the evolutionary selection of the low-frequency mutations by comparing our genomic data to current global mutational spectra. Therefore, this study aimed to provide additional information and context to early-onset symptoms and clinical features for the improved risk prediction of patients diagnosed with COVID-19 using a combination of statistical and ML techniques. At the same time, this study highlights the importance of genomic surveillance in identifying SARS-CoV-2 genome mutations, which can undergo evolutionary selection in the future.

METHODOLOGY

Data Acquisition and Pre-processing

The data used in the study were collected from 257 confirmed COVID-19 patients from DY Patil group of hospitals in Pune, Maharashtra, India. Patients were admitted in the hospital between July and September 2020. A confirmed COVID-19 case was defined by a positive real-time polymerase chain reaction (RT-PCR) test for SARS-CoV-2 infection. The patient records were collected and anonymized at the data warehouse of CSIR-IGIB. The electronic hospital data used in the study included multiple demographics, vitals, and biochemical test reports pertaining to the COVID-19 patients. This comprehensive dataset encompasses vital signs and patient demographic data on oxygen saturation, respiratory rate, body mass index (BMI), age, gender, comorbidities, and respiratory support levels. Detailed blood test reports of the levels of C-reactive protein (CRP), interleukin 6 (IL-6), total leukocyte count (TLC), D-dimer, and lactate dehydrogenase, among others, were also included in the study, with a total of 31 different test parameters. The data were then curated to exclude patients with missing values of critical parameters. The patients were categorized as *severe* or *mild* based on their disease outcomes, i.e., discharged or deceased and ventilatory support requirement, as respiratory failure is a well-known indicator of COVID-19 severity. All patients requiring ventilatory support or deceased were considered to be severe; the rest were categorized as mild. This resulted in a curated list of 175 patients, which has been used in this study.

RT-qPCR

To obtain SARS-CoV-2 viral RNA, upper or lower respiratory tract secretions were used for obtaining naso/oropharyngeal swabs, which were preserved in a viral transport media (VTM) solution; for patients with a productive cough, liquified sputum samples were obtained to extract RNA. RNA extraction was performed using a silica column-based RNA extraction kit for cell-free bodily fluids (QIAmp viral mini kit, cat. no 52906; Qiagen, Hilden, Germany). Of the VTM solution, 200 µl was processed for lysing and viral enrichment, in accordance with the protocol in the kit (QIAamp Viral RNA Mini Handbook). RNA was eluted in RNase-free water after washing with wash buffers. Quantitative real-time polymerase chain reaction (RT-qPCR) for SARS-CoV-2 detection was performed using the TRUPCR SARS-CoV-2 kit (cat. no 3B304; 3B BlackBio Biotech India Ltd., Bhopal, India). For RT-qPCR, 10 µl RNA was added to 15 µl of the reaction mixture in accordance with the kit protocol. The qPCR reaction was run on Rotor-Gene Q (Qiagen) using the recommended cycling conditions. To designate a patient as positive, a cycle threshold (C_t) value of ≤35 was considered.

SARS-CoV-2 Whole-Genome Sequencing

RNA was checked for the presence of sufficient quality and quantity for sequencing. A sequencing library was prepared from the RNA samples for sequencing on the Oxford Nanopore or Illumina-MiSeq platforms. Briefly, double-stranded cDNA was synthesized using 100 ng of total RNA. Herein, first-strand cDNA was synthesized using Superscript IV (cat. no. 18091050; Thermo Fisher Scientific, Waltham, MA, USA) and second-strand using DNA polymerase-I large (Klenow) fragment (cat. no. M0210S; New England Biolabs, Ipswich, MA, USA) after RNase H digestion of RNA in the first-strand. Purification of the double-stranded cDNA was carried out using AMPure XP beads (cat. no. A63881; Beckman Coulter, Brea, CA, USA).

Further sequencing library was prepared according to the Oxford Nanopore Technology (ONT) library preparation protocol *PCR tiling of COVID-19 virus* (version: PTC_9096_v109revE_06Feb2020) for Oxford Nanopore sequencing. Here, 100 ng of the purified cDNA, 200 ng of multiplexed PCR amplicons, and 200 ng of end-prepped samples were taken to perform ARTIC multiplex PCR, end repair, and barcode ligation, respectively. The final library was quantified on a Qubit High Sensitivity DNA kit (cat. no. Q32854) and sequenced using the MinION Mk1C platform.

For sequencing on the Illumina MiSeq platform, the sequencing library was prepared using the Illumina DNA Prep with Enrichment protocol (doc. no. 1000000048041v05; Illumina, San Diego, CA, USA). For this purpose, 100 ng of

purified cDNA was used to prepare the library following tagmentation, indexing, enrichment, PCR amplification, and purification. Enrichment was performed using the Illumina Respiratory Virus Oligo Panel (cat no. 20042472; Illumina). The quality and the quantity of the sequencing library were checked using the Agilent 2100 Bioanalyzer with high sensitivity DNA chip (cat. no. 5067-4626; Agilent, Santa Clara, CA, USA) and the Qubit dsDNA High Sensitivity DNA kit, respectively. A loading concentration of 10 pM was prepared by denaturing and diluting the libraries in accordance with the MiSeq System Denature and Dilute Libraries Guide (document no. 15039740, v10; Illumina). Sequencing was performed on the MiSeq system using the MiSeq Reagent Kit v3 (150 cycles) at 2 × 75 bp read length.

Sequencing Data Analysis

To analyze MinION raw fast5 files until variant calling, the ARTIC end-to-end pipeline was used. Base calling was performed using Guppy basecaller v3.2.4 (https://nanoporetech.com/nanopore-sequencing-data-analysis) with a quality cutoff Phred score of >7 on a GPU-Linux accelerated computing machine. Demultiplexed fastq reads were normalized by a read length of 300–500 bp for further downstream analysis and aligned to the SARS-CoV-2 reference (MN908947.3) using the aligner Minimap2 v2.17 (Li, 2018). To index the raw fast5 files for variant calling and to create consensus fasta, Nanopolish v0.13.3 (Loman et al., 2015), SAMtools v1.7, and BCFtools v1.8 (Danecek et al., 2021) were used over the normalized minimap2 output.

All fastq files generated from Illumina sequencing were checked for quality using FastQC v0.11.9. A threshold of Phred score >20 was used for filtering the reads from all samples. Subsequently, adapter trimming was performed using TrimGalore v0.6.6 and alignment of the sequences was performed using HISAT2 v2.2.1 (Kim et al., 2019) on human data build hg38 (Kim et al., 2015). SAMtools v1.12 was used to remove aligned sequences. Henceforth, only unaligned sequences were taken into consideration. BCFTools v1.12 was used to generate the consensus fasta and variant calling.

Phylogenetic Analysis

The Wuhan reference genome for SARS-CoV-2 (NC_045512.2) was used to perform multiple sequence alignments of 92 SARS-CoV-2 genomes using MAFFT (v7.475) (Katoh et al., 2002). The alignment was manually trimmed and a phylogenetic tree was constructed using MEGA-X (Tamura et al., 2007). SARS-CoV-2 clades were assigned using Nextclade (https://clades.nextstrain.org/). The phylogenetic analysis was visualized with FIGTREE software (http://tree.bio.ed.ac.uk/software/figtree/).

Mutation Analysis

The vcf file was used to obtain the top most frequent and least frequent mutations in the samples, and snpEff v5.0 (Cingolani et al., 2012) was utilized to perform variant annotation such as the variant definitions. The SnpEff database was created with "SnpEff build" using the Wuhan reference NC_045512.2. Furthermore, global frequency of the mutations was checked against a global dataset available at 2019 Novel Coronavirus Resource (2019nCoVR), CNCB (Song et al., 2020). Once the annotated VCF was generated, a lollipop plot representing the low-frequency (lower quartile) and-high frequency (upper quartile) mutations was generated in R v4.1.0 using the g3viz (Guo, 2021), rtracklayer (Michael Lawrence, 2017), and trackViewer (Ou and Zhu, 2019) packages, followed by data visualization using the ggplot2 package (ggplot2 version 3.3.5, 2021). Inkscape was utilized to modify the figures (Draw Freely| Inkscape). Variant position along the SARS-CoV-2 genome is indicated in the plot, which was used to compare the high- and low-frequency mutations of the cohort study with the global frequency.

Statistical Analysis

Continuous and categorical variables are represented as median (interquartile range, IQR) and *n* (%). We applied the point-biserial correlation to compare continuous and dichotomous variables. Fisher's exact test was used to comparing categorical variables of gender, breathlessness, cough, diabetes, fever, hypertension, heart conditions, and presence of other comorbidities. Using these tests, the *p*-values of all features were calculated. Multiple testing correction was done using the Bonferroni test with an alpha value of 0.05. Data pre-processing was done using pandas (https://pandas.pydata.org/pandas-docs/stable/) and NumPy (Bisong, 2019). Statistical analysis was conducted with SciPy (SciPy v1.7.1; https://docs.scipy.org/doc/scipy/reference/) and statsmodels (https://www.researchgate.net/publication/264891066_Statsmodels_Econometric_and_Statistical_Modeling_with_Python), and the findings are visually represented using the Matplotlib (Matplotlib 3.4.3; https://matplotlib.org/) and Seaborn (Waskom, 2021) libraries in Python (Python.org).

Machine Learning Pipeline

The pipeline used to build the ML models in this study is described in **Figure 1**. Broadly, the curated dataset was divided into seven folds over seven iterations, six folds for training and one fold for testing in each iteration, thus covering the whole dataset. A value between 3 and 10 is generally selected for the number of folds, with a higher value leading to less bias. We chose to divide into seven folds because the dataset size is 175, 25 patients in each fold being ideal. This is the outer loop. In each iteration, all of the 31 different variables were passed into the function for building the ML models. Feature selection was done on the training folds using the Extra Trees Classifier, in which we selected the five most important features to train the model. For hyperparameter tuning, GridSearchCV, on the six training folds, was performed in the inner loop. ML algorithms of logistic regression, random forest, XGBoost, SVM, and multilayer perceptron were tested in this study. All five ML algorithms are trained on six folds and tested on the seventh, over seven iterations. After all seven iterations, the evaluation metrics were averaged out to report the final performance of the model. This method is known as nested cross-validation (CV). To avoid information leaking into the test set and overfitting of data, nested CV effectively uses a series of different train–test set splits in each iteration. Nested CV is the preferred way to evaluate and compare tuned ML models and has been used before in

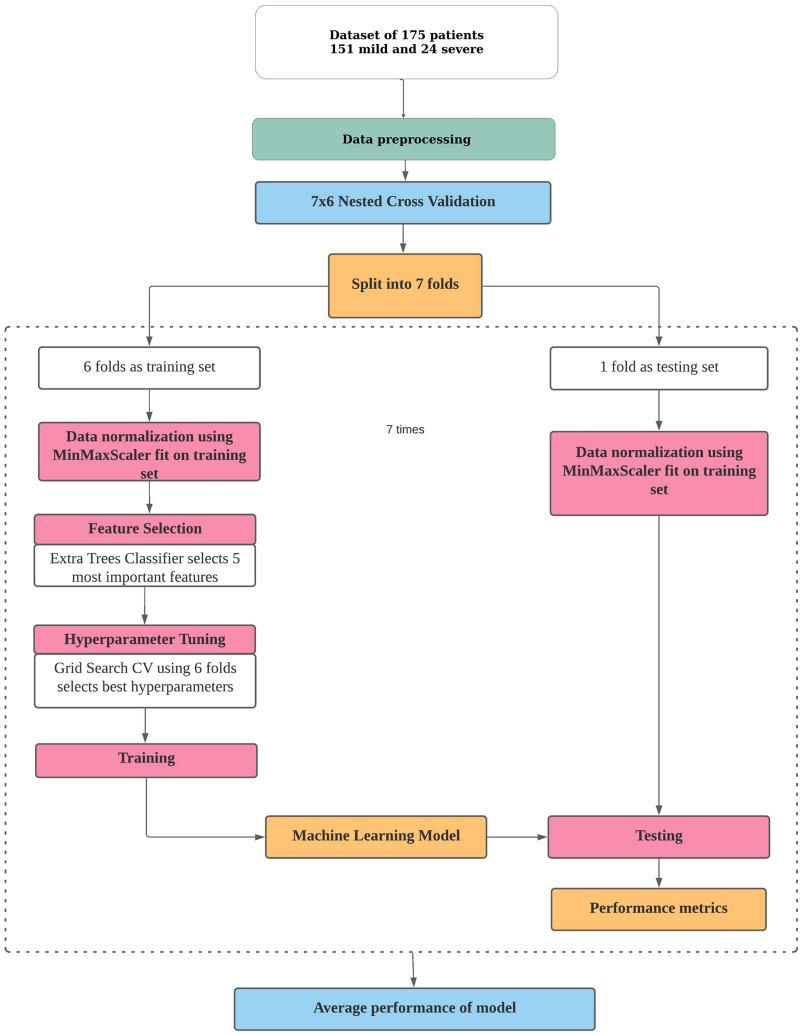

FIGURE 1 | Pipeline for building a machine learning model for risk prediction. The processes of data pre-processing, data normalization, feature selection, hyperparameter tuning, and training and testing of a model in nested cross-validation are depicted.

clinical settings (Gupta V. K. et al., 2021). **Figure 2** demonstrates the workflow of the nested CV algorithm. A detailed description of the steps used in building the models is given in the following sections.

Data Normalization

Data normalization was performed using MinMaxScaler of scikit-learn library in Python, which performs a linear transformation on the original data. It was fitted on the training set and transformed on the training and test sets using the same fit.

Feature Selection

Feature selection was done on the training folds using the ExtraTreesClassifier (Extremely Randomised Random Tree Classifier), which is present in the scikit-learn ensemble methods. It is a type of ensemble learning technique that accumulates multiple correlated trees in a forest and generates the classification result. Each feature was ordered in descending order according to its Gini importance, from which the five most important features were selected for the model.

Hyperparameter Tuning

To perform hyperparameter tuning, grid search cross-validation over six folds of the current training set was performed. GridSearchCV is a package of scikit-learn that selects the best hyperparameters from a range of listed hyperparameters by trying all combinations of the values passed. They were scored based on the F1 scores (one of our major evaluation metrics).

Evaluation Metrics

The following metrics were recorded to assess the predictive performance of the supervised models. The formulae for the calculation of all metrics are given below.

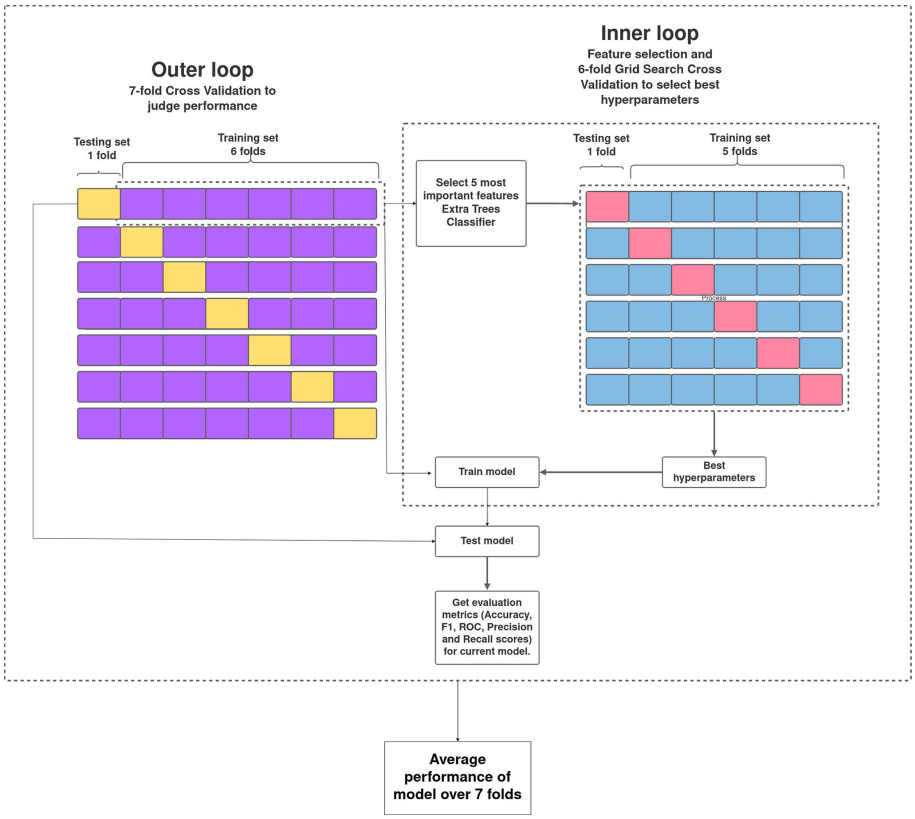

FIGURE 2 | Overview of nested cross-validation. The outer loop is for feature selection and average performance of each model on the different training and testing folds, and the inner loop is for hyperparameter tuning using GridSearchCV.

- **Accuracy score**: Accuracy is the fraction of predictions that the model got right.

$$\text{Accuracy} = \frac{\text{Number of correct predictions}}{\text{Total number of predictions}}$$

- **Precision**: Precision indicates what proportion of the positive predictions was correct. It is the number of correct positive results divided by the number of positive results predicted by the classifier.

$$\text{Precision} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

- **Recall**: Recall denotes what proportion of the actual positives was identified correctly. It is the number of correct positive results divided by the number of all relevant samples.

$$\text{Recall} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

- **F1 score**: The F1 score is the harmonic mean between precision and recall. The range for the F1 score is [0, 1]. It indicates how precise the classifier is (how many instances it classifies correctly) and how robust it is (it does not miss a significant number of instances).

$$\text{F1 Score} = 2 * \frac{\text{Precision} * \text{Recall}}{\text{Precision} + \text{Recall}}$$

- **ROC score**: The receiver operator characteristic (ROC) curve is a probability curve that plots the true-positive rate (what proportion of the positive class got identified correctly) against the false-positive rate (what proportion of the negative class got incorrectly classified) at various threshold values and essentially separates the "signal" from the "noise." The area under the ROC curve (AUC) is a measure of the ability of a classifier to distinguish between classes and is used as a summary of the ROC curve.

RESULTS

Patient Clinical and Demographic Characteristics

The initial dataset containing 257 patients was intensively curated to exclude patients with missing data on important parameters, resulting in a set comprising 175 patients with 31 different clinical parameters, which was used in the study. In this dataset, 24 patients were classified as severe and 151 as mild

COVID-19 patients. The median age of the patients was 46 years, ranging from 5 to 92 years. Males comprise a higher percentage of the patients, constituting nearly 62%. The most common clinical features were fever (48.57%), cough (42.28%), and breathlessness (37.71%). The average respiratory rate was seen to be 14–34 breaths per minute (median = 20). The hematological parameters showed TLC counts from 2,500 to 25,200 cells/mm^3, alkaline phosphatase median of 68, and CRP median of 12mg/L. A summary of the available parameters is presented in **Table 1**. Histograms of all continuous parameters are shown in **Supplementary Figures 1–3**. The diversity in the clinical presentations of the patients across various clinical parameters and the severity are illustrated in **Figure 3**.

Symptoms Associated With Disease Severity

To understand the significance of the clinical presentations of patients and the possibility of developing severe disease, we analyzed the statistical correlations of patients' symptoms and comorbidities across mild and severe individuals. We observed symptoms such as breathlessness (p = 5.29E−09) and cough (p = 6.00E−04) to be significantly correlated with patients developing severe COVID-19. At the same time, comorbidities did not have a significant correlation with the disease severity. It may be mentioned that comorbidities are a diverse set of conditions that may have differential roles in modulating the disease. The differential abundance of these factors across mild and severe patients is highlighted in **Figure 4**.

Figure 4 shows the differential number of patients included in our study with symptoms ranging from breathlessness, cough, fever, hypertension, diabetes, and heart disease.

A closer look at the clinical data also revealed that 22 out of 66 patients reporting breathlessness developed severe disease, while only 2 out of 109 patients not suffering from breathlessness developed severe disease. To further understand the significance of patients' disease symptoms in delineating disease severity, we calculated the combined effect of symptoms on severity prediction. Unsurprisingly, we noted that the presence of all three symptoms—cough, fever, and breathlessness—increased the probability of contracting severe disease (**Table 2**).

TABLE 1 | Clinical summary of patients positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Patient features	Cohort (n = 175)	Mild (n = 151)	Severe (n = 24)	p-values
Age	46.00 (5–92)	43 (5–85)	70 (33–92)	**5.61E–06**[a]
Gender				1.77E–01[b]
Female	68 (38.85%)	62 (35.42%)	6 (3.42%)	
Male	107 (61.14%)	89 (50.85%)	18 (10.28%)	
Temperature	98.10 (95.70–102.00)	98 (95.7–102.0)	98.6 (96.7–100.0)	1.86E–01[a]
BMI	22.70 (18.70–17.40)	22.8 (18.9–27.4)	22.4 (18.7–27.2)	3.32E–01[a]
SpO_2	98.00 (94.00–100.00)	97.0 (94.0–100)	98.0 (94.0–100)	7.73E–01[a]
Respiratory rate (per minute)	20.00 (14.00–34.00)	18.0 (14.0–34.0)	28.0 (16.0–32.0)	**1.39E–17**[a]
Presence of symptoms	144 (82.28%)	120 (68.57%)	24 (13.71%)	NA
Fever	85 (48.57%)	73 (41.71%)	12 (6.85%)	1.00E+00[b]
Cough	74 (42.28%)	56 (32%)	18 (10.28%)	**6.00E–04**[b]
Breathlessness	66 (37.71%)	44 (25.14%)	12 (6.85%)	**5.29E–09**[b]
Presence of comorbidities	84 (48.00%)	66 (37.71%)	18 (10.28%)	7.00E–03[b]
Hypertension	58 (33.14%)	45 (25.71%)	13 (7.42%)	3.30E–02[b]
Heart condition	21 (12.00%)	16 (9.14%)	5 (2.85%)	1.74E–01[b]
Diabetes	56 (32.00%)	45 (25.71%)	11 (6.28%)	1.57E–01[b]
Hemoglobin	13.70 (4.5–18.6)	13.60 (0–18.6)	13.95 (9.7–18.2)	6.84E–01[a]
TLC count	7,100.00 (2,500–25,200)	6,500.0 (2,500.0–24,700.0)	10,350.0 (5,200.0–25,200.0)	**4.21E–05**[a]
Platelet count	214,000.00 (73,000.00–1,820,000)	214,000.0 (73,000.0–546,000.0)	208,000.0 (73,000.0–1,820,000.0)	5.20E–02[a]
Random blood sugar	114.00 (74.00–432.00)	111.0 (74.0–432.0)	125.0 (83.0–401.0)	6.59E–01[a]
Urea	26.70 (13.50–176.00)	25.9 (13.5–165.5)	48.0 (25.2–176.0)	**2.68E–05**[a]
Creatine	1 (0.30–5800)	0.90 (0.3–5800.0)	1.05 (0.8–12.5)	6.99E–01[a]
Sodium	140 (124–159)	141.0 (124.0–159.0)	138.0 (127.0–146.0)	1.80E–02[a]
Potassium	4.30 (2.90–104.00)	4.30 (3.0–104.0)	4.45 (2.9–104.0)	1.18E–01[a]
Chloride	105.25 (1.10–125)	108.0 (1.1–125.0)	106.0 (88.0–111.0)	4.27E–01[a]
Total bilirubin	0.60 (0.30–4.20)	0.6 (0.3–4.2)	0.6 (0.3–2.4)	2.18E–01[a]
Direct bilirubin	0.20 (0.10–2.20)	0.2 (0.1–2.2)	0.2 (0.1–1.2)	6.21E–01[a]
SGOT	34.20 (0.20–474.20)	33.6 (0.4–285.0)	54.7 (0.2–474.2)	4.84E–03[a]
SGPT	31.20 (3.80–190)	31.0 (11.0–190.0)	40.7 (3.8–190.0)	1.86E–01[a]
Total proteins	6.40 (3.60–12.60)	6.5 (4.8–12.6)	6.0 (3.6–7.4)	8.00E–03[a]
Albumin	3.50 (2.60–6.60)	3.5 (2.6–6.5)	3.4 (2.7–6.6)	7.67E–01[a]
Alkaline phosphatase	68.00 (3.80–320.90)	67.8 (3.8–237.0)	75.1 (32.4–320.9)	1.28E–02[a]
C-reactive protein	12.00 (0.10–381)	5.67 (0.1–381.0)	126.25 (12.0–168.9)	**3.89E–11**[a]

Outlier trimming was performed for SGOT, thus removing one outlier. Data are shown as median (IQR) or n (%). Significant parameters found after multiple correction testing are shown in bold. TLC, total leukocyte count; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase. The table highlights the spectrum of a multitude of clinical parameters across mild and severe patients and the p-values of every parameter in predicting disease severity.

[a]*Point-biserial correlation.*

[b]*Fisher's exact test.*

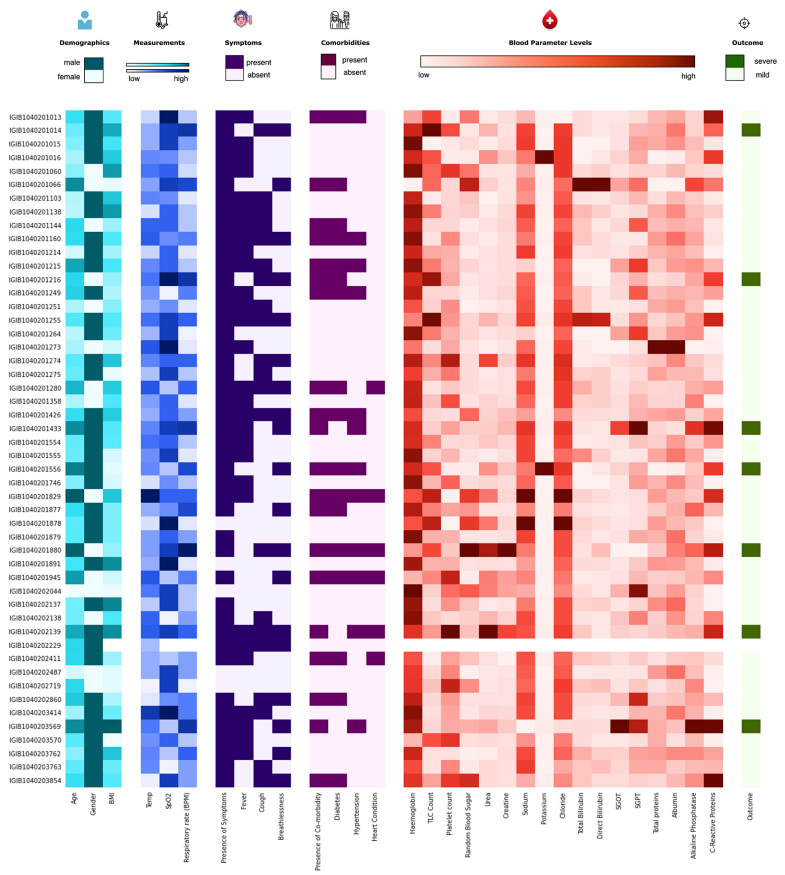

FIGURE 3 | Distribution of the clinical parameters across severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) patients. The figure highlights the clinical heterogeneity across various clinical parameters in COVID-19 patients. These include demographic, clinical, and blood parameters and disease outcomes.

Statistical Correlation Analysis of All Clinical Characteristics

To identify the clinical parameters associated with disease severity, statistical tests for correlation were used on the curated dataset of 31 parameters across 175 patients. We found that 13 parameters had a p-value <0.05, namely, age, alkaline phosphatase, breathlessness, CRP, SGOT, cough, hypertension, comorbidities, respiratory rate, sodium, TLC count, total proteins, and urea (**Table 1**). Upon further scrutiny of the significance threshold by performing multiple testing correlation, a p-value <0.0016 was considered significant, thus resulting in the identification of seven highly significant factors associated with severity. These factors are age (65.41 ± 16.75, p = 5.61E−06), breathlessness (p = 5.29E−09), CRP level (115.84 ± 45.81 mg/L, p = 3.89E−11), respiratory rate (26.5 ± 3.78 breaths/minute, p = 1.39E−17), TLC count (11,887.5 ± 6,378.108, p = 4.21E−05), coughing (p = 6.00E−04), and blood urea level (60.88 ± 42.21 mg/dl, p = 2.68E−05). We also noted that, in our dataset, there were no patients with severe clinical disease that were completely free of symptoms: cough, fever, and breathlessness. To understand the association of the clinical parameters and disease severity, bar graphs are plotted for all parameters in **Supplementary Figures 4–8**. The order of statistical significance of the 31 parameters are shown in **Figure 5A**, and significant correlations are shown in **Figure 5B**.

We observed that patients above the age of 60 years were more prone toward having severe COVID-19. It is important to note that only 8 out of 130 people below 60 years had severe disease, while 16 out of 45 people above 60 years had severe disease outcomes. **Figure 5B** highlights that, for patients with a respiratory rate above 25 breaths/minute, the outcome was most likely to be severe. Similarly, it was observed that a CRP level above 100 mg/L was correlated with severity, albeit with a few outliers.

Machine Learning Model Development

Five different ML algorithms were used to develop viable predictive models for disease severity. All the models were tested using nested CV and their average performance metrics reported. For the final ROC curve, the ROC curve for each iteration of nested CV was plotted and averaged to obtain the final curves (**Supplementary Figure 9**).

The statistical results previously obtained tallied with the features that were selected for most models using ExtraTrees

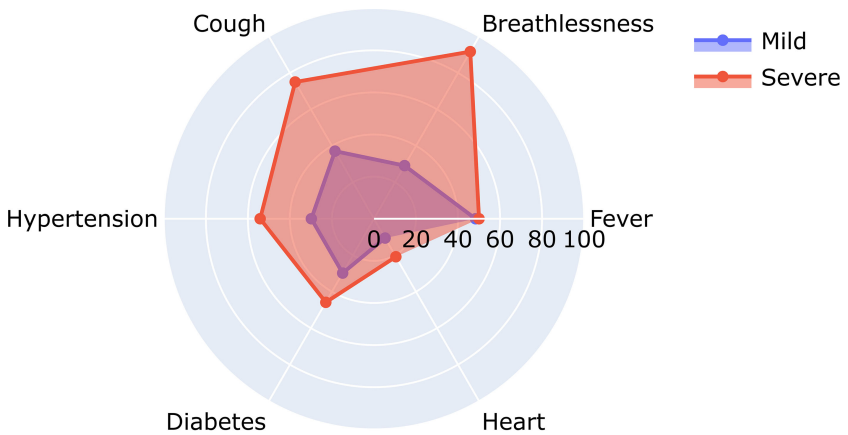

FIGURE 4 | Relative abundance of symptoms in severe and mild coronavirus disease 2019 (COVID-19) patients.

TABLE 2 | Correlation between severity and presence of multiple symptoms.

Fever	Cough	Breathlessness	No. of patients with these symptoms	Probability of severe outcome in our cohort
Absent	Absent	Absent	41	0
Absent	Absent	Present	13	0.23
Absent	Present	Absent	8	0
Absent	Present	Present	28	0.32
Present	Absent	Absent	37	0
Present	Absent	Present	10	0.3
Present	Present	Absent	23	0.09
Present	Present	Present	15	0.47

The table highlights the combinatorial effect of the most prominent disease symptoms and how it increases the probability of severe disease outcomes in the presence of multiple symptoms.

Classifier. Every model showed more than 90% accuracy, but their performance was judged by comparing the ROC AUC scores and F1 scores, as these measures are better suited for an imbalanced dataset. It was observed that most models performed similarly to each other with respect to the ROC-AUC scores (in the range of 0.90–0.93), but multilayer perceptron stood out with an ROC score of 0.96. In terms of the F1 scores, SVM performed the best (F1 score = 0.793), followed closely by multilayer perceptron (F1 score = 0.791), with a difference of just 0.002. It must be noted that the results would vary slightly with each test run, so it can be said that, for our test run, multilayer perceptron performed the best overall with the second best F1 score of 0.791 (very close to that of the first being 0.793) and the best ROC score of 0.96. The scores of each model across various performance metrics are shown in **Supplementary Table 1**. A detailed comparative report of the model performances is shown in **Figure 6**, with the confusion matrices, ROC curve, and F1 scores of each model.

SARS-CoV-2 Phylogenetic Analysis

SARS-CoV-2-positive nasopharyngeal RNA samples from 92 patients out of 257 in the patient cohort with sufficient quantity and quality of RNA were available for viral genome sequencing. To understand the SARS-CoV-2 genomic diversity in our patient cohort, genome sequencing and analysis were performed on these RNA samples. Phylogenetic analysis showed that the majority of the samples belonged to clades 19A (47.8%), 20A (11.9%), and 20B (40.2%) (**Figure 7**). This is consistent with the SARS-CoV-2 genomic surveillance observations in India during a similar period of the pandemic (Banu et al., 2020; Kumar et al., 2020; Joshi et al., 2021). The clades and genome coverage of all sequenced patient samples are listed in **Supplementary Table 2**.

SARS-CoV-2 Mutation Analysis

To further observe the diversity of mutations captured in our samples, we performed mutational analysis vis-a-vis comparison with the global frequency and genomic distribution of the observed mutations. Individual mutation level analysis revealed 422 unique mutations in our sample set. Further analysis toward the global frequency of all these mutations during SARS-CoV-2 surveillance with 21,51,254 globally shared sequence data revealed that eight mutations present in low frequencies (lower quartile) in our dataset are now present in higher frequencies (>35%) when compared with the global mutational frequency data. The evolutionary selection of these mutations over the period of the pandemic (from May 2020 to Sept 2021) and their presence in the VOCs and VOIs indicate their potential functional significance. Orthogonally, we noticed five highly frequent mutations in our dataset (upper quartile) that are now present in very low frequency (<1%) in current global data (**Figure 8**). These mutations can possibly have a detrimental effect on the improved transmission characteristics observed in the latest variants spreading across the globe. We also noticed six mutations in our dataset (D614G, P4715L, R203R, R203K, C15279T, and Q57H) that have currently been designated as clade-defining mutations of the VOCs and VOIs of SARS-CoV-2, namely, 20I (Alpha), 21A (Delta), 21B (Kappa), and 20H (Beta). All mutations were then annotated on the SARS-CoV-2 reference genome to identify the presence of the mutations in different regions of the SARS-CoV-2 genome. We observed 67% of the

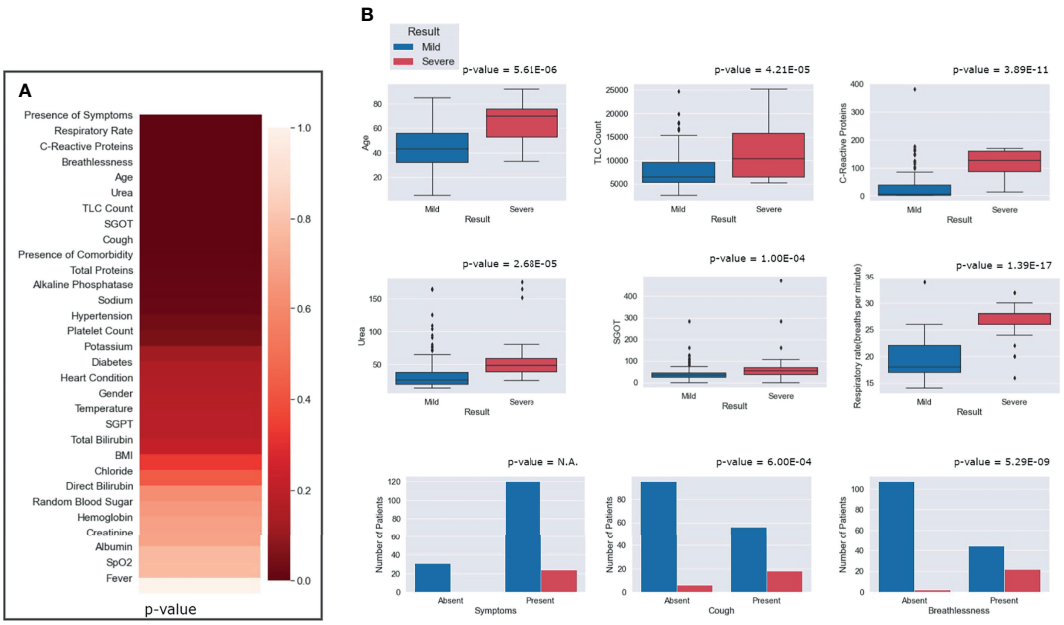

FIGURE 5 | Statistical correlations of the clinical parameters and clinical severity. **(A)** Ranking of the parameters according to predictive significance. **(B)** Depiction of the differential abundance of clinical parameters across mild and severe patients. For the presence of any symptom, cough and breathlessness, the *p*-value is between mild and severe patients with respect to the presence and absence of symptoms.

mutations to be present in the ORF1ab region and 11.76% of the mutations to be present in the spike region of the SARS-CoV-2 genome. It is interesting to note that, due to the size of the SARS-CoV-2 spike region being around one-fifth of the ORF1ab region, we saw equal mutation rates in the spike and ORF1ab regions, which were around 1.31 and 1.34, respectively. We also observed a significant number of mutations present in other regions of the genome (**Supplementary Table 3**).

DISCUSSION

COVID-19 has spread around the globe with rapidly emerging VOCs and VOIs reducing the efficacy of global vaccination efforts, which has transformed the pandemic to an ever-looming threat to global healthcare. Therefore, meaningful surveillance efforts and an efficient, applicable COVID-19 risk prediction model based on the Indian cohort are warranted. In this study, we analyzed 175 patient clinical data with 31 different parameters, which included vital signs and patient demographic data on oxygen saturation, respiratory rate, BMI, age, gender, comorbidities, and respiratory support levels. Detailed blood test reports pertaining to the levels of CRP, IL-6, TLC, D-dimer, and lactate dehydrogenase were also included in the study. We observed that symptoms such as breathlessness and cough segregated toward severe outcomes in due course of disease progression. The significance of these symptoms has been previously reported by different studies (Burke et al., 2020; Ioannou et al., 2020), whereas the presence of symptoms such as cough, fever, and breathlessness together significantly increased the probability of the patient developing severe COVID-19. This has been shown in other cohorts, but not in India (Ioannou et al., 2020). To further improve the identification of risk factors associated with COVID-19, we analyzed every biochemical test parameter for its severity predictive significance. We observed that seven parameters—age above 60 years, breathlessness, CRP level above 100 mg/L, cough, respiratory rate above 25 breaths/minute, TLC count above 10,000, and blood urea level above 40 mg/dl—showed a statistically significant ($p < 0.0016$) correlation with disease severity. Some of these parameters have been individually reported to be of predictive significance in COVID-19: CRP levels (Ali, 2020), blood urea levels signifying kidney involvement (Cheng et al., 2020b), TLC (Zhao et al., 2020), and old age (Mueller et al., 2020). Using these factors in a combined manner can help to assess the risk of patients developing severe disease. Although due to the relatively small patient cohort in the study, albeit detailed clinical parameters, it would be important to validate the findings in a larger Indian dataset to further iterate the findings and highlight the significance of these clinical parameters in predicting COVID-19 severity and clinical outcomes.

To enable a robust risk stratification procedure to be implemented in clinical settings, five ML models for risk stratification were developed. Herein, we observed that multilayer perceptron outperformed all other models, with an ROC score 0.96 and an F1 score 0.791. Upon close observation of the characteristics of the other models, it became clear that a simpler model such as logistic regression (F1 score = 0.51) cannot

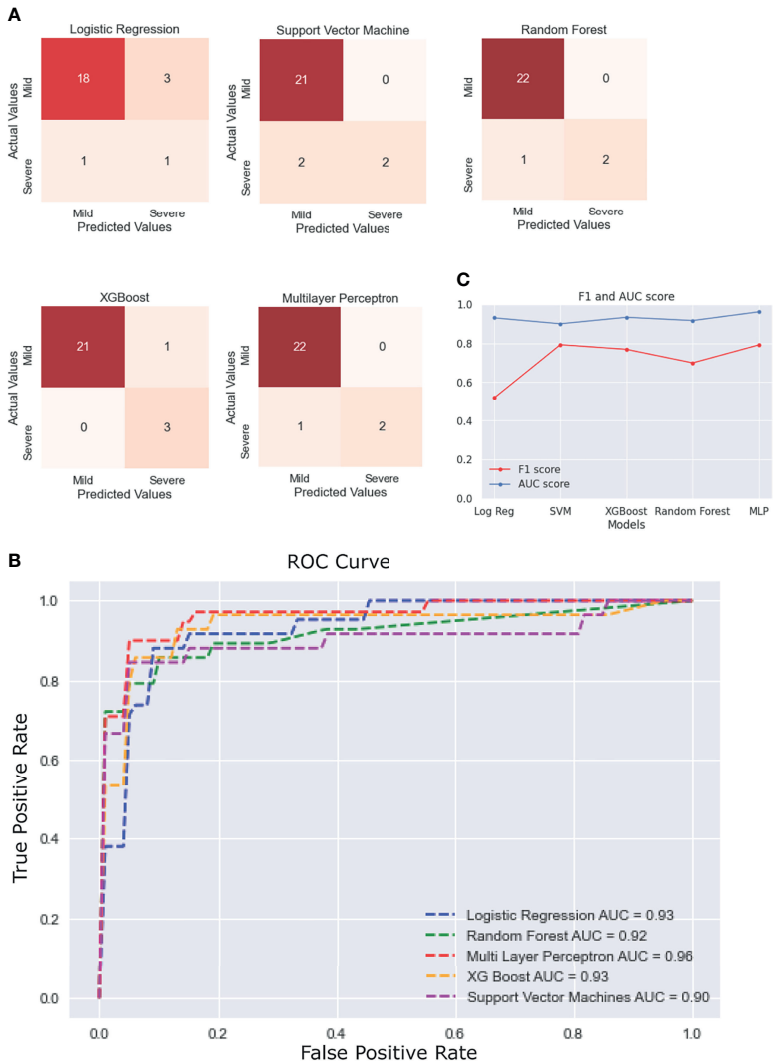

FIGURE 6 | Performance of the different machine learning (ML) models. **(A)** Confusion matrices of the last fold in the nested cross-validation (CV) for each model. **(B)** Plot of the average receiver operating characteristic (ROC) curve of each model. **(C)** Line plot for the F1 and area under the curve (AUC) scores for each model.

capture the complexity of the dataset, even after selecting the best features and tuning the hyperparameters. In various other studies with similar models (Prakash, 2020; Gupta V. K. et al., 2021), it was observed that logistic regression underperformed. In our study, random forest, multilayer perceptron, XGBoost, and SVM performed much better and had similar scores, so we can use the results of the four combined to arrive at a prediction. The only plausible limitation of the approach is the relatively small sample size, which may limit how well the models built here generalize and the lack of repeatability of the ML-generated results. However, it is important to mention that the ML pipeline described adapts to and can be applied as a method for larger datasets, when available, making it a novel approach to the problem.

In our genomic analysis of 92 patients, we identified clades 19A, 20A, and 20B. To help stratify the SARS-CoV-2 mutations with respect to potential functional significance, we accessed the current global frequency of all 422 mutations identified in our dataset. Herein, we observed a few mutations that have a significant frequency flip for their occurrence. We noticed eight mutations present in extremely low frequencies in our dataset ($n \leq 2$ patients), but were highly abundant in the global dataset (>35%). All of these mutations are now seen predominantly in VOC 20I Alpha. The mutations C3267T (T1001I) (Public Health England, 2021; Castonguay et al., 2021; Srivastava et al., 2021), A28111G (Y73C) (Public Health England, 2021, C23271A (A570D) (Public Health England, 2021), C913T (S216S) (Castonguay et al., 2021), T28282A (D3E) (He et al., 2021), G28280C (D3H) (Castonguay et al., 2021; He et al., 2021), and A28281T (D3V) (Castonguay et al., 2021) are reported in 20I Alpha clade. Of these, C15279T (H5005H) is a clade-defining

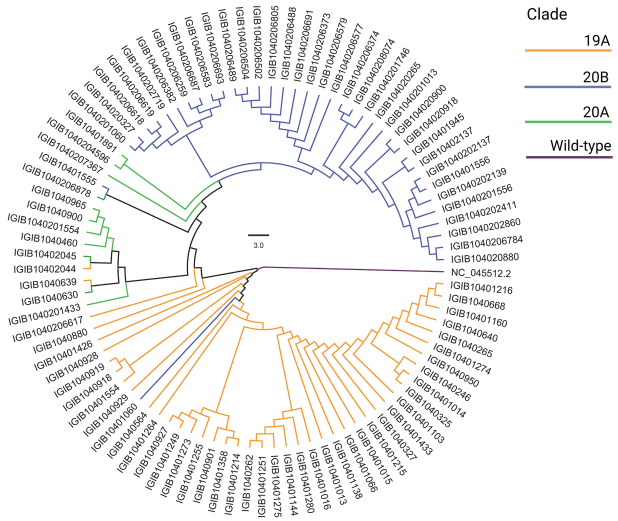

FIGURE 7 | Phylogenetic analysis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomes. The distribution of SARS-CoV-2 clades among 92 coronavirus disease 2019 (COVID-19) patients compared with the wild-type strain is shown.

mutation for 20I (Hadfield et al., 2018). The identification of these mutations associated with 20I in the Indian cohorts around the period from July to September 2020 is an interesting finding for further evolutionary analysis of clade 20I, as it is believed to have originated from the UK where it was first discovered on September 20, 2020 (Public Health England, 2021). Orthogonally, five of the mutations highly abundant in our dataset were seen to be sparsely present in the global data (<1% frequency). The literature reviews of these mutations—C13730T (A4489V) (Banu et al., 2020; Alai et al., 2021), (C6312A) T2016K (Banu et al., 2020; Sarkar et al., 2021), C6310A (S2015R) (Kumar et al., 2020), C5700A (A1812D) (Joshi et al., 2021; Srivastava et al., 2021), and C23929T (Y789Y) (Banu et al., 2020; Joshi et al., 2021; Sarkar et al., 2021)—reiterate this finding as they showed that all of these mutations were highly prevalent during the initial phase of the pandemic in India as a part of clades 19A, 20A, and 20B, whereas these mutations are not a part of the currently circulating clades, even in India, such as 21A (Delta) (Dhar et al., 2021; Shastri et al., 2021). This possibly indicates the detrimental effect of these mutations in viral transmission characteristics. Our effort toward mutational analysis beyond strain identification of the SARS-CoV-2 variants can provide epidemiological context to help prioritize SARS-CoV-2 mutations for functional analysis. However, cohort-specific genomic analysis provides valuable insights into the evolving genomic characteristics of the virus. Further validation toward the prevalence of the identified mutations can be done by continuous monitoring of the regional genomic trends of COVID-19 patients at the same time point and beyond.

An accurate risk stratification model for segregating disease-specific patient populations based on detailed clinical parameters can help in the rapid screening and resource allocation in healthcare facilities. Cohort-specific changes can be present between patients of different ethnicities (Ali, 2020), therefore necessitating the development of cohort-specific models for country-specific healthcare settings. Our study hereby provides an approach and method to converge the diversity of the clinical variables observed in the early phases of COVID-19 into a few consequential diagnostic variables for severity prediction. This ML model, upon further validation in larger patient cohorts across India, can be implemented by a clinician using an interactive dashboard at a healthcare facility in the future. A brief outline of the approach and future perspectives of this study are summarized graphically in **Figure 9**.

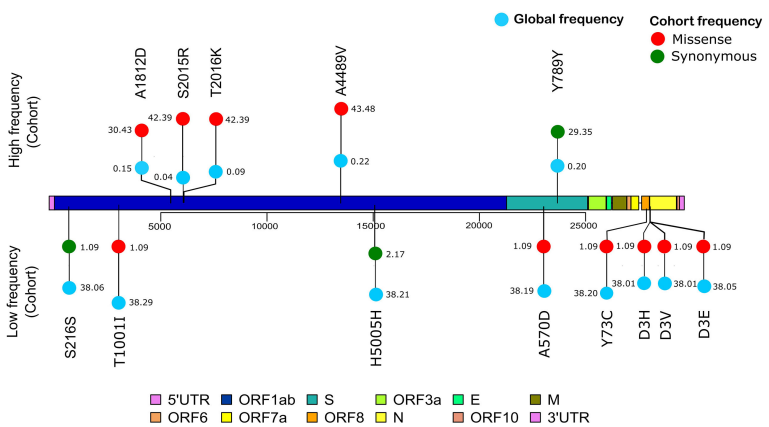

FIGURE 8 | Mutation frequency analysis with frequency flip. The frequency of mutations with significant frequency differences between the global and our study cohort are shown. Global frequency is represented as *blue circles* and the missense mutation frequency in our cohort is shown as *red circles*. Synonymous mutation frequencies in our cohort, S216S (C913T), H5005H (C15279T), and Y789Y (C23929T), are shown as *green circles*.

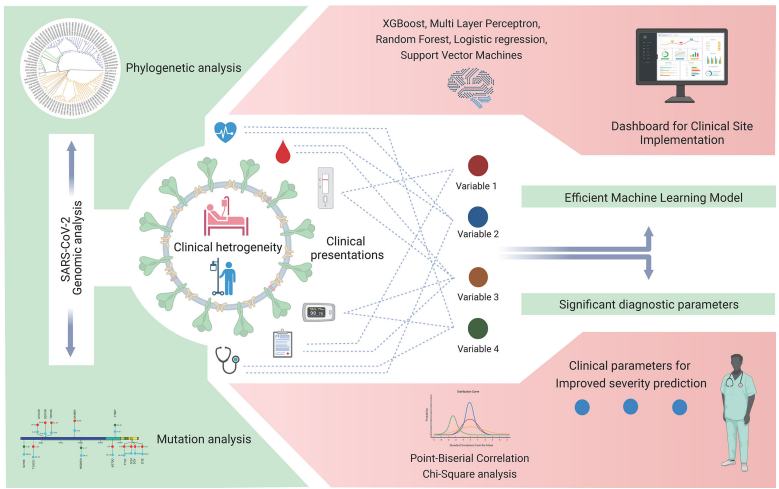

FIGURE 9 | Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomic and coronavirus disease 2019 (COVID-19) clinical data analyses for variant detection and patient severity classification. Clinical data analysis using a machine learning model for the identification of crucial clinical variables for risk stratification and potential development of a stand-alone dashboard for healthcare implementation.

CONCLUSION

The findings of this study highlight the integrative analysis of the diverse clinical data, SARS-CoV-2 genomic mutations, its future relevance when compared with the global frequency of the mutations, and the use of ML to reduce the dimensionality of the data in order to identify key features associated with disease severity. The findings regarding the low-frequency mutations being part of future VOCs and VOIs provide a framework for closely monitoring the low-frequency mutations for their future functional importance in transmission and immune escape.

DATA AVAILABILITY STATEMENT

The clinical dataset collected and analyzed as a part of this study is attached as **Supplementary Document 1**. The SARS-CoV-2 Genomic data used in the study has been uploaded to GISAID with accession numbers EPI_ISL_4503527 to EPI_ISL_450361, and EPI_ISL_4518814. The code for the NestedCV method used in the study for building and analysing various risk prediction models is available at https://github.com/INGEN-HOPE/NestedCV.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

SB, AP, AK, RM, JV, PD and PC performed the analysis. SB, AP, AK, and RP wrote the manuscript. AK and RP designed, conceptualized, implemented, and coordinated the study, along with inferences of the results, and wrote the manuscript. SS, RK, and MJ shared the clinical samples and clinical data. All authors contributed to the article and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.783961/full#supplementary-material

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REVIEW
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Experimental Models of COVID-19

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COVID-19 is the most consequential pandemic of the 21st century. Since the earliest stage of the 2019-2020 epidemic, animal models have been useful in understanding the etiopathogenesis of SARS-CoV-2 infection and rapid development of vaccines/drugs to prevent, treat or eradicate SARS-CoV-2 infection. Early SARS-CoV-1 research using immortalized *in-vitro* cell lines have aided in understanding different cells and receptors needed for SARS-CoV-2 infection and, due to their ability to be easily manipulated, continue to broaden our understanding of COVID-19 disease in *in-vivo* models. The scientific community determined animal models as the most useful models which could demonstrate viral infection, replication, transmission, and spectrum of illness as seen in human populations. Until now, there have not been well-described animal models of SARS-CoV-2 infection although transgenic mouse models (i.e. mice with humanized ACE2 receptors with humanized receptors) have been proposed. Additionally, there are only limited facilities (Biosafety level 3 laboratories) available to contribute research to aid in eventually exterminating SARS-CoV-2 infection around the world. This review summarizes the most successful animal models of SARS-CoV-2 infection including studies in Non-Human Primates (NHPs) which were found to be susceptible to infection and transmitted the virus similarly to humans (e.g., Rhesus macaques, Cynomolgus, and African Green Monkeys), and animal models that do not require Biosafety level 3 laboratories (e.g., Mouse Hepatitis Virus models of COVID-19, Ferret model, Syrian Hamster model). Balancing safety, mimicking human COVID-19 and robustness of the animal model, the Murine Hepatitis Virus-1 Murine model currently represents the most optimal model for SARS-CoV-2/COVID19 research. Exploring future animal models will aid researchers/scientists in discovering the mechanisms of SARS-CoV-2 infection and in identifying therapies to prevent or treat COVID-19.

Keywords: SARS-CoV-2, COVID-19, experimental models of COVID-19, pathology, variants of concern, MHV-1, pneumonia, *in vitro* model

1 INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also referred to as human coronavirus 19 (HCoV-19), is the virus that causes coronavirus disease (COVID-19). SARS-CoV-2 is believed to be a respiratory virus although it exerts a severe impact on other organs resulting in multi-organ failure (Mokhtari et al., 2020; Zaim et al., 2020; Farooqui, 2021; Loganathan et al., 2021; Paidas et al., 2021);. SARS-CoV-2 is a positive single stranded RNA virus, with a single linear RNA segment that causes more severe disease than any other coronavirus. The US National Institutes of Health described it as the successor to SARS-CoV-1, the virus that caused the SARS 2002 outbreak. SARS-CoV-2 was first identified in the city of Wuhan, Hubei, China. The World Health Organization declared the outbreak a Public Health Emergency of International Concern on 30th January, 2020 and SARS-CoV-2 was subsequently declared as a pandemic on 11th March 2020.

SARS-CoV-2 is an airborne virus that infects its host by first binding respiratory epithelium in the upper airways. At this time, the host may complain of non-specific flu-like symptoms (i.e. fever, fatigue, rhinorrhea). However, it is common for hosts to remain asymptomatic during an extended incubation period of 5-14 days (Hu et al., 2021). During the incubation period, asymptomatic transmission is possible and the virus itself has been shown to be highly infectious in close quarters and poorly ventilated areas. Within the host, the virus then migrates to the lower airways where it binds to a receptor of a membrane protein that regulates the renin-angiotensin system [(angiotensin converting enzyme 2 (ACE-2)], allowing viral uptake within the cell; systemic infection then ensues (Hu et al., 2021). The host's own cellular-mediated immune response most likely is responsible for the severe illness that patients can experience when infected (Wang et al., 2020). Most characteristic of the SARS-CoV-2 virus is the atypical "walking" pneumonia, causing patients to appear healthy & lucid at their baseline health while at the same time harbor dangerously low oxygen saturation levels in the severely hypoxic range, emphasizing the importance of diagnostic imaging such as CT in aiding clinical diagnosis (Larici et al., 2020).

Due to the virus's specific tropism with host interaction, there are also symptoms that can help differentiate from the common cold or influenza, such as neurological symptoms (i.e. anosmia, encephalopathy) and GI symptoms (i.e. diarrhea, weight loss) (Huang et al., 2020). The most severely infected patients may present to emergency departments and indicate treatment for Acute Respiratory Distress Syndrome (ARDS) (Qiu et al., 2020). Current treatment protocols for moderate and severe disease are based upon success with steroids, laying the patient prone, and other therapies (Malin et al., 2020; Sood et al., 2020) (i.e. Remdesivir). Vaccine development has been the most crucial development in COVID-19 prophylaxis, especially in the highly exposed (i.e. emergency rooms) as well as the most vulnerable populations (i.e. nursing homes).

There has been debate as to whether proximity to an infected person, duration of exposure, or viral load is the primary determinant for risk of infection. Comorbidities, advanced age, and immunosuppression were the highest risk factors for severe illness from COVID-19. However, the virus has also caused severe disease in seemingly healthy younger populations and caused lasting effects which continue to debilitate patients long after disease remission, hence long COVID (Yong, 2021). Studies to understand the epidemiology of SARS-CoV-2 infection in children have been limited (Rankin et al., 2021). Children have been shown to be able to transmit the virus at much lower rates than adolescents, but with no data suggesting increased susceptibility to disease (Meyer et al., 2021). However, there is debate on whether children are able to transmit the virus asymptomatically, which could affect household transmission. Conversations in the public sector regarding in-person classroom settings are hotly debated in today's government halls and public forums.

By comparing the DNA to prior coronaviruses (i.e. SARS-CoV-1 & MERS), SARS-CoV-2 has been shown to be most similar in structure and transmission to the SARS-CoV-1 virus. The virus most likely originated in bat hosts, and may or may not have infected an intermediate host, such as pangolins (Goh et al., 2020). Regardless of SARS CoV-2's exact infectious route, human cells have shown to be highly tropic to the virus, specifically epithelial cells with human ACE-2 receptors, which line organs such as respiratory and GI tracts. Understanding how the virus infects its hosts is crucial in the production of vaccines that can help prevent disease (Chaudhari et al., 2021).

1.1 Variants of SARS-CoV-2

Many variants of SARS-CoV-2 have been identified, which are grouped into the much larger clades. Different clade nomenclatures have been proposed. Nextstrain divides the variants into five clades (19A, 19B, 20A, 20B, and 20C), while GISAID divides them into seven (L, O, V, S, G, GH, and GR). Several notable variants of SARS-CoV-2 emerged in late 2020. The World Health Organization has currently declared four variants of concern, which are as follows:

Alpha: Lineage B.1.1.7, found to be derived from the SARS-CoV-2 20B/GR clade, emerged in the United Kingdom in September 2020, with evidence of increased transmissibility and virulence. Notable mutations at the viral S gene include N501Y, an asparagine to tyrosine amino acid substitution at position 501, and P681H, a proline to histidine substitution at position 681. An E484K mutation, glutamate to lysine substitution at position 484 of the RBD, in some lineage B.1.1.7 virions has been noted and also tracked by various public health agencies (Tang et al., 2021). Researchers are currently investigating the etiology of lineage B.1.1.7 and hypothesize that these mutations may have occurred due to prolonged infection of an immunocompromised host (Choi et al., 2020). Such mutations, especially the E484K arising in South Africa, are concerning for their possible gain-of-function ability to confer advantage against current convalescent plasma treatments as well as vaccines.

Beta: Lineage B.1.351 cases were first reported in South Africa on December 2020 (Hoffmann et al., 2021), with evidence of increased transmissibility and changes to antigenicity, with some public health officials raising alarms about its impact on the efficacy of some vaccines. Notable mutations include K417N,

E484K and N501Y, with 12 total mutations and one deletion compared to the original Wuhan strain, and most mutations in the Spike protein domain, causing concern for a possible gain-of-function escape from neutralizing antibodies (Rees-Spear et al., 2021). There have been promising studies making use of monoclonal antibodies that may serve as therapeutics in treating mutated variants of concern (Du et al., 2021).

Gamma: Lineage P.1, or B.1.1.28.1, emerged in Brazil in November 2020, also with evidence of increased transmissibility and virulence, alongside changes to antigenicity. Similar concerns about vaccine efficacy have been raised due to P.1's high number of accumulated mutations in the S protein which is the suggested cause for a rapid increase in hospital admissions. Notable mutations also include K417N, E484K and N501Y, a group of mutations that are highly suspected for variants ability to escape antibody-mediated immunity (Rees-Spear et al., 2021).

Delta: Lineage B.1.617.2 emerged in India on October 2020. There is also evidence of increased transmissibility and changes to antigenicity due to mutations in the gene encoding the SARS-CoV-2 Spike protein. Of the 17 mutations found in the Delta genome, four are of major concern: D614G substitution is also found in other highly transmissible variants, T478K substitution, L452R substitution correlates with higher affinity to the ACE2 host receptor, & P681R may be responsible for increased infectivity of host cells (Lazarevic et al., 2021; Lustig et al., 2021). A recent cohort study in England found increased hospital admission or emergency care in patients infected with delta variant compared to those infected with alpha variant, suggesting that outbreaks of delta variant in unvaccinated patients may lead to higher burdens on healthcare systems compared to alpha variant cases (Twohig et al., 2021). Other notable variants include 6 other WHO-designated variants under investigation and Cluster 5, which emerged among mink in Denmark and resulted in a mink euthanasia campaign rendering it virtually extinct (Larsen, 2021). See **Figure 1** for SARS-CoV-2 variants and its mutation points.

More recently discovered variants include Lambda (C.37) - a variant of interest first reported to WHO from Peru on August 2020, Mu (B.1.621) - a variant of interest first reported to WHO from Colombia on January 2021, and Omicron (B.1.1.529) - a variant of concern first reported to WHO from South Africa and Botswana on November 2021. Additionally, there are a number of variants under monitoring by the CDC. These include C.36 +L452R, B.1.1.318, P.1+P681H, B.1.617.2+K417N, C.1.2, B.1.617.2+E484X, B.1.617.2+Q613H, B.1.617.2+Q677H, and B.1.640. Based on the mode of transmission and rates of worldwide spread, new variants will continue to emerge.

Viral structure and predictions: Phylogenetic analyses of SARS-CoV-2 variants shed light on the mutations responsible for more infectious strains. The structure of the virion can be determined through analysis of its coding regions. These include basic components which comprise coronaviridae: Spike protein (S),

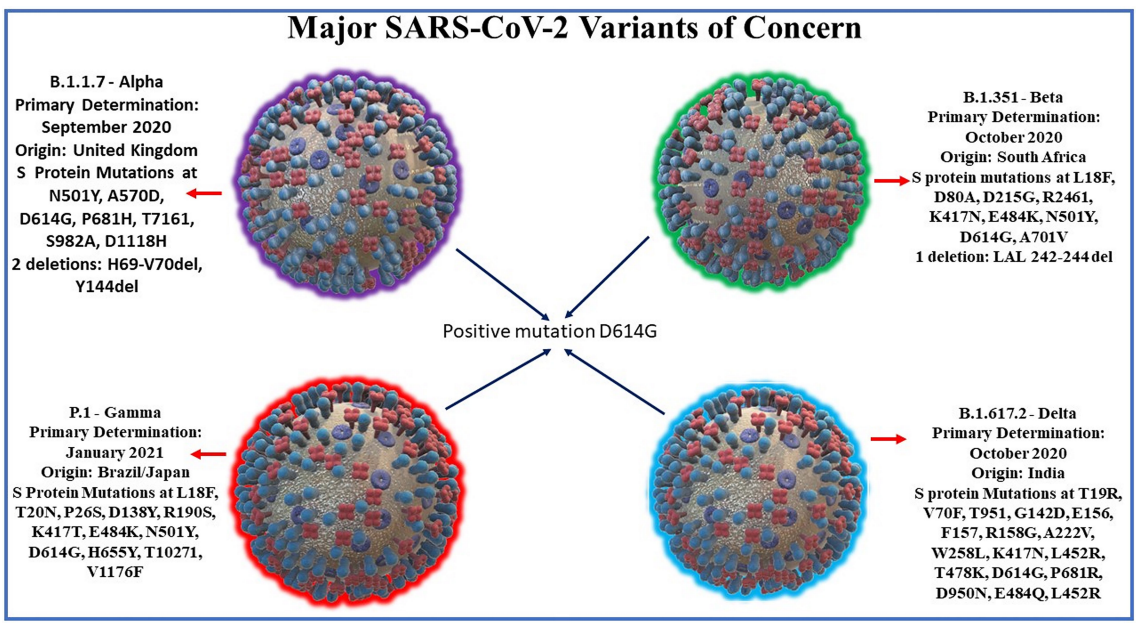

FIGURE 1 | Major SARS-CoV-2 variants along with their dates and location of primary determination and their characteristic spike protein mutation sites. SARS-CoV-2 variants share a common mutation in D614G of the spike protein. Notable spike mutations: Alpha variant mutations include N501Y, an asparagine to tyrosine amino acid substitution at position 501, and P681H, a proline to histidine substitution at position 681have been found to be preserved in subsequent variants. The Beta variant E484K mutation, glutamate to lysine substitution at position 484 of the RBD arising from South Africa may lead to possible gain-of-function ability to confer advantage against current convalescent plasma treatments. Beta & Gamma (Brazil/Japan) lineage mutations also include K417N and N501Y and may together lead to possible gain-of-function escape from neutralizing antibodies. Delta variant, first discovered in India 2020, mutations include L452R substitution correlates with higher affinity to the ACE2 host receptor, & P681R may be responsible for increased infectivity of host cells.

Membrane protein (M), Envelope protein (E), Nucleoprotein (N), and Ribonucleic Acid (RNA). The Receptor Binding Domain (RBD) found on Spike protein residues is responsible for binding of the ACE-2 receptor, allowing the virion cell entry into its host. Further, Hoffman et al. (2020) reported that SARS-CoV-2 cell entry was dependent on the presence of both the ACE2 receptor and a serine protease TMPRSS2. Choi et al. (2020) conducted phylogenetic analyses in an immunocompromised patient who succumbed to persistent infection and found that viral evolution was found to be rooted in amino acid changes predominantly in the spike gene and receptor-binding domain. A small portion of the entire viral genome was responsible for the majority of observed changes in viral persistence. The spike protein itself can further be divided into its two subunits: 1) S1 subunit, the globular receptor binding domain containing the receptor binding motif, responsible for binding to the host cell receptor; 2) S2 subunit, the stalk fusion domain, responsible for the fusion of viral and cellular membranes (Mittal et al., 2020). Studies have shown that the S1 subunit of the RBD has 10 to 20 fold higher affinity for the ACE-2 receptor when compared to SARS-CoV-1 RBD (Wrapp et al., 2020). The HR1 and HR2 domains which comprise the S2 subunit have been found to be highly stable, further suggesting the importance of the Spike protein in the infectivity of SARS-CoV-2 (Xia et al., 2020). SARS-CoV-2 entry requires cleavage of the Spike protein at the S1/S2 cleavage site, and this cleavage is carried out by furin protease (Peacock et al., 2021). The P681H mutation at the PRRAR furin cleavage site, a polybasic insertion provides a selective advantage to SARS-CoV-2 in human airway epithelial cells, allowing more favorable binding of furin to the S protein, and thus enhanced membrane fusion (Mohammad et al., 2021). See **Figure 2** for structural and functional aspects of SARS-CoV-2 spike protein. Recent studies have shown/suggested other possible receptors for SARS-CoV-2. These include 1) a transmembrane glycoprotein CD147 and a receptor on host cells commonly known as basic immunoglobulin (Basigin) or extracellular matrix metalloproteinase inducer (EMMPRIN), 2) a transmembrane protein Neuropilin-1 (NRP-1), 3) an ectopeptidase dipeptidyl peptidase 4 (DPP4) also known as CD26, 4) alanyl aminopeptidase (ANPEP), 5) glutamyl aminopeptidase (ENPEP), and 6) angiotensin II receptor type 2 (AGTR2) (see Masre et al., 2021 and references therein). Further, studies have also identified and explored intersection genes of niacin such as Bcl-2-like 1 (BCL2L1), prostaglandin-endoperoxide synthase 2 (PTGS2), interlukin-1-β, interferon gamma, plasminogen activator inhibitor-1 (SERPINE1) and COVID-19 (Li et al., 2021), probably involved in viral spread and can be targeted for potential therapy.

2 ANIMAL MODELS OF COVID 19

Important elements in vaccine production and drug testing include the selection of animal models based upon specific

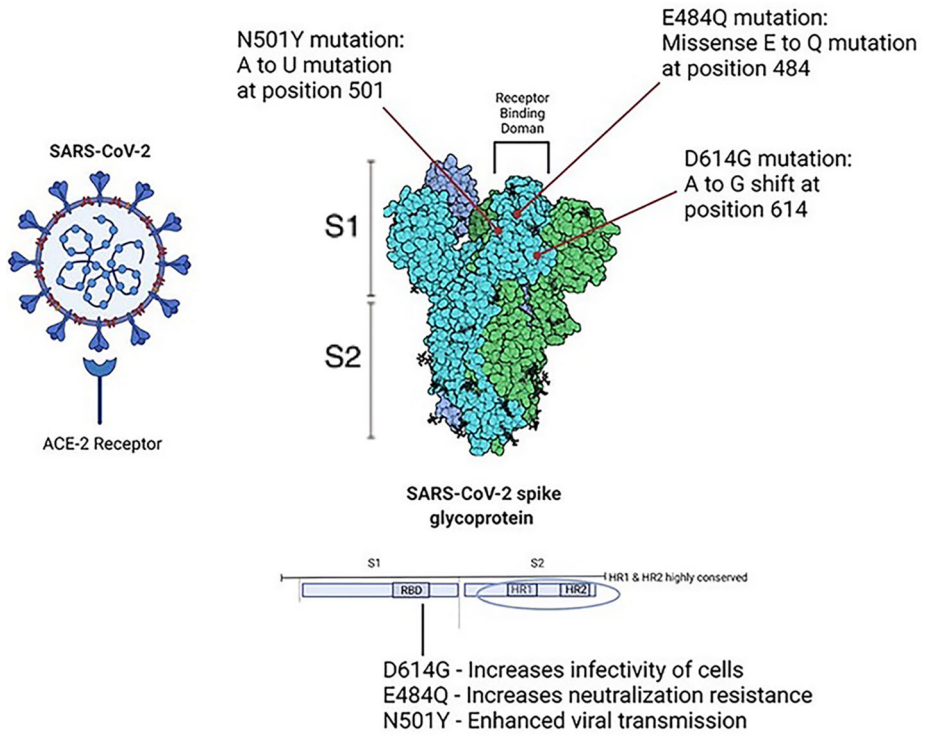

FIGURE 2 | The structural and functional aspects of SARS-CoV-2. Point mutations in Spike protein have been shown to increase survival of SARS-CoV-2 virion from host immune response. D614G is related to increased infectivity of cells, E484Q has been shown to increase neutralization resistance, and N501 Y has been shown to cause enhanced viral transmission.

criteria (Davidson et al., 1987): They must be able to: 1) replicate life cycle of the pathogen (i.e. incubation period similar to humans); 2) Demonstrate similar symptoms as seen in humans when infected with SARS-CoV-2; 3) recapitulate the illness brought on by physiological viral dosing as seen in humans. (It would not be useful for animal models to only exhibit infection when given a dose unlikely to be seen clinically); 4) transmit the viral pathogen in same manner as in humans (i.e. virus can infect respiratory tract of animal model); and 5) measure the immune response of the animal model (i.e. acute phase reactants vs seroconversion); 6) enable low cost and highly practical models which allow for models and results to be easily reproduced in a lab setting; and 7) provide acceptable ecological as well as ethical consequences during experimentation.

Laboratory mice/rats are the most commonly used animals in preclinical research, as well as the most useful and appropriate resource for mechanistic investigations. Additionally, they are easy to handle, and their size, and high reproduction number further assist in identifying the therapeutic potential of drugs. The SARS-CoV-2 spike protein requires the human ACE2 receptor (hACE2) for cellular entry and infection (Wang et al., 2020). Wan et al. demonstrated that exposure of wild-type mice to SARS-CoV-2 lack the ability to be infected due to differences in their ACE2 receptors (Wan et al., 2020). Mouse models were genetically engineered to express hACE2 by manipulating different promoter regions and were successfully infected with SARS CoV-2, making them useful for vaccine and therapeutic research. A disadvantage of these mouse models includes their limited availability.

2.1 K18-hACE2 Transgenic Mouse Model

Transgenic expression of hACE2 in mice allows for infection following exposure to SARS- CoV-2. Winkler et al. (2020) were able to demonstrate that mice genetically engineered to express epithelial cell cytokeratin-18 promoter (k-18) hACE2 gene, originally used in SARS research, were able to be infected similarly to human hosts (Winkler et al., 2020). Expression of this gene allowed hACE2 to be expressed in multiple tissues after mice were inoculated with SARS-CoV-2 in the intranasal epithelial passages, similar to SARS-CoV-2 tropism in human respiratory passages. Viral RNA was detected consistently in these tissues and showed pathological evidence of severe infection which allowed researchers to compare their findings with human subjects. Winkler noted that while this model is useful in studying severe SARS-CoV-2 infection in humans, some limitations of this model included the risk of ectopic hACE2 expression which changes the cellular tropism of the virus. Another limitation is that hACE2 is expressed at non-physiological levels due to unique k18 promoter in this model. Furthermore, this model does not take into account the complex interplay of hACE2 expression that can be seen in humans with certain comorbidities such as hypertension, cardiovascular disease and diabetes, as well as obesity, COPD & liver disease; diseases that have been shown to increase susceptibility to SARS-CoV-2 infection and increase the risk for development of COVID-19. (Ejaz et al., 2020).

Seibert et al. (2021) made use of the k18-hACE2 model to better understand the effect of SARS-CoV-2 infection on lung and GI microbiome diversity, an important factor in the immune system of infected hosts. Additionally, Liu et al. (2021) used this model when they reverse-engineered SARS-CoV-2 clones that could be used to further understand viral pathogenesis, as well as COVID-19 variants. Arce and Costoya (2021) further investigated the usefulness and limitations of the k18-hACE2 model and found that extrapulmonary infection symptoms manifest in a mild manner due to the high concentration of ACE2 receptors in lung tissue, and thus would not be useful in understanding the severe clinical symptoms that can be seen in COVID-19 patients.

k18-hACE2 transgenic mice have also been used in research of the pathogenesis of SARS-CoV-2 infection (Zheng et al., 2021). This study reproduced the histopathological lung disease, in a dose-dependent manner, as was seen in humans. These authors further demonstrated SARS-CoV-2's ability to replicate in the sinonasal epithelium of the k18-hACE2 model and also supporting cells of olfactory neurons (not the olfactory neurons themselves), causing anosmia which was a common clinical symptom reported in humans associated with SARS-CoV-2 infection (seen more in females than males). This study also investigated the usefulness of convalescent plasma from COVID-19 patients in preventing mortality and clinical symptoms at certain levels of inoculum ($<10^5$) (Zheng et al., 2021). However, as noted above, the limitation in the use of transgenic mouse models to study COVID-19 pathogenesis is that there is a risk of ectopic hACE2 expression, causing a change in the cellular tropism of the virus and thus a decrease in the usefulness of the model itself.

2.2 Mouse ACE2 Promoter With Human ACE2 Coding Sequence

Bao et al. (2020) were able to demonstrate the pathogenesis of SARS CoV-2 in transgenic mice [adult/aged male and female mice (6-11 month old)] which expressed hACE2 by way of the mouse ACE2 promoter. This model was produced by injection of mouse ACE2 promoter linked with human ACE2 coding gene into pronuclei of ICR mice fertilized ova. Bao found that ACE2 was mostly expressed in lungs, GI tract (intestines), kidneys and heart. Both wild-type and hACE2 transgenic mice were then inoculated with SARS-CoV-2 HB01 strain at 50% tissue culture infected dose (TCID50) and found weight changes, clinical symptoms, and death. The mice were then dissected at differing days post-infection so as to compare the histopathological changes in infected tissues (Bao et al., 2020). Limitations noted in this model were lack of symptoms which would not be useful in the study of clinical symptoms.

2.3 Endogenous Mouse ACE2 Promoter Model

One of the strategies scientists have employed to increase the specificity of the SARS-CoV-2 spike protein to endogenous mACE2 is to perform sequential passage of the virus in the

animal model, thus over time causing an increase in viral tropism for the receptor, which results in infection and mortality in these mice, as seen in humans. Zhang et al. (2021) were able to demonstrate the usefulness of this model. Both aged (12 months) and young (2 months) mice were infected intranasally with 1 x 10^5 of TCID50 of SARS-CoV-2 and collected samples of tissue & blood 3, 5, and 7 days post infection. Samples were analyzed for evidence of infection and viral replication. Notable findings of the study were the aged BALB/c model's ability to support viral replication in airways (lungs, trachea and nasal epithelium), and develop interstitial pneumonia, and neutralizing antibodies. These authors further demonstrated a rapid adaptation of SARS-CoV-2 to aged BALB/c mice (Zhang et al., 2021), the delayed viral clearance seen in older populations, and shed light on the immune response to infection. This study also noted the model's usefulness in creating mouse-adapted strains, as well as aiding in understanding of COVID-19 disease in older human populations. Limitations in this study included mice with only mild clinical symptoms compared to transgenic models.

Huang et al. (2021) were able to use the endogenous mouse model in their study in which they serially passaged SARS-CoV-2 until a mouse–adapted strain was obtained. Using lab analyses (complete deep gene sequencing, indirect immunofluorescence analysis, and interferometry), the authors were able to demonstrate that two mutations, Q493K and Q98H, in the RBD of the Spike protein of a mouse-adapted strain of SARS-CoV-2 (WBP-1) resulted in an increased affinity for mouse ACE2, causing increased infectivity and severe COVID-19. They were also able to use this model to demonstrate the effectiveness of a TLR7/8 agonist at protecting mice against WBP-1; a useful finding in understanding the pathogenesis of variant strains, as well as mechanisms of possible therapies (Huang et al., 2021). See **Figure 3** for comparison of animal models.

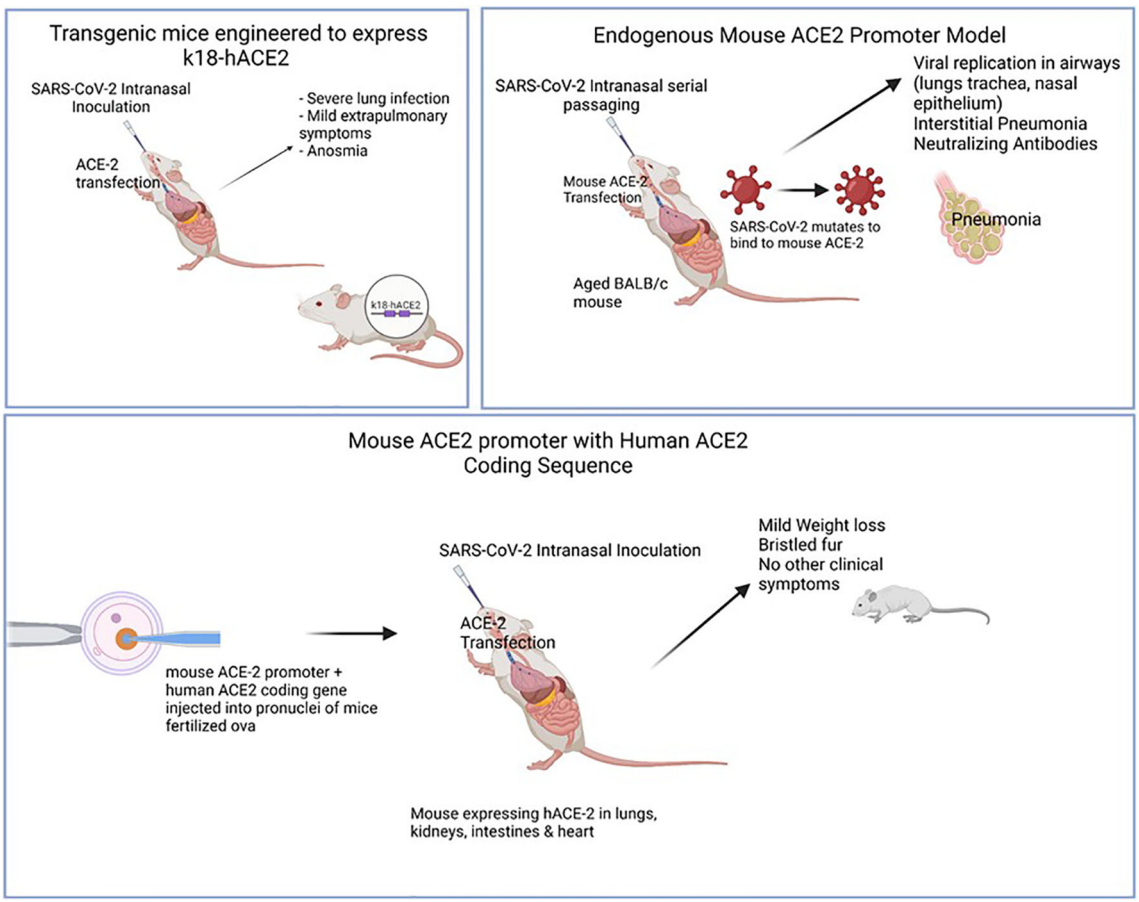

FIGURE 3 | Illustrates various animal models of COVID-19 and the mode of infection. Models were useful in displaying different levels of clinical disease severity once sensitized to SARS-CoV-2 infection. Top left: Mice engineered to express hACE2 gene using k18 promoter. SARS-CoV-2 intranasal inoculation & ACE-2 transfection was shown to cause clinical symptoms (severe lung infection, anosmia) with mild extrapulmonary symptoms (GI, neurological). Top right: Aged, wild-type mice were serially passaged with intranasal SARS-CoV-2. Over time, viral generations eventually were able to bind to mACE-2 leading to clinical interstitial pneumonia and positive titers of neutralizing antibodies. Bottom: Mouse ACE-2 promoter with Human ACE-2 coding sequence were injected into pronuclei leading to mACE-2/hACE-2 susceptible to SARS-CoV-2 infection. hACE-2 receptors were expressed in lungs, kidneys, intestines & heart with mild clinical symptoms (mild weight loss, bristled fur).

2.4 Adenovirus hACE2 Mouse Model for SARS-CoV-2 Infection

Hassan et al. (2020) developed the Adenovirus Human-ACE2 Mouse Model (Adv-hACE2) for SARS-CoV-2 infection by demonstrating the effectiveness of transduction in inducing the expression of hACE2 in mice. Young and adult mice (between 3-4 weeks old or 8-10 weeks old) were used in this study. Mice infected intranasally with SARS-CoV-2 (2.5 x10^8 PFU) in the Adenovirus hACE2 Model showed a greater weight loss, clinical symptoms, and pathology in lung sections in addition to the absence of IFN signaling. While further investigation is needed, these findings suggest the possible protective effects of IFN signaling against viral SARS-CoV-2 infection.

It was concluded that benefits to this model would be the ability to quickly sensitize different species of mice due to the rapid deployability of the adenovirus delivery system for expressing hACE2 in mice, thus potentially accelerating the pace of vaccines and therapeutic drug developments, as well as advancing to NHP and human trials. The costly and time-consuming procedure of genetically engineering transgenic mice to express hACE-2 could greatly be reduced by simply infecting wild-type lab mice with an Adenovirus vector leading to expression of hACE-2 and thus increasing susceptibility to SARS-CoV-2 infection. Han et al. (2021) further supported the Adv-hACE2 model in SARS-CoV-2 research by independently demonstrating its ability to be rapidly deployed in all transgenic and wild-type mice, proving Adv-hACE2 to be a beneficial model in testing vaccine candidates and therapies. More recently, Rai et al. (2021) used the Adv-hACE2 model in SARS-CoV-2 research to study the effects of obesity on COVID-19 disease severity and noted significant changes in cytokine expression in infected obese mice compared to lean mice, further proving the relative usefulness of this model in current COVID-19 research, Noted limitations would be differences in concentration of hACE2 expression in tissues from mouse-to-mouse, local and temporally-limited induced receptor expression, as well as inflammation of airways with Adv inoculation. Further limitations noted in this study were the induction of receptor density localized in lungs/low number in extrapulmonary organs making this Adv model less helpful in studies to treat extrapulmonary COVID-19 manifestations. See **Figure 4**.

2.5 Mouse Hepatitis Virus Model

Murine hepatitis virus strains have been shown to produce a clinically relevant model of severe acute respiratory syndrome in mice. The ability of a large number of murine coronavirus strains, including MHV-1, MHV-3, MHV-A59, MHV-JHM, and MHV-S, has been tested in various mice strains (from 6-8

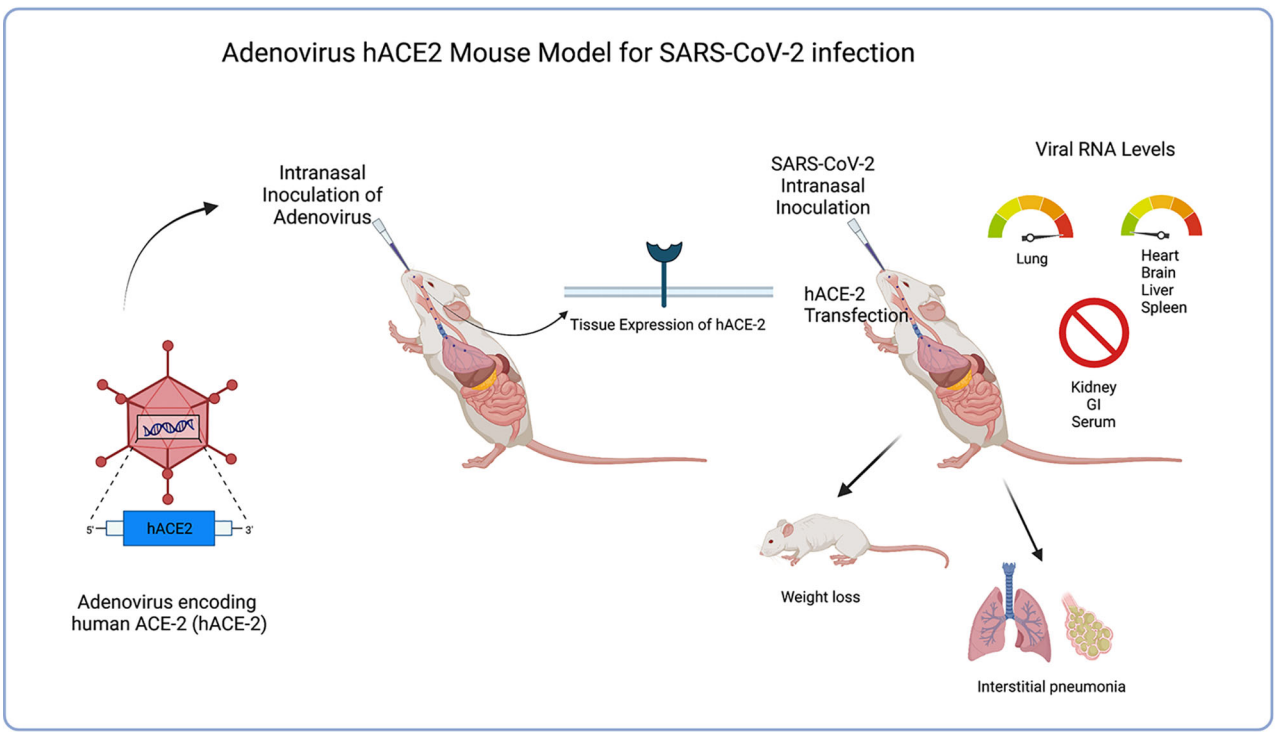

FIGURE 4 | Illustrates the process of Adenovirus hACE2 mouse model transgenesis as well as clinical symptoms of model after SARS-CoV-2 inoculation. Adv-hACE-2 mice showed greater weight loss, clinical symptoms (interstitial pneumonia, weight loss), and pathology in lung sections in addition to the absence of IFN signaling. Elevated viral RNA levels are seen in lung tissue, but low levels are shown in other tissues (heart, brain, liver spleen) while other tissues are not tropic (kidney, GI, serum). Benefits of this model include the ability to quickly sensitize different species of mice due to the rapid deployability of the adenovirus delivery system for expressing hACE2.

week old Female BALB/cJ, C57BL/6J A/J, and C3H/St mice) for whether these viruses produce SARS-like pathology.

2.5.1 MHV-1 Virus

De Albuquerque et al. (2006) were able to produce clinical symptoms and pathology with the MHV-1 strain in their research in further understanding SARS and concluded in their studies that A/J mice were highly susceptible to MHV-1 infection and provided a useful model in human SARS so as to understand pathogenesis and for use in treatment innovation. Paidas et al. (2021) were recently published for data gathered which provide support for the MHV-1 model as a clinically important model for use in COVID-19 research. MHV-1 infected female mice were observed post-inoculation. These mice were then euthanized and their tissue histopathology were examined. Six different disease stages based on clinical progression of symptoms were established prior to inoculation to aid in observation of the infected models. 40% of inoculated mice exhibited clinical symptoms by day 2, with the number of symptomatic mice increasing until up to 75% of mice had signs of clinical disease by 7-12 days. The majority of MHV-1-infected mice initially developed only mild pulmonary disease (Stage I: drowsiness + lack of movement) similar to that seen in BALB/cJ mice by day 2 post-infection. These mice progressed to Stage II symptoms (ruffled fur + altered hind limb posture) noted on day 3 post-inoculation and further to Stage III (ruffled fur + mildly labored breathing) by day 4 & 5. In the majority of infected mice, pulmonary disease progressed to Stage IV (ruffled fur + inactivity + moderately labored breathing + tremor) on day 6, and by days 7-12 Stage V and VI of clinical disease were noted in the infected mice (ruffled fur + obviously labored breathing and lethargy; moribund state + death). See **Figures 5A, B**.

Histopathological examination of MHV-1-infected mice at day 7 showed inflammation (i.e. granular degeneration of cells, and migration of leukocytes into the lungs), along with proteinaceous debris filling the alveolar spaces with fibrillar to granular eosinophilic protein strands caused by progressive breakdown of the capillary wall and epithelial integrity, which permits leakage of protein rich edematous fluid into the alveoli, and the presence of hemosiderin-laden macrophages (indicating pulmonary congestion with dilated capillaries and leakage of blood into alveolar spaces). Furthermore, peribronchiolar interstitial infiltration, bronchiole epithelial cell necrosis and necrotic cell debris within alveolar lumens, alveolar exudation, hyaline membrane formation and alveolar hemorrhage with red blood cells within the alveolar space and interstitial edema are all characteristic features of infected lungs observed in humans with SARS-CoV-2 infection (Paidas et al., 2021).

Examination of the livers of MHV-1-infected A/J mice showed near normal histology to day 6, but on day 7 just prior to death, there was severe hepatic congestion, hepatocyte degeneration, severe periportal hepatocellular necrosis with pyknotic nuclei, ballooned hepatocytes, vacuolation, presence of piecemeal necrosis, as well as hemorrhagic changes. Ground glass hepatocytes show voluminous, abundant, granular cytoplasm, with peripheral cytoplasmic clearing and central nuclei, and apoptotic (acidophil) bodies, as well as absent hepatocytes replaced by abundant inflammatory cells. Condensation and dark staining of the cytoplasm and absence of the nucleus, fatty changes, binucleated hepatocytes, and activated Kupffer cells were also observed in MHV-1 exposed mice livers, compatible with lung and heart failure as described in humans.

Upon examination of the MHV-1 infected mice brain, we observed congested blood vessels, perivascular cavitation (suggestive of edema), pericellular halos, vacuolation of neuropils, darkly stained nuclei and pyknotic nuclei amid associated vacuolation of the neuropil, and acute eosinophilic necrosis (Paidas et al., 2021). MHV-1 infected mice brain hippocampus showed necrotic neuron with fragmented nucleus and vacuolation. The heart of MHV-1 infected mice showed severe interstitial edema, vascular congestion and

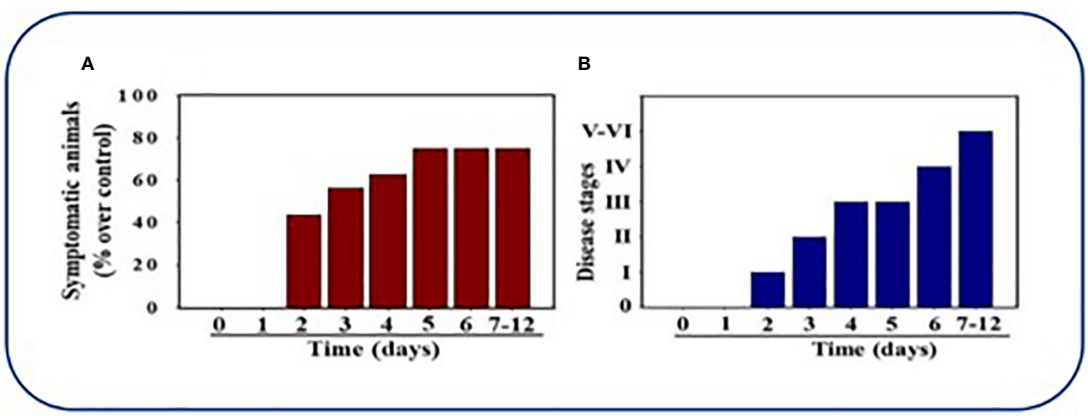

FIGURE 5 | Mice infected by MHV-1 inoculation. **(A)** 40% of mice were symptomatic by Day 2 post-infection and exhibition of clinical signs progressively increased to 75%. **(B)** Mice inoculated by MHV-1 exhibited clinical signs starting at 2 days post-infection. Mild to moderate stage (I-III) of clinical symptoms were seen on days 2-4. Severe sickness (IV-VI) was seen in mice after 6 days. Reproduced with permission from Paidas et al, Viruses; Published by MDPI, 2021.

dilation, and red blood cells infiltrating between degenerative myocardial fibers while tubular epithelial cell degenerative changes, peritubular vessel congestion, proximal and distal tubular necrosis, hemorrhage in interstitial tissue, and vacuolation of renal tubules were observed in MHV-1 exposed mice kidneys, which are identical to that observed in humans associated with SARS-CoV-2 (Paidas et al., 2021). The histopathology observed in this study, as well as the timeline for the progression of clinical symptoms, the high viral load recovered from infected tissues, and the ability to study this model in a Biosafety Level 2 lab further support the use of MHV-1 model in further viral research. These findings collectively suggest that MHV-1 infection in A/J mice is a suitable model to study SARS-CoV-2. See **Figure 6** for organs histology in infected and uninfected mice.

2.5.2 MHV-3

MHV-3 also produces pulmonary lesions, while these changes were milder than those caused by MHV-1 and did not have the characteristic features described in SARS-CoV-2 infection in humans. Additionally, MHV-3-infected mice all developed severe hepatic necrosis and died of liver failure by day 10. Further, A/J mice are resistant to MHV-3 infection, thus MHV-3 did not represent relevant models of SARS-CoV-2.

2.5.3. MHV-A59

Similar to MHV-3, MHV-A59 also produces pulmonary lesions, while these changes were also milder than those caused by MHV-1, and did not have the characteristics of SARS-CoV-2 infection in humans. MHV-A59-infected mice also developed severe hepatic necrosis and died of liver failure by day 10. These findings clearly suggests that both MHV-3 and MHV-A59 strains did not represent relevant models of SARS-CoV-2.

2.5.4. MHV-JHM

MHV-JHM strain, first isolated from a paralyzed mouse in 1949 (Bailey et al., 1949), has been shown to be highly neurotropic, inducing encephalomyelitis and demyelination while weakly affecting liver and lungs cells. De Albuquerque et al. (2006) recently demonstrated how nasal inoculation of BALB/c mice with MHV-JHM failed to produce lung or liver pathology. Other neuropathic effects of neurotropic MHV infection have been studied and include demyelination of CNS leading to overactive bladder (similar to MS), paralysis of lower limbs and muscle wasting (Perlman et al., 1987; Lane et al., 1998; McMillan et al., 2014). MHV-JHM's increased neuropathogenesis is associated with elevated cytokine levels (IFN-β, IL-1β, IL-6, CCL3, CCL4) (Rempel et al., 2004a). The high neurovirulent lethality of JHM has been associated with a diminished CD8 T-cell immune response to infection (Rempel et al., 2004a). Mice with chronic MHV-JHM infection exhibited astrocyte production of cytokines (TNF-α, IL-1β, and IL-6) in the spinal cord (Sun et al., 1995). MHV-JHM virus has been proven to be recoverable from uterus, placenta and fetus of infected BALB/c mice during all three trimesters of pregnancy (Barthold et al., 1988). MHV-JHM and other highly virulent neurotropic strains, can be considered useful in research regarding the mechanisms of viral entry into the CNS, but due to low pneumotropism, are poor model candidates for COVID19 research.

MHV-S: Barthold and Smith (1983) infected 3-week-old mice with MHV-S and analyzed tissues 49 days after inoculation and it was found that 1 week after infection, virus was recovered from brain and lung tissue of most mice and the liver of one mouse. Lesions included olfactory mucosal necrosis, infiltrates and vacuolation of the brain, pulmonary perivascular infiltrates, focal interstitial pneumonia and hepatitis; seroconversion was detected 10 days post-infection and serum titers peaked at 28 days post-infection. While the MHV-S strain seems to have tropism comparable to the MHV-1 strain, which most exhibits the clinical disease progression and histopathology seen in COVID-19 patients, it has been shown by Körner et al. (2020) that MHV-S has low virulence and is detected at low percentages in infected tissue, limiting its usefulness as a COVID-19 model when compared to more virulent MHV strains.

2.6 Syrian Hamster Model

Hamsters, specifically the Golden Syrian Hamster, have been used in SARS-CoV and MERS CoV studies, as well as multiple respiratory infectious diseases including influenza virus. Sia et al. (2020) performed studies to test for pathogenesis of SARS-CoV-2 in 4-5-week-old male Syrian Hamsters. These authors infected the hamsters intranasally $8x10^4$ TCID50 with SARS-CoV-2 virus isolated from cells of a COVID-19 patient from Hong Kong. Sectioned tissue samples were obtained and analyzed for pathology 2-, 5- and 7-days post-infection (DPI) and compared with control studies. The authors of this study concluded that the Syrian hamster was an effective animal model in studying the effects of COVID-19 for several reasons. These include the possibility to observe a consistent progression of illness and clearance of SARS-CoV-2 from 2 to 7 days. Peak viral loads were noted on 2 DPI, detected at bronchial epithelium which then declined 5 DPI (peak viral antigen detected at type II pneumocytes), and was not detectable by 7 DPI. Monocyte infiltration and CD3 T lymphocytes were noted in bronchial epithelium and consolidation changes in the lungs were seen during the course of infection and clearance of the virus. They were also able to observe infection of nasal olfactory neurons which correlate to anosmia in COVID-19 patients (**Figure 7**). Sia et al. were also able to demonstrate transmission of SARS-CoV-2 between inoculated hamsters and naïve hamsters, in a short period after inoculation, which is useful in understanding transmission of SARS-CoV-2 by aerosol droplet and fomites. The fast clearance of the virus in hamsters can also shed light on the immune response mechanism and defense against COVID-19. While there was viral antigen noted in epithelial cells of the duodenum and colon at 2 DPI, which correlates to COVID-19 patients, researchers noted that there were no histopathological changes in samples of inoculated or contact hamster brains, livers, hearts or kidneys. This is a limitation of the hamster model as it would not be useful to study the extrapulmonary pathologies that are observed in patients associated with COVID-19. Yang et al. (2021) recently used the Syrian hamster model to explore biomarkers of COVID-19. Of note, elevated levels of amylase, lipase, GOT/GTP ratio, as well as viral replication in liver and

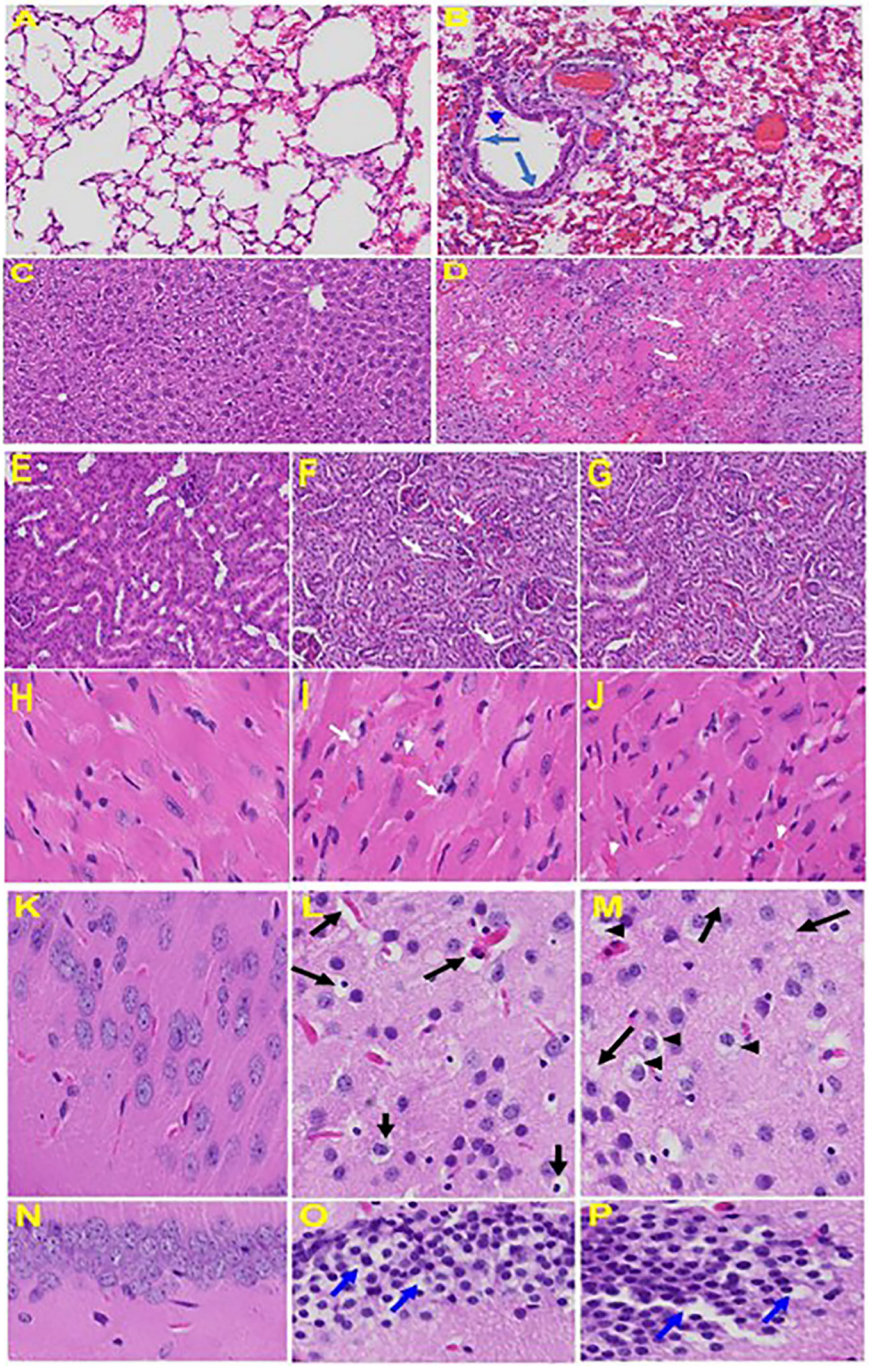

FIGURE 6 | Organs histology in infected and uninfected mice: Representative histological images of haematoxylin and eosin (H&E) stained lung tissue sections of normal mouse **(A)** and infected mouse lung **(B)**. MHV-1-infected mice lung shows arterial endothelial swelling (hypertrophy, long arrow), inflammation and granular degeneration of cells (short arrow) and migration of leukocytes (arrowhead) into lung. Peribronchiolar interstitial infiltration, bronchiole epithelial cell necrosis and necrotic cell debris within alveolar lumens, alveolar exudation, infiltration, hyaline membrane formation and alveolar hemorrhage with red blood cells within the alveolar space and interstitial edema were observed in MHV-1-infected mice. Liver, kidney, heart, and brain tissue degenerative changes were observed in MHV-1-infected mice. MHV-1-infected mice at day 6 showed liver hepatocytes degeneration, severe cells necrosis [**(D)**, long arrows] and hemorrhagic changes (short arrows) when compared to uninfected mice **(C)**; Uninfected kidney **(E)** and tubular epithelial cells degenerative changes and vacuolation, and peritubular vessels congestion **(F, G)**; Severe interstitial edema (arrows), vascular congestion and dilation (arrow heads) and red blood cells infiltrating between degenerative myocardial fibers were seen in the MHV-1 infected mice heart **(I, J)**, as compared to uninfected mice **(H)**. Vacuolation of cerebral matrix [arrows, **(L, M)**] and cytotoxic edema [arrow heads, **(L, M)**], congested blood vessels with perivascular edema [long arrows, **(L, M)**], as well as cytotoxic edema (short arrows) were seen in MHV-1infected mice brain cortex, and in hippocampus (O&P). Reproduced with permission from Paidas et al, Viruses; Published by MDPI, 2021.

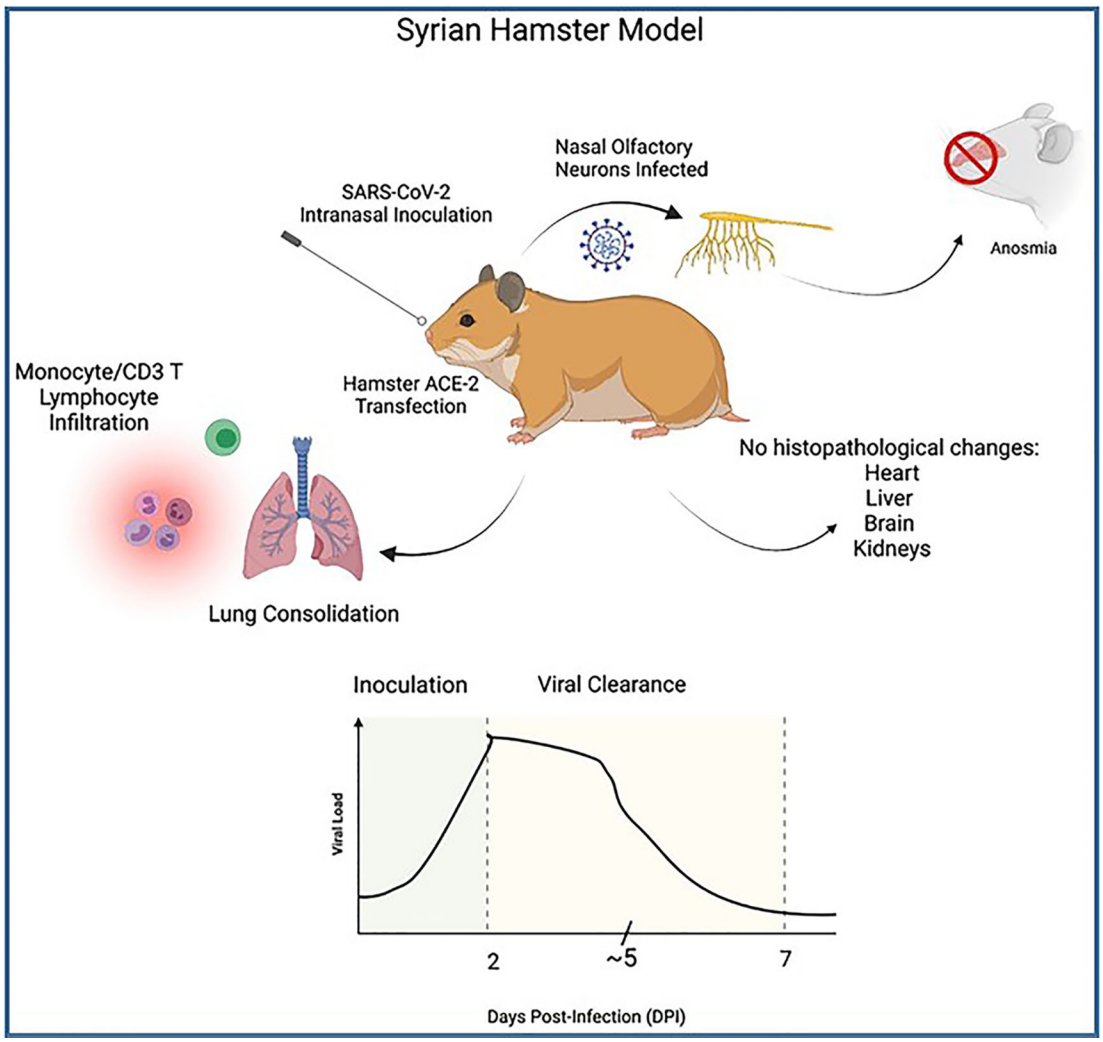

FIGURE 7 | Illustrates the process of Syrian hamster model SARS-CoV-2 inoculation as well as clinical symptoms. Top: Wild-type Hamster ACE-2 are readily susceptible to SARS-CoV-2 infection, particularly nasal olfactory neurons, leading to anosmia. Innate and cell-mediated infiltration were seen at lung with consolidation noted, however no histopathology was seen in extrapulmonary tissues despite viral antigen noted at duodenal and colon epithelia. Bottom: Graph illustrates viral course of SARS-CoV-2 infection in Syrian hamster model. High titers of viral RNA are noted 2 days post-inoculation (DPI), with steady decline of viral load 5 DPI and sharp decline and viral clearance at approximately 7 DPI.

pancreatic tissues were observed in this study suggesting a useful model in the study of the effects of comorbidities on COVID-19 as patients with chronic liver and pancreas disease have been shown to have increased susceptibility to infection and thus face increased risk of mortality to COVID-19.

Notable advantages to the use of hamsters in COVID-19 would be that which is shared with similarly sized models (high reproduction rate, ability to thrive in small housing allows large number of subjects to be studied at a time). Olfactory neurons of the hamster model are readily infected by SARS-CoV-2 which may be useful in studies of COVID-19 patients experiencing lingering anosmia after they have cleared SARS-CoV-2. Disadvantages that were noted based on the studies observed in this review include lack of extrapulmonary manifestations of common COVID-19 that is seen in human patients, and that SARS-CoV-2 is quickly cleared limiting the hamster's usefulness as a model for severe COVID-19.

2.7 Ferret Models

The ferret model of SARS-CoV-2 infection was first used by Kim et al. (2020). Ferret model was suitable for this goal due to ferret ACE2 having specific SARS-CoV binding proteins, which at the time had been shown to be homologous to SARS-CoV-2 binding proteins. Kim et al. performed 3 trials, inoculating 2 ferrets intranasally with $10^{5.5}$ TCID50 of NMC-nCoV02 strain from a COVID-19 patient. The study observed different modes of viral infection, direct inoculation vs direct contact vs indirect contact, between the infected ferrets and naïve ferrets. The study noted

elevated body temperature changes in all infected ferrets between 2 and 8 DPI, with return to normal body temperature on 8 DPI. Infected ferrets showed mild respiratory symptoms (i.e. occasional cough), no changes in body weight, and no mortalities were observed as had been seen in murine models or humans associated with SARS-CoV-2 infection. It was noted that all direct contact ferrets (which had been housed with directly infected ferrets) experienced elevated body temperature changes 4-6 days post-contact, and no detectable changes in body weight (**Figure 8**).

Benefits of the ferret model would be their natural susceptibility to respiratory viruses such as SARS-CoV-2, a clear advantage compared to transgenic mice models. Another benefit of the ferret model includes the ability to reproduce the human condition of viral infections due to proportional respiratory tract anatomy of the upper and lower airways, as well as the number of terminal bronchioles and density of submucosal glands. Ferrets are also able to produce high titers of viral loads in upper airways and infect naïve ferrets by contact transmission which would be useful in understanding human viral transmission. Limitations of ferret model use would be low viral titers in the lungs and mild clinical symptoms, findings that would not be of much use to understand lung pathology or severe COVID-19 cases. The study did take note that prolonged infection in the lungs at lower viral levels may help in understanding transmission in asymptomatic carriers, a useful model in vaccine experimentation & research.

Corroborating, extensive studies by (Shi et al. 2020) using multiple animal species for supporting infection with SARS-CoV-2 concluded that ferrets were highly susceptible in their upper airways (nasal turbinate, soft palate, and tonsils) but not lung tissue, and may affect the GI tract. Researchers noted more studies were needed to see if male ferrets were more susceptible than females, as is seen in COVID-19 patients.

2.8 Non-Human Primate Models

Expansion of SARS-CoV-2 infection studies to non-human primates (NHPs) were explored in an effort to optimize animal models used in COVID-19 research, considering limitations that had been seen in rodents. The benefit of using NHP models is their close physiological relation to the human immune response during infections. This review will focus on studies involved with 3 of the more promising NHP models: Rhesus Macaques, Cynomolgus Macaques and African Green Monkeys.

2.8.1 Rhesus Macaques

Rhesus Macaques were used in the study of Munster et al. (2020) in order to assess their usefulness as animal models in the study of SARS-CoV-2. In this study 8 adult macaques (4 males and 4 females between 4-6 years-old) were inoculated *via* a combination of routes (intranasal, intraocular, intratracheal and oral) with a dilution of the virus. The animals were split

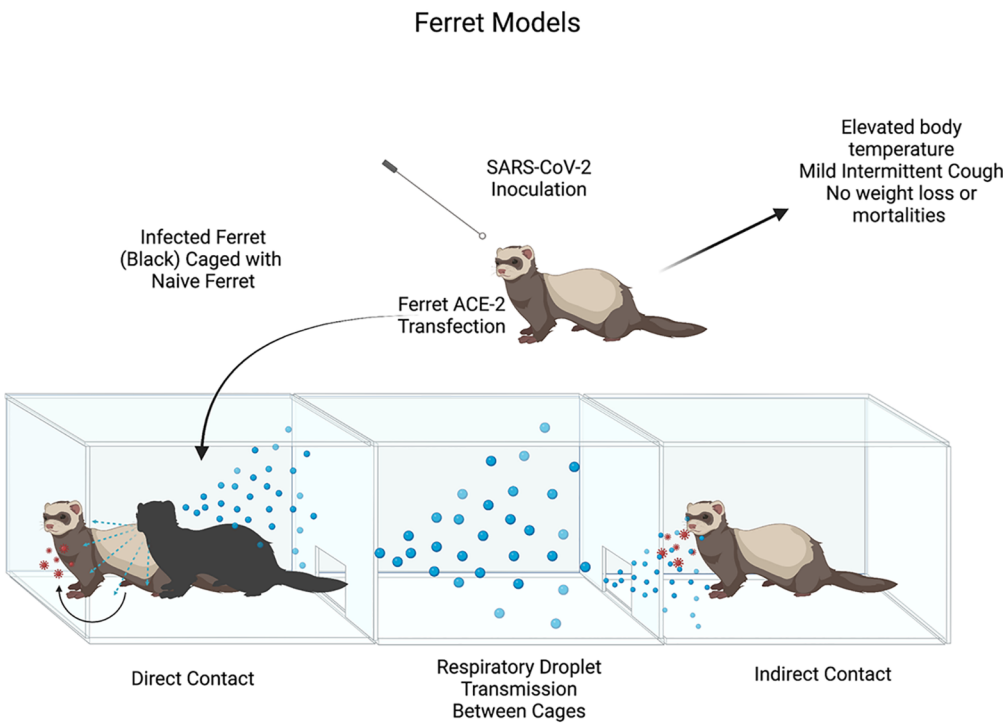

FIGURE 8 | Illustrates the process of Ferret model SARS-CoV-2 inoculation and clinical symptoms post-infection. Illustration of SARS-CoV-2 Ferret Direct vs. Indirect transmission experiment. Ferret ACE-2 receptors are readily susceptible to SARS-CoV-2 infection with mild clinical signs seen in in infected ferrets (hyperthermia, mild cough). High titers of viral loads in upper airways provide evidence for direct & indirect contact leading to viral infection though respiratory droplet transmission.

into two groups (3 DPI and 21 DPI) and observed for clinical signs. Clinical exams were performed on days 0, 1, 3, 5, 7, 10, 12, 14, 17, 21 and reviewed clinical factors such as bodyweight & temperature as well as chest x-rays. Bronchiolar lavages and histopathological changes from collected tissues were also performed (Munster et al., 2020).

The results of the Munster study showed transient moderate COVID-19 disease as is seen in humans. Of note, pulmonary infiltrates were seen in all test macaques. The rhesus macaque model viral pattern of shedding from upper and lower respiratory tracts was also shown to reflect human viral spread and, similar to humans, there was evidence of viral shedding after resolution of clinical symptoms and radiographic findings. These authors were also able to demonstrate a seroconversion timeframe similar to humans, with IgG antibodies detected 7-10 DPI.

Additional studies by McMahan et al. (2021) using rhesus macaques to understand the immune response to infection suggested a threshold for neutralizing antibody titers which protects against illness in this animal model and the contribution of cell-mediated immunity against infection. This study supports the use of this model in understanding vaccine development and the importance of stimulating humoral immunity in the host.

Further studies by Shaan Lakshmanappa et al. (2021) made use of the rhesus macaque model in their research of CD4 helper T-cell response to SARS-CoV-2 infection. The authors were able to provide evidence of follicular T helper cell generation and Germinal Center stimulation, leading to early production of IgG antibodies. Histopathology lesions in this study showed mild to moderate extensive interstitial pneumonia. However, there were no signs of weight loss, fever, or clinical disease seen in infected macaques and there were no presentations of acute respiratory distress syndrome (ARDS). Benefits of the rhesus macaque model include the displayed high levels of viremia, which can be useful in immunological studies, but due to the lack of clinical disease presentations, limits the model's usefulness in understanding clinical illness of COVID19 patients.

Supporting studies by Blair et al. (2021) provided additional evidence for the utilization of rhesus macaques in COVID19 study due to their ability to exhibit mild clinical disease similar to humans. The authors were able to use this model to demonstrate that the highest levels of SARS-CoV-2 viral replication are found in the pharynx and nasal cavity of the host, as is seen in COVID19 patients. The study also showed high viral RNA loads in feces, similar to humans. Multiple routes of infection were used in their rhesus macaque study and it was shown that despite large differences in exposure doses, there were no significant differences in viral RNA loads or kinetics. See **Figure 9**.

2.8.2 Cynomolgus Macaques

The Rockx et al. study (2020) was the first to confirm the basis for the use of cynomolgus macaques as an animal model for SARS-CoV-2 research. This was accomplished by comparing and contrasting histopathology and clinical signs of SARS-CoV-2 with two similar coronaviruses, SARS-CoV and MERS CoV. In their study design, two groups of 4 Cynomolgus Macaques (young adult, 4-5 years- old, and older animals, 15-20 years of age) were inoculated with a SARS-CoV-2 strain by both intratracheal (IT) and intranasal (IN) routes. Only one of the animals showed clinical signs on examination (serous nasal discharge 14 DPI), while no animals exhibited significant weight loss as seen in humans associated with SARS-CoV-2 infection and by 14 DPI all animals had seroconverted antibodies specific to SARS-CoV-2. They were also able to demonstrate viral shedding through mucosal swabbing, RT-qPCR and viral cultures. Of note, aged animals exhibited higher viral levels in nasal swabs, when compared to the younger age group.

Histopathological signs noted at consolidated lung tissue, of both age groups, involved alveoli and bronchiolar luminal tissue which revealed exudative fluid, fibrin, cellular debris and immune cells (mostly alveolar macrophages). As has been seen in other studies, there was evidence of type II pneumocyte hyperplasia with type I pneumocytes being primarily affected.

Researchers concluded that the diffuse alveolar damage (DAD) seen, along with SARS-CoV-2 antigen expression at these lesions, are evidence for the SARS-CoV-2 virus causing the characteristic histopathology that can also be seen in human COVID-19 cases. The data provided in the study showed evidence of cynomolgus macaques usefulness as an animal model for SARS-CoV-2, most specifically their ability to demonstrate upper airway infection & disease transmission and lower airway lung disease. Salguero et al. (2021) further reinforced the usefulness of the cynomolgus macaque model in pathogenesis and histopathological research.

2.8.3 African Green Monkeys

The African Green Monkey (AGM) model, demonstrated by Woolsey et al. (2020), was performed after the success of the cynomolgus and rhesus macaque models demonstrated by the Rockx (2020) and Salguero (2021) studies. AGM was investigated in their immune response to SARS-CoV-2 due to their ability to support the highest levels of replication of SARS-CoV-1 compared to the other NHPs.

In the Woolsey et al. study, the authors inoculated six adult AGMs with 5 x 10^5 of SARS-CoV-2 isolated from a COVID-19 patient in Italy by both intratracheal and intranasal routes. Three AGMs were euthanized at 5 DPI while the other 3 were observed throughout the experiment (for 21 days). Blood samples were collected from all monkeys on days 0, 2, 3, 4 & 5 and continued on days 7, 9, 12, 15, and 21 for the remaining 3 subjects. The AGMs were analyzed for clinical signs, blood inflammatory markers, immune responses, markers of organ function, chest radiographs and for histopathology.

This study found that there were no signs of notable clinical illness observed in the AGMs, however there were indications of systemic response to infection as was evident by elevated inflammatory markers, leukocytes, and thrombocytes. Elevated temperatures were seen in two animals. All animals held after 5 DPI seroconverted, with two having high IgG titers while one

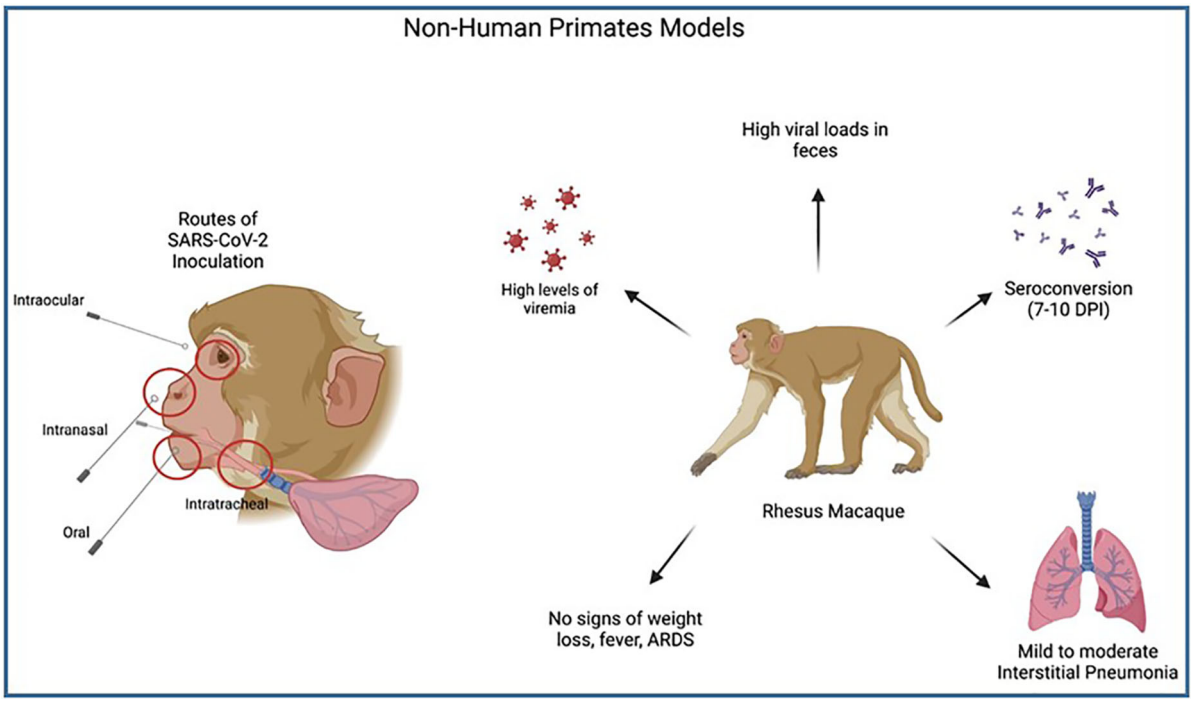

FIGURE 9 | Illustrates the process of Non-Human Primate model SARS-CoV-2 inoculation and clinical symptoms post-infection. Multiple routes of SARS-CoV-2 inoculation allow researchers to understand which area of the upper airways are most susceptible to SARS-CoV-2 infection, with the highest levels found in the pharynx and nasal cavities. A common NHP model, Rhesus macaques, have been shown to have high levels of viremia & viral loads in feces, with seroconversion comparable to humans and mild clinical symptoms (mild-moderate interstitial pneumonia). No systemic symptoms or ARDS were seen in infected macaques making this model less useful in studying severe COVID-19.

animal held at 21 DPI had low level IgG titers. All AGMs exhibited viral pneumonia and pulmonary consolidation seen on radiographs, as was seen in other NHP studies (Hartman et al., 2020). These findings suggest that factors other than elevated inflammatory markers, leukocytes, and thrombocytes may have been responsible for lung consolidation and it is recommended not to rely solely on chest radiographs for primary diagnosis of COVID-19.

This study also showed evidence of GI involvement in the infected subjects, with all subjects showing abnormalities in the small intestine despite no signs of GI distress. This can be attributed to ACE-2 receptors expressed at the ileum and colon which would also be sites of entry for SARS- CoV-2, which has also been reported in human cases.

Another interesting finding in the data of this study is the similarities in the inflammatory immune response markers in AGMs and humans and the correlation of disease severity and the levels of cytokine markers. Fibrinogen levels were also elevated in most of the infected AGMs, another finding seen in human cases, and may be implicated in thrombosis and ischemic vascular events. A significant advantage of the AGM model is the spectrum of AGM host response to SARS-CoV-2 infection, as is seen in human cases. Such similarities in phenotypic manifestations of COVID-19 in AGMs compared to humans lends support for non-human primates to serve as models in COVID-19 research. Examining COVID-19 biomarkers such as tissue viral load and histopathology in infected AGM tissue can aid in comparing the efficacy of vaccines and therapeutics.

AGMs, and NHPs in general, are more difficult models for use in animal research as they legally have a special status that requires strong justification for their use, they are in need of larger enclosure spaces, must be kept in social groups, have a more varied diet that is more costly to maintain, and need to be habituated to housing & transportation so as to reduce stress levels which can compromise scientific data (Bushmitz, 2014).

2.8.4 Baboons

A recent study compared acute SARS-CoV-2 infection in young and old rhesus macaques and baboons and old marmosets (Singh et al., 2021). Macaques, baboons and old marmosets were infected by multiple routes (ocular, intratracheal and intranasal) with sixth-passage, fully sequenced and authenticated virus at a target dose of 1.05×10^6 PFU/per animal. SARS-CoV-2 viral RNA (vRNA) was detected early in all species at 3 dpi, and declined thereafter at variable rates. The authors found that baboons had prolonged viral RNA shedding and substantially more lung inflammation compared with macaques. Further, inflammation in bronchoalveolar lavage (BAL) was increased in old versus young baboons.

Histopathologic analysis of infected baboons revealed extensive interstitial lymphocytes, plasma cells, lesser macrophages and eosinophils expanding the alveolar septa and alveolar spaces filled with macrophages. Alveolar wall thickening by interstitial deposits of collagen, alveoli lined by occasional type II pneumocytes and alveolar spaces containing syncytial cells and alveolar macrophages were also observed. Using techniques like CT imaging, immunophenotyping, alveolar/peripheral cytokine responses and immunohistochemical analyses, the authors delineated cellular immune responses to SARS-CoV-2 infection in macaque and baboon lungs, including innate and adaptive immune cells and a prominent Type I-interferon response. The authors concluded that acute respiratory distress in baboons recapitulates the progression of COVID-19 in humans, making them suitable as models to test vaccines and therapies.

2.9 Cat Model

Studies have utilized cat models in SARS-CoV-2 research due their close proximity with humans and reports of viral human-to-cat transmission. Shi et al. (2020) investigated SARS-CoV-2 replication and respiratory droplet transmission in their 2020 study and provided evidence of respiratory droplet transmission as well as viral replication in inoculated cats. A total of 7 young adult cats (aged 6 to 9 months) were used in the study and intranasally inoculated with 10^5 PFU of SARS-CoV-2 isolate. In four cats, viral replication in organs was examined after euthanasia on days 3 & 6 post-infection, two cats for each DPI. To study droplet transmission, 3 cats were placed in separate cages with an isolator. An uninfected cat was placed in a cage adjacent to the infected cats' cages. The authors reported that a limitation of this cat model would be aggressive behavior caused difficulty in collecting regular nasal washings and so feces were collected with viral RNA examined in organs after euthanasia.

It was found that the animals that were euthanized 3 days post-infection had detectable viral RNA in the upper airways (nasal turbinate, soft palate, tonsils, trachea), lower airways (lungs) and small intestines. Both animals euthanized on day 6 post-infection showed no detectable viral RNA in lung samples, while viral RNA was detected in both animals' nasal turbinates, soft palate and tonsils. The respiratory droplet transmission study showed viral RNA present in feces of two cats 3 DPI and all three cats 5 DPI. Viral RNA was also detected in feces of the exposed cat, providing evidence of feline-to-feline respiratory droplet transmission. The results of this study provided evidence that SARS-CoV-2 is able to replicate efficiently in cats, younger cats were found to be more vulnerable to infection than older cats, and SARS-CoV-2 was found to be transmitted effectively through respiratory droplet transmission, as is seen in COVID-19 patient studies.

Based on the outcomes of the reviewed cat model studies, advantages to this model's use would be that it is readily susceptible to SARS-CoV-2 infection which is preferable to the use of a transgenic model which would be more costly and time-consuming. Younger animals showed increased susceptibility to infection increases this model's usefulness in studying mild pediatric COVID19 but limits its functionality as a model for COVID19 in older populations. High titers of viral RNA is readily detected 3 days post-infection which is accelerated relative to other explored models and may lend itself to use in therapeutic research. Notable disadvantages would be difficulty in handling the model itself due to cats known aggressive nature, making recovering i.e. nasal samples difficult. Cats are also at a higher level of regulation in scientific research and require sufficient housing, food, and resources, making the cat model costly for smaller labs to afford.

3 *IN VITRO* MODELS OF SARS-COV-2 INFECTION

In vitro cell lines have been used in several studies to aid in the understanding of pathogenesis, tropism and treatment of respiratory virus infections (Rijsbergen et al., 2021). Immortalized cell lines are able to help researchers discover the different cells needed for the infections seen in *in-vivo* models and are able to be manipulated. Through previous SARS-CoV studies performed (i.e. Ren et al., 2006), there has been a clear understanding of the cell lines with the highest concentration of the ACE-2 receptor at their apical side which is needed for SARS-CoV-2 infection. High concentration of ACE-2 receptors was found to correlate with high rates of cell infection. Cell lines such as apical plasma membrane of polarized respiratory epithelial cells, colon carcinoma cell line (Caco-2) (Pascoal et al., 2021), a lung carcinoma cell line (Calu-3) (Kanimozhi et al., 2021) and Vero E6 cells (Zupin et al., 2021) have been put forward as candidates for models in COVID19 research. Human cancer cell lines were screened for the SARS-CoV-2 cellular entry factors ACE2 and TMPRSS2 based on RNA-seq data of the Cancer Cell Line Encyclopedia (CCLE). These findings suggest that surface expression of ACE2 in polarized epithelial cells function as receptor for severe acute respiratory syndrome.

Vero E6: Vero E6 cell lines, extracted from kidney epithelial cells of African Green Monkeys, are used in the testing of various agents and drugs. Due to the fact that these cells are obtained from normal epithelial cells, and not immortal cell lines, they retain many normal cell functions and are used regularly in viral studies, most notably recent and on-going SARS-CoV-2 research (Prieto, 2002). The usefulness of Vero E6 in SARS-CoV-2 studies is highlighted by this cell line's high ACE2 receptor expression which cause this cell line to be vulnerable to SARS-CoV-2 infection, which can be enhanced by engineering Vero E6 to express the TMPRSS2 protein which is needed for S protein priming (Matsuyama et al., 2020). This model was also used to aid in a SARS-CoV-2 pathogenesis study in which the accumulation of lipids was seen in infected *in vitro* Vero E6 cells as well as lungs of COVID19 patients, suggesting that lipids are involved in SARS-CoV-2 pathogenesis (Nardacci et al., 2021). Vero E6 cells were recently used in a study determining the *in vitro* minimal exposure time and viral concentration needed to establish persistent SARS-CoV-2 infection (Zupin

et al., 2021). Vero E6 cell lines have also been used in multiple studies of SARS-CoV-2 repurposed antivirals (Canal et al., 2021, Leneva et al., 2021), protease inhibitors (Cui and Jia, 2021; Han et al., 2021) as well as inhibiting peptides (Kelesidis et al., 2021). This cell line model was used in research investigating the indirect inhibition of the ACE-2 receptor as a possible SARS-CoV-2 therapeutic (Tanimoto et al., 2021) along with human polyclonal IgG (Gilliland et al., 2021). Treating SARS-CoV-2 infection with repurposed drugs has been an area of intense research due to the speed at which these therapeutics can be approved and implemented into the clinical setting. Vero E6 cell lines were used in the investigations of Losartan (Nejat et al., 2021), Doxycycline (Gendrot et al., 2020), and Fluoxetine (Dechaumes et al., 2021). Scientists have cautioned the use of Vero E6 cell lines in serial propagation of SARS-CoV-2 due its ability to rapidly generate virus variants relative to other cell lines (Funnell et al., 2021).

Caco-2: Caco-2 cell lines, an immortal cell line derived from human colorectal adenocarcinoma cells, are actively used in many areas of research, particularly in gastrointestinal studies such as the Pascoal et al. study (2021) which used the Caco-2 model to conclude that changes in microbiota and short-chain fatty acids produced do not interfere with SARS-CoV-2 intestinal infection. Caco-2 cells have been particularly useful in SARS-CoV-2 research due to the fact that unlike Vero E6, which also expresses ACE-2, Caco-2 is able to express the TMPRSS2 coreceptor endogenously, unlike Vero E6 in which TMPRSS2 expression must be induced (Lee et al., 2020). Caco-2 cell line has shown to be useful in areas of research investigating the nature of SARS-CoV-2 such as comparing & contrasting the kinetics of variants *in vitro* (Touret et al., 2021), the role of integrins in SARS-CoV-2 pathogenesis (Nader et al., 2021), and SARS-CoV-2 transcriptional & post-transcriptional processing dynamics in infected cells (Chang et al., 2021). Similar to Vero E6 cell lines, Caco-2 was also used in studies investigating 6 repurposed drugs in the treatment of SARS-CoV-2 infection with activity against SARS-CoV-2 (i.e. Amiodarone, Lactoferrin, Remdesivir) (Mirabelli et al., 2021). The use of miRNA targeting the SARS-CoV-2 TMPRSS2 coreceptor to prevent cell entry has also been studied using the Caco-2 cell line. (Kaur et al., 2021)

Huh-7: Huh-7, derived from human colorectal adenocarcinoma, is another popular cell line model that has had uses in multiple areas of viral and drug research. The Huh-7 cell line was found to also exhibit ACE-2 receptor protein expression (Kaye, 2006) and so it has been selected in several SARS-CoV-2 studies including investigations of anti-HCV drugs against SARS-CoV-2 (Sacramento et al., 2021), repurposing of viral inhibitors (Puhl et al., 2021), testing of an immunomodulating herbal extract (Roshdy et al., 2020) and traditional Chinese medicine effect on signaling pathways involved in SARS-CoV-2 pathogenesis (Ma et al., 2020).

A549: A549, derived from Human lung adenocarcinoma, is a cell line that similar to previously discussed cell lines, is sensitive to SARS-CoV-2 infection and has been made useful in SARS-CoV-2 investigations. A549 has been most recently used in studies concerning viral replication cycle kinetics (Bernhauerová et al., 2021), SARS-CoV-2 accessory proteins (Silvas et al., 2021), Ebola and Marburg Virus Inhibitors (Puhl et al., 2021), infection-induced promoter hypomethylation (Muhammad et al., 2021), SARS-CoV-2 protease $3CL^{pro}$ inhibitors (de Vries et al., 2021), and 7 FDA-approved antivirals (Weston et al., 2020).

There were shared similar advantages and disadvantages between the use of cell lines when compared to the use of animal models. Cell lines were found to be cost-effective in that there is no required housing of cages, food and low lab maintenance costs. Experiments are also easier to perform and immortal cell lines have the benefit of an unlimited supply as well as bypassing the ethical and legal drawbacks of animal model use. Disadvantages that ultimately limit the usefulness of cell line as a model for COVID19 research include the problem of results of *in-vitro* studies not being reproducible in *in-vivo* studies due to the complex interplay of multiple physiological processes that include multiple (i.e. inflammatory cytokines, signaling molecules, hormone dynamic changes). Genetically modified tissues may also not behave similarly to *in-vivo* tissues and so *in-vitro* has been relegated to use in understanding protein-receptor relationships.

In the Wurtz et al. study (2021), 34 cell lines derived from both animal and human tissues were investigated for supportive infection of SARS-CoV-2. While this study proposed the use of various cell lines for SARS-CoV-2, Vero E6, Caco-2, Huh-7, Calu-3, and A549 cell lines are more representative due to similar factors. Refer to **Table 1** for cell line data.

4 CONCLUSION

Reliable animal models are important in the development of vaccines and therapeutics. Optimizing the animal model in the study and research of a specific pathogen can streamline research efforts towards vaccines and cures. SARS-CoV-2 vaccine & therapeutic development was accelerated due to the worldwide effort to share resources and data. *In-vitro* cell lines aided in the understanding of the interaction between the human ACE-2 receptor and SARS-CoV-2 spike protein which was first discovered in prior SARS-CoV research. Using this foundational research and understanding of coronavirus pathogenesis, *in-vivo* research would be the next step in combating COVID-19 and animal models would need to be thoroughly investigated for optimal use in therapeutic and vaccine exploration. Of the animal models discussed in this review, there were many benefits and limitations to consider depending on the goal of the intended research of each study Refer to **Table 2** for summarized conclusions of the review conclusions of the review. Most models were able to demonstrate transmission, pathogenesis, and histopathology similar to human cases. There were nuances to be explored within each animal model that would shed light on COVID-19. Infection with SARS-CoV-2 in hamsters demonstrated demographic differences in response as seen in humans (males and elderly experience more severe illness vs

TABLE 1 | Cell lines susceptible to SARS-CoV-2 infection.

No.	Cell line	ACE-2 (Presence or Absence)	Effectiveness	Reference
1	Vero E6	Presence	Yes	(Cui and Jia (2021); Dechaumes et al., (2021); Funnell et al., (2021); Gendrot et al., (2020); Gilliland et al., (2021); Han et al., (2021); Leneva et al., (2021); Kelesidis et al., (2021); Matsuyama et al., (2020); Nardacci et al., (2021); Nejat et al., (2021); Prieto et al., (2002); Tanimoto et al., (2021); Wurtz et al., (2021); Zupin et al., (2021))
2	Caco-2	Presence	Yes	(Chang et al., (2021); Kaur et al., (2021); Lee et al., (2020); Mirabelli et al., (2021); Nader et al., (2021); Pascoal et al., (2021); Touret et al., (2021); Wurtz et al., (2021)
3	Huh-7	Presence	Yes	(Kaye et al., (2006); Ma et al., (2020); Puhl et al., (2020); Roshdy et al., (2020); Sacramento et al., (2021); Wurtz et al., (2021))
4	Calu-3	Presence	Yes	(Kanimozhi et al., (2021); Wurtz et al., (2021))
5	A549	Absence	No	(Bernhauerová et al., (2021); de Vries et al, (2021); Muhammad et al., (2021); Puhl et al., (2021); Silvas et al, (2021); Weston et al., (2020); Wurtz et al., (2021))
6	HEK-293T	Presence	No	Wurtz et al., (2021)
7	LNCaP	Absence	No	Wurtz et al., (2021)
8	Aa23	Absence	No	Wurtz et al., (2021)
9	C6/36	Absence	No	Wurtz et al., (2021)
10	S2	Absence	No	Wurtz et al., (2021)
11	ISE6	Absence	No	Wurtz et al., (2021)
12	IPL-LD-65Y	Absence	No	Wurtz et al., (2021)
13	BGM	Presence	Yes	Wurtz et al., (2021)
14	VERO/hSLAM	Presence	Yes	Wurtz et al., (2021)
15	MA104	Presence	Yes	Wurtz et al., (2021)
16	VERO81	Absence	No	Wurtz et al., (2021)
17	LLC-MK2	Presence	Yes	Wurtz et al., (2021)
18	HT-29	Absence	No	Wurtz et al., (2021)
19	HELA	Absence	No	Wurtz et al., (2021)
20	HCT-8	Absence	No	Wurtz et al., (2021)
21	HEP-2	Absence	No	Wurtz et al., (2021)
22	ECV304	Absence	No	Wurtz et al., (2021)
23	HL-60	Absence	No	Wurtz et al., (2021)
24	MRC5	Absence	No	Wurtz et al., (2021)
25	THP1	Absence	No	Wurtz et al., (2021)
26	BHK21	Absence	No	Wurtz et al., (2021)
27	McCoy	Absence	No	Wurtz et al., (2021)
28	L929	Absence	No	Wurtz et al., (2021)
29	P388D1	Absence	No	Wurtz et al., (2021)
30	RAW 264.7	Absence	No	Wurtz et al., (2021)
31	BA 886	Absence	No	Wurtz et al., (2021)
32	MDCK	Absence	No	Wurtz et al., (2021)
33	DH82	Absence	No	Wurtz et al., (2021)
34	OA3.Ts	Absence	No	Wurtz et al., (2021)
35	MDOK	Absence	No	Wurtz et al., (2021)
36	R05T	Absence	No	Wurtz et al., (2021)
37	R06E	Absence	No	Wurtz et al., (2021)
38	TB1 Lu	Absence	No	Wurtz et al., (2021)
39	XTC-2	Absence	No	Wurtz et al., (2021)

ACE-2 presence increases both susceptibility to infection and cell line's effectiveness in use for COVID-19 research and has been shown to correlate with the presence of ACE-2 receptors in cell line tissue. The cell lines that have been found to be effective, and most useful in COVID-19 research include VeroE6, Caco-2, A549, Huh-7, & Calu-3.

females and young). Studies in hamsters can be completed quickly and in a cost-effective manner. Ferrets were shown to be readily infected without the need for transgenics (such as mice species), however infection was mostly restricted to upper respiratory passages, which would not lend this model to lung pathology research. Ferret models would be useful in research into viral shedding from nasal and oropharyngeal epithelium and asymptomatic transmission in COVID-19 patients. Transgenic mice were shown to be useful in the understanding of mild extrapulmonary disease, infectious encephalitis, thrombosis and anosmia. Adv-hACE2 mice were shown to be the most useful murine model due to their ability to quickly infect any and all lab mice, regardless of genetic predisposition to infection.

TABLE 2 | Experimental models of SARS-CoV-2 with characteristic features, advantages and disadvantages.

Experimental Model	Characteristics	Advantages	Disadvantages	References
Non-Human Primates				
Cynomolgus Macaques	Histopathological signs noted at consolidated lung tissue involved alveoli and bronchiolar luminal tissue which revealed exudative fluid, fibrin, cellular debris and immune cells (mostly alveolar macrophages). Aged animals exhibited higher viral levels at nasal swabs, when compared to the younger age group	• Physiologically closest in similarity to humans • Timeline of COVID19 clinical symptoms and seroconversion similar to humans Aged Rhesus macaques & Baboons highly susceptible to COVID19, useful for studying older age and SARS-coV-2 infection AGMs good model for inflammatory biomarkers of COVID19	• Detailed regulation for housing NHPs requires sufficient housing, food, and resources – extremely costly and most difficult model to maintain • Slow reproduction rate • AGMs poor model for clinical COVID19 and other NHP models lack any clinical presentation of disease despite high viremia	(Rockx et al., 2020; Salguero et al., 2021)
Rhesus Macaques	Seroconversion timeframe similar to humans, with IgG antibodies detected 7-10 DPI. Highest levels of SARS-CoV-2 viral replication are found in the pharynx and nasal cavity of the host			(Blair et al., 2021; McMahan et al., 2021; Munster et al., 2020; Shaan et al., 2021)
African Green Monkeys	Viral pneumonia and pulmonary consolidation seen on radiographs of all subjects. No signs of notable clinical symptoms. Elevated inflammatory markers, leukocytes, fibrinogen and thrombocytes. Elevated temperatures seen in few subjects.			(Bushmitz et al., 2013; Hartman et al., 2020; Rockx et al., 2020; Salguero et al., 2021; Woolsey et al., 2020)
Baboons	Prolonged viral RNA shedding and substantial lung inflammation. Extensive interstitial lymphocytes, plasma cells, lesser macrophages and eosinophils expanding the alveolar septa and alveolar spaces filled with macrophages. Alveolar wall thickening by interstitial deposits of collagen, alveoli lined by occasional type II pneumocytes and alveolar spaces containing syncytial cells and alveolar macrophage.			(Singh et al., 2021)
Ferret	Respiratory tract anatomy of the upper and lower airways proportional to humans, as well as the number of terminal bronchioles and density of submucosal glands. Produce high titers of viral loads in upper airways	• Direct contact transmission significant • Do not require spacious housing due to small size • Highly susceptible to SARS-CoV-2 infection • Prolonged infection at lower levels in lungs may be useful in understanding asymptomatic COVID19	• Low viral titers in the lungs and mild clinical symptoms, poor model for severe COVID19	(Kim et al., 2020; Shi et al., 2020)
Syrian Hamster	• Monocyte infiltration and CD3 T lymphocytes noted in bronchial epithelium • Consolidation changes in the lungs seen during the course of infection and clearance of the virus. Able to observe infection of nasal olfactory neurons which correlate to anosmia in COVID-19 patients	• Able to reproduce quickly Direct contact transmission significant Do not require spacious housing due to small size Infection of nasal olfactory neurons may be useful in COVID-19 patients exhibiting anosmia post-infection	• Virus is quickly cleared, not a good model for severe COVID19 • Do not manifest common extrapulmonary symptoms seen in humans	(Sia et al., 2020; Yang et al., 2021)
Mouse Models				
K18-hACE2 Transgenic Mice	Epithelial cell cytokeratin-18 promoter (k-18) hACE2 gene	• All mouse models able to reproduce quickly • Do not require spacious housing	• Producing transgenic mice can be costly • Most mouse models are not able to produce severe clinical symptoms	(Arce and Costoya, 2021; Ejaz et al., 2020; Liu et al., 2021; Seibert et al., 2021; Winkler et al., 2020; Zheng et al., 2021)
mACE2-hACE2	Mouse ACE-2 promoter with hACE-2 coding sequence			(Bao et al., 2020)

(Continued)

TABLE 2 | Continued

Experimental Model	Characteristics	Advantages	Disadvantages	References
Transgenic Mice		due to small size • K18-hACE2 model useful for mild extrapulmonary clinical COVID (i.e. anosmia) Adv-hACE2 model able to infect mice readily without trangenesis MHV model able to be studied in BSL-2 lab	seen in COVID19 • Some transgenic mouse models (k18-hACE2, mACE2-hACE2) may have ectopic hACE2 expression limiting their usefulness	
Endogenous mACE2 Transgenic Mice	Sequential passaging of SARS-CoV-2 virus over time causes an increase in viral tropism for the mouse ACE-2 receptor			(Huang et al., 2021; Zhang et al., 2021)
Adv-hACE2 Transgenic Mice	Transduction infection of mouse with Adenovirus with hACE-2 gene			(Goh et al., 2020; Hartman et al., 2020; Qiu et al., 2020)
Mouse Hepatitis Virus Model*	MHV virus (Coronavirus family) produces identical SARS-CoV-2/COVID19 tissue pathology and clinical symptoms compared to humans			(Bailey et al., 1949; Barthold and Smith, 1983; Davidson et al., 1987; Barthold et al., 1988; Rempel et al., 2004a; Kim et al., 2020; McMahan et al., 2021; Nejat et al., 2021; Rees-Spear et al., 2021; Singh et al., 2021)
Cat	Feline-to-feline respiratory droplet transmission & viral replication	• Wild-types susceptible to SARS-CoV-2 infection Younger animals more vulnerable to infection Viral RNA detectable 3 DPI	• Aggressive behavior caused difficulty in collecting regular nasal washings – feces collected Detailed regulation for housing cats requires sufficient housing, food, and resources - costly	(Shi et al., 2020)
***In Vitro* Cell Lines**				
Vero E6	Kidney epithelial cells of African Green Monkey	• Cost effective compared to housing of animal models • Easy to use • Unlimited supply	• Genetically modified tissues may not reproduce physiological *in-vivo* state Limited uses for clinical research Results do not take into account complex physiological interplay seen in *in-vivo*	(Cui and Jia, 2021; Dechaumes et al., 2021; Funnell et al., 2021; Gendrot et al., 2020; Gilliland et al., 2021; Han et al., 2021; Leneva et al., 2021; Kelesidis et al., 2021; Matsuyama et al., 2020; Nardacci et al., 2021; Nejat et al., 2021; Prieto et al., 2002; Tanimoto et al., 2021; Wurtz et al., 2021; Zupin et al., 2021)
Caco-2	Human colorectal adenocarcinoma cells			(Chang et al., 2021; Kaur et al., 2021; Lee et al., 2020; Mirabelli et al., 2021; Nader et al., 2021; Pascoal et al., 2021; Touret et al., 2021; Wurtz et al., 2021)
Huh-7	Human hepatocellular carcinoma			(Kaye et al., 2006; Ma et al., 2020; Puhl et al., 2020; Roshdy et al., 2020; Sacramento et al., 2021; Wurtz et al., 2021)
A549	Human lung adenocarcinoma			(de Vries et al., 2021; Bernhauerová et al., 2021; Muhammad et al., 2021; Puhl et al., 2021; Silvas et al., 2021; Weston et al., 2020; Wurtz et al., 2021)

NHP models were ultimately shown to be the most representative of COVID-19 disease process as seen in humans. Baboons have been shown in studies to support SARS-CoV-2 replication and may be useful in understanding the effects of comorbidities, such as cardiovascular disease, and their interactions with the virus. Macaques and African green monkeys were shown to support a high level of viral replication at low viral load and display a similar spectrum of clinical disease as is seen in human patients, allowing research to study mild to severe illness and work towards therapeutics to combat these effects of infection, yet NHP models are an under-utilized model in research due to their high costs.

Of the models investigated in this study, it is the suggestion of this review that the Murine Hepatitis Virus-1 model be considered an excellent and most optimal research model in future COVID19 studies. The MHV-1 virus model is able to replicate the life cycle of a beta coronavirus at physiological doses with analogous histopathology of infected tissues as well as represent the broad spectrum of clinical disease seen in COVID19 patients, lending this model to further our understanding of this illness. The MHV-1 model is also able to replicate viral transmission seen in humans and has similar pneumotropism as well as extrapulmonary effects seen in human patients. Immunological host response and

seroconversion is proportional to that seen in human studies and most importantly the MHV-1 model is able to be used at low cost, is highly practical, and most importantly can be studied in a Biosafety Level-2 lab setting, and provides acceptable ecological, as well as ethical consequences during experimentation. Balancing safety, mimicking human COVID-19 and robustness of the animal model, the Murine Hepatitis Virus-1 model currently represents the most suitable model for SARS-CoV-2/COVID19 research.

AUTHOR CONTRIBUTIONS

LC-C, MP, and AJ contributed to conception and design of the review. All authors contributed to the writing of the manuscript.

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Analyzing the Effect of Vaccination Over COVID Cases and Deaths in Asian Countries Using Machine Learning Models

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Coronavirus Disease 2019 (COVID-19) is spreading across the world, and vaccinations are running parallel. Coronavirus has mutated into a triple-mutated virus, rendering it deadlier than before. It spreads quickly from person to person by contact and nasal or pharyngeal droplets. The COVID-19 database 'Our World in Data' was analyzed from February 24, 2020, to September 26, 2021, and predictions on the COVID positives and their mortality rate were made. Factors such as Vaccine data for the First and Second Dose vaccinated individuals and COVID positives that influence the fluctuations in the COVID-19 death ratio were investigated and linear regression analysis was performed. Based on vaccination doses (partial or complete vaccinated), models are created to estimate the number of patients who die from COVID infection. The estimation of variance in the datasets was investigated using Karl Pearson's coefficient. For COVID-19 cases and vaccination doses, a quartic polynomial regression model was also created. This predictor model helps to predict the number of deaths due to COVID-19 and determine the susceptibility to COVID-19 infection based on the number of vaccine doses received. SVM was used to analyze the efficacy of models generated.

Keywords: linear regression, machine learning, COVID-19, polynomial distribution, OLS regression, Support Vector Machine (SVM)

INTRODUCTION

Pandemics have been created throughout history; pandemics have been caused by new strains of viruses, such as influenza, increasing sickness, death, and destruction in the countries. Some of the acknowledged pandemics include the Spanish flu (1918), the Asian flu (1957), the Hong Kong flu (1968), and the Swine flu (2009), each varying in the morbidity and mortality rates (Akin and Gözel, 2020).

Coronavirus, or SARS-CoV-2, which causes Coronavirus Disease (COVID-19), first surfaced in the United States with COVID-19 outbreaks in Wuhan, China, during December 2019 and spread throughout the globe in less than a month like a forest fire. The first confirmed case in the United States was reported on January 20, 2020, (Holshue et al., 2020). As of May 14, 2021, there are 161,846,189 instances of COVID-19 and 3,359,004 persons who have died due to this disease worldwide (Worldometer, 2021). The coronavirus disease 2019 (COVID-19) pandemic, which is caused by severe acute respiratory syndrome and has spread to 220 countries, has resulted in over 63 million laboratory-confirmed cases and over 1.4 million deaths [World Health Organization (WHO)], causing widespread social and economic disruption.

Coronaviruses are members of the Coronaviridae family, which is part of the Nidovirales order (Enjuanes et al., 2006). Coronaviruses are programmed to successfully adjust to a wide range of hosts (Mammals and Aves) and tissue tropism and have the ability to adapt to novel environments through mutation and recombination (Graham and Baric, 2010). Coronaviruses are positive-stranded RNA viruses. The RNA-dependent RNA synthesis in Nidovirales involves a discontinuous step during the development of sgRNA mRNAs (Enjuanes et al., 2006). A helical capsid consisting of nucleocapsid proteins encases the viral genome. The three major structural proteins found in the virus include: the Membrane protein (M), and the Envelope protein (E), both assist in virion assembly, while the Spike protein (S) aids in the entry of the virion into the host cells (Li, 2013). SARS-CoV-2 has many copies of the S glycoprotein on its surface, which gives the virus its unique crown form. ORF1 3′ is dependent on the S protein's ectodomain's tropism-altering interchangeability and the inherent capability of coronavirus-targeted recombination (de Haan et al., 2008). The S glycoprotein of SARS-CoV-2 is divided into two domains: an amino-terminal S1 domain-containing 200-amino acids receptor-binding domain (RBD), and a carboxy-terminal S2 domain-containing a potential fusion peptide, two heptad repeat (HR) domains, and a transmembrane (TM) domain that forms a trimer (Bosch et al., 2008).

The S1 and S2 subunits of the S glycoprotein are important for the interaction between host and virus, which impacts the range of the host (Naqvi et al., 2020). An investigation also discovered that spike protein had several alterations in its structure. Destabilizing mutations have been discovered in the SARS-CoV-2 replication machinery enzyme and S, E, and N structural proteins which cause an increase in infectivity rate (Mohammad et al., 2021). It has been established that an elegant coronavirus reverse-genetics technique can effectively introduce mutations in SARS-CoV-2 genomic regions. The cellular proteases cathepsins, HAT (human airway trypsin-like protease), and TMPRSS2 (transmembrane protease serine-2) are responsible for penetration of SARS-CoV-2 within the host cell and splitting up the spike protein (Upadhyay et al., 2020). The angiotensin 1-converting enzyme 2 (ACE2) molecules on the host cells act as a receptor for SARS-CoV2 (Li et al., 2003). This initiates the entry of the virus into the host cell, which in turn leads to COVID-19 infection. Even though a lot of different research has been conducted on COVID, we are still unsure of the mutation that causes the COVID genome to mutate. Any information on the source or rate at which the COVID virus' genome is altering will be extremely useful in preventing the virus' future development (Asrani et al., 2020).

COVID-19 symptoms include fever, cough, chest tightness or pain, tiredness, and sore throat. However, asymptomatic infections have also been reported. In some studies, it was found that during the infection, the white blood cell counts of the patient were found to be normal (71.4%) or decreased (28.6%) in nearly 70% of the patients, and lymphocytopenia was found in 50% (7/14) of them (Su et al., 2020). Dr. Sakoulas estimated that 80% of COVID-19 participants will not require medical attention, 15% may require non-ICU medical care, and 5% may require ICU stays (Dr. Sakoulas, 2020). Pneumonia puts patients' lives in jeopardy. COVID-19 appears to be milder in children than in adults. Approximately 90% of pediatric patients are diagnosed with asymptomatic, mild, or moderate disease. According to the statistics, one-quarter of COVID-19 deaths occurred in older adults aged 70–79 years. Up to two-thirds occurred in those over 80 years, regardless of the incidence of the disease or the completeness with which deaths were recorded across nations (Calderón-Larrañaga et al., 2020).

The selection of appropriate specimens is crucial for identifying COVID-19 infected individuals in the majority of cases. The nasopharyngeal swab is commonly used for diagnosis. Still, lower respiratory tract specimens such as sputum and broncho-alveolar lavage (BAL) may also be used to increase the chances of detection (Loeffelholz and Tang, 2020). Antibodies against SARS-CoV-2 are detected using an enzyme-linked immunosorbent assay (ELISA) based on the recombinant nucleocapsid protein of SARS-CoV-2 in patients with confirmed or suspected COVID-19 within 3–40 days after symptom onset (Xiang et al., 2020). An immunological test is essential in establishing whether or not a person has COVID-19, but only in the early stages of infection (Asrani et al., 2021a). COVID-19 serodiagnosis with IgM and IgG ELISA offers high sensitivity and specificity for COVID-19 detection, which improves the accuracy of diagnosis. Researchers discovered that the sensitivity and specificity of IgM detection in confirmed COVID-19 patients was 77.3 and 100%, respectively, and that the sensitivity and specificity of IgG detection were 83.3 and 95.0%, respectively. The sensitivity and specificity for IgM in suspected COVID-19 cases were 87.5 and 100%, respectively, and 70.8 and 96.6% for IgG. As a result, the increased specificity of IgG and IgM detection improves accuracy and may aid in diagnosing COVID-19 patients (Xiang et al., 2020). Serological testing is crucial in this pandemic to discover active cases. The pandemic due to the coronavirus (COVID-19) has underlined the necessity for precise and speedy COVID-19 detection, and real-time RT-PCR is the most often used method for coronavirus identification (Asrani et al., 2021b). Molecular testing remains the "gold standard" for relevant case diagnosis (Xu et al., 2020). Other approaches that have been developed include loop-mediated isothermal amplification, clustered regularly

interspaced short palindromic repeats (CRISPR), and multiplex isothermal amplification followed by microarray detection (Loeffelholz and Tang, 2020).

In this pandemic, the most prevalent care techniques for severe cases include artificial breathing, ICU admission, and supportive care (such as bed rest, adequate nutrition, avoidance of dehydration, electrolyte and acid–base balance maintenance, antibiotics, and isolation of patients suspected or proven to have the infection), according to the WHO guidelines (Liu et al., 2020). Mass media and Public health communication contribute significantly to the pandemics from SARS in 2003, H1N1 in 2009, and MERS in 2012 (Anwar et al., 2020). At the time of COVID infection, media coverage of hardships, lockdown, and quarantine generated stress and fear in individuals. Many false media and false therapies have also been proven to be wrong, and we have all learned about them as a result of mass media coverage. From educating individuals on COVID procedures to social distance, the media plays an important part in the world today. We may also discuss the issues that will arise if the media does not perform its part. An Indonesian investigation discovered that their awareness of preventative measures was limited (Adella Halim et al., 2020). So we may conclude the role of message transmission among individuals. As a result, more emphasis should be placed on educating people rather than focusing on cleaning and maintaining the environment (Rizki et al., 2021).

Various therapies were tested to treat COVID infection which includes: use of probiotics which consists of living microorganisms that benefit human health. Probiotics show a close association with the respiratory system so can be used as a remedy to treat the infection. There are also several pre-existing medicines such as remdesivir, tocilizumab, and other existing and prospective candidates that can be considered for COVID treatment. Several anti-inflammatory like Colchicine and antimalarial drugs such as Hydroxychloroquine and chloroquine (Kumari et al., 2020) were also be tested for the treatment of COVID infection (Asrani et al., 2022). The plant produces secondary metabolites which are generated against viral infection. So considering the fact these metabolites can be targeted using nanoparticles made of carbohydrates and lipids and can be tested for COVID infection (Singh et al., 2021). Therapeutic targets include enzymes involved in viral replication and transcription, helicases and proteases, host cell proteases. Inhibition of host cell endocytosis, anti-sense RNA and ribozyme, neutralizing antibodies, mAbs targeting host cell receptors, or interfering with the S1 RBD domain have been reported (Yong et al., 2019). Focus on the vaccination process is the need of the hour to save lives, as the death rate from COVID-19 is rising every day. To compete with the current situation, more ventilators, oxygen supplies, and ICU beds are needed. However, up to 6.7% of cases might be severe. Patients under the age of one year and those with underlying problems are at a higher risk of developing severe disease. Although several pediatric reports have been published, the epidemiology and clinical patterns of COVID-19, and also treatment options, remain unknown (Tezer and Bedir Demirdağ, 2020). Pregnant women are also COVID vulnerable since there is a decrease in lymphocytes, NKG2A inhibitory receptors, and an increase in ACE2, IL-8, IL-10, and IP-10 during pregnancy, indicating a higher risk of developing COVID-19 (Phoswa and Khaliq, 2020). Various other candidate drugs such as tocilizumab, an immunosuppressive drug, were also tested for COVID-19 use (Asrani and Hassan, 2021; Hariyanto et al., 2021) but did not succeed. A drug named Dexamethasone, a glucocorticoid treatment was also studied, and it was discovered that the death rate was reduced due to its use (Horby et al., 2021). To help and end this pandemic, the production of a safe and efficient vaccine is critical. So, we can either target the Spike protein domain or the RBD domain for vaccine production (refer to **Figure 1**).

The Pfizer-BioNTech COVID-19 mRNA vaccine was the first vaccine approved by the WHO's Strategic Advisory Group of Experts on Immunization (SAGE) as safe and reliable. As we know, healthcare workers are continuously working with COVID patients, so they must be kept in the priority group before vaccinating the population. The COVID-19 vaccine from Pfizer BioNTech has a 95% efficacy against symptomatic SARS-CoV-2 infection. The Janssen Ad26.CoV2.S COVID-19 vaccine was also introduced, which is 85.4% effective against the South African and Brazilian strains of COVID-19. The Oxford/AstraZeneca COVID-19 vaccine (AZD1222) is a non-replicating vector-based vaccine that has been listed for emergency use by the WHO. One version is developed by

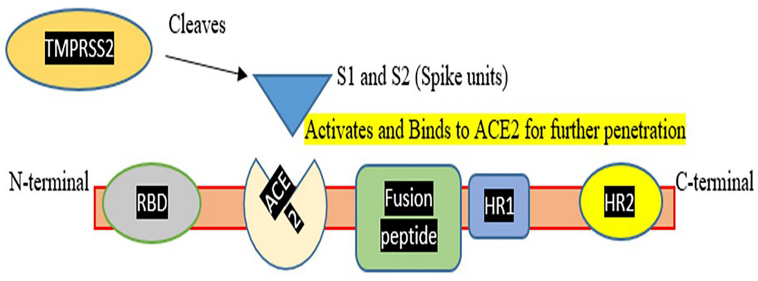

FIGURE 1 | Represents a Corona spike viral fusion protein with N and C terminals, as well as different domains and Spike assembly for hydrolytic cleavage. TMPRSS2, which aids Spike protein activation and penetration, was also demonstrated. This figure demonstrates that how TMPRSS2 cleaves spike protein so that it activates the spike protein for binding to the ACE2 receptor and mediating entry of virus in the host cell.

AstraZeneca-SKBio (Republic of Korea) and the other by the Serum Institute of India (AZD1222). Against symptomatic SARS-CoV-2 infection, this vaccine has a 63.09% efficacy rate. Also in use is the Moderna (mRNA-1273) vaccine. The Moderna vaccine has been shown to protect against COVID-19 with an efficacy of about 92%. The efficacy of the Moderna mRNA vaccine is unaffected by the recent SARS-CoV-2 versions, such as B.1.1.7 and 501Y.V2. Bharat Biotech developed the inactivated BBV152 vaccine with 80% efficacy against COVID-19 (World Health Organization). Sinovac was the first vaccine used in Indonesia against COVID infection (Heriyanto et al., 2021). In Brazil trials, it was identified that Sinovac should be administered in 2 doses and shows the efficacy of 51% against symptomatic SARS-CoV-2 infection and it is 100% effective against severe COVID-19 infection after receiving a 2nd dose (Organization World Health, 2021). Sputnik V has an efficacy rate of 91.6% which has been authorized for use in 70 countries around the world but yet not approved by the U.N. Health (Jones and Roy, 2021). There are also Sinopharm Beijing and Sinopharm Wuhan, both of which have a 79% effectiveness rate against symptomatic COVID strains. The Pfizer vaccine has the greatest efficacy rate of all vaccinations approved by the WHO. The majority of vaccines are based on a buffer dose, which means that two doses must be administered to an individual to develop enough antibodies in the individual's body to protect against COVID infection. Buffer doses play a significant role in COVID vaccinations because they provide a threshold at which enough antibodies are developed to fight COVID infection (Kathy, 2021). To forecast trends in COVID instances, we require an online database to evaluate future predictions or COVID patterns. To find patterns in COVID cases or fatalities with other parameters, the data must be classified into distinct classifiers based on the availability of the data. And, after the parameters are specified, the data in the model must be updated daily (Wang, 2021). This will result in an accurate predictor model. The major goal of the study is to compare the effects of vaccinations on vaccinated and unvaccinated people. This research will conclude on the effectiveness of vaccines and the necessity for a buffer dose to build enough antibodies in a person to protect against COVID infection. By applying numerous machine learning models, we will analyze the accuracy of the model and compute the percentage decrease in COVID cases and deaths as a result of immunizations.

MATERIALS AND METHOD

Source of Data

The data used in this work were obtained from the publicly released database 'Our World in Data' (Mathieu et al., 2021). The data are publically released and available at www.ourworldindata.org/covid-vaccinations. The data include the number of COVID-19 confirmed cases, number of deaths, and vaccination doses that are given to people in 48 Asian countries from February 24, 2020, to September 26, 2021. However, the database gets updated daily on the website and gives all the information related to the COVID pandemic.

Data Pre-Processing and Normalization

Data cleaning and pre-processing is a crucial step before starting any analysis as it makes our data reliable for further calculations. Data taken from online databases offer structured data on a day-to-day basis. All variables were converted into monthly data, namely, COVID cases, deaths, and people vaccinated for dosage 1 and dosage 2. The data were normalized to fit it within a range and perform statistical computations using *Standard scaler and min–max scaler*. After data standardization and normalization, logarithmic transformations were also performed so data could become reliable for analysis. Following the data cleaning, statistical calculations were performed using linear, polynomial, OLS regression models and a support vector machine to assess the effect of vaccination over COVID cases and deaths and check the accuracy of the model. All analysis was performed on the Python platform.

Statistical Analysis

Statistical analysis is essential in verifying assumptions and demonstrating them to create a concrete conclusion about a study. This study focuses on the efficacy of vaccination over COVID cases and COVID deaths. The linear regression analysis will investigate the relevance of vaccinations, followed by polynomial and OLS regression models and SVM models. This will provide information about the effectiveness of being immunized.

Linear Regression

In linear regression, two variables are employed: one is the dependent variable (plotted on the y-axis) on which the prediction is based, and the other is an independent variable (plotted on the x-axis) utilized to make the prediction. Variable-based prediction might be univariate (based on one variable) or multivariate (based on several variables) (Moore et al., 2013). A regression line is a straight line that explains how the dependent variable changes with the change in the independent variable. To contrast the model predictions against several sets of field data, we use vaccine dose data to calculate the number of COVID-19 cases and the number of people dying considering the above factors. We fit the model with

$$\text{Regression line, } y = mx + c, \tag{1}$$

where c is an intercept and m is the slope and y is the dependent variable and x is the independent (explanatory) variable r^2 is the coefficient of determination which is calculated by Karl Pearson's coefficient (r) (Calkins, 2005). This coefficient indicates how many variations are explained by the variable being predicted. The greater the slope, the greater the correlation between the variables, and the greater the ability to explain fluctuations in other variables. Linear regression improves prediction since it focuses on situations with one or more predictor variables (in our study, vaccine data for First Dose and Second Dose) and one outcome variable (Marill, 2004).

$$\text{Multiple linear regression, } y = ax + bz + c, \tag{2}$$

where a and b are coefficients of regression, c is the intercept while having x and z as multiple explanatory variables.

The outcome variable, *y*, is a linear function of each predictor variable, *x*, and *z*, forcing the regression model to be a straight line (Marill, 2004). For a good model, the *r-value* should lie in 0.5 to 1.0 so this score gives a good correlation and a good predictor. Regression *analysis* is also used to predict the *p-value* for significance testing. The statistical inference approach is based on a complex network that includes assumptions about how data was gathered and analyzed and how the research results were presented.

RMSE and r^2-Value

The *RMSE* is the square root mean error. This error value gives an idea about the fitness of the model, i.e., how the values deviate from the true value. *RMSE* is an absolute measure of fit, while *R-squared* is a relative measure of fit. *RMSE* can be interpreted as the standard deviation of the unexplained variance since it is the square root of the variance. It has the advantage of being in the same units as the answer variable. The lower the *RMSE* value the better will be the prediction. If the main goal of the model is prediction, the *RMSE* is the essential criterion for fit because it is a valid standard of how well the model predicts the response.

$$Mean\ Square\ Error = True\ value - Predicted\ value \quad (3)$$

$$Root\ mean\ square\ error = (True\ value - Predicted\ value)^2 \quad (4)$$

We square the error because the estimate can be above or below the true value, resulting in a negative or positive difference. If we didn't square the errors, the sum might fall due to negative differences rather than a strong model fit. Lower values of *RMSE* indicate a better fit.

Polynomial Regression

For a good predictor model, polynomial regression is the best approach. Some points which do not fit in linear regression fit best in polynomial regression. If the linear regression is underfitting, then we plot polynomial regression to increase complexity in the model by increasing the power of the features and making them new features. All models for degree quadratic, cubic and quartic were constructed using python, and based upon the *RMSE* value, the best was selected. Equation (5) shows a polynomial regression curve of degree 4.

$$\text{Polynomial regression of degree4, } y = x^4 + ax^3 + bx^2 + cx + d, \quad (5)$$

where, *a*, *b*, and *c* are coefficients of regression and *d* is the intercept. The polynomial curve can be studied for the complexities of COVID cases and deaths based upon vaccination Dosage 1 and Dosage 2.

OLS Regression Model and P-Value Interpretation

The *p-value* is the "probabilities" of hypotheses. When we perform statistical significance analysis based upon the hypothesis we have designed; if there is a condition in which we have a *p-value* that is very low like 0.0 (although it is not exactly 0), that means that there is a strong correlation between the coefficients and the target (Princeton University). Statistical "significance tests" based on this concept have been a central part of statistical analyses for centuries (Stigler Stephen, 2003). Traditional *p-value* and statistical significance notions have emphasized null hypotheses, treating all other assumptions used to calculate the *p-value* as if they were true. Recognizing that the other assumptions are always suspect, if not outright false, we'll look at the *p-value* in a broader sense as a statistical overview of the compatibility between observed results and what we would expect to see if the entire statistical model (all the assumptions used to compute the *p-value*) were correct. And then, we have an alternate hypothesis in which it is opposite to the null hypothesis. Based on *p-value*, i.e.,

If *p-value* >0.5; so, we accept null hypothesis
If *p-value* <0.5; so we reject null hypothesis

And we can make a concrete statement about our study do have relevance or significance or not. In logical terms, we can say that the *p-value* tests all assumptions related to the model developed, not only focusing on the target hypothesis, i.e., the null hypothesis. OLS regression uses *Student's t distribution* for calculating class intervals from which *p-value* can be interpreted.

To perform the statistical analysis, we can use the OLS regression model, which stands for ordinary least square regression used to compute unknown parameters in the Regression model (linear or polynomial). The method of OLS provides minimum-variance mean-unbiased estimation when the errors have finite variances and are normally distributed. OLS is the maximum likelihood estimator. The (squared) vertical distance from each data point to the line is reduced overall data points by using OLS regression to match a line to bivariate data. The equation:

$$bOLS = Cov(x, y)/var \quad (6)$$

describes the slope of this axis *(x)*. As a result, OLS slopes change whether either the way *x* and *y* covary or the variance of the *x*-axis variable changes (Sokal and Rohlf, 2012). This OLS regression calculates a *p-value* which is easy to interpret based on all variables. OLS model was chosen as it gives a reasonable interpretation of models generated and a better way to access the model's relevancy. Using the stats model package, the OLS regression was calculated and the hypothesis generated as stated below:

Null hypothesis = There is no significant effect of people vaccinated over COVID cases and COVID deaths

Alternate hypothesis = There is a significant effect of people vaccinated over COVID cases and COVID deaths.

Using this model, we can be clearer about the significance of vaccinations.

Support Vector Machine Algorithm

Calculating the accuracy of a model-designed support vector machine (SVM) algorithm is a good measure. It is a supervised learning algorithm that classifies data into 2 classes based upon which training is done and then, using that, future learning

classifications are made. These algorithms are more efficient as their performance is high. Using SVM, a hyperplane can be plotted between datasets which are called a decision boundary. Based upon that classifier, classification can be performed. This is an advanced version of the linear and polynomial regression model analyzed above. By using SVM we can make some predictions and these predictions can be compared with the actual values and in the last, the accuracy of the model can also be obtained (Bruno, 2017). SVM regression *analysis* will complete our *analysis* and tell us about the efficacy of the model and decrease the error value of the model by making it more precise.

RESULTS

Linear Regression Analysis

The study was done by taking monthly cumulative data of 48 countries from the Asian continent. **Figure 2** is plotted using Tableau (Tableau Software, 2003) software which shows the COVID cases in Asian countries. The color bar designated the incidence of the color scheme based upon the prevalence of COVID cases in a country. The overall COVID cases prediction was performed on the class intervals at 95% confidence and p-value interpreted on for First Dose was 0.0914 [0.087–0.096] (p-value <0.05) and for Second Dose of vaccination was 0.2654 [0.250–0.281] (p-value <0.05). After this, the analysis of the overall death prediction rate on vaccination First Dose was 0.0012 (95% Class interval [0.001–0.001]. Overall death prediction rate on vaccination Second Dose was 0.0034 (95% Class interval [0.003–0.004]. Linear regression analysis revealed the vaccination; these show a positive correlation between variables (vaccination Dose 1 and Dose 2) and target (COVID cases, Deaths). A mathematical model that underpins the approach encapsulates the entire set of assumptions. This model is a statistical representation of data variability, and in theory, it accurately captures all data variability sources. In our model—based upon vaccination doses, i.e., First Dose (partially vaccinated) and Second Dose (fully vaccinated), COVID cases and COVID deaths are calculated and a linear regression analysis was performed. All the models constructed were developed on the python platform. The regression line was plotted for the model designed. Some predictions were performed like if we have 9.5 individuals who were vaccinated for First Dose, 5.5 individuals vaccinated for Second Dose, so we can calculate the decrease in COVID cases and COVID deaths using this linear regression equation and calculating a percentage decrease in the number of cases and deaths upon taking dose 1 and dose 2. For better analysis, the r-value was calculated for each independent variable.

Figure 3A represents the linear regression model plotted for the number of COVID cases and the number of people vaccinated for the First Dose. This model shows a strong correlation between both the target and variable and the *r-value* is 0.2176. The *RMSE* value is 0.8844 is significantly less, so this is a good predictor. Equation (7) represents the regression line equation for calculating COVID cases based upon the First dose of vaccine. From the regression graph, we can conclude that as the number of vaccination doses increases, there was no effect on COVID cases initially. Still, with time there is a decrease in COVID cases as the graph clearly shows that reaching towards more vaccinated individuals, the cases are decreasing.

$$\begin{aligned} &\textit{Number of COVID cases} \\ &= 0.46658304 * \textit{Number of people vaccinated for First dose} \\ &\quad + 2.46406751e^{-18} \end{aligned} \tag{7}$$

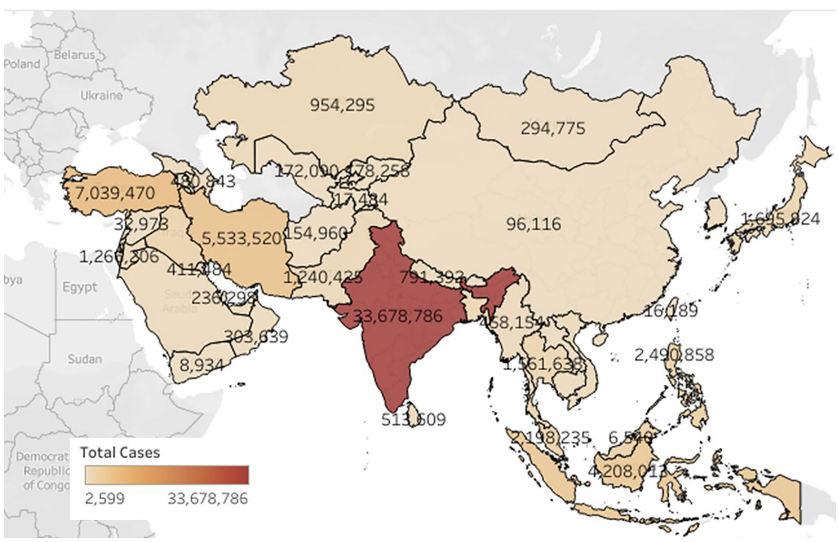

FIGURE 2 | Represents the prevalence of COVID-19 cases in the Asian continent.

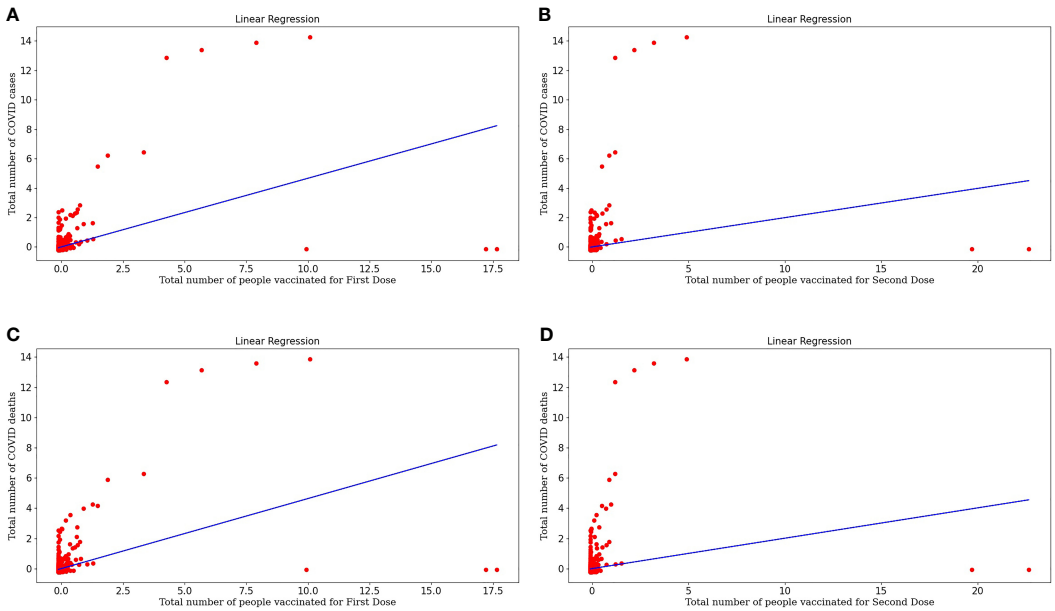

FIGURE 3 | **(A)** Represents linear regression model plotted for Number of COVID-19 cases and Number of people vaccinated for the First Dose. **(B)** Represents linear regression model plotted for Number of COVID-19 cases and Number of people vaccinated for Second Dose. **(C)** Represents linear regression model plotted for Number of COVID-19 deaths and Number of people vaccinated for the First Dose **(D)** Represents linear regression model plotted for Number of COVID-19 deaths and Number of people vaccinated for Second Dose.

Figure 3B represents the linear regression model plotted for the number of COVID cases and the number of people vaccinated for the Second Dose. This model shows a good correlation between both the target and variable and the *r-value* is 0.0395 and *RMSE* value is 0.9800, which is less so this model fits well. Equation (8) represents the regression line equation for calculating COVID cases based upon the Second dose of vaccine. From the regression graph, we can conclude that as the number of Second vaccination doses increases, COVID cases decrease. The straight-line plot shows that with an increase in doses, there is a decrease in COVID cases. Also, Dose 2 is more effective in decreasing cases as there are few red spots on the graph, designating more decrement in cases upon taking Dose 2 than Dose 1 of the vaccine because taking the second dose after the first dose generates enough amount of antibodies against COVID infection.

$$\textit{Number of COVID cases} = 0.19890426 * \textit{Number of people vaccinated for the Second dose} - 1.18585991e^{-17} \quad (8)$$

Figure 3C represents the linear regression model plotted for the Number of COVID deaths and the number of people vaccinated for the First Dose. This model shows a good correlation between both the target and variable and the *r-value* is 0.2147 and the *RMSE* value is 0.8861 which is less so this model fits well. Equation (9) represents the regression line equation for calculating COVID deaths based upon the First dose of vaccine. From the regression graph, we can conclude that as the number of vaccination doses increases, there is a decrease in COVID deaths and vaccination plays a critical role in the decrement of COVID cases.

$$\textit{Number of COVID deaths} = 0.46342108 * \textit{Number of people vaccinated for First dose} + 1.71500253e^{-17} \quad (9)$$

Figure 3D represents the linear regression model plotted for the Number of COVID deaths and the number of people vaccinated for the Second Dose. This model also shows a good correlation between both the target and variable and the *r-value* is 0.0403 and the *RMSE* value is 0.9795 which is less so this is a moderately fitted model. Equation (10) represents the regression line equation for calculating COVID deaths based upon the Second dose of vaccine. From the regression graph, we can conclude that as the number of Dose2 vaccinated individuals increases, there is a huge decrease in deaths. This makes a concluding statement about the effectiveness of vaccinations, especially two doses that act as a buffer against COVID infection.

$$\textit{Number of COVID deaths} = 0.200961 * \textit{Number of people vaccinated for Second dose} + 2.97482365e^{-18} \quad (10)$$

Root Mean Square Error and r-Value Analysis

RMSE is a good measure of how accurately the model predicts the response. RMSE value doesn't lie in any range but should be less than the sample size. So a lesser RMSE value will be a good fit. **Table 1** shows the RMSE and r-value calculated for each model using a linear regression model.

From the *r-value*, we can say that the data variable explains this percentage of variability and tells us about the relationship between variables.

Polynomial Regression and P-Value Testing

All models designed above for linear regression have a low mean squared error value. But the linear regression results were not satisfactory to make a concrete decision as some data points are far away from the linear regression line so the polynomial regression was performed for quadratic, cubic, and quartic degrees.

After designing all the degree models for polynomial regression we concluded that the quartic degree polynomial fits better for our model, as it has the lowest *RMSE* value. Also, the resulting polynomial curve can be extrapolated or interpolated to predict death based on the number of people who have been vaccinated. We used this prediction model and generated 70% testing and 30% training data sets using the sklearn package.

Equation (11) is a polynomial equation that can be used to calculate the number of individuals diagnosed as COVID positive, considering the effect of the first dose of vaccine *(x)*, where 0.04421746 is the intercept and all other multiplications with x are the coefficients of the polynomial equation. Also, **Figure 4A** represents the polynomial regression model for quartic degree plotted for people vaccinated for First Dose plotted against the number of COVID positive patients. From the above polynomial regression curve, we can identify a pattern about vaccinations, i.e., initially, we can see more data sets, i.e., more COVID cases and there were no vaccinations available. But with the time the vaccinations started, and then there was a decrease in cases with an increase in vaccinations as with time we can see a decrease in color spots in the regression curve plotted.

$$\begin{aligned}&\textit{Number of COVID positive patients}\\&= 1.55622435 * x^4 + 0.63234385 * x^3 - 0.11061507 * x^2\\&\quad + 0.00396629 * x + 0.04421746\end{aligned} \tag{11}$$

Equation (12) is a polynomial equation that can be used to calculate the number of individuals diagnosed as COVID positive, considering the effect of the second dose of vaccine *(x)*, where 0.16024788 is the intercept and all other multiplications with x are the coefficients of the polynomial equation. **Figure 4B** represents the polynomial regression model for quartic degree plotted for people vaccinated for the Second dose of vaccine plotted against the number of COVID positive patients. Polynomial regression curves are more reliable than linear regression curves as they consider the complexities of variables. From the regression, curve plotted we can conclude that with an increase in Dose 2, there is a sharp decrease in COVID cases which tells about the effectiveness of the buffer dose after the first dose.

$$\begin{aligned}&\textit{Number of COVID positive patients upon getting both vaccine doses}\\&= 3.60870165e^{+00} * x^4 + 7.78207554e^{-02} * x^3\\&\quad - 3.20635017e^{-02} * x^2 + 9.52339532e^{-04} * x\\&\quad + 0.16024788\end{aligned} \tag{12}$$

Equation (13) is a polynomial equation that can be used to calculate the number of individuals who died considering the effect of the first dose of vaccine.

$$\begin{aligned}&\textit{Number of people died due to COVID upon taking Dose}1\\&= 1.80295049 * x^4 + 0.51069827 * x^3 - 0.09657263 * x^2\\&\quad + 0.00351539 * x + 0.06844712\end{aligned} \tag{13}$$

where x is the number of vaccinated people partially with a complex of power 4 and 0.06844712 is the intercept and all other multiplications with x are the coefficients of the polynomial equation. **Figure 4C** represents the Polynomial regression model for quartic degree plotted for people vaccinated for First Dose plotted against the number of COVID deaths. Polynomial regression curve plotted for COVID deaths and vaccination doses, we can conclude that with an increase in Dose 1, COVID deaths decrease.

$$\begin{aligned}&\textit{Number of people died due to COVID upon taking Dose}2\\&= 3.76222600e^{+00} * x^4 - 8.88558776e^{-03} * x^3\\&\quad - 2.48292596e^{-02} * x^2 + 7.89026296e^{-04} * x\\&\quad + 0.17096195\end{aligned} \tag{14}$$

Equation (14) is a polynomial equation that can calculate the number of deaths considering the effect of dose 2, where x is the number of people who are entirely vaccinated with a complex of

TABLE 1 | Representing *r-value* and *RMSE* error value for all variables used and model designed for a linear regression model.

Model Serial number	Models Designed	*RMSE* value	r-value (Variability explained by data)
1.	Number of Cases and Dose 1 vaccinated individuals	0.88447	0.2176
2.	Number of Cases and Dose 2 vaccinated individuals	0.9800	0.0395
3.	Number of Deaths and Dose 1 vaccinated individuals	0.9800	0.2147
4.	Number of Deaths and Dose 2 vaccinated individuals	0.9795	0.0403

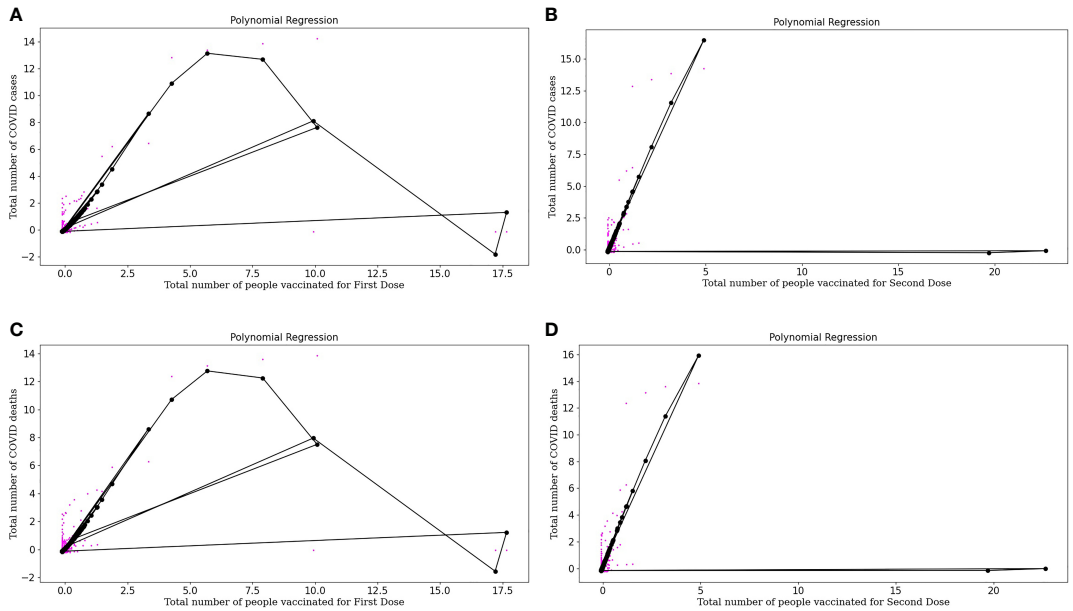

FIGURE 4 | **(A)** Represents Polynomial regression model for quartic degree plotted for People vaccinated for First doses plotted against Number of COVID-19 positive patients. **(B)** Represents Polynomial regression model for quartic degree plotted for People vaccinated for Second doses plotted against Number of COVID-19 positive patients. **(C)** Represents Polynomial regression model for quartic degree plotted for People vaccinated for First doses plotted against Number of COVID-19 deaths. **(D)** Represents Polynomial regression model for quartic degree plotted for People vaccinated for Second Dose (both dose of vaccine) and Number of COVID-19 deaths.

power 4 and 0.17096195 is the intercept and all other multiplications with x are the coefficients of a polynomial equation. **Figure 4D** represents the polynomial regression model for quartic degree plotted for people vaccinated for Second Dose (both doses of vaccine) and the number of COVID deaths. From the regression curve plotted, we can conclude that with an increase in Dose 2, there is a sharp decrease in COVID deaths which tells us about the effectiveness of buffer dose.

After fitting the curve for degree 4 the prediction was made based upon the number of people vaccinated. Based upon the above problem and the prediction model developed, we predicted the number of people who are diagnosed COVID-19 positive and also who died based upon the vaccination doses received. **Table 2** represents the *RMSE* and r-values for the models designed regarding COVID cases and COVID deaths for the First Dose and Second Dose vaccinated individuals. If 9.5 people are vaccinated for First Dose, then the number of people who die due to COVID will be = 4.4020 number of people and when some people received their Second Dose, the number of people who died is reduced to 1.1058.

Upon estimating this we also predicted the number of cases, i.e., they are decreasing upon vaccination. So, an *analysis* was made on the prediction of COVID cases. We found that if 9.5 people are vaccinated for First Dose, then the upcoming cases were 4.43 and if they are vaccinated for Second Dose, these cases will reduce to 1.093. So this concludes that upon vaccination rate of COVID-19 infection will also reduce to 75.319% and the COVID death rate will also reduce to 74.89%.

As a result of this estimate, we can conclude that vaccination is necessary to reduce the death risk and the infection rate, so more vaccination centers should be established for people to be vaccinated. From the above analysis, we can make a concrete statement about the necessity of vaccination. After this, we can perform *OLS regression* for better understanding and hypothesis testing. Polynomial regression model revealed about the COVID cases which reduced up to 74.89% upon vaccination and death rate which reduced to 75.31% after receiving the Second dose of vaccination which is a great decrease in death number which makes a concrete statement that being vaccinated decreases the risk of death due to COVID. The null hypothesis was rejected after analyzing the *p-value* using the OLS regression algorithm as the *p-value* (0.05) >0.00. We can say there is a significant effect of vaccination over COVID cases and death numbers. Using *Student's t distribution* test, the *p-value* was calculated. This tells us about the behavior of the sample mean on the number of observations. Using the OLS regression model using a 95% confidence interval, the class interval was checked. The coefficients lie between these confidence intervals, so we can say that the *p-value* is either 0.05 or 0 so the OLS regression model *p-value* was calculated. It is 0.00, which is less than 0.05, so the null hypothesis is rejected. We can say there is a significant effect of people vaccinated over non-vaccinated for COVID death and cases. This whole study shows that after being vaccinated for both, the Doses of vaccine, the death rate, and COVID cases will decrease, making a significant difference. The accuracy of the models used may be measured by overlaying the

TABLE 2 | Representing *r-value* and RMSE error value for all variables used in the polynomial regression model.

Model Serial number	Model Designed	Degree of polynomial Fitted best	*RMSE* value	r-value
1.	Number of Cases and Dose 1 vaccinated individuals	Degree 4	0.4678	0.7811
2.	Number of Cases and Dose 2 vaccinated individuals	Degree 4	0.5270	0.7221
3.	Number of COVID deaths and Dose 1 vaccinated individuals	Degree 4	0.4884	0.7614
4.	Number of COVID deaths and Dose 2 vaccinated individuals	Degree 4	0.5379	0.7106

predicted and actual datasets, and for this, a more advanced regression approach, such as Support Vector Regression, will be used for further analysis.

Support Vector Regression Analysis

Using SVM, the accuracy of the model was tested and it showed 61.45 and 67.96% accuracy rates for COVID cases and COVID deaths over Dosage 1 and Dosage 2 vaccinated individuals, respectively, which is a good performance score for the model designed. **Table 3** represents the *RMSE* error value and the *r-value* calculated for the model.

The SVM model is best in all the models discussed above, and the root means the squared error is also reduced. This makes our model more reliable. To verify the performance of the model created using SVM accuracy rate calculation is the most crucial step. The accuracy rate of the model was calculated for 30% testing datasets and 70% training datasets and it was found that the model generated using SVM for Prediction of COVID cases and deaths corresponding to Dosage 1 vaccinated population was 61.45% and for Prediction of COVID cases and deaths corresponding to Dosage 2 vaccinated population was 67.96%. This accuracy rate is excellent and we can conclude that our Support Vector Machine model shows good performance and accurate results.

Figure 5A represents the support vector machine model for people vaccinated for the First Dose and the number of COVID cases. In the above graph, we can study the original and predicted dataset pattern by considering the spots on the plot.

Figure 5B represents the support vector machine model for people vaccinated for the Second Dose and the number of COVID cases. From the plot, we can conclude about the model's efficacy, and the predicted dataset is following the slightly same pattern as the points of the original dataset. So, this tells us about the excellent performance of the model.

Figure 5C represents the support vector machine model for people vaccinated for the First Dose and the number of COVID deaths.

Figure 5D represents the support vector machine model for people vaccinated for the Second Dose and the number of COVID deaths. In these plots, original datasets and predicted datasets are plotted in blue and crimson colors, respectively. As the performance of the model is high, our predictor model is good and there is an effect of vaccinations over the decrease in the rate of COVID cases and deaths. By plotting to scatter plots for support vector regression, we can conclude that our predicted datasets and original datasets are overlapping. Hence, we can say that our predicted dataset is good. SVM regression is more accurate and also decreases the error value. Support vector regression makes the model more reliable and stable.

Performance of All the Models Analyzed

We have used Linear Regression, Polynomial Regression, and Support Vector Machine algorithms to analyze the COVID data and make concluding remarks. Initially, in linear regression results, we observed that some data points are far away from the regression line. However, when we drew a polynomial curve, the findings revealed that all data points were considered, resulting in a more impactful analysis. **Table 4** below represents the *RMSE* value and *r-value* calculated for each model using different machine learning algorithms. After analyzing all models designed using different machine learning algorithms, we identified that SVM is the best of all and it gives more accurate results. The *RMSE* error value is minimum and hence, the performance of the model is high.

DISCUSSION

People will benefit from such a model by lowering their risk of COVID-19 infection, which reduces to 75.31 and a 74.89% decrement in the death rate on getting fully vaccinated. We may argue that prediction is constrained since we used data from an online database that is not unique to a patient but is a summary of everyday COVID events. We might have stronger predictions if we had person-level data outside the scope of the work we discussed above. Various cells in the data are null, or data might not be available for that day, making interpretation difficult. As the pandemic progresses, this model will require an update, recalibration, and redesigning. Our study isn't meant to evaluate the genuine issue of COVID cases and vaccination,

TABLE 3 | Representing *r-value* and *RMSE* error value for all variables used in the support vector regression model.

Model Serial number	Model Designed	*RMSE* value	*r-value*
1.	Number of Cases and Dose 1 vaccinated individuals	0.4051	0.4537
2.	Number of Cases and Dose 2 vaccinated individuals	0.4746	0.2496
3.	Number of COVID deaths and Dose 1 vaccinated individuals	0.4772	0.4743
4.	Number of COVID deaths and Dose 2 vaccinated individuals	0.5402	0.2810

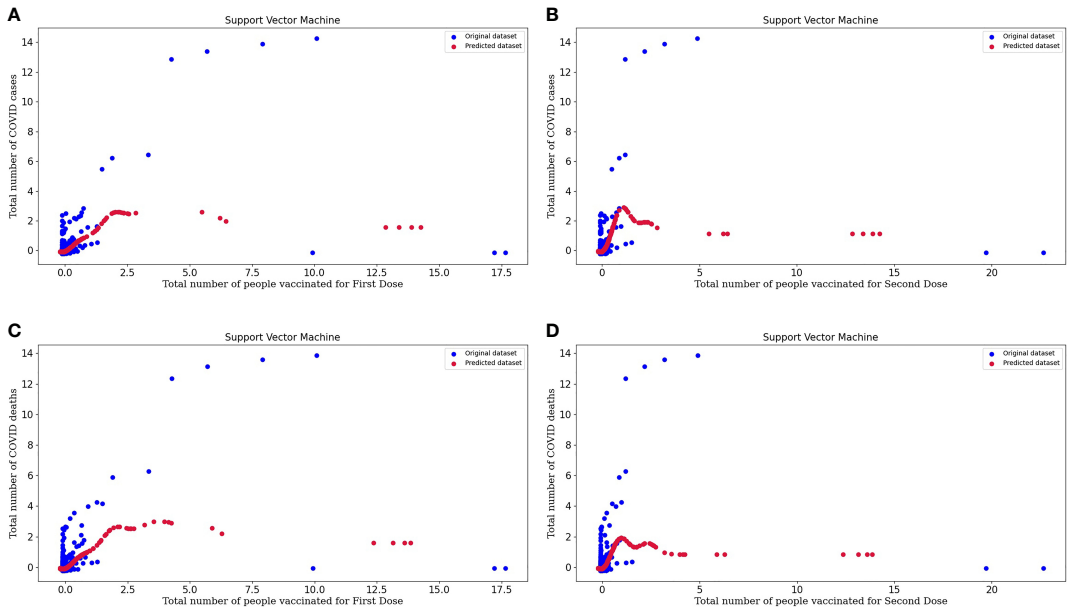

FIGURE 5 | **(A)** Represents the Support vector machine model for People vaccinated for the First Dose and the Number of COVID-19 cases. **(B)** Represents the Support vector machine model for People vaccinated for the Second Dose and the Number of COVID-19 cases. **(C)** Represents the Support vector machine model for People vaccinated for the First Dose and the Number of COVID-19 deaths. **(D)** Represents the Support vector machine model for People vaccinated for the Second Dose and the Number of COVID-19 deaths.

TABLE 4 | Represents the comparison of *r-value* and *RMSE* error value for all models analyzed using a different machine learning algorithm.

Models	Linear Regression		Polynomial Regression		Support Vector Machine	
	***RMSE* value**	***r-value***	***RMSE* value**	***r-value***	***RMSE* value**	***r-value***
Number of Cases and Dose 1 vaccinated individuals	0.88447	0.2176	0.4678	0.7811	0.4051	0.4537
Number of Cases and Dose 2 vaccinated individuals	0.9800	0.0395	0.5270	0.7221	0.4746	0.2496
Number of deaths and Dose 1 vaccinated individuals	0.9800	0.2147	0.4884	0.7614	0.4772	0.4743
Number of deaths and Dose 2 vaccinated individuals	0.9795	0.0403	0.5379	0.7106	0.5402	0.2810

which would necessitate a community-based approach to healthcare delivery research, which is not possible due to data limitations. As we all know, certain vaccinations do not require a buffer dose of vaccination while others do. To ensure data reliability, only Asian countries were included in the analysis, because the majority of Asian countries employ buffer dose vaccines. Other factors such as lockdowns, social distancing, and improved healthcare facilities might also significantly affect the death rate. Still, these factors have not been included in the present study.

CONCLUSION

A predictor model is being developed that calculates the number of people getting infected for COVID-19 upon taking vaccinations and who died due to COVID-19 based upon the vaccination doses. This estimate is based on people who received their first or both doses of vaccine and can conclude the need for vaccination. The vaccination process has started is going on in the world currently. We can consider two types of population: one that has encountered COVID-19 and the other that is not COVID positive. There are numerous challenges with getting vaccinated nowadays. Therefore we may divide our population into three groups: those who have got First Dose, those who delay taking Second Dose, and those who have already got the Second Dose. The immunization panel stated that a COVID patient develops antibodies naturally over 100–180 days. They are immune and should be vaccinated after 6 months of recovery. The population should be vaccinated since the body will generate an immune response after the first dosage, increasing immunogenicity and vaccination efficacy. We may also claim that Second Dose is required since it will produce a sufficient level of antibody response in our bodies, allowing patients to fight infection more effectively than non-vaccinated people. Nowadays, the second buffer dose duration time is increased by the WHO and also by the institutes which are developing a vaccine as if the second dose is taken after an interval of 6 to 8 weeks, then it is more efficient. Now it has been approved according to the

United Kingdom COVID-19 working group. The dosing interval can be increased to 12–16 weeks. Also, a study published in an international medical journal reported that we should increase the dosing interval of the vaccine. This interval will increase the efficacy of the vaccine from 55.1 to 81.3%, which is quite higher in building immune response (Calisher et al., 2020). It was also reported that receiving the first dose of vaccine raises immunity by 64% while receiving the second dosage raises immunity by 94% (Centre of Disease Control and Prevention). Vaccination is critical, especially both dosages, to build enough immunological response in a person's body to render them immunity against COVID-19 infection. People will benefit from such a model by lowering their risk of COVID-19 infection, which reduces to 75.31% on getting fully vaccinated and the death rate. We have demonstrated a 75.31% decrease in COVID cases and a 74.89% decrement in the death rate upon getting fully vaccinated. After analyzing various machine learning algorithms in the above study, we identified that the high SVM model performance predicts more accurate results. As a result, we can make a clearer statement regarding the necessity for vaccines, and we can recommend that the number of vaccine doses and vaccination facilities must be increased. We must overcome the inevitability of a shortage of funding, vaccine output, and inequity in health care. Vaccination is critical, especially both dosages, to build immunity against COVID-19 infection and lower the risk of death.

DATA AVAILABILITY STATEMENT

Data used for this article are available at: www.ourworldindata.org/covid-vaccination.

AUTHOR CONTRIBUTIONS

VR, IKS, and AS conceived and designed research. VR, MB, T, PS, RA, MFA, AH, MIH, IKS, and AS collected and analyzed the data. VR, MB, IKS, MFA, PS, RA, AH, AS, and MIH, wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SARS-CoV-2: Recent Variants and Clinical Efficacy of Antibody-Based Therapy

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Multiple variants of SARS-CoV-2 have emerged and are now prevalent at the global level. Currently designated variants of concern (VOCs) are B.1.1.7, B1.351, P.1, B.1.617.2 variants and B.1.1.529. Possible options for VOC are urgently required as they carry mutations in the virus spike protein that allow them to spread more easily and cause more serious illness. The primary targets for most therapeutic methods against SARS-CoV-2 are the S (Spike) protein and RBD (Receptor-Binding Domain), which alter the binding to ACE2 (Angiotensin-Converting Enzyme 2). The most popular of these strategies involves the use of drug development targeting the RBD and the NTD (N-terminal domain) of the spike protein and multiple epitopes of the S protein. Various types of mutations have been observed in the RBDs of B.1.1.7, B1.351, P. and B.1.620. The incidence of RBD mutations increases the binding affinity to the ACE2 receptor. The high binding affinity of RBD and ACE2 has provided a structural basis for future evaluation of antibodies and drug development. Here we discuss the variants of SARS-CoV-2 and recent updates on the clinical evaluation of antibody-based treatment options. Presently, most of the antibody-based treatments have been effective in patients with SARS-CoV-2. However, there are still significant challenges in verifying independence, and the need for further clinical evaluation.

Keywords: SARS-CoV-2, variant, antibody, treatment, efficacy, neutralization

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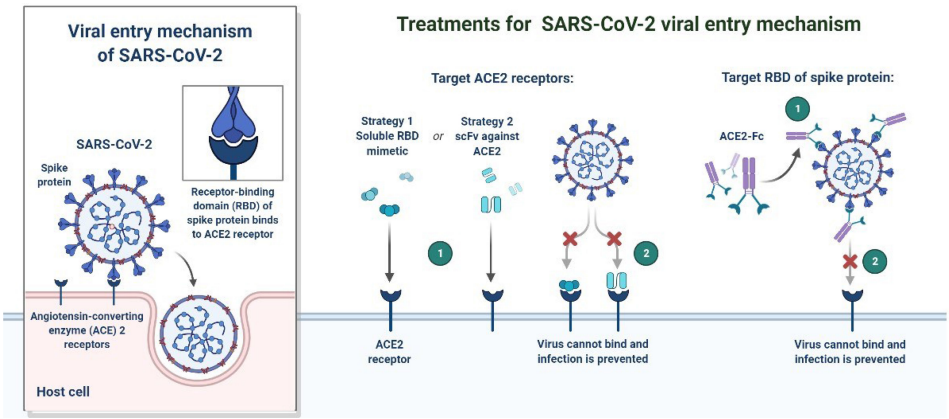

GRAPHICAL ABSTRACT |

INTRODUCTION

In December 2019, a non-specific case of respiratory disorder was reported in Wuhan, Hubei Province, Republic of China, and it was transmitted from human to human (Chen et al., 2020). SARS-CoV-2, a coronavirus, is found in more than 200 nations and territories around the world. Coronaviruses are divided into four groups: Alpha (B.1.1.7), Beta (B1.351), Gamma (P.1), Delta (D.1) and Omicron (B.1.1.529). Human coronaviruses are Alpha and Beta coronaviruses (Singh et al., 2020a). Bats are hosts to the largest number of viral genotypes of coronaviruses. Coronaviruses and their characteristics are shown in **Table 1** (Singh et al., 2020b). SARS-CoV-2 has genetic markers that have been linked to a potentially increased risk (Singh et al., 2021). New variants may elude medical treatments (Haimei, 2020). Researchers from the field at a global level were notified of the emergence of a SARS-CoV-2 variant (Kar et al., 2021). More than half of the total genomic sequencing of SARS-CoV-2 was carried out in the UK. Researchers from the field have identified eight global clades and classified them as S, O, L, V, G, GH, GR, and major lineages such as A, B, B.1, B.1.1, B.1.177, and B.1.1.7 have been identified (Koyama et al., 2020; Nayak et al., 2021). The Omicron variant, known as lineage B.1.1.529, was proclaimed a variant of concern by the World Health Organization on November 26, 2021 (Callaway, 2021). There are over 30 mutations in the variant, some of which are worrisome. The number of cases in line B.1.1.529 is increasing in all regions of South Africa. First discovered in South Africa, this new strain is now spread to more than 10 countries, including Canada, the United Kingdom, the Netherlands, Denmark and Australia. Concerns are growing around the world that the new strain will be more resistant to vaccine protection, prompting concerns that the pandemic and associated lockdown restrictions will last considerably longer than planned (Callaway, 2021). Research on Omicron has begun around the globe, but it is not yet clear if this new COVID variant is more transmissible than other previous variants such as Alpha, Kappa, Delta, etc. (Callaway, 2021). Mutations found in other VOCs include the N501Y mutation, which improves the binding of peplomer proteins to cell receptors, and the D614G mutation, which is thought to increase viral replication, both of which can increase viral infectivity. There is a sex. Others include the K417N and T478K mutations. These help the virus evade neutralizing antibodies produced by vaccination or previous infections. Researchers have discovered B.1.1.529 with 43 peplomer mutations in Rome (Callaway, 2021). The SARS-CoV-2 protein recognizes host cells and is the primary target of the body's immune response. In November, cases increased rapidly in many countries, especially schools and adolescents. Variants have spike mutations that allow detection by genotyping tests that provide much faster results than genomic sequencing. The new variant of coronavirus reportedly has more than 30 mutations in the spike protein region and therefore has the potential to develop immune escape mechanisms. Most vaccines form antibodies against the spike protein, and so many mutations in the spike protein region may lead to a decreased efficacy of therapeutic options. The effectiveness of SARS-CoV-2 therapeutic developments is affected by the new emergent variants at the global level. Antibodies against the surface of the SARS-CoV-2 are commonly used to neutralize infection (Wang et al., 2020; Diamond et al., 2021). Most of the drugs are targeted towards the receptor binding domain (RBD) of the spike protein, and multiple epitopes of the S protein, as shown in **Figure 1**.

As the protein SARS-CoV-2 and its RBD have been shown *in vitro* in cell culture, neutralization of the mAb against them effectively inhibits the binding of the virus to the host receptor, human angiotensin converting enzyme (hACE2), and thus is a major target of the mAb. Blocks viruses from invading cells (Yang et al., 2021). Some antibodies bound outside the RBD may also neutralize the virus *in vitro* using an undefined mechanism. Some of the neutralizing antibodies passively protect SARS-CoV-2 infected animal models with high efficacy (Sette and Crotty, 2021). Longitudinal studies evaluating the onset and duration of viral shedding and antibody response are needed in asymptomatic, mild, or severe patients. Here we discuss the emergence of variants of SARS-CoV-2 and the clinical evaluation of antibody-based treatment options. Presently, most of the antibody-based

TABLE 1 | List of important pathogenic coronaviruses their host organisms, genera name, and associated clinical manifestations.

S. No.	Name	Host organism	Genera name	Clinical manifestations
1	Feline infectious peritonitis virus	Cat	Alpha	Vasculitis, fever, serositis, with or without effusions
2	Camel alphacoronavirus isolate camel/Riyadh	Camel	Alpha	Asymptomatic
3	Canine CoV/TU336/F/2008	Dog	Alpha	Diarrhea and mild clinical signs
4	SeACoV-CH/GD-01	Pig	Alpha	Acute and severe diarrhea and vomiting
5	TGEV/PUR46-MAD	Pig	Alpha	Diarrhea
6	PRCV/ISU-1	Pig	Alpha	Mild respiratory tract infections (RTIs)
7	PEDV/ZJU-G1-2013	Pig	Alpha	Severe watery diarrhea
8	Human CoV-NL63	Human	Alpha	Mild RTIs
9	Human CoV-229E	Human	Alpha	Mild RTIs
10	MHV-A59	Mouse	Beta	Severe lung injuries and acute pneumonia
11	Equine CoV/Obihiro12-1	Horse	Beta	Leucopenia, fever, and anorexia
12	Bovine CoV/ENT	Cow	Beta	Diarrhea
13	MERS-CoV	Human	Beta	Severe acute respiratory syndrome
14	SARS-CoV	Human	Beta	Severe acute respiratory syndrome
15	Human CoV-OC43	Human	Beta	Mild RTIs
16	Human CoV-HKU1	Human	Beta	Pneumonia
17	IBV	Chicken	Gamma	Severe respiratory disease
18	Beluga Whale CoV/SW1	Whale	Gamma	Terminal acute liver failure and pulmonary disease
19	Sparrow coronavirus HKU17	Sparrow	Delta	Respiratory disease
20	Bulbul coronavirus HKU11	Bulbul	Delta	Respiratory disease

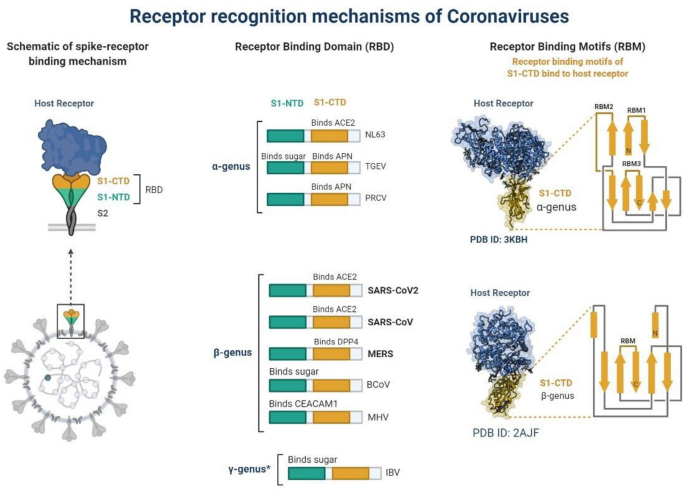

FIGURE 1 | Schematic representation of receptor recognition and binding, spike receptor binding mechanism, receptor binding domain and receptor binding motifs. * γ-genus is belong to Avian infectious bronchitis virus (IBV).

treatments have been effective in patients with SARS-CoV-2. However, there are still significant challenges in verifying independence, and a need for further clinical evaluation.

ANTIBODY-BASED TREATMENT OPTIONS

The appearance of novel SARS-CoV-2 variants has been observed all over the world, hampering the drug development process (**Tables 2**, **3**). New variants of current therapeutic options are required to maintain clinical efficacy (Sette and Crotty, 2021). More clinical investigations are required for FDA approval against emerging variants. Bamlanivimab and etesevimab, will expected stagger in efforts to improvement full FDA approval given the antiviral resistance observed against B.1.351, P.1. and B.1.526 (Doggrell, 2021). Optimization are required for its monoclonal antibody (mAb) to prove effective against the UK B.1.1.7 variant. Bamlanivimab have been observed less effective against most of the variants, but improved efficacy was observed in combination with etesevimab (Focosi et al., 2021). The FDA has cancelled the EUA for bamlanivimab as monotherapy. Combo of casirivimab/imdevimab has been observed more effective against new variants of SARS-CoV-2. Phase-III clinical trial data of casirivimab and imdevimab has been observed effective against new variants (Taylor et al., 2021).

TABLE 2 | Relative risk level for variants of concern (VOC).

Identification	WHO level	Alpha	Alpha	Beta	Delta	Gamma
	Phylogenetic Assignment of Named Global Outbreak (PANGO) Lineages	B.1.1.7	Alpha with E484K	B.1.351	B.1.617.2	P.1
	Public Health England (PHE)	VOC–20DEC–01	VOC–21Feb–02	VOC–20DEC–02	VOC–21APR–02	VOC –21JAN –02
	Nextstrain clade	20I (V1)	20I (V1)	20H (V2)	21A	20J (V3)
Emergence	**First outbreak**	United Kingdom	United Kingdom	South Africa	India	Brazil
	Earliest sample	20 Sep 2020	26 Jan 2021	May 2020	Oct 2020	Nov 2020
	Designated VOC	18 Dec 2020	5 Feb 2021	14 Jan 2021	6 May 2021	15 Jan 2021
Changes relative to previously circulating variants at the time and place of emergence	**Notable mutation**	69–70del, N501Y, P681H	E484K, 69–70del, N501Y, P681H	K417N, E484K, N501Y	L452R, T478K, P681R	K417T, E484K, N501Y
	Transmissibility	+29% (24–33%)	+29% (24–33%)	+25% (20–30%)	+97% (76–117%)	+38% (29–48%)
	Hospitalization	+52% (47–57%)	+52% (47–57%)	Under investigation	+85% (39–147%)	Possibly increased
	Mortality	+59% (44–74%) CFR 0.06% for <50 age group, 4.8% for >50 age group	+59% (44–74%) CFR 0.06% for <50 age group, 4.8% for >50 age group	Possibly increased	+137% (50–230%) CFR 0.04% for <50 age group unvaccinated, 6.5% for >50 age group unvaccinated	+50% (50% CrI, 20–90%)
Neutralizing antibody activity	**From natural infection**	Minimal reduction	Considerably reduced	Reduced, T cell response elicited by D614G virus remains effective	Reinfections happened, with smaller occurrence rate than vaccinated infections	Efficacy reduction for non-severe disease
	Vaccination	Minimal reduction	Considerably reduced	Efficacy: reduced against symptomatic disease, retained against severe disease	Efficacy reduction for non-severe disease	Retained by many

SOTROVIMAB

Sotrovimab (VIR-7831), an antibody drug, is based on the entry of coronavirus into the body. Data from phase III clinical trials revealed that this medicine lowers the rate of hospitalization and death (Gupta et al., 2021). In a recent study published in The New England Journal of Medicine, researchers theorized that a monoclonal antibody that neutralizes all SARS-CoV-2 would target a highly conserved epitope that would remain effective as SARS-CoV-2 mutates (Aschenbrenner, 2021). In the phase III, multicenter, double-blind, placebo-controlled study, SARS-CoV-2 Monoclonal Antibody Efficacy Trial–Intent to Care Early (COMET-ICE), Researchers evaluated the impact of a single intravenous infusion of sotrovimab 500 mg on mild-to-moderate SARS-CoV-2 in high-risk, non-hospitalized patients (Cheng et al., 2021). The risk of severe SARS-CoV-2 is higher in patients over 55 years old or in those who have diabetes, obesity, chronic kidney disease, chronic obstructive pulmonary disease, congestive heart failure, or moderate-to-severe asthma. One-time infusions of 500 mg of sotrovimab or placebo saline were given randomly to the patients (1:1). Primary outcomes were the percentage of patients who died or spent more than 24 hours in the hospital. A 72-day follow-up was averaged for the sotrovimab and placebo groups in the intention-to-treat population. Overall, 1% (3/291) of patients in the sotrovimab group and 7% (21/292) of patients in the placebo group had disease progression requiring hospitalization or death. In high-risk adults with symptomatic SARS-CoV-2, a single 500 mg dose of sotrovimab was found to minimize the probability of hospitalization or mortality by 85% (Aschenbrenner, 2021; Cheng et al., 2021; Gupta et al., 2021). COMET-ICE, which compared monoclonal antibodies to SARS-CoV-2 and the subsequent variants, was apparent as evidence that sotrovimab neutralized SARS-CoV-2 and its variants. Sotrovimab has also shown efficacity against variant lineages B.1.1.7, B.1.351, P.1, B.1.617, B.1.427/B.1.429 and B.1.526. Preclinical data suggest it could both block viral entry into healthy cells and clear infected cells by binding to an epitope on SARS-CoV-2 that's participated with SARS-CoV-1 (Cheng et al., 2021; Gupta et al., 2021).

LENZILUMAB

Lenzilumab is an engineered anti-human granulocyte-macrophage colony-stimulating factor (GM-CSF) monoclonal antibody designed to prevent and treat cytokine release syndrome preceding lung dysfunction and acute respiratory distress syndrome in serious SARS-CoV-2 infection cases (Bonaventura et al., 2020; Temesgen et al., 2021b). Lenzilumab aced the Phase III LIVE-AIR trial (NCT04351152), a 54 relative

TABLE 3 | List of variants for further monitoring.

Pango lineage	GISAID clade	Date of designation	Comments
R.1	GR	07-04-2021	It has found in more than 30 countries, E484K and W152L mutation have been observed, it may cause immune escape.
B.1.466.2	GH	28-04-2021	First sampled in Indonesia, in Nov 2021.
B.1.1.318	GR	02-06-2021	Detected in the UK, it was named Fin-796H after found in Finland with E484K and D796H mutations originate from Nigeria.
B.1.1.519	GR	02-06-2021	Variants Under Monitoring (VUM) in Nov 2021.
C.36.3	GR	16-06-2021	VUM in Nov 2021.
B.1.214.2	G	30-06-2021	VUM in Nov 2021.
B.1.427 B.1.429	GH/ 452R.V1	06-07-2021	VUM in Nov 2021. Epsilon, first sample was observed in the United States.
B.1.1.523	GR	14-07-2021	VUM in Nov 2021.multiple countries
B.1.619	G	14-07-2021	VUM in Nov 2021.multiple countries
B.1.620	G	14-07-2021	Detected in Lithuania, Central Africa, North America, France and Belgium, the lineage contains an E484K, P681H, S477N and D614G mutation
C.1.2	GR	01-09-2021	It was detected in England and China, Portugal, Switzerland, Democratic Republic of the Congo (DRC), Mauritius, and New Zealand with multiple substitutions C136F, R190S, D215G, Y449H, N484K, N501Y, H655Y, N679K and T859N and deletions (Y144del, L242-A243del) in the spike protein.
B.1.617.1	G/ 452R.V3	20-09-2021	Kappa
B.1.562	GH/ 253G.V1	20-09-2021	Iota
B.1.525	G/ 484K.V3	20-09-2021	Eta
B.1.630	GH	12-10-2021	Identified in March 2021, Dominican Republic.
B.1.1.529	GR/ 484A, 200	24-11-2021	Named Omicron by the WHO, identified in November 2021 in more than 15 countries.

enhancement in the liability of survival without ventilation (SWOV) vs. placebo (Temesgen et al., 2021a). SWOV liability bettered by 92 in actors who entered both corticosteroids and Gilead Lores remdesivir (Veklury), and triple in cases under 85 times of age with a C-reactive protein position of< 150 mg/L. In the NIAID- patronized, placebo- controlled Phase II ACTIV-5 Big Effect Trial (NCT04583969), lenzilumab is being studied alone and in combination with Veklury to help and treat cytokine storms. Lenzilumab is also being researched for a variety of other indications. In May, Lenzilumab Humanigen submitted an application to the FDA for an emergency use authorization (EUA) for lenzilumab to treat SARS-CoV-2 patients hospitalized. Lenzilumab has been proven to be effective against the B.1.1.7, P.1, B.1.617, B.1.427/B.1.429, and B.1.526 variant lineages (Bonaventura et al., 2020;Temesgen et al., 2021b).

BAMLANIVIMAB

Bamlanivimab (LY-CoV555) is a recombinant human IgG1 mAb, that prevents viral attachment and penetration into human cells while also neutralizing the virus (Kuritzkes, 2021). The Journal of the American Medical Association released the results of a Phase 3 study of bamlanivimab among residents and staff in long-term care facilities (NCT04497987) (Dougan et al., 2021). The emergency use of LY-CoV555 700 mg in combination with etesevimab (LY-CoV016) 1400 mg has been expanded by the FDA to include post-exposure prophylaxis (PEP) to prevent SARS-CoV-2 infection or symptomatic SARS-CoV-2 infection (**Table 4**). These antibodies, which have been demonstrated to be effective against the extremely contagious Delta variant, can now be used to protect some of the most vulnerable people who are exposed to the virus with this expanded authorization (Dougan et al., 2021; Nathan et al., 2021). Bamlanivimab and etesevimab jointly retain neutralizing activity against the Alpha and Delta forms, according to pseudovirus and authentic virus studies. Because both the P.1 and B.1.351 variants exhibit reduced sensitivity to bamlanivimab and etesevimab, the distribution of bamlanivimab with etesevimab has been halted in the United States. However, in areas with low prevalence of these and other variants that have lowered susceptibility to bamlanivimab and etesevimab, the distribution of the agents has been reinstated in states (Dougan et al., 2021; Nathan et al., 2021).

AZD7442

AstraZeneca has developed two antibody cocktails known as AZD7442, which have been shown to have potent responses against SARS-CoV-2 (Mahase, 2021). In a clinical trial including 5000 volunteers, AZD7442 was reported to be 77% effective in a patient with SARS-CoV-2. AstraZeneca reported the results in August 2021 (Dong et al., 2021). That continuity would make it especially useful to immunocompromised cases who do not get important protection from vaccines (Dong et al., 2021). The federal government has reached an agreement with the company to order up to 700,000 doses of the treatment this year, but it will first need to be authorized (Dong et al., 2021). AZD7442 has been shown to be effective against the variant lineages B.1.1.7, P.1, B.1.617, B.1.427/B.1.429, and B.1.526 (Dong et al., 2021).

TABLE 4 | Recent updates on clinical data of anti-SARS-CoV-2 selected monoclonal antibodies.

Double-blind, randomized controlled trial in SARS-CoV-2 patients with mild- to -moderate	Phase	Dose concentration	Inclusion criteria	Interventions compared to placebo	Participant characteristics	Interpretation (compared to placebo)	Primary end-point	Primary outcomes (SARS-CoV-2-related hospitalizations over days)
Bamlanivimab (BAM)	Double-Blind, Phase 3	700 mg + Etesevimab + 1,400 mg in Nonhospitalized	Aged ≥12 years At high risk for severe SARS-CoV-2 patient.	BAM 700 mg + ETE 1,400 mg (n = 511) Within 3 days of a positive SARS-CoV-2, Placebo (n = 258).	Median age 56 years; 30% ≥65, 76% mild and 24% had moderate SARS-CoV-2 patient.	5% absolute reduction and 87% relative reduction in SARS-CoV-2-related hospitalizations.	defined as ≥24 hours of acute care.	Day 29: 0 in BAM plus ETE arm vs. 4 (1.6%) in placebo arm; P = 0.01.
Bamlanivimab with Etesevimab	Phase 3	Bamlanivimab 2,800 mg Plus Etesevimab (ETE) 2,800 mg in Nonhospitalized patients	Aged ≥12 years At high risk for severe SARS-CoV-2 or hospitalization	In 3 days of a positive SARS-CoV-2 patient, BAM 2,800 mg with ETE 2,800 mg (n = 518); Placebo (n = 517).	Mean age 53.8 years; 31% ≥65 years; 52% female; 48% male	Placebo with 4.8% absolute reduction and 70% relative in hospitalized patients.	Proportion of patients with SARS-CoV-2-related hospitalization	Day 7: 9.8% in BAM plus ETE arm vs. 29.5% in placebo arm (P < 0.001)
Casirivimab (CAS) Plus Imdevimab (IMD) in Nonhospitalized	Phase 3	Aged ≥18 years with SARS-CoV-2 positive; Symptom onset within 7 days of randomization; analysis only: ≥1 risk factor for severe SARS-CoV-2.	Single IV (intravenous) infusion of CAS 600 mg with IMD 600 mg (n = 736) or placebo (n = 748); CAS 1,200 mg plus IMD 1,200 mg (n = 1,355) or placebo (n = 1,341).	CAS 600 mg plus IMD 600 mg (n = 736) or placebo (n = 748), CAS 1,200 mg plus IMD 1,200 mg (n = 1,355) or placebo (n = 1,341).	Median age 50 years; 35% Hispanic/Latinx; 5% Black/ African American.	CAS 600 mg with IMD 600 mg was associated with 2.2% absolute reduction and 70% relative risk reduction in SARS-CoV-2 Patients.	Proportion of patients with SARS-CoV-2-related hospitalization through Day 29.	Day 29,7 (1.0%) in CAS 600 mg with IMD 600 mg arm vs. 24 (3.2%) in placebo arm (P = 0.002). 18 (1.3%) in CAS 1,200 mg plus IMD 1,200 mg arm vs. 62 (4.6%) in placebo arm (P < 0.001).
Sotrovimab (SOT) in Non-hospitalized patients with mild -to-moderate SARS-CoV-2	Phase -III	SOT 500 mg, Placebo (n = 292)	Aged ≥18 years with ≥1 comorbidity, aged ≥55 years, Symptom onset ≤5 days Laboratory-confirmed SARS-CoV-2.	SOT 500 mg IV (n = 291) Placebo (n = 292)	Median age 53 years; 22% ≥65 years 63% Hispanic/ Latinx; 7% Black/African American	Receipt of SOT was associated with 6% absolute reduction and 85% relative risk reduction.	Proportion of patients with all-cause hospitalization or death by Day 29	Day 29: 3 (1%) in SOT arm vs. 21 (7%) in placebo arm (P = 0.002).

BRII-196/BRII-198

BRII-196/BRII-198 is a SARS-CoV-2 negativing monoclonal antibody combination remedy (Yang et al., 2020). Preliminary *in vitro* evidence suggests continued antiviral activity against commonly circulating variants from the U.K. and South Africa (Yang et al., 2020). A phase 1 study completed dosing and follow-up by providing safety profiles and human pharmacokinetic profiles for two separate antibodies (Baral et al., 2021). Combination therapy consisting of BRII196 and BRII198 was originally investigated in the April 2021 NIAID ACTIV3 study (NCT04501978) in inpatients. However, it did not meet the pre-determined performance criteria required for Phase 3 entry. As part of an ongoing NIH ACTIV2 trial (NCT04518410), a mixture of BRII196 and BRII198 antibodies is in phase 3 clinical trials (Baral et al., 2021).

CERC-002

CERC002 is a fully human monoclonal antibody against LIGHT or TNFSF14 (a member of the tumor necrosis factor superfamily 14) (Haljasmägi et al., 2020). He is currently being tested for SARS-CoV-2 ARDS due to Crohn's disease and cytokine storm. This study will evaluate the efficacy and safety of CERC002 in patients with severe SARS-CoV-2 for 28 days as a single dose in addition to standard treatment (Perlin et al., 2020). CERC002 increased survival by day 28 and the number of people without respiratory failure in hospitalized patients with mild to moderate SARS-CoV-2-associated pneumonia (ARDS) compared to placebo (83.9% versus 64.5%, P = 0.044). Efficacy was highest in the predefined patient subgroup 60 years and older (76.5% versus 47.1%, P = 0.042), which is the population most vulnerable to serious complications and death from SARS-CoV-2 infection (Rodriguez-Perez et al., 2021). There was an approximately 50% reduction in mortality with CERC002 compared to placebo on both the first 28 days and 60 days (7.7% vs. 14.3% at 28 days and 10.8% vs. 22.5% at 60 days) (Rodriguez-Perez et al., 2021). In the final efficacy data for the phase 2 study (NCT04412057), Cerecor showed that more COVID19 patients with acute respiratory distress who received a single dose of CERC002 instead of placebo were alive and not

experiencing dyspnea during the 28-day study period. The efficacy was highest in patients over the age of 60 who frequently suffered from other inflammatory diseases.

SAB-185

SAB185 is a therapeutic candidate for neutralizing polyclonal antibodies to treat non-hospitalized mild-to-moderate SARS-CoV-2 patients (Winkler et al., 2021). The candidate is being evaluated in the ACTIV2 trial conducted by NIAID, which is part of the NIH in collaboration with the AIDS Clinical Trial Group. SAB185 is a fully human polyclonal candidate antibody designed to confer passive immunity. The first patient in the NIAID-sponsored Phase II/III ACTIV2 study (NCT04518410) received a dose of SAB185 in April after a previous trial demonstrated the safety of an antibody with a half-life of 25-28 days. SAB185, the second drug to enter Phase 3 and the first candidate for polyclonal antibody therapy in ACTIV2, is evaluating several research drugs to treat early symptoms of SARS-CoV-2 in non-hospitalized individuals (Liu et al., 2021). SAB185 was transferred to Phase II as part of the Phase III ACTIV2 trial after meeting all required termination criteria. SAB185 effectively neutralizes viruses containing SARS-CoV-2 spikes with S477N, E484K, and N501Y mutations. This virus has been associated with the outbreak and outbreak of SARS-CoV-2 in several countries, leading to antibody resistance (Liu et al., 2021). WHO has identified several VOCs with mutations in the spike protein SAB185 was tested in BSL2 medium using a lentiviral pseudo virus experiment containing a stable 293T cell line expressing human ACE2 and TMPRSS2. Data collected from 221 patients in study SG016 Phase II (NCT04385095) showed that 33 patients with severe or severe dyspnoea and received SNG001 were 3.41 times more likely to recover than patients who received placebo. *In vitro* data show that SNG001 exhibits antiviral activity against two strains of COVID19, B.1.1.7 and B.1.351 (Saeed et al., 2020). The results show that SAB185 retained neutralizing ability against several strains of SARS-CoV-2-like virus, including delta, kappa and lambda variants, which is displacing other VOCs in many countries and regions around the world (Liu et al., 2021).

CASIRIVIMAB/IMDEVIMAB

In outpatients with mild to moderate SARS-CoV-2, a placebo-controlled randomized trial looked at different dosages of casirivimab plus imdevimab (Razonable et al., 2021). FDA simplified the EUA for casirivimab plus imdevimab, reducing the approved dose for single intravenous infusion from casirivimab 1200 mg plus imdevimab 1200 mg to casirivimab 600 mg plus imdevimab 600 mg (NCT04425629) (Deeks, 2021; Razonable et al., 2021). Participants included were 18 years of age or older, tested positive for SARS-CoV-2, and had at least one risk factor for developing severe SARS-CoV-2. Results showed a 2.2% overall reduction and a 70% reduction in hospitalizations or deaths when taking casirivimab 600 mg plus imdevimab 600 mg. These results are similar to those observed with an intravenous infusion of casirivirab 1200 mg plus imdevimab 1200 mg, which resulted in an absolute 3.3° reduction in hospitalizations or deaths and a 71% relative decrease (NCT04519437), also found to be active against delta variant (O'Brien et al., 2021).

REGDANVIMAB

Regdanvimab (CTP59) blocks the RBD interaction region of ACE2 in one direction. Therefore, CTP59 has the potential to be a promising treatment candidate for COVID19 (Kim et al., 2021). In September 2021, the Korean Ministry of Food and Drugs (MFDS) treated patients over the age of 50 with mild COVID19 and approved Regdanvimab in adults with at least one underlying disease and moderate disease symptoms. This approval is based on the first part of a global Phase 2/3 study showing a 54% reduction in progression to severe COVID 19 in patients with mild to moderate symptoms and a 68% reduction in patients over 50 years of age. In October 2021, the European Medicines Agency (EMA) began considering a marketing authorization application for this mAb for the treatment of adults with COVID19 who do not require additional oxygen therapy and are at high risk of developing severe COVID19. Did. The dose of Regdanvimab is a single intravenous infusion of 40 mg/kg (Syed, 2021). A double-blind, placebo-controlled, randomized, phase II study, BLAZE4 (NCT04634409), found the efficacy of other mAbs, including bumlanivimab (700 mg) and sotrovimab (500 mg), for the treatment of symptoms. We are evaluating safety. Low-risk, non-hospitalized COVID 19 patients. Preliminary results showed that bablanivimab/sotrovimab (700/500 mg) showed 70% (p <0> 5.27; day 7 cycle threshold <27.5 vs. placebo) (Syed, 2021). A recent study compared and evaluated all published studies investigating SARS-CoV-2 neutralized mAbs (single or combined vs. active comparator, placebo, or no intervention) for the treatment of patients with COVID19 and "to the evidence. I evaluated "trust". About preventive use). The authors conclude that the available evidence is insufficient to draw meaningful conclusions about treatment with SARS-CoV-2-neutralized mAbs (Kim et al., 2021; Syed, 2021)

INTERFERONS

Interferons are produced by our cells naturally against viral infection. Interferons have strong effects on the immune system, stimulating it to attack invaders while also inhibiting it to avoid damaging the body's own tissues (Felgenhauer et al., 2020). Injecting synthetic interferons is now a standard treatment for several immune disorders. Interferon's approach to storming our bodies, enthused researchers to see whether an improvement in interferon might help in the early-stage infection of patients with SARS-CoV-2 (Della-Torre et al., 2020). Preliminary investigations in cells and mice have

yielded reassuring results that have led to clinical trials (Murugan et al., 2021). On October 20, 2021, Synairgen proclaimed that the drug was moving forward into a Phase III clinical trial in mild- to -moderate SARS-CoV-2 patients. Sarilumab and tocilizumab are two classes of FDA-approved IL-6 inhibitors (**Table 5**).

SARILUMAB

Sarilumab is a monoclonal antibody that has been evaluated for off-label usage in the treatment of SARS-CoV-2. It binds to both membrane-bound and soluble IL-6 receptors with significant affinity (Gremese et al., 2020). Sarilumab 400 mg is reconstituted in 100 cc of 0.9% NaCl and administered as an hour long IV infusion. The SQ formulation was utilized to produce the IV infusion in the randomized, embedded, multifactorial adaptive platform trial for community-acquired pneumonia (REMAP-CAP) trial. In a revised report of the REMAP-CAP trial, sarilumab and tocilizumab were equally effective in improving survival and reducing time to organ supply. Patients receiving dexamethasone and sarilumab had lower mortality than patients in the control group who received dexamethasone plus placebo, had shorter time to discharge from the ICU, and had more days without organ support (https://www.covid19treatmentguidelines.nih.gov/). The combination of sarilumab and dexamethasone (n = 483) is 99% and 98% likely to be inferior to tocilizumab (n = 943) with dexamethasone in terms of days without organ support and days of death, respectively. REMAPCAP studies have shown that tocilizumab and sarilumab show similar efficacy in treating inpatients with COVID 19, but the panel recommends the use of sarilumab only if tocilizumab is not available or applicable. A single 400 mg dose of sarilumab for injection of SQ was reconstituted with normal saline (50 or 100 ml) and intravenously over 1 hour in the REMAPCAP study (https://www.covid19treatmentguidelines.nih.gov/). It was administered as an internal infusion. Recommendations for COVID19 treatment for the IL6 inhibitors sarilumab and tocilizumab in hospitalized patients requiring oxygenation, high flow oxygen, non-invasive ventilation or invasive ventilation (https://www.covid19treatmentguidelines.nih.gov/).

TOCILIZUMAB

Tocilizumab is a monoclonal antibody against interleukin-6 receptor-alpha that is used for inflammatory diseases, improved consequences have been observed in patients with severe SARS-CoV-2 pneumonia (**Figure 2**) (Samaee et al., 2020; Stone et al., 2020). Tocilizumab showed a slower progression of the disease, as well as a sharp decrease in temperature and mechanical ventilation. In the STOPCOVID study, tofacitinib was associated with a lower risk of respiratory failure and death

TABLE 5 | Recent updates on clinical evaluation of selected interleukin-6 inhibitors.

Open-Label RCT in hospitalized patients with SARS-CoV-2	Key inclusion criteria	Participant characteristics (PCR-confirmed SARS-CoV-2 infection)	Key limitations	Interventions	Primary outcomes	Key secondary endpoints
Tocilizumab	Oxygen saturation (SpO2) <92% on room air or receipt of supplemental oxygen C-reactive protein (CRP)≥75 mg/L	Mean age 63.6 years; 67% male; 76% White, 41% on HFNC or non-invasive ventilation,14% on IMV,82% on corticosteroids.	Arbitrary enrollment cut off at CRP ≥75 mg/L Difficult to define exact subset of patients in RECOVERY cohort who were subsequently selected for secondary randomization/tocilizumab trial	800 mg tocilizumab) and probable second dose (n = 2,022), Usual care (n = 2,094).	Day 28 mortality was lower in tocilizumab arm than in usual care arm (31% vs. 35%; rate ratio 0.85; 95% CI, 0.76–0.94; P = 0.003)	Among those not on IMV at enrollment, receipt of IMV (invasive mechanical ventilation) or death.
Tocilizumab and Sarilumab	Receipt of IMV, noninvasive ventilation, or cardiovascular support.	Mean age 60 years; Median time from ICU admission until enrollment was 14 hours	Enrollment in tocilizumab and sarilumab arms was partially nonconcurrent with SOC (Standard of care) arm.	Tocilizumab 8 mg/kg and possible second dose, plus SOC (n = 952)	1.46 (95% CrI, 1.13–1.87).	66% in tocilizumab arm and 63% in SOC arm (aOR 1.42; 95% CrI, 1.05–1.93).
Sarilumab	Aged ≥18 years; SARS-CoV-2 pneumonia.	Median age 59 years; 63% male; 77% White; 36% Hispanic/Latinx; 39% on HFNC, IMV, or non-invasive mechanical ventilation.	Only 20% of patients received corticosteroids.	There was no benefit of sarilumab in hospitalized adults with SARS-CoV-2 in time to clinical improvement.	No difference in median time to clinical Improvement among the sarilumab arms.	(92% in placebo arm vs. 90% in sarilumab 200 mg arm vs. 92% in sarilumab 400 mg arm).

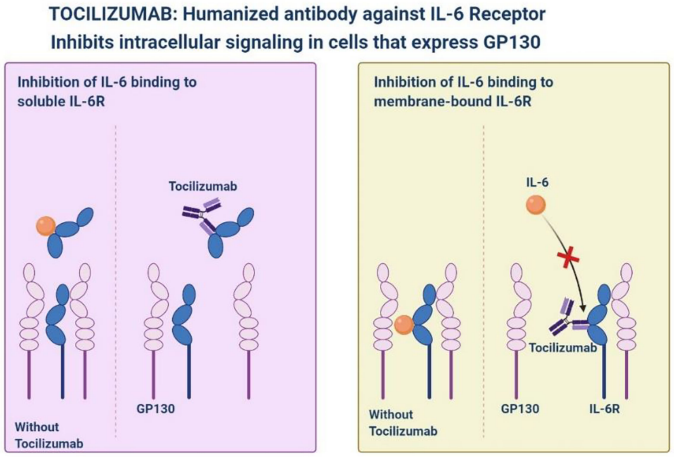

FIGURE 2 | Inhibition of intracellular signaling by Tocilizumab Humanized Antibody against IL-6.

(hazard ratio 0.63, 95% CI 0.41–0.97). Within 28 days, 5.5% of patients in the placebo group (n = 145) and 2.8% of patients in the tofacitinib group (n = 144) (hazard rate 0.49, 95% CI, 0.15-1.63) had all-cause mortality. About 80% of participants in each group also received corticosteroids. Serious adverse events occurred in 14.2% of participants in the tofacitinib group and 12.0% of participants in the placebo group (Hermine et al., 2021). STOPCOVID study found that tofacitinib plus steroids improved outcomes in hospitalized SARS-CoV-2 patients.

A MONOCLONAL ANTIBODY APPROVED AGAINST SARS-CoV-2 BY EMERGENCY USE AUTHORIZATIONS (EUAs)

EUA of mAbs against SARS-CoV-2 were due to the context declared emergency without available alternatives (Aschenbrenner, 2021). EUA is a mechanism used by the FDA to facilitate making products available quickly during a public health emergency; this differs from FDA approval, which is an independent, scientifically reviewed approval for medical products, drugs, and vaccines, based on substantial clinical data and evidence (Bonaventura et al., 2020; Temesgen et al., 2021b). The use of SARS-CoV-2 neutralizing antibodies has not been authorized by the FDA-EUAs for patient hospitalized for SARS-CoV-2 or for those requiring oxygen therapy due to SARS-CoV-2 or patient who are on chronic oxygen therapy due to an underlying condition not related to SARS-CoV-2 that require an increase in oxygen flow rate from baseline (Taylor et al., 2021). Furthermore, the FDA EUAs indicates that all approved mAbs may be associated with worse clinical outcomes when administered to hospitalized patients with SARS-CoV-2 requiring high flow oxygen or mechanical ventilation. In the bamlanivimab plus etesevimab arm, the trial showed a 4.8% absolute reduction and a 70% relative reduction in hospitalizations due to SARS-CoV-2 or deaths from any cause. The authorized dosage of 700/1400 mg lower than the dosage tested in BLAZE-1 is based on initial results (Dougan et al., 2021). Sotrovimab is supported by the results of an interim analysis of an ongoing multicenter, double-blind, Phase 3 COMETICE trial (NCT04545060) (Gupta et al., 2021). The main limitation of these studies is the reported result of environmental heterogeneity, making it difficult to make appropriate comparisons are shown in **Table 6**.

PERSONALIZED CELL THERAPIES TO COMBAT SARS-CoV-2

Personalized medicine plays an important role in the treatment of 19 cases of severe COVID (Khoury et al., 2020; Toor et al., 2021). The idea of cell-based treatment has not been accepted by some scientific communities due to some concerns about the lack of satisfactory clinical research. Nonetheless, MSC and its clinical results show the safety and efficacy of this therapeutic approach in some diseases, especially immune-inflammatory and some incurable diseases (**Figure 3**) (Khoury et al., 2020; Li et al., 2020). With promising results, clinical trials are ongoing. Currently, there are no approved cell-based therapies to prevent or treat patients with SARS-CoV-2 virus, and various clinical studies are underway. Recently, MSCs (Mesenchymal Stem Cells) have attracted clinical trials because of their immunomodulatory properties (Toor et al., 2021). Moreover, as long as the MSC is clinical and time consuming and costly, the MSC remains suspicious.

In mRNA vaccines, single-strand RNA (ssRNA) and double-strand RNA (dsRNA) is recognized by endosomes and cytosols. which is an important part of the natural immune response to the virus (Park et al., 2021). Endosomal Toll-like receptors bind to endosome ssRNA and inflammasome components such as MDA5, RIGI, NOD2 and PKR. Inflammasome components activate the

TABLE 6 | Randomized clinical trials supporting mAbs approved by FDA EUAs.

Monoclonal antibody	Clinical trial number	Study Design	Methods		Results		References
			Intervention:	Primary endpoint	Number of Participants	Primary outcome	
Bamlanivimab plus etesevimab	(Trial Number NCT04427501)	Double-blind, phase 3 randomized clinical trial in outpatients with mild to moderate SARS-CoV-2 who are at high risk for progressing to severe SARS-CoV-2 and/or hospitalization	Single intravenous infusion of etesevimab 2800 mg - Placebo with amlanivimab 2800 mg+	Proportion of participants with SARS-CoV-2 related hospitalization or death by any cause by day 29	bamlanivimab + etesevimab (*n* = 518) - placebo (*n* = 517)	Proportion of participants with SARS-CoV-2 related hospitalization in the bamlanivimab + etesevimab	Dougan et al., 2021
Casirivimab plus imdevimab	NCT04425629	Double-blind, Phase 3 RCT in outpatients with mild to moderate SARS-CoV-2	Single intravenous infusion of: - casirivimab 600 mg + imdevimab 600 mg - casirivimab 1200 mg + imdevimab 1200 mg	Proportion of patients with SARS-CoV-2-related hospitalization or all-cause death through Day 29	SARS-CoV-2-related hospitalization or all-cause death through Day 29	Casirivimab 600 mg + imdevimab 600 mg (*n* = 736) ; 7 of 736 (1.0%) in casirivimab 600 mg plus imdevimab 600 mg; 18 of 1355 (1.3%) in casirivima62 of 1341	Bierle et al., 2021
Sotrovimab	NCT04545060	Double-blind, Phase 1/2/3 RCT in outpatients with mild to moderate SARS-CoV-2	Sotrovimab 500 mg IV - Placebo	Proportion of patients with hospitalization or death from any cause by Day 29	Proportion of patients with hospitalization or death from any cause by Day 29	Sotrovimab (*n* = 291) placebo (*n* = 292); There an 85% relative risk reduction in all-cause hospitalizations	Gupta et al., 2021

production of interferons and inflammation Mediators. Current vaccines contain purified *in vitro* transcriptional single-stranded mRNA with nucleotides modified to reduce binding to TLRs and immune sensors, thus inhibiting overproduction of type I interferon and cell translation. Functions are limited. LNP carriers further protect mRNA, target delivery to lymphatic vessels, and promote protein translation in lymphatic vessels. Preclinical and early results from human studies show that both vaccines produce anti-S protein IgG and virus-specific neutralizing antibody responses months after vaccination, but T cell data not been completely elucidated (Teijaro and Farber, 2021).

PROPHYLACTIC USE OF mAb AGAINST SARS-CoV-2

Vaccines are the most effective way for most people to protect themselves from COVID 19. For the past two years, as the only possible solution to the further spread and recurrence of SARS-CoV-2, the entire scientific community has focused on researching, developing, and ultimately manufacturing safe and effective vaccines (Liz et al., 2020; Nathan et al., 2021). Vaccine development can take years or even decades, but aggressive efforts to screen multiple COVID19 vaccine candidates simultaneously can significantly reduce the overall time required for the development process. MAb is currently an alternative preventive route for COVID19 and may provide short-term prophylaxis to those who have not yet been vaccinated or who do not respond appropriately to vaccination, such as immunocompromised patients. In addition, mAb may be useful if the circulating mutant virus is not adequately covered by vaccination protection (Dong et al., 2021). The PROVENT study was conducted on subjects who would benefit from long-acting antibody prevention because of an increased risk of inadequate response to active immunization or an increased risk of SARS-CoV-2 infection.

MONITORING RESISTANCE TO mAbs AMONG THE NEW VARIANTS

Monitoring resistance to mAbs among new mutants is important in deciding whether to discontinue some of the newly developed mAbs or investigate different combinations. Mutations in SARS-CoV-2 peplomer and clinical mAb resistance profile in VOC It is summarized in **Table 7**.

CONCLUSION

The idea behind the development of SARS-CoV-2 Abs was that enhanced neutralization efficacy would equate to more therapeutic benefit. Development of deactivating cross-reactive human Abs to conserved epitopes on SARS-CoV-2 that can impede infection by emerging SARS-CoV-2 outbreaks. Identification of such conserved epitopes is also essential for the layout of broadly reactive vaccines to thwart future SARS-related coronavirus infections. Monoclonal antibodies and neutralizing antibodies targeting SARS-CoV-2 virus antigens have shown promising results in treating SARS-CoV-2 patients and controlling disease progression. To improve treatment

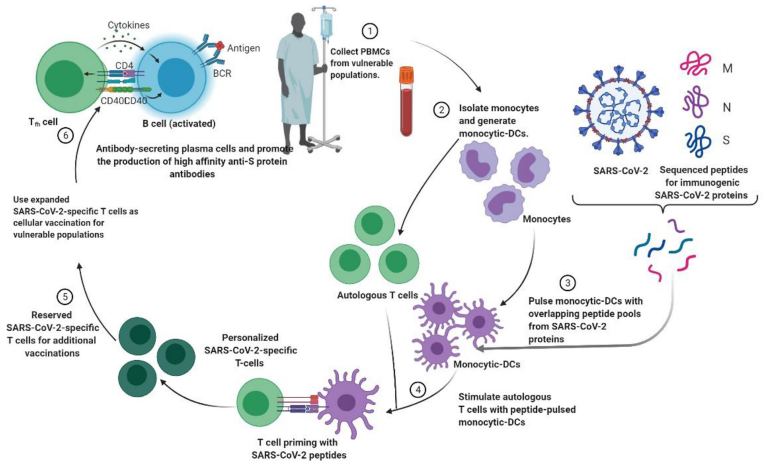

FIGURE 3 | Strategies to generate tailored virus-specific T cells as potential therapeutics for prophylaxis and/or treatment of SARS-CoV-2 infection among vulnerable populations. Monocyte DCs from individuals are treated with SARS-CoV-2 peptide and then used to prime T cells from the same individual to generate SARS-CoV-2-specific T cells. These T cells can be cryopreserved or injected into vulnerable people to prevent or treat SARS-CoV-2.

TABLE 7 | Mutations of SARS-CoV-2 S in VOC and resistance profile of clinical mAbs.

VOC	Bamlanivimab	Etesevimab	Casirivimab	Indevimab	Sotrovimab	Cilgavimab	Tixagevimab	Regdanvimab
B.1.351 (South Africa)	Resistant	R	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Poorly neutralized or not neutralized
B.1.1.7 (UK)	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
P.1 (Brazil)	Resistant	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Poorly neutralized or not neutralized
B.1.1.258 (Scotland)	Sensitive	Not known	Sensitive	Resistant	Sensitive	Not known	Not known	Poorly neutralized or not neutralized
B.1.526 (New York)	Potential Sensitive pot	Potential Sensitive pot	Potential Sensitive pot	Potential Sensitive pot	Sensitive	Potential Sensitive pot	Potential Sensitive pot	Not known
B.1.617.1 (India)	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Potential Sensitive pot	Potential Sensitive pot	Not known
B.1.525 (Nigeria)	Poorly neutralized or not neutralized	Poorly neutralized or not neutralized	Potential Sensitive pot	Potential Sensitive pot	Sensitive	Potential Sensitive pot	Potential Sensitive pot	Not known
B.1.429 (California)	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Poorly neutralized or not neutralized

options, an effective understanding of the competent therapeutic characteristics of antibody-based treatments, primarily neutralizing monoclonal antibodies and establishing their therapeutic or prophylactic applications against SARS-CoV-2, is required. In addition, among other potential therapeutic strategies, personalized viral-specific T cells can be generated to prevent infections among populations at risk and/or treat SARS-CoV-2 infections.

AUTHOR CONTRIBUTIONS

DDS and DKY conceived and designed the project, collected data from the literature. DDS, AS, H-JL, and DKY analyzed the data and wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Impact of Age and Sex on COVID-19 Severity Assessed From Radiologic and Clinical Findings

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Background: Data on the epidemiological characteristics and clinical features of COVID-19 in patients of different ages and sex are limited. Existing studies have mainly focused on the pediatric and elderly population.

Objective: Assess whether age and sex interact with other risk factors to influence the severity of SARS-CoV-2 infection.

Material and Methods: The study sample included all consecutive patients who satisfied the inclusion criteria and who were treated from 24 February to 1 July 2020 in Dubai Mediclinic Parkview (560 cases) and Al Ain Hospital (605 cases), United Arab Emirates. We compared disease severity estimated from the radiological findings among patients of different age groups and sex. To analyze factors associated with an increased risk of severe disease, we conducted uni- and multivariate regression analyses. Specifically, age, sex, laboratory findings, and personal risk factors were used to predict moderate and severe COVID-19 with conventional machine learning methods.

Results: Need for O_2 supplementation was positively correlated with age. Intensive care was required more often for men of all ages ($p < 0.01$). Males were more likely to have at least moderate disease severity ($p = 0.0083$). These findings were aligned with the results of biochemical findings and suggest a direct correlation between older age and male sex with a severe course of the disease. In young males (18–39 years), the percentage of the lung parenchyma covered with consolidation and the density characteristics of lesions were higher than those of other age groups; however, there was no marked sex difference in middle-aged (40–64 years) and older adults (≥65 years). From the univariate analysis, the risk of the non-mild COVID-19 was significantly higher ($p < 0.05$) in midlife adults and older adults compared to young adults. The multivariate analysis provided similar findings.

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Conclusion: Age and sex were important predictors of disease severity in the set of data typically collected on admission. Sexual dissimilarities reduced with age. Age disparities were more pronounced if studied with the clinical markers of disease severity than with the radiological markers. The impact of sex on the clinical markers was more evident than that of age in our study.

Keywords: COVID-19, viral pneumonia, age, sex, machine learning, radiomics, risk stratification, severity

1 INTRODUCTION

Although there is a wide body of literature on COVID-19, data on the epidemiological characteristics and clinical features of patients of different age and sex are limited. Extensive studies were conducted to cover mainly pediatric (Lu et al., 2020) and elderly populations (Liu et al., 2020a). These studies seldom include the laboratory findings and radiomics of the patients. Commonly, researchers analyze the association of demographic factors and underlying diseases with hospitalization for COVID-19 (Hsu et al., 2020; Sapey et al., 2020; Deeb et al., 2021).

Gaining an insight into the risk factors for non-mild disease is useful for risk stratification and proper management. For this, practitioners should know whether demographics (e.g., age, sex, ethnicity, nationality) are predictors of the severity and outcomes of COVID-19. Research on this issue is challenging as it requires an observational study covering all levels of disease severity. Commonly, the asymptomatic or mildly symptomatic cases remain unstudied as non-severe patients are not admitted to the hospital. This impacts risk assessment because a study cohort is not representative of the entire population in terms of age. The same issue accounts for the disparities in age-specific COVID-19 mortality rates reported in distinct locations (e.g., in China and Korea) (Dudley and Lee, 2020). However, the results obtained in the present study are free of such limitations because the datasets for our study were collected when all the COVID-19 patients verified with reverse transcription-polymerase chain reaction (PCR) were hospitalized and treated in the in-patient clinic at the beginning of the epidemic in the United Arab Emirates (UAE). In this way, we obtained a comprehensive dataset that was representative of the UAE population with regard to age and sex.

Abbreviations: ACE-I, angiotensin-converting-enzyme inhibitors; adm, admission to the hospital; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; ARB, angiotensin-II receptor blockers; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; CK, creatine kinase; CRRT, continuous renal replacement therapy; DIC, disseminated intravascular coagulation GCS, Glasgow coma scale; G6PDH, glucose-6-phosphate dehydrogenase; GGO, ground glass opacity; GGTP, gamma-glutamyl transpeptidase; GI, gastrointestina; HD, hemodialysis; HIV, human immunodeficiency virus; ICU, intensive care unit; IL-6, interleukin-6; LDH, lactate dehydrogenase; LMWH, low molecular weighted heparins; MERS-CoV, Middle East respiratory syndrome coronavirus; NN, neural network; NSAID, non-steroid anti-inflammatory drugs; PCR, reverse transcription-polymerase chain reaction; RBC, red blood cells; ROC, receiver operating characteristic; SD, standard deviation; SOFA, sequential organ failure assessment; SOB, shortness of breath; SpO2, oxygen saturation; WBC, white blood cells.

1.1 Age-Related Features of COVID-19

Age appears to be a strong risk factor for COVID-19 severity and outcomes, as the percentage of immunocompromised people in a population is linked with the age structure of that population. Below is a brief overview of studies on the age-related features of COVID-19.

Children are not as prone to severe forms of COVID-19 compared to adults. This comes from an analysis of SARS-CoV-2 viral load by patient age (Jones et al., 2021). They are underrepresented in study cohorts so that they seem to be less susceptible to the disease (Lee et al., 2020).

There are controversial findings about the group of young adults. The authors assume that reduced compliance with social distancing in young adults may impact the age-specific rate of morbidity and mortality (Dudley and Lee, 2020).

Elderly patients with COVID-19 are more likely to progress to severe disease (Liu et al., 2020a). Reasonably, age-related comorbidities are the leading reason for the increased mortality observed in this age group (Wang et al., 2020; Yang et al., 2020; Zhou et al., 2020). However, physicians should not necessarily extrapolate the age-related tendencies from the population to the individual level. Otherwise, a patient can be considered either high or low risk based on their age rather than on their actual health status which might lead to improper risk assessment, suboptimal resource allocation, and inadequate patient management.

One of the limitations of the previous studies is a focus on midlife adults and elderly people. Exclusion of younger adults means it is not possible to explore the age-related features of COVID-19 across all age groups (Alkhouli et al., 2020; Bertsimas et al., 2020; Covino et al., 2020). Another common limitation in earlier work on age-related aspects of COVID-19 is that authors do not adjust study samples to account for other risk factors which also correlate with age (e.g., diabetes, hypertension, and other background diseases) (Dudley and Lee, 2020; Guan et al., 2020). The outcomes of these studies cannot be generalized because of the limited study samples (Colombi et al., 2020; Hariyanto et al., 2020; Habuza et al., 2021). Finally, it is not clear whether the verification of SARS-CoV-2 infection with nasal and pharyngeal swab is performed properly in all cases with the symptoms and combined with radiological findings suggestive of COVID-19. This can be a source of false positive findings (Luo et al., 2020; Liu et al., 2020a). Another point of concern is that the number of studies on COVID-19 severity in the elderly is disproportionately higher than those on young adults (Costagliola et al., 2021). As such, the impact of age on COVID-19 disease severity has not yet been studied properly.

1.2 Sex as a Stratifying Factor

Sex dissimilarity in infectious diseases. A growing body of literature indicates that infectious diseases can affect men and women at a different level. The reasons for these sex differences are related to socioeconomic status, gender inequities, including occupational exposure (Roberts et al., 1998; Tolhurst et al., 2002; Morgan and Klein, 2019), and a sex gap in immune response (Whitacre et al., 1999).

Women are more likely to present with a wide range of specific and non-specific inflammatory (e.g., upper respiratory tract infection, oral and dental conditions) and autoimmune diseases (Schlagenhauf et al., 2010). The immune modulating effect of the sex hormones may underlie these findings (Whitacre et al., 1999).

There is a sex bias in COVID-19, especially at the early stage of the disease (Takahashi et al., 2020). Sex-specific features of the innate and adaptive immune systems (e.g., a higher number of CD4+ T cells, more robust CD8+ T cell cytotoxic activity, and increased B cell production of immunoglobulin) may account for an advantage in the defense against COVID-19 in females. Women are more likely to synthesize higher levels of antibodies against an inactivated influenza vaccine (Peckham et al., 2020). Furthermore, there are sex disparities in physiological responses to viral diseases. The immune system of females has been reported to be twice as strong as that of males (Klein, 2012).

The sex disparity in the efficiency of the immune response correlates with the disease outcomes; i.e., mortality for COVID-19 is twice as common in males (Williamson et al., 2020). Some researchers have associated these findings with genes allocated in the X chromosome (Pontecorvi et al., 2020).

Estrogens may potentiate immune activities of vitamin D, thus improving infection outcomes (Pagano et al., 2020). Conversely, male sex hormones make men vulnerable to COVID-19 and worsen the disease prognosis. First, they are thought to promote viral entry by increasing the activity of the ACE2 receptor—the entry point for the SARS-CoV-2 coronavirus. Second, testosterone exerts immunosuppressive effects and may blunt antibody response. Men may benefit from stimulants of T-cell immune responses and anti-testosterones. Estrogens can be administered to reduce COVID-19 disease severity (Wray and Arrowsmith, 2021).

Researchers aimed to study sex-related differences in COVID-19-associated mortality in multinational cohorts. For this, they used data from national registries or hospitals. Male and female cohorts were not equal in the sets of parameters: age, chronic obstructive pulmonary disease, nicotine dependence, and the total number of comorbidities, including obesity and heart failure (Alkhouli et al., 2020). As the sex groups were not identical, new studies are required to justify a lower mortality from COVID-19 for women and to understand the factors accounting for this sex difference.

Socioeconomic aspects. After adjusting the death rate to socioeconomic factors, authors found that mortality is higher in men in disparate ethnic groups. The relative risk of death from COVID-19 in men compared to women ranges from 1.3 to 3.5 times in different ethnicities (Islam et al., 2020). It remains unknown if socioeconomic inequality accounts for the sex disproportion in the outcomes of COVID-19 (Islam et al., 2020). Reports on the socioeconomic risk factors of COVID-19 are limited. Many studies have focused on the reverse impact of COVID-19 on gender equality (Alon et al., 2020) and the gender gap in work hours (Jin et al., 2020). A study from China showed that men and women have equal chances of getting the disease. However, men with COVID-19 are at a greater risk of worse outcomes and death, independent of age (Jin et al., 2020).

Taking into consideration the results and limitations of recent studies, we aimed to address differential susceptibility of men and women to COVID-19 in order to develop sex-specific intervention strategies. More epidemiological studies should be done throughout the world to compare the burden of the viral disease on human communities in desperate regions (Crighton et al., 2007).

2 OBJECTIVES

To investigate whether age and sex interact with other risk factors to influence the severity of SARS-CoV-2 infection, we addressed the following objectives:

- Compare the severity of COVID-19 assessed from the radiological findings in patients of different ages and sex.
- Explore the differences between people of distinct age groups and sex with regard to disease severity estimated from the clinical and laboratory findings.
- Measure the informative value of sex and age along with the laboratory findings and personal risk factors for predicting the severity of COVID-19.

3 MATERIALS AND METHODS

3.1 Study Design and Sample

The current study analyzed retrospective data obtained as a part of standard primary and secondary care. The study sample included all consecutive patients who satisfied the inclusion criteria (see below section) and who were treated from February 24 to July 01, 2020, in Dubai Mediclinic Parkview (560 cases) or Al Ain Hospital (AAH; 605 cases). The demographic characteristics of both the study cohorts are described in **Table 1**. AAH provides medical care to the second largest city in the Abu Dhabi Emirate. The inclusion criteria were as follows: age 18 years or older; in-patient admission; SARS-CoV-2-positive real-time reverse transcription PCR from nasopharyngeal swabs only, at the hospital site. As mentioned in our previous paper (Statsenko et al., 2021), the novel features of the study are that due to the UAE-wide COVID-19 regulations at the time of the study period all patients with COVID-19 verified by PCR were hospitalized, and we observed all the disease forms (from mild to severe) with a broad spectrum of analyses and radiologic examinations performed. Examinations were conducted in all cases regardless of disease severity.

TABLE 1 | Characteristics of study samples.

Study center	Abu Dhabi Emirate Dataset						Dubai Emirate Dataset					
	Al Ain Hospital						Dubai Mediclinic Parkview Hospital					
	DEMOGRAPHICS											
	Total		Female		Male		Total		Female		Male	
	n = 605		n = 86		n = 519		n = 560		n = 189		n = 371	
- 18–39 yo	341	56.36%	47	54.65%	294	56.65%	292	52.14%	119	62.96%	173	46.63%
- 40–64 yo	253	41.81%	33	38.37%	220	42.39%	236	42.14%	55	29.10%	181	48.79%
- ≥65 yo	11	1.81%	6	6.98%	5	0.96%	32	5.71%	15	7.94%	17	4.58%
	SEVERITY OF COVID-19											
Asymptomatic/mild	357	59.01%	54	62.79%	303	58.38%	343	61.25%	129	68.25%	214	57.68%
Moderate	215	35.54%	28	32.56%	187	36.03%	88	15.71%	29	15.34%	59	15.9%
Severe	31	5.12%	4	4.65%	27	5.20%	83	14.82%	20	10.58%	63	16.98%
Critical	2	0.33%	0	0.00%	2	0.39%	46	8.21%	11	5.82%	35	9.43%

Criteria of severity level	Radiological criteria	Clinical criteria
- *Mild form*	Lung involvement < 5% total lung volume	Clinical symptoms of upper respiratory tract infection and no signs of pneumonia
- *Moderate form*	Lung involvement [5%, 25%) total lung volume	Fever and respiratory symptoms with radiological findings of pneumonia
- *Severe form*	Lung involvement [25%, 50%) total lung volume	ARDS: respiratory rate > 30/min, SpO2 <93% at rest, P/F ratio <300
- *Critical form*	Lung involvement ≥ 50% total lung volume	Any of the following: P/F ratio < 200, sepsis, multiorgan failure, GCS<13

The criteria for assessing disease severity differed by study site (Abu Dhabi versus Dubai) due to the data available at the time of the retrospective study. In the dataset from Al Ain Hospital, the assessment was based on the percentage of the damage to the lungs. Contrarily, in the dataset from Mediclinic Parkview Hospital, the severity level was measured in accordance with the clinical and biochemical signs (see *Methods Used*). The information from electronic health records was summarized using a standardized data collection form adapted from ISARIC Rapid Case Record Form (Harrison et al., 2020).

3.2 Patient and Public Involvement

There was no patient involvement as the data were collected retrospectively from the medical record system and PACS server.

3.3 Methods Used

To address the first objective, we divided the study samples into groups and used descriptive statistics. The range of years corresponding to the age groups was 18–39 years for young adults, 40–64 for midlife adults, and 65 years and over for older adults.

As the variables of the datasets were distributed non-normally, we utilized non-parametric tests for the analysis. In the age groups, the relationships between the continuous features were assessed with the Kruskal–Wallis test. With this test, we also examined the sex-related differences within each age group.

This part of our study was conducted with the dataset from AAH. We used radiomics to estimate disease severity as, evidently, the level of the lung involvement in CT correlates with disease severity. In analogy to the existing scoring systems (e.g., lung CT score), we applied the following thresholds: mild cases had <5%, moderate $\in$ [5, 25), severe $\in$ [25, 50), and critical ones ≥50% lung involvement.

To collect the radiomics data for the entire lung and their lobes, we applied lung masks. The masks were segmented with the deep learning U-net model trained on a large and diverse dataset (Hofmanninger et al., 2020). Ground glass opacity (GGO), consolidation, and pleural effusion are the most common types of the lung lesions in COVID-19. These lesions were segmented with the CT Thorax COVID-19 model from MedSeg tool (COVID-19 CT Segmentation Dataset, 2021). By multiplying the number of voxels in the mask by the voxel size, we received the total lung volume as well as the volumes of the lung lobes. We also segmented pathology lesions. To calculate the mean density, its standard deviation, and entropy, we utilized the fslstats tool from the FSL framework (Jenkinson et al., 2012). The characteristics of density were in Hounsfield units (HU). Finally, all volume variables were normalized or expressed as a percentage to the total lung volume. To study the association of the radiomical features, clinical signs, laboratory findings, comorbidities, and complications with age, sex, and disease severity, we calculated Pearson's correlation coefficients and assessed their significance.

To address the second objective, we used the same methods of descriptive statistics as in the first objective but with a different dataset. This allowed us to verify the findings. In the Dubai Mediclinic Parkview Hospital sample, disease severity was determined according to the National Guidelines National Emergency Crisis and Disasters Management Authority (2020) in the following way: *an asymptomatic form* referred to a patient with no symptoms; *a mild form*—clinical symptoms of upper respiratory tract infection and no signs of pneumonia; *a moderate form*—fever and respiratory symptoms with radiological findings of pneumonia; *a severe form*—any of the following criteria: respiratory distress (RR > 30/min), oxygen saturation <93% at rest, P/F ratio of less than 300; and *a critical form* fitted any of the following criteria: ARDS (P/F ratio < 200), sepsis, multiorgan failure, and altered level of consciousness (GCS < 13).

We followed the same procedure as in the first objective to assess the association between the clinical and laboratory findings in both datasets by computing Pearson's correlation coefficients.

To achieve the third objective, we used a set of statistical approaches. First, we used univariate and multivariate analyses to investigate which factors were associated with the non-mild form of COVID-19 (see **Table 2**). This allowed us to calculate the adjusted odds ratio associated with age and sex used either as single

TABLE 2 | Univariate and multivariate analyses of age and sex factors on severity of the disease (severity is at least moderate).

Variable	Severity assessed radiologically, Abu Dhabi Emirate Dataset			Severity assessed clinically, Dubai Emirate Dataset		
	aOR	95% CI	p	aOR	95% CI	p
			Univariate analysis			
Age	1.028	[1.014–1.044]	**<0.001**	1.08	[1.06–1.10]	**<0.001**
Young adults, 18–39 yo	Ref.	–	–	Ref.	–	–
Midlife adults, 40–64 yo	1.52475	[1.09–2.12]	**0.01265**	5.55	[3.47–9.14]	**<0.001**
Older adults, ≥ 65 yo	4.72629	[1.34–21.88]	**0.02364**	17.05	[7.62–39.83]	**<0.001**
Females	Ref.	–	–	Ref.	–	–
Males	1.2	[0.76–1.94]	0.44	1.82	[1.18–2.9]	**0.0083**
			Multivariate analysis			
Age	1.02	[1.00–1.03]	*0.06869*	1.07	[1.05–1.10]	**2.55e-09**
Sex	1.36	[0.81–2.32]	0.25640	1.93	[1.02–3.82]	*0.050473*
BMI	1.01	[0.99–1.02]	0.31163	1.11	[1.03–1.19]	**0.003459**
SpO_2	0.00	[0.00–0.85]	*0.06421*	0.61	[0.53–0.70]	**8.49e-11**
Systolic blood pressure	1.00	[0.99–1.02]	0.43913	1.00	[0.98–1.02]	0.95765
Diastolic blood pressure	0.98	[0.97–1.00]	**0.01982**	0.99	[0.95–1.02]	0.365582
Heart rate	1.01	[1.00–1.03]	**0.03488**	1.01	[0.99–1.04]	0.246975
Temperature	1.33	[0.83–2.14]	0.23986	2.62	[1.79–3.91]	**1.25e-06**
Breath rate	1.10	[0.97–1.26]	0.15006			
HCO3	0.93	[0.84–1.02]	0.12393			
Serum potassium	1.34	[0.82–2.18]	0.24068			
Anion gap	0.96	[0.87–1.07]	0.46646			

aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; SpO2, oxygen saturation.
Significant associations between variables and disease severity ($p < 0.05$) are marked in bold font.

predictors or in combination with the data typically collected on admission, i.e., the results of the physical examination (body mass index, blood pressure, body temperature, heart rate, breath rate) and hypoxia markers (SpO2, HCO3, serum potassium, anion gap).

Then, we employed machine learning (ML) classification models. To start with, all the informative features from AAH were ranked by their potential to predict disease severity. To assess the importance of the features fed into the ML models as predictors, we employed four ensemble tree-based estimators such as AdaBoost, Gradient Boosting, Random Forest, and Extra Trees. These models were trained on the whole dataset and used to rank the features in ascending order concerning their predictive potential. As the dataset was unbalanced due to common reasons (severe and critical disease forms are less common than the mild ones), we built a binary classification to distinguish the mild disease from the other forms (357 vs. 248 cases in AAH; 431 vs. 129 in Dubai). To test the impact of age and sex on the disease course, we utilized different sets of predictors. Particularly, we merged the data on age and sex with the laboratory findings from the AAH dataset. In the Dubai Mediclinic dataset, we selected personal risk factors, including background diseases, age, and sex, to show the most informative features. To provide further evidence of the importance of age and sex as predictors of COVID-19 severity, we compared the accuracy of machine learning models built with and without them (see **Table 3**).

We used the computational power of the Linux Ubuntu 18.04 NVIDIA DGX-1 deep learning server with 40 CPU cores and 8x NVIDIA Tesla V100 GPU with 32 GB memory each, accessed with a web-based multiuser concurrent job scheduling system (Habuza et al., 2020). The experimental work was conducted using programming languages R, Python, and its libraries for deep learning, data processing, and data visualization, such as tensorflow-gpu, keras, Sklearn, SciPy, NumPy, Pandas, Matplotlib, and Seaborn.

4 RESULTS

4.1 Sex and Age Disparities in Disease Severity Assessed From Radiomics

As expected, the lung volume was smaller in women and in older adults (see **Table 4**). The interpretation of age- and sex-specific differences in some radiomics data (e.g., lungs entropy, density) was challenging because of an unclear clinical value of the data. In young and middle-aged adults, there was a marked sex disproportion in the location of the center of gravity along the axial axis. This may have resulted from a more severe lung involvement in men and up–down gradient in the distribution of the lung lesions in COVID-19-associated pneumonia.

The portion of mild cases decreased dramatically with age. The severity of COVID-19 assessed from the percentage of lung involvement did not differ between sexes. The distribution of the lung lesions over specific lobes was common for distinct age groups and sexes.

Men had a greater lung volume compared to women ($p \leq 0.0179$). The portion of the lung with emphysematous changes was larger in men. Sex disparity in the lung involvement was significant in young adults: there were differences in the density characteristics of ground glass opacity (GGO) and consolidation. The data reflect a more severe lung involvement in men; i.e., the lesions were denser in males compared to their counterparts of the same age ($p < 0.05$). The total involvement of the left lower lobe, right middle lobe, and right lower lobe and the percentage of the lung parenchyma covered with consolidation were higher in men.

TABLE 3 | Performance of classification models predicting severity of COVID-19 from laboratory findings (Abu Dhabi dataset) or individual risk factors (Dubai dataset) with and without age and sex.

ML method		Prediction of severity from laboratory findings, age and sex					Prediction of disease severity from individual risk factors inclusively age and sex				
		Precision	Recall	F1	AUC	Acc	Precision	Recall	F1	AUC	Acc
AdaBoost	w/o	0.63	0.64	0.62	0.6444	0.64	0.72	0.73	0.72	0.641	0.73
	with	0.64	0.64	0.63	0.6585	0.64	0.72	0.74	0.73	0.7222	0.74
ExtraTrees	w/o	0.68	0.68	0.65	0.6658	0.68	0.73	0.76	0.74	0.6824	0.76
	with	0.71	0.69	0.67	0.6728	0.69	0.74	0.76	0.75	0.7452	0.76
Random forest	w/o	0.72	0.69	0.66	0.6633	0.69	0.73	0.78	0.73	0.709	0.78
	with	0.72	0.69	0.66	0.6887	0.69	0.74	0.78	0.73	0.7998	0.78
NN	w/o	0.70	0.71	0.70	0.7547	0.71	0.76	0.79	0.76	0.7208	0.79
	with	0.79	0.77	0.76	0.7906	0.77	0.80	0.81	0.80	0.8134	0.81
SVM (linear)	w/o	0.66	0.66	0.63	0.6465	0.66	0.68	0.76	0.69	0.6656	0.76
	with	0.67	0.67	0.64	0.6534	0.67	0.71	0.76	0.71	0.7806	0.76
LR	w/o	0.65	0.66	0.64	0.655	0.66	0.74	0.78	0.74	0.7239	0.78
	with	0.66	0.66	0.65	0.6643	0.66	0.74	0.78	0.74	0.7863	0.78
Gain after adding age and sex to predictors		+3%	+1.33%	+1.83%	+1.64%	+1.33%	+1.5%	+0.5%	+1.33%	**+8.41%**	+0.5%

Significant differences ($p < 0.05$) between models performance with and without such predictors as age and sex are marked in bold font.
AUC, area under the receiver operating characteristic curve; Acc, accuracy; LR, logistic regression; ML, machine learning; SVM, support vector machine; w/o, without.

In older adults, there were no marked differences between sexes. In midlife adults, such differences were also minimal with the exception of a statistically larger center of gravity along the axial axis in males ($p = 0.0002$). The results suggest that dissimilarities in the hormonal status may underlie differences between sexes in COVID-19. As the level of hormonal activity reduces with age, the sex disparities in the disease also decreases.

Pearson's correlation coefficients show the relationship between age, sex, and radiomical findings (see **Figure 1**). The level of severity assessed with the percentage of the lung involvement had a strong positive correlation with age ($r = 0.16$, $p = 0.0001$). However, there was no statistical association between the severity marker and sex ($r = 0.003$, $p = 0.44$).

4.2 Differences Between Sex and Age Groups in COVID-19 Severity as per Clinical Data and Laboratory Findings

4.2.1 Laboratory Findings

From our data, the majority of findings were within the reference norm as the mild form of COVID-19 was most prevalent in the study cohort (see **Supplemental Tables 1, 2**) The biochemical findings in both datasets support the relationships identified from radiomics and briefly described in *Sex and Age Disparities in Disease Severity Assessed From Rediomics*. The statistical analysis of the laboratory data supports the trend toward worsening of the SARS-CoV-2 infection with age. Sexual dissimilarities were also pronounced at the age of active hormonal changes and decreased with age.

The analysis of the coagulatory system and biochemical markers illustrated the common observations in both study cohorts. The level of CRP is an important marker of disease severity, and it showed the same association with advancing age as the radiological markers of lung involvement. The majority of substrates demonstrate a greater count in men than in women, although a significant difference ($p < 0.05$) was observed only for a few laboratory findings. This suggests that, in general, men appear to suffer more severe cases and die of the disease at greater rates. Older patients are more likely to have hypercoagulation and clot formation as they have a higher level of APTT, D-Dimer, and fibrinogen compared to the younger groups ($p < 0.001$). Moreover, the level of D-dimer deviated from the clinical reference values (>0.5 ug/l) only in older adults.

An increase in lactate dehydrogenase (LDH) and creatine kinase (CK) activity may reflect energy deficiency caused by hypoxia. The levels of these enzymes were lower in young adults than in midlife or older-aged patients ($p \leq 0.003$). Although there was a marked difference in the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) between the three age groups, it was not possible to confirm a direct association between the elevated levels of these enzymes with age as midlife adults had the highest enzyme activity. The Dubai-based cohort demonstrated a profound difference in the enzyme level between sexes and between midlife and young adults ($p < 0.001$). The variance of the platelet, white blood cell (WBC), and red blood cell (RBC) count among patients of distinct age was not clinically relevant.

These were associations between age, sex, and the major clinical parameters and laboratory findings studied in two clinics. The markers of the disease progression indicate worsening of the disease course with advancing age. This was true for the numerical values of the laboratory findings (see **Figure 2A**) and appearance of the clinical symptoms (see **Figure 2B**). The level of clinical severity collected in Dubai dataset expressed a moderate-strength positive correlation with age ($r = 0.41$, $p < 0.0001$).

From the same data, there was a very weak association of male sex with severe disease ($r = 0.10$, $p = 0.13$).

4.2.2 Clinical Findings, Patient Management, and Outcomes

In the dataset from Dubai, it was possible to assess the severity of COVID-19 directly from the clinical data (*"Clinical severity"*) and indirectly from *"Onset of hospitalization days"*, *"Duration of viral*

TABLE 4 | Radiomics data on lung involvement f subjects with regard to age groups and gender, Abu Dhabi Emirate.

	Total	Both sexes				All ages			18-39 years			40-64 years			≥65 years		
		18-39 n_1=341 (56.36%)	40-64 n_2=253 (41.82%)	≥65 n_3=11 (1.82%)	p_{1-3}	Female n_4=86 (14.21%)	Male n_5=519 (85.79%)	p_{4-5}	Female n_6=47 (13.78%)	Male n_7=294 (86.22%)	p_{6-7}	Female n_8=33 (13.04%)	Male n_9=220 (86.96%)	p_{8-9}	Female n_{10}=6 (54.55%)	Male n_{11}=5 (45.45%)	p_{10-11}
GENERAL LUNG RADIOMICS																	
Lung volume, *L*	3.4 [2.62-3.96]	3.44 ± 1.08	3.37 ± 0.99	3.02 ± 1.24	0.2157	2.68 ± 0.82	3.52 ± 1.03	**<0.001**	2.82 ± 0.93	3.54 ± 1.07	**<0.001**	2.57 ± 0.66	3.49 ± 0.97	**<0.001**	2.21 ± 0.3	3.98 ± 1.25	**0.0179**
Lung entropy	0.71 [0.69-0.74]	0.71 ± 0.03	0.71 ± 0.04	0.74 ± 0.06*	0.0636	0.71 ± 0.05	0.71 ± 0.04	0.2814	0.71 ± 0.03	0.71 ± 0.03	0.4965	0.7 ± 0.05	0.71 ± 0.04	0.171	0.72 ± 0.07	0.78 ± 0.04	0.1177
Density																	
- maximal	664.27 [547.0-700.0]	645.37 ± 236.11*	692.11 ± 234.5*	610.0 ± 126.04	**0.0013**	657.86 ± 192.45	665.34 ± 241.46	0.2057	626.0 ± 143.67	648.47 ± 247.57	0.3197	706.42 ± 244.93	689.96 ± 232.82	0.196	640.33 ± 134.02	573.6 ± 104.71	0.324
- mean	-70.16 [-85.06–52.14]	-73.42 ± 25.46*	-65.88 ± 22.34*	-67.22 ± 27.64	**0.0024**	-68.46 ± 24.31	-70.44 ± 24.56	0.2273	-75.43 ± 24.4	-73.1 ± 25.61	0.2369	-60.36 ± 20.43	-66.71 ± 22.49	0.0597	-58.41 ± 25.88	-77.8 ± 25.93	0.0603
- std deviation	221.02 [190.24-250.95]	226.56 ± 41.97*	214.02 ± 40.32*	210.11 ± 47.36	**0.0033**	217.02 ± 42.4	221.68 ± 41.75	0.1633	229.23 ± 40.59	226.14 ± 42.17	0.3064	203.87 ± 38.57	215.54 ± 40.35	0.053	193.61 ± 44.89	229.9 ± 42.4	0.0855
Center of gravity																	
- lateral axis (x)	255.71 [255.24-256.09]	255.74 ± 0.86	255.66 ± 0.72	255.75 ± 0.82	0.8183	255.76 ± 0.82	255.7 ± 0.8	0.232	255.79 ± 0.87	255.73 ± 0.85	0.3087	255.71 ± 0.7	255.66 ± 0.72	0.2689	255.8 ± 1.01	255.69 ± 0.51	0.4636
- anterior-posterior axis (y)	256.68 [255.49-257.7]	256.78 ± 2.03	256.52 ± 1.75	257.1 ± 1.56	0.1201	257.33 ± 1.78	256.57 ± 1.91	**<0.001**	257.8 ± 1.79	256.62 ± 2.01	**<0.001**	256.6 ± 1.59	256.51 ± 1.77	0.1877	257.74 ± 1.33	256.33 ± 1.45	0.0855
- axial axis (z)	154.62 [143.66-165.03]	153.89 ± 15.27	156.06 ± 15.77	144.33 ± 15.91*	**0.0398**	145.26 ± 14.67	156.17 ± 15.19	**<0.001**	145.13 ± 14.76	155.29 ± 14.88	**<0.001**	146.72 ± 15.06	157.46 ± 15.39	**0.0002**	138.19 ± 8.38	151.71 ± 19.3	0.0855
SEVERITY LEVEL ASSESSED FROM LUNG INVOLVEMENT																	
Severity level																	
- mild	357 (59.01%)	218 (63.93%)*	136 (53.75%)*	3 (27.27%)		54 (62.79%)	303 (58.38%)		34 (72.34%)	184 (62.59%)		18 (54.55%)	118 (53.64%)		2 (33.33%)	1 (20.0%)	
- moderate	215 (35.54%)	113 (33.14%)	97 (38.34%)	5 (45.45%)	**0.0005**	28 (32.56%)	187 (36.03%)	0.833	13 (27.66%)	100 (34.01%)	0.2598	12 (36.36%)	85 (38.64%)	0.9075	3 (50.0%)	2 (40.0%)	
- severe	31 (5.12%)	10 (2.93%)*	18 (7.11%)	3 (27.27%)*		4 (4.65%)	27 (5.2%)		0 (0.0%)	10 (3.4%)		3 (9.09%)	15 (6.82%)		1 (16.67%)	2 (40.0%)	
- critical	2 (0.33%)	0 (0.0%)	2 (0.79%)	0 (0.0%)		0 (0.0%)	2 (0.39%)					0 (0.0%)	2 (0.91%)				
LUNG INVOLVEMENT																	
Involvement, %	6.71 [0.81-9.24]	5.07 ± 6.79*	8.55 ± 10.58*	15.36 ± 11.95*	**<0.001**	6.7 ± 7.93	6.71 ± 9.09	0.3984	3.79 ± 4.24	5.27 ± 7.1	0.1181	9.47 ± 9.28	8.42 ± 10.76	0.1406	14.28 ± 10.98	16.66 ± 12.9	0.3921
Emphysema, %	0.65 [0.06-0.59]	0.68 ± 1.5	0.61 ± 1.17	0.6 ± 1.16	0.9977	0.43 ± 0.9	0.69 ± 1.43	**0.0021**	0.53 ± 1.13	0.71 ± 1.55	0.0534	0.32 ± 0.52	0.65 ± 1.24	**0.0126**	0.22 ± 0.23	1.07 ± 1.58	0.1577
GGO, %	5.43 [0.45-7.87]	4.13 ± 5.77*	6.83 ± 8.28*	13.71 ± 10.68*	**<0.001**	5.88 ± 7.19	5.35 ± 7.25	0.1682	3.41 ± 4.05	4.24 ± 5.99	0.3505	8.07 ± 8.28	6.64 ± 8.27	0.0979	13.19 ± 10.42	14.34 ± 10.94	0.4636
- max density, *HU*	11.05 [-0.0–0.0]	7.03 ± 20.54*	15.92 ± 29.08*	23.82 ± 29.35*	**<0.001**	5.31 ± 14.64	12.0 ± 26.29	**0.0225**	1.23 ± 8.37	7.95 ± 21.72	**0.0061**	10.18 ± 19.64	16.78 ± 30.15	0.168	10.5 ± 10.56	39.8 ± 35.96	0.1461
- mean density, *HU*	-2.4 [-3.67–0.26]	-1.9 ± 2.16*	-2.9 ± 3.2*	-6.27 ± 4.95*	**<0.001**	-2.59 ± 2.83	-2.37 ± 2.8	0.1970	-1.76 ± 1.93	-1.92 ± 2.19	0.3720	-3.26 ± 3.04	-2.85 ± 3.22	0.1434	-5.36 ± 4.38	-7.37 ± 5.35	0.3921
- std density, *HU*	32.07 [13.38-47.59]	28.88 ± 18.82*	35.4 ± 22.45*	54.04 ± 24.47*	**0.0001**	33.7 ± 21.06	31.8 ± 20.96	0.2202	27.74 ± 18.37	29.06 ± 18.88	0.3496	39.02 ± 21.59	34.86 ± 22.53	0.1523	51.1 ± 20.38	57.58 ± 28.21	0.3921
Consolidation, %	1.28 [0.14-1.08]	0.94 ± 1.97*	1.73 ± 3.74*	1.65 ± 1.78	**<0.001**	0.82 ± 1.43	1.36 ± 3.04	**0.0016**	0.38 ± 0.59	1.03 ± 2.09	**<0.001**	1.4 ± 2.01	1.78 ± 3.93	0.3827	1.09 ± 0.89	2.31 ± 2.28	0.324

(Continued)

TABLE 4 | Continued

	Total	Both sexes				All ages			18-39 years			40-64 years			≥65 years		
		18-39 n_1=341 (56.36%)	40-64 n_2=253 (41.82%)	≥65 n_3=11 (1.82%)	p_{1-3}	Female n_4=86 (14.21%)	Male n_5=519 (85.79%)	p_{4-5}	Female n_6=47 (13.78%)	Male n_7=294 (86.22%)	p_{6-7}	Female n_8=33 (13.04%)	Male n_9=220 (86.96%)	p_{8-9}	Female n_{10}=6 (54.55%)	Male n_{11}=5 (45.45%)	p_{10-11}
- max density, *HU*	145.31 [89.0-174.0]	126.05 ± 105.04*	169.0 ± 158.88*	197.55 ± 96.18*	**<0.001**	112.51 ± 105.53	150.74 ± 135.18	**0.0005**	79.91 ± 62.2	133.42 ± 108.56	**<0.001**	149.12 ± 140.93	171.98 ± 161.19	0.1792	166.5 ± 38.43	234.8 ± 126.63	0.2614
- mean density, *HU*	-0.15 [-0.09–0.01]	-0.08 ± 0.19*	-0.24 ± 0.62*	-0.3 ± 0.38*	**<0.001**	-0.08 ± 0.18	-0.16 ± 0.46	**0.0289**	-0.03 ± 0.06	-0.09 ± 0.21	**0.0022**	-0.14 ± 0.26	-0.25 ± 0.65	0.3827	-0.16 ± 0.2	-0.46 ± 0.47	0.1177
- std deviation, *HU*	4.89 [1.9-5.82]	3.76 ± 3.33*	6.28 ± 6.19*	7.97 ± 4.84*	**<0.001**	3.77 ± 3.21	5.08 ± 5.14	**0.0092**	2.52 ± 1.85	3.96 ± 3.47	**0.0008**	5.13 ± 3.83	6.45 ± 6.45	0.2689	5.97 ± 3.58	10.37 ± 5.05	0.1177
SPECIFIC LOBE INVOLVEMENT																	
Left upper lobe																	
- GGO, %	1.73 [0.15-2.47]	1.44 ± 2.13*	2.04 ± 2.62*	3.5 ± 3.21*	**0.0041**	2.06 ± 2.51	1.68 ± 2.38	**0.0221**	1.36 ± 1.58	1.45 ± 2.21	0.1985	2.74 ± 3.08	1.94 ± 2.52	0.0556	3.68 ± 3.08	3.29 ± 3.34	0.4636
- consolidation, %	0.17 [0.01-0.11]	0.1 ± 0.27*	0.26 ± 0.54*	0.24 ± 0.3*	**<0.001**	0.11 ± 0.23	0.18 ± 0.44	**0.0249**	0.03 ± 0.08	0.12 ± 0.29	**0.0011**	0.2 ± 0.29	0.26 ± 0.57	0.4614	0.23 ± 0.33	0.25 ± 0.26	0.4636
Left lower lobe																	
- GGO, %	0.45 [0.01-0.25]	0.24 ± 0.81*	0.66 ± 1.37*	2.14 ± 2.14*	**<0.001**	0.5 ± 1.02	0.44 ± 1.17	0.1373	0.15 ± 0.38	0.25 ± 0.85	0.3118	0.75 ± 1.1	0.65 ± 1.4	0.0795	1.89 ± 1.96	2.44 ± 2.3	0.4636
- consolidation, %	0.09 [0.0-0.03]	0.04 ± 0.18*	0.15 ± 0.43*	0.11 ± 0.14*	**<0.001**	0.07 ± 0.23	0.09 ± 0.33	0.1883	0.01 ± 0.01	0.04 ± 0.19	**0.0034**	0.17 ± 0.34	0.14 ± 0.44	0.1602	0.08 ± 0.1	0.14 ± 0.16	0.3921
Right upper lobe																	
- GGO, %	0.51 [0.01-0.38]	0.26 ± 0.63*	0.77 ± 1.59*	2.41 ± 2.94*	**<0.001**	0.49 ± 1.06	0.52 ± 1.28	0.381	0.13 ± 0.17	0.28 ± 0.68	0.1725	0.69 ± 1.01	0.78 ± 1.66	0.2317	2.22 ± 2.47	2.64 ± 3.41	0.4636
- consolidation, %	0.07 [0.0-0.02]	0.03 ± 0.2*	0.12 ± 0.4*	0.08 ± 0.07*	**<0.001**	0.03 ± 0.07	0.08 ± 0.32	0.1703	0.0 ± 0.01	0.04 ± 0.22	**0.0172**	0.06 ± 0.09	0.13 ± 0.42	0.4584	0.07 ± 0.06	0.08 ± 0.07	0.4636
Right middle lobe																	
- GGO, %	0.11 [0.0-0.04]	0.05 ± 0.18*	0.17 ± 0.41*	0.63 ± 0.73*	**<0.001**	0.11 ± 0.35	0.11 ± 0.32	0.222	0.02 ± 0.04	0.05 ± 0.19	0.4297	0.18 ± 0.43	0.17 ± 0.41	0.1828	0.5 ± 0.68	0.79 ± 0.77	0.2614
- consolidation, %	0.02 [0.0-0.01]	0.01 ± 0.03*	0.04 ± 0.15*	0.04 ± 0.05	**<0.001**	0.02 ± 0.08	0.02 ± 0.1	0.0849	0.0 ± 0.0	0.01 ± 0.04	**0.0287**	0.04 ± 0.12	0.04 ± 0.15	0.4237	0.05 ± 0.07	0.03 ± 0.03	**0.5**
Right lower lobe																	
- GGO, %	2.48 [0.13-4.0]	2.05 ± 2.62*	2.99 ± 3.29*	4.43 ± 2.8*	**0.0001**	2.57 ± 3.11	2.47 ± 2.95	0.4147	1.66 ± 2.24	2.11 ± 2.67	0.1583	3.52 ± 3.68	2.91 ± 3.22	0.1762	4.52 ± 3.03	4.32 ± 2.48	0.4636
- consolidation, %	0.17 [0.0-0.1]	0.1 ± 0.36*	0.27 ± 0.64*	0.2 ± 0.17*	**<0.001**	0.1 ± 0.27	0.19 ± 0.53	0.0825	0.02 ± 0.05	0.12 ± 0.39	**0.0099**	0.19 ± 0.4	0.28 ± 0.67	0.4316	0.12 ± 0.1	0.29 ± 0.19	0.0603

[a]*Statistical data are expressed as IQR, Median ± SD, or the absolute number of cases and their percentage in the sample studied.*

If the distribution of a variable differs significantly ($p < 0.05$) in an age group plotted against all the other ones, its value is marked with an asterisk.

Disparities in the distribution of data across age and sex groups are presented with p-values in separate columns. The p-value is marked in bold if the difference between the groups is statistically significant ($p < 0.05$).

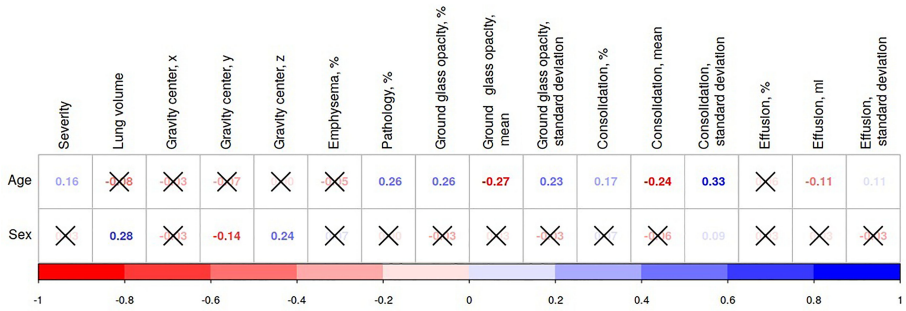

FIGURE 1 | Association of radiomical features with age and sex in the datasets from the Emirate of Abu Dhabi. If an association between variables is significant ($p < 0.05$), the values of Pearson's correlation coefficients for it are presented in the diagram; otherwise, the values are crossed out.

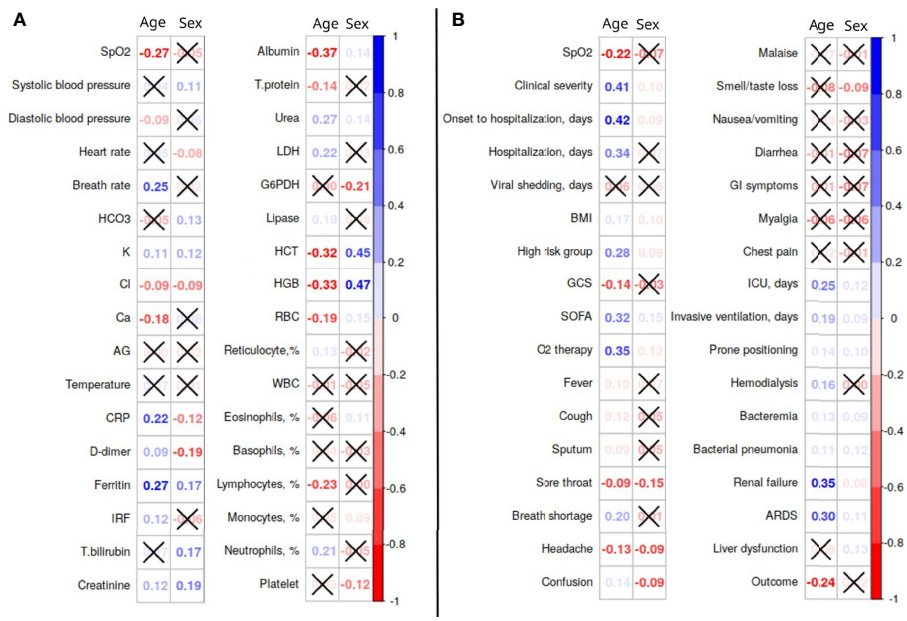

FIGURE 2 | Association of clinical and laboratory features with age and sex in the datasets from the Emirate of Abu Dhabi **(A)** and Dubai **(B)**. AG, anion gap; ARDS, acute respiratory distress syndrome; BMI, body mass index; CRP, C-reactive protein; G6PDH, glucose-6-phosphate dehydrogenase; GCS, Glasgow Coma Scale; GI, gastrointestinal; HGB, hemoglobin; HCT, hematocrit; ICU, intensive care unit; IRF, immature reticulocyte fraction; LDH, lactate dehydrogenase; RBC, red blood cells; SpO2, oxygen saturation; SOFA, sequential organ failure assessment; T.bilirubin, total bilirubin; T.protein, total protein; WBC, white blood cells.

shading", *"Disease outcomes"*, symptoms, etc. (see **Supplemental Table 2**). The study data shows that in the age range from 18 to 39 years the disease was more severe in males ($p = 0.328$), but there was no evidence on sex dissimilarities from the indirect markers ($p > 0.05$).

Hypertension was the prevailing comorbidity (20.54%) in the study sample. There was no pronounced sex difference in the prevalence of individuals suffering from hypertension and diabetes mellitus. Comparison of young and older adults shows that more midlife subjects were classified in a severe or critical condition. Disease severity was positively associated with the following background diseases: diabetes mellitus ($r = 0.29$, $p < 0.0001$), hypertension ($r = 0.21$, $p < 0.0001$), chronic kidney ($r = 0.15$, $p = 0.0003$), and cardiac disease ($r = 0.12$, $p = 0.0041$; see **Figure 3** and **Supplemental Table 3**). There was a weak negative association between current smoking and the severity of COVID-19 ($r = -0.09$, $p = 0.043$). This might explain the fact that smoking was twice as common in young adults as in midlife adults (8.22% vs. 4.24%) and there was no marked correlation between current smoking and COVID-19 severity inside age groups ($p > 0.05$).

Male subjects in the age group of 18–39 years had a statistically higher mean BMI. Noticeably, while the BMI of male patients was consistent throughout age groups, the BMI of women increased with age. For instance, in the age bracket of 65 years and over, the

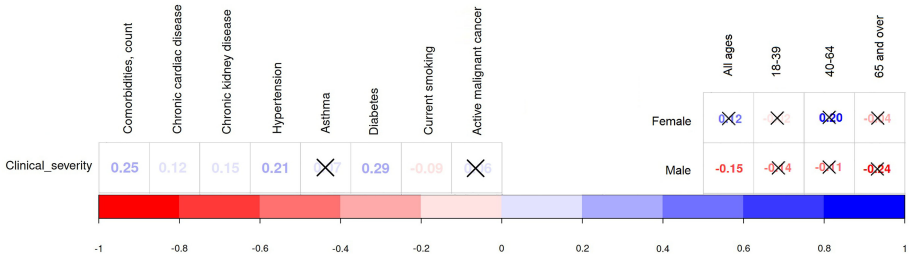

FIGURE 3 | Association of clinical severity with comorbidities (Dubai dataset).

BMI was higher than the BMI of males, $p = 0.0179$. Young males had a higher body temperature (°C) than their female counterparts (37.0 ± 0.67 vs 37.0 ± 0.47; $p < 0.0211$). Both systolic and diastolic blood pressures were significantly higher in adult males with the p-value varying from less than 0.0001 to 0.0179.

The respiratory rate was slightly higher in males, except for older adults where female patients displayed higher values of breaths per minute (22.0 ± 6.29 vs. 18.0 ± 2.22; $p < 0.0129$). The same relationship was observed for SOFA score; specifically, SOFA score was higher in male patients (0.30 ± 0.94 for young adults and 1.28 ± 2.32 for midlife adults) than in females (0.04 ± 0.20 and 0.42 ± 1.15, respectively) with the p value ranging from 0.0016 to 0.0046. Although they are statistically significant, these differences are hard to interpret in all age groups because they have no notable clinical value.

There was no large difference in the number of comorbidities in any study cohorts, but some were more prevalent in certain age groups. We also analyzed statistics on smoking which is a risk factor for background diseases worsening COVID-19 outcomes (e.g., lung and cardiovascular pathology). Thus, male subjects in the age group of 18–39 years showed a higher percentage of current smokers (12.72 vs. 1.68%; $p < 0.0004$). This trend was not observed in the 40–64-year age group with any significant difference; however, older-aged female subjects had a higher prevalence of asthma compared to men (10.91 vs. 3.31%; $p < 0.0357$). Marked differences between sexes were apparent in chronic cardiac disease. Its prevalence in male patients was much higher than females (13.33 vs. 58.82%; $p < 0.0118$).

Out of 560 patients analyzed, the majority (76.96%) of patients had either an asymptomatic or mild disease form, followed by the severe cases (14.82%), and the fewest patients were in critical condition (8.21%). Young adults were either asymptomatic or had a mild disease form in 90.75% of cases, midlife adults in 65.25%, and older adults only in 37.5% of cases. Only 6.85% of young adults had a severe case of the disease, while 21.61% of midlife and 37.5% of older adults were classified as severe cases. Out of all age groups, older adults had the highest portion of critical cases (25%), followed by midlife adults (13.14%) and young adults (2.4%). 0_2 supplementation was required in 14.64% of cases, and it was most frequently administered to older adults both on admission (43.75%) and later in the clinics (53.12%). The need for O_2 supplementation was directly correlated with age. Male patients of all age groups were considerably more likely to require O_2 supplementation with the p-value from 0.0034 to 0.0257. Among all patients, 56 (10%) were admitted to the ICU directly, and 72 (12.86%) were transferred there later when their condition deteriorated. Older adults were more likely to be admitted to the ICU than midlife and young adults ($p < 0.001$). Male patients were admitted to the ICU more frequently than female patients were ($p < 0.0027$). The time between the onset of the disease and hospitalization as well as between the onset and positive PCR was the longest for older adults followed by midlife and then young adults ($p < 0.001$). The opposite tendency was observed for young adults ($p < 0.0102$). There was evidence of a correlation between age and the duration of the disease ($p < 0.0009$). Male patients had a longer duration of disease in the 40–64- ($p < 0.0259$) and above 65-year-old ($p < 0.0467$) age groups. The outcome of the disease was reported to be fatal for 15 (2.68%) patients (4.24% of midlife adults and 15.62% of older adults). Older males were more likely to survive compared to females (100% vs. 66.67%, $p < 0.0149$).

There was a noticeable positive correlation between the patients' age and complications as well as between the latter and disease severity (see **Figure 4**). The most common complications that patients experienced were acute respiratory distress syndrome (ARDS)—76 (13.57%) cases, bacterial pneumonia—15 (2.68%), liver dysfunction—54 (9.64%), acute renal injury—47 (8.39%), septic shock—25 (4.46%), and seizure—5 (0.89%). Out of 123 (21.96%) patients who experience these complications, 17 were older adults (53.12% of the age group), 73—midlife adults (30.93%), and 33—young adults (11.3%). Young and midlife males were more likely to present complications than females from the same age groups (p-value ranges from 0.0012 to 0.0499). Older patients also experienced cardiac complications far more frequently: as cardiac arrhythmia (12.5% vs. 4.24% in midlife adults and 0.34% in and young adults; $p < 0.001$), cardiac arrest (12.5% vs. 4.24% in midlife adults; $p < 0.001$). There were significant associations ($p < 0.001$) between disease severity and the total number of complications ($r = 0.76$), ARDS ($r = 0.82$), any cardiac complication ($r = 0.64$), including myocarditis ($r = 0.16$),

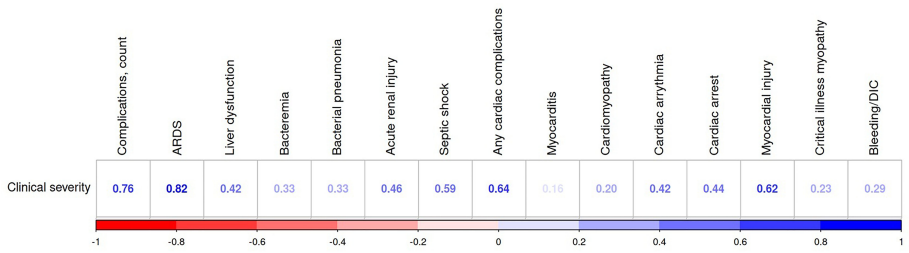

FIGURE 4 | Association of clinical severity with COVID-19 complications (Dubai dataset). All the associations are significant (p<0.05). ARDC, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation.

septic shock (r = 0.59), acute renal injury (r = 0.46), and liver dysfunction (r = 0.42).

There were marked differences in symptoms among age and sex groups. Cough and fever were more frequent symptoms in midlife adults (p < 0.001 and 0.0108), sputum and shortness of breath in older adults (p = 0.0228 and 0.001), and headache in young adults (p = 0.021). Symptoms such as sore throat, headache, and loss of smell or taste were more common in females (p = 0007, 0.0445, and 0.0396). The tendency was especially notable in young and midlife adults.

4.3 Forecast of COVID-19 Severity From Age and Sex Along With Laboratory Findings and Personal Risk Factors

The risk of the non-mild form of COVID-19 increased with advancing age considerably (see **Table 2**). From the radiological findings, the risk of the non-mild COVID-19 was 1.5 times higher in midlife adults (aOR = 1.52475; p = 0.01265) and 4.7 times greater in older adults (aOR = 4.72629; p = 0.02364) compared to young adults. This relationship was more pronounced when we analyzed the clinical markers of disease severity for midlife adults (aOR = 5.55; p < 0.001) and older adults (aOR = 17.05; p < 0.001) with young adults as a reference. Age disparities were more pronounced when studied with the clinical markers of disease severity than with the radiological markers. The higher sensitivity of the clinical markers for our study was even more evident in sex-related differences: men were more likely to develop a non-mild form of COVID-19 as per clinical classification (aOR = 1.82; p = 0.0083), but there was no strong argument to support this statement when the radiologic features were in use (aOR = 1.2; p = 0.44).

The multivariate analysis provided similar findings. In general, age and sex were important predictors of disease severity among the set of data typically collected on admission.

The comparison of the informative value of age and sex with the laboratory findings showed age to be on the list of the top valuable predictors, and sex to be less important (see **Figure 5A**). Patients' age was the most important feature among individual risk factors (see **Figure 5B**). In the list of predictors ranked by their informative gain, age was followed by BMI, ethnicity, total number of comorbidities and, finally, sex. Recently, we published a detailed analysis of ethnic disparities of COVID-19 (Al Zahmi et al., 2022).

The prediction of the disease progression was more accurate after considering sex and age of the individuals. The area under the curve value increased pronouncedly (p < 0.05) after we added these predictors to the model forecasting disease severity from individual risk factors (+8.41%; see **Table 3** and **Figure 6**).

5 DISCUSSION

The results of our study indicate a higher severity of COVID-19 disease in men and in older patients. With regard to sex dissimilarities, the number of male patients exceeded the number of female patients in both study centers allocated in different Emirates. These findings are similar to the results of other studies on age and sex features of various infectious diseases held in the past. They show differences in the response to infectious diseases between sexes and among age groups. For example, a retrospective analysis of hospitalization for pneumonia and influenza illustrates higher rates of admission in men of all age groups. This is also true for patients aged 65 years and over (Crighton et al., 2007). Moreover, the coronavirus that caused MERS in 2012–2018 affected mostly men (Das et al., 2021) and in the UAE more than 70% patients diagnosed with MERS were males (Ahmadzadeh et al., 2020).

5.1 Risk Factors and Comorbidities Associated With Age and Sex

Our study reports hypertension as the most common comorbidity in COVID-19 patients followed by diabetes. Similar studies have reported an increased risk of mortality from COVID-19 in patients with underlying heart conditions, e.g., with hypertension in males over 55 years of age and with coronary artery disease in females under 65 years (Aghajani et al., 2021). Hypertension, obesity, and chronic kidney disease were the predictors of mortality in another study (Jun et al., 2021). Patients diagnosed with COVID-19 commonly suffer from such diseases as hypertension (62.9%), hyperlipidemia (47.7%), and diabetes (39.8%) (Guerson-Gil et al., 2021). Male patients tend to have more background diseases. The spectrum of the background diseases also differs between sexes. In a study, peripheral vascular disease, dementia, chronic pulmonary disease, connective tissue disease, peptic ulcer disease and malignancies were common in women suffering from COVID-19, whereas a history of hypertension, hemiplegia, or myocardial

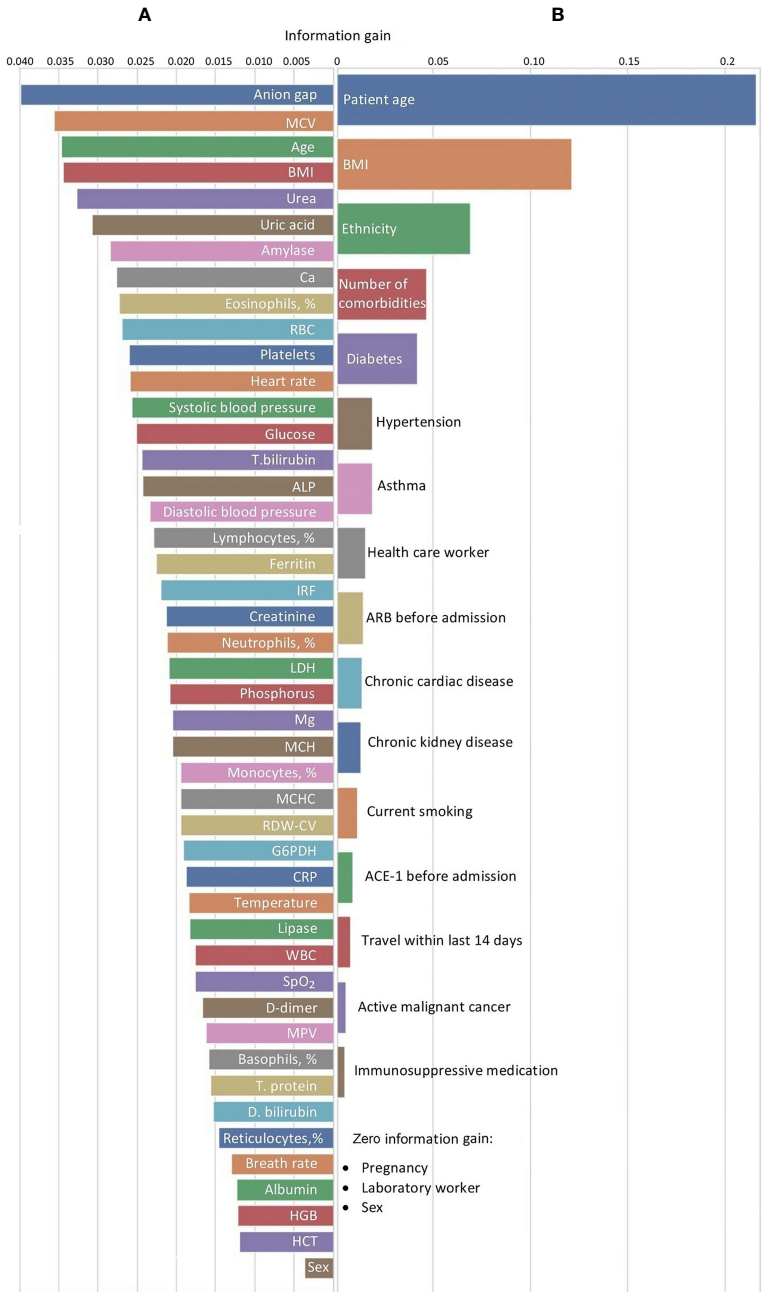

FIGURE 5 | Feature selection for predicting disease severity assessed with **(A)** laboratory findings or **(B)** clinical criteria in Dubai dataset. ACE-I, angiotensin-converting-enzyme inhibitors; ALP, alkaline phosphatase; ARB, angiotensin-II receptor blockers; ARDS, acute respiratory distress syndrome; BMI, body mass index; CRP, C-reactive protein; D.bilirubin, direct bilirubin; G6PDH, glucose-6-phosphate dehydrogenase; GCS, Glasgow coma scale; GI, gastrointestinal; HGB, hemoglobin; HCT, hematocrit; ICU, intensive care unit; IRF, immature reticulocyte fraction; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin level; MCHC, mean corpuscular hemoglobin concentration; MPV, mean platelet volume; RBC, red blood cells; RDW-CV, red blood cell distribution width; SpO2, oxygen saturation; SOFA, sequential organ failure assessment; T. bilirubin, total bilirubin; T. protein, total protein; WBC, white blood cells.

infarction was more typical of men infected with SARS-CoV-2 (Cho et al., 2021).

Obesity is a risk factor for admission to ICU and intubation for coronavirus pneumonia in both sexes (Jun et al., 2021). In the present study the mean BMI ranges were within the interval of 27–28 kg/m2 in both hospital cohorts. This refers to overweight or pre-obesity. On average, patients of both sexes aged above 65 years had a BMI greater than 31 kg/m2 indicating obesity.

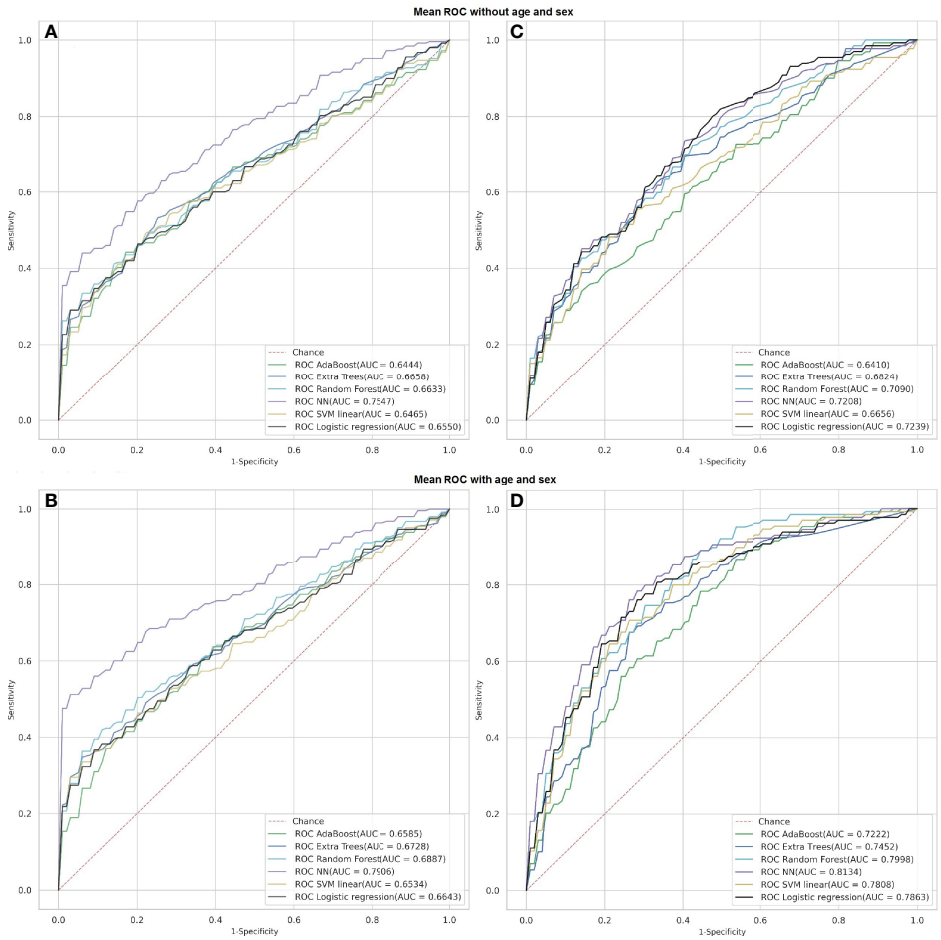

FIGURE 6 | Receiver operating characteristics of classification models predicting severity of COVID-19 from laboratory findings (**A, B**; Abu Dhabi dataset) or individual risk factors (**C, D**; Dubai dataset) with and without age and sex.

Recent studies suggested that the level of obesity correlated independently with the in-hospital mortality in patients with COVID-19. Moreover, people with a BMI above 25 kg/m2 are at an increased risk of developing severe pneumonia and the in-hospital mortality was higher in underweight patients and in individuals with morbid obesity (Guerson-Gil et al., 2021).

5.2 Radiologic Findings in COVID-19 With Regard to Age and Sex

In the patients with COVID-19, the involvement of the lung parenchyma varies between sexes and among ages. From our study, age bias in the lung involvement seemed to be stronger than sex bias (see **Figure 1**).

5.2.1 Sex Differences in Radiologic Findings in COVID-19

The present study demonstrates a significant sex disparity of the lung involvement in young adults. The data reflect a more severe lung involvement in men, i.e., the lesions are denser in men compared to women of the same age ($p < 0.05$). Although we did not assess the distribution of lesions across the lung space, our findings are compliant with the results of recent studies on related issues (Dangis et al., 2020). In contrast to these studies, we resort to the analysis of radiomics features extracted from semiautomatic segmentation of the lung lesions and lobes because this approach seems to be the most comprehensive one.

A study on CT appearance of COVID-19 presents differences between male and female patients of two age groups: below and above 60 years. There is a considerable difference in the peripheral involvement of the lung lobes (100% in males vs. 88.2% in females) as well as in the peribronchovascular distribution of the lesions (18.2% vs. 41.2%). In the age group below 60 years, the anterior distribution of the lesions was more common in men (66.7% vs. 41.7% in women). In each lung lobe, the CT score was higher in male patients. This leads to higher numbers of the total lung CT score in men (11 vs. 6.5) (Moradi et al., 2020). Other scientists observed unilateral pneumonia with ground-glass opacification mostly in women. They detected bilateral pneumonia more often in men (Wang et al., 2021).

5.2.2 Age-Related Dissimilarities in Radiologic Appearance of COVID-19

Lung involvement in COVID-19 in diverse age groups has only been studied in a few studies. Previous work reported the lung involvement at the level of 36.4% in the young group versus 67.9% in the group above 60 years of age (Zhu et al. 2020). These data are consistent with our findings which show the percentage of the lung involvement in young adults to be approximately twice as low as in midlife adults and three times as low as in older adults. In line with this, several studies show bilateral lung involvement to be more common in older adults (Li et al., 2020; Fan et al., 2020; Zhao et al., 2020). Single focal lesions are characteristic for patients under 35 years of age, diffuse ones for the individuals aged 60 years and over (Fan et al., 2020; Liu et al., 2020b).

Both sexes present with a marked association between age and such a metric as a "combined lung severity and pulmonary score." Notably, men of the age range from 50 to 79 years portray a higher score value. Conversely, the score values were higher for females aged 80 years and over (Borghesi et al., 2020b). In another type of coronavirus pneumonia, scientists also observed a positive association between the chest radiographic score and the disease progression in patients diagnosed with MERS (Das et al., 2021). A recent study also provided evidence for a distinct age bias in asymptomatic and symptomatic patients with COVID-19. In symptomatic patients, the total lung score in the elderly was higher than in the young patients (8.5 vs. 5.0; p = 0.07). In contrast, the score did not differ significantly in the asymptomatic group (Mori et al., 2021). The value of the aforementioned studies is limited as almost all of them did not consider background diseases that may confound the results.

The lung density. In our study, the density of the lesions was higher in men and in older adults. Notably, men were more often in a severe or critical condition than women. According to a prior chest evaluation report on patients with COVID-19-associated pneumonia, critical cases had a higher mean density of the lung compared to the ordinary cases and this was most likely accounted for by interstitial changes (Lyu et al., 2020).

The lung lobe involvement is another radiomical feature under analysis. According to our data, the involvement of both right and left lower lobes at the early disease phase was approximately equal in both sexes and all ages. As the disease progresses, we noted age- and sex-specific differences in this feature. A supposed reason for this is that the lobar distribution of the ground glass opacities (GGO) reflects the COVID-19 progression. The right and left lower lobes were affected most commonly, particularly in 42% and 38% of cases, respectively. Other researchers have also shown that opacification in any lobe correlates with the risk of hospitalization and intubation except for the one allocated in the left lower lobe (Toussie et al., 2020). The lung opacities in COVID-19 are commonly bilateral, peripheral, and basilar in distribution. At the early stage of the disease, they predominantly occupy the right lower lobe predominantly (Shi et al., 2020). This finding can be related to the thick and short physiological structure of the right lower lobe bronchus, which allows the virus to enter this area easily (Chen et al., 2020). However, other research gives the opposite data that the left lower lobe is affected most commonly in all age groups (Li et al., 2020).

The total number of the affected lobes and the presence of mild pleural thickening correlates with the age of patients (Wang et al., 2020; Liu et al., 2020b). The lesions are distributed across multiple lobes, predominantly in the posterior and peripheral parts of the lungs (Wang et al., 2020).

Some authors show a profound age difference in the involvement of the peripheral parts of the right upper and middle lobes. They report such a difference between the young (<60 years) and older adult (≥60 years) symptomatic patients (Mori et al., 2021). This is in line with another study that shows a variance in distribution of lesions across subpleural parts of the lung. In the midlife patients, it is twice as large as in the young and older adults infected with SARS-CoV-2 (Fan et al., 2020).

Lung lesions. In our study, the portion of the lung volume occupied with GGO was higher than the one affected in the form of consolidation. This tendency was true for all the age groups and reflects the known dynamics of the radiologic changes over the disease course. Chest CT examinations in an in-patient clinic in Wuhan (China) elucidated distinct lung opacification patterns with regard to age. In the group below 60 years, the proportion of patients with consolidation, pure GGO, and GGO with consolidation in the peripheral area was 1: 2.1: 3.4. The ratio for the group above 60 years is 1: 2.5: 4.2, respectively (Zhu et al., 2020). There is a slight increase in consolidation of the inflammatory exudate in the alveoli of patients aged above 60 years (Fan et al., 2020). Researchers presume that the appearance of the pure GGO is slightly lower than that of its combination with consolidation. This happens because the pure GGO is present in the early stage of the disease. The old-age group is characterized by a higher proportion of extensive involvement of the lung lobes (71.4% vs. 36.4%, p = 0.009) and a higher incidence of subpleural line and pleural thickening (50.0% vs. 25.0%, and 71.4% vs. 40.9%, p = 0.030 and p = 0.011, respectively) (Zhu et al., 2020).

5.3 Biochemical Response to COVID-19 With Regard to Age and Sex

5.3.1 Sex Disparities in Biochemical Response to COVID-19

Biochemical substrates. The results we observed on the study cohorts from hospitals in two Emirates support the findings obtained on a multinational database of 41 global healthcare organizations (Alkhouli et al., 2020). From the analysis, the level of stress and inflammatory markers (CRP, fasting glucose levels), the end products of protein metabolism (urea nitrogen, creatinine), and heme metabolism (bilirubin) are disproportionally higher in men. This presents sex disparities in the response to the disease. Both in our study and in the study based on the large multinational cohort, the level of albumin was slightly lower in men. The additive value of our study is the uniformly based sample consisting of all the patients admitted subsequently to the hospitals regardless of disease severity. This allows us to verify the aforementioned sex disparities and to justify their statistical significance.

Biochemical enzymes. In our study, the levels of ALT, AST, and LDH were higher in males. No significant difference between sexes was found for the level of alkaline phosphatase (AP). In addition, we did not observe considerable difference in the level of either LDH or AP. Despite this, the levels of amylase, lipase, and glucose-6-

phosphate dehydrogenase (G6PDH) were markedly higher in men, thus justifying the disparities in metabolic changes between males and females. Similarly, a multinational study elucidated sex-related differences in the activity of serum enzymes (Alkhouli et al., 2020).

Electrolytes. Our data showed a pronounced difference in the blood level of potassium and bicarbonate, both reflecting a more severe level of hypoxia in men. This may be well explained by the sex dissimilarity in the lung involvement seen on CT (see **Table 4**). The multinational registry shows the concentration of bicarbonate to be approximately equal in both sexes and the level of sodium to be slightly higher in men. Also, there was no considerable difference in calcium, magnesium, and chloride levels in that study (Alkhouli et al., 2020). Thus, the electrolyte disbalance provides evidence for the respiratory changes that are more pronounced in male patients.

Hematologic findings. No notable differences related to COVID-19 were found between the sexes in hematological findings either in our study or in the broad study that involved 6,387 men and 8,325 women (Alkhouli et al., 2020). The same was true for the platelets and the WBC count (Alkhouli et al., 2020). However, another study on sex differences in the immune resistance to SARS-CoV-2 found an association of the weak T cell response with worse disease outcome in males. Conversely, high innate levels of cytokines (e.g., IL-1, IL-6) were predictive of a mild disease form in women (Takahashi et al., 2020). Cytokines rise in patients diagnosed with COVID-19, and the levels of IL-4 and sCD40L cytokines were higher in men than in women in our study. The association between these types of cytokines shows differences between female and male cytokine responses (Petrey et al., 2021). A recent study conducted in China showed higher lymphocyte and lipoprotein levels in females (Sha et al., 2021).

Clinical symptoms. A few facts are found on the variance in the clinical appearance of COVID-19 with regard to age (Liu et al., 2020b). In our study, a sore throat and headache in women are almost twice as common as in men (40.21 vs. 25.88%; $p < 0.0007$ and 24.87 vs. 17.52%; $p < 0.0445$). A similar study reported fatigue at the level of 11% in the elderly and only 7% in the younger and middle-age groups (Liu et al., 2020a).

5.3.2 Dissimilarities in the Response to COVID-19 Among Age Groups

Coagulogram. Much research has been done to find out what accounts for a larger number of complications and a higher mortality rate in the elderly. Some manage to link age with procoagulant changes in the blood clotting system. For instance, a few studies reported that coagulation deviations can be associated with older age (Sayad and Rahimi, 2020). Such coagulogram abnormalities may lead to thrombotic complications and endanger infected individuals even more (Giannis et al., 2020). A comparison of two age groups (below and above 60 years old) shows a markedly higher APTT level in the older group. Patients aged above 60 years had a higher level of fibrinogen than in the younger group (Peng et al., 2021). A conflicting finding in a study from Tehran does not seem to be representative because of a small sample size. In that study of patients with severe COVID-19, the fibrinogen level was 4.9 ± 1.9 g/l in 6 patients aged less than 60 years versus 2.4 ± 0.6 g/l in 10 patients aged above 60 years ($p = 0.08$) (Sayad and Rahimi, 2020). Researchers observed a positive association of the level of D-dimer with advancing age (Zhao et al., 2020; Peng et al., 2021). However, the above mentioned Iranian study reported an opposite tendency (Sayad and Rahimi, 2020).

Biochemical findings. The general outcome of our research and other studies is that the age-related disparities in biochemical findings are analogous to the sex-related dissimilarities. The risk of metabolic dysregulation is higher in older adults than in young adults. In an equivalent way, male sex is associated with abnormal levels of a set of biochemical parameters. For instance, a study showed a significantly lower level of *troponin* in the younger group (Peng et al., 2021). There was an age-related bias in the level of *electrolytes* indicative of hypoxia (e.g., potassium) (Zhao et al., 2020; Peng et al., 2021).

Laboratory markers of the protein metabolism have evident age-related bias in COVID-19. The levels of total protein and albumin are significantly higher in younger patients (Zhao et al., 2020; Liu et al., 2021; Peng et al., 2021). Contrarily, in the older patients researchers noted a significantly higher concentration of the end products of the protein and heme metabolism, i.e., urea (Peng et al., 2021), blood urea nitrogen (Zhao et al., 2020; Liu et al., 2021), creatinine (Zhao et al., 2020; Peng et al., 2021), total bilirubin (Zhao et al., 2020; Peng et al., 2021), and direct bilirubin (Zhao et al., 2020). However, there are some conflicting findings on the association of the total bilirubin (Liu et al., 2021) and direct bilirubin (Peng et al., 2021) with advancing age. Besides the difference in disease severity, an appreciably higher glomerular filtration rate in the young patients may explain these facts (Liu et al., 2021).

Enzymatic activity is subject to age. However, there are conflicting findings on the activity of ALT (Liu et al., 2020b; Liu et al., 2021; Peng et al., 2021; AST Zhao et al., 2020; Liu et al., 2021; Peng et al., 2021), ALP (Liu et al., 2021), LDH (Peng et al., 2021), GGTP (Liu et al., 2021), and CK (Zhao et al., 2020; Liu et al., 2021; Peng et al., 2021). Supposedly, the high variance in the individual enzymatic activity induced by pathology accounts for the discrepancies among independent studies.

Inflammatory markers. In analogy to our study, other studies justify age and sex as valuable predictors of disease severity with distinct approaches to data analysis. Similar to our study, the level of CRP on admission and during the disease course was higher in the older patients (Zhao et al., 2020; Peng et al., 2021). CRP fluctuations are independent risk factors for ICU admission in young and middle-aged severe patients in contrast to the older ones (Liu et al., 2021).

Cytokines. In our study, the individual variance in the level of interleukin-6 (IL-6) was considerable among patients. A recent study found evidence to suggest that elderly patients were prone to a cytokine storm (Xu et al., 2021). Because of the high variance, there are conflicting findings on whether the age difference in IL-6 is notable (Zhao et al., 2020) or not (Peng et al., 2021). A study shows greater pulmonary concentrations of proinflammatory cytokines in men than in women (Conti and Younes, 2020). The opposite tendency was observed in influenza A infection (Morgan and Klein, 2019). Further studies on this issue are required to verify the results.

Hematologic findings. *Platelets*. From our data, the platelet count did not vary among the age groups. In a study conducted in China, an absolute count of platelets did not differ considerably in patients of different ages (Peng et al., 2021). The same tendency was observed in severe patients of two age groups with a threshold of 60 years (Liu et al., 2021). Identical results were reported by a research group from Iran (Sayad and Rahimi, 2020). In a study that included patients of three age groups (<60 years; 60–74 years; ≥75 years), the middle-aged patients had a remarkably higher platelet count compared to the younger ones (215 vs. 209), whereas the oldest patients showed the lowest (186) platelet count (Zhao et al., 2020).

WBC. We did not observe any associations between WBC and age; however, one study reported a marked increase in the WBC count with advancing age (Zhao et al., 2020), while others failed to observe this age-related pattern in WBC (Peng et al., 2021); Liu et al., 2021). Despite the discrepancies, the mean leukocyte count for the groups ranges within the reference diapason. Thus, the dissimilarities have no clinical value.

Neutrophils and monocytes are an issue in our study as the data on them are also discrepant. Previous research has reported that either the absolute count of neutrophils and monocytes was not associated with age (Peng et al., 2021) or it rises with advancing age (Zhao et al., 2020; Liu et al., 2021). Our findings support the second statement.

The absolute lymphocyte count has been shown to be negatively associated with age (Zhao et al., 2020; Liu et al., 2021; Peng et al., 2021). Consistently, a retrospective study reported a markedly lower percentage of lymphocytes in the older group 19.15 (10.58–26.93) than in the young and middle-aged group 28.95 (24.45–33.58) (Liu et al., 2020a). Another reference shows an appreciably lower percentage of lymphocytes in older adults compared to younger adult patients (24.4% vs. 26.5%; $p = 0.02$) (Mori et al., 2021).

The red blood cells count and the level of hemoglobin decreased with advancing age in our study as well as in reports of other studies (Liu et al., 2020b). However, another study reported a slight difference in the indices across the age (Peng et al., 2021).

5.4 Informative Value of Sex and Age in Prediction of COVID-19 Severity

In analogy to our findings, other studies with distinct approaches to data analysis have reported evidence to suggest age and sex as valuable predictors of disease severity. In a study on prediction of the in-hospital mortality for COVID-19, researchers highlighted that the patients' age conferred the highest risk of death. Furthermore, a multivariate analysis shows that age along with a CXR severity index and immunosuppression were strongly associated with the in-hospital mortality (Borghesi et al., 2020a). Similarly, older adults were more frequently affected during a MERS outbreak in Saudi Arabia, and the 30-days mortality was higher for that age group Ahmed (2017).

A low oxygen saturation level (SpO_2) is a known predictor of mortality or a rapid progression of COVID-19 to more severe forms requiring hospitalization and ICU admission (Covino et al., 2020; Xie et al., 2020). To forecast an adverse outcome in COVID-19, previous researchers built a multivariate model based on age and other covariates (e.g., oxygen saturation, asthma). The accuracy of the model was lower than in our study, and the authors did not assess the informative value of age in the prediction (Goodacre et al., 2021). Scientists and clinical specialists observe high severity of the disease or death mostly in patients with the oxygenation level below 90%. For example, a retrospective analysis of clinical data of patients from China indicates that the SpO_2 level below or equal to 90% is a predictor of the severe and critical disease. According to the study, older patients (61–78 years) had a lower oxygenation status compared to the middle-aged adults (40–63 years) (Xie et al., 2020). In another study, the saturation level correlated negatively with age. Patients in a severe or critical condition have a mean SpO_2 level of 90.25%, while in other cases it is 97% (Mi et al., 2020). Another research group states that the oxygen saturation on admission below 90% is strongly associated with the in-hospital mortality for COVID-19, but the SpO_2 level was not age dependent (Mejía et al., 2020).

Inconsistent findings and models with weak performance justify a necessity for a more precise analysis that would elucidate the impact of age on the level of oxygen saturation which is predictive of disease outcomes (Goodacre et al., 2021).

6 STRENGTH AND LIMITATIONS

There were several strengths to the study. Firstly, the study cohort was representative of the COVID-19 population in Dubai including all adult age groups and disease severity enabling us to calculate actual risk estimates. *Secondly*, it was a multicentered study which compared data from two hospitals functioning in separate Emirates. Hospitals in each Emirate report to a designated health authority of a distinct emirate, i.e., Dubai Health Authority and Department of Health of Abu Dhabi. This justifies them as independent centers for clinical services and studies. At the same time, the diagnostics and treatment were performed in full accordance with the common "National Guidelines for Clinical Management and Treatment of COVID-19" (National Emergency Crisis and Disasters Management Authority, 2020).

The main limitation of our study was the retrospective design. For this reason, we could not unify the settings of the study for both hospitals and collected the data available in their medical information systems.

Another limitation is that we resorted to different ways of assessing disease severity in the datasets used. Thus, the impact of age and sex on disease severity can be assessed only within the framework of each sample. However, the level of severity is not transferable between the datasets used.

7 CONCLUSION

Our study highlights several important novel findings that have clear clinical implications for the treatment and management of COVID-19 patients across a spectrum of age groups and disease severity:

- The need for 0_2 supplementation was directly correlated with age. Intensive care was required more often for men than for women of all ages ($p < 0.01$). These facts mirror the results of

biochemical findings and may justify a direct correlation of older age and male sex with a severe course of the disease.
- Laboratory data justify the trend toward worsening of the SARS-CoV-2 infection with age. The portion of mild cases decreases dramatically with age while the percentage of severe cases rises. Biochemical findings in both datasets support the tendencies identified from radiomics. Sexual dissimilarities were pronounced in young adults and reduced with age.
- The severity of COVID-19 assessed from the percentage of the total lung involvement did not differ between sexes. However, in young male adults, both the percentage of the lung parenchyma covered with consolidation and the density characteristics of lesions were higher. These facts suggest a more severe lung involvement in men. In midlife and older adults, no marked differences between sexes were found.
- From the univariate analysis, the risk of the non-mild COVID-19 was higher in midlife adults and older adults compared to young adults. Age disparities were more pronounced if studied with the clinical markers of disease severity than with the radiological markers. The higher sensitivity of clinical markers in our study is even more evident in sex-related differences. The multivariate analysis provides similar findings.
- Age and sex should be considered while forecasting the severity of COVID-19. This can improve management of patients, personnel, and equipment in real clinical settings.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The datasets generated for this study are available on request at the site of Big Data Analytics Center https://bi-dac.com.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Department of Health Abu Dhabi (reference number: DOH/CVDC/2020/889), Mediclinic Middle East Research and Ethics Committee (reference number MCME.CR.104.MPAR.2020), and Dubai Scientific Research Ethics Committee of Dubai Health Authority (protocol number DSREC-05/2020_25). The committees approved the study for the retrospective analysis of the data obtained as a standard of care. No potentially identifiable personal information is presented in the study. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

All authors contributed to the creation of the article as follows: JAK, FAZ, and YS formulated the objectives. JAK, FAZ, RA, HE, MaL, NS, RS, DK, and SN collected the datasets. YS, DS, and GS wrote the manuscript; TH performed the statistical analysis, prepared the figures and tables for data presentation and illustration, KG, MiL, TL, TA, AB and AD contributed to the literature review and data analysis. All the authors have reviewed the manuscript and vouched for the accuracy and completeness of the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.777070/full#supplementary-material

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Ethnicity-Specific Features of COVID-19 Among Arabs, Africans, South Asians, East Asians, and Caucasians in the United Arab Emirates

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Background: Dubai (United Arab Emirates; UAE) has a multi-national population which makes it exceptionally interesting study sample because of its unique demographic factors.

Objective: To stratify the risk factors for the multinational society of the UAE.

Methods: A retrospective chart review of 560 patients sequentially admitted to inpatient care with laboratory confirmed COVID-19 was conducted. We studied patients' demographics, clinical features, laboratory results, disease severity, and outcomes. The parameters were compared across different ethnic groups using tree-based estimators to rank the ethnicity-specific disease features. We trained ML classification algorithms to build a model of ethnic specificity of COVID-19 based on clinical presentation and laboratory findings on admission.

Results: Out of 560 patients, 43.6% were South Asians, 26.4% Middle Easterns, 16.8% East Asians, 10.7% Caucasians, and 2.5% are under others. UAE nationals represented half of the Middle Eastern patients, and 13% of the entire cohort. Hypertension was the most common comorbidity in COVID-19 patients. Subjective complaint of fever and cough were the chief presenting symptoms. Two-thirds of the patients had either a mild disease or were asymptomatic. Only 20% of the entire cohort needed oxygen therapy, and 12% needed ICU admission. Forty patients (~7%) needed invasive ventilation and fifteen patients died (2.7%). We observed differences in disease severity among different ethnic groups. Caucasian or East-Asian COVID-19 patients tended to have a more severe disease despite a lower risk profile. In contrast to this, Middle Eastern COVID-19 patients had a higher risk factor profile, but they did not differ markedly in disease severity from the other ethnic groups. There was no noticeable difference between the Middle Eastern

subethnicities—Arabs and Africans—in disease severity (p = 0.81). However, there were disparities in the SOFA score, D-dimer (p = 0.015), fibrinogen (p = 0.007), and background diseases (hypertension, p = 0.003; diabetes and smoking, p = 0.045) between the subethnicities.

Conclusion: We observed variations in disease severity among different ethnic groups. The high accuracy (average AUC = 0.9586) of the ethnicity classification model based on the laboratory and clinical findings suggests the presence of ethnic-specific disease features. Larger studies are needed to explore the role of ethnicity in COVID-19 disease features.

Keywords: COVID-19, ethnicity, Middle East, Gulf region, UAE, machine learning, viral pneumonia, host organism

1 INTRODUCTION

During the pandemic, the impact of coronavirus disease 2019 (COVID-19) on society varied considerably from country to country. To compare different nations, researchers estimated the impact with case fatality ratio (CFR). In the middle of 2020, the CFR was 3.7% in mainland China, 15.1% in the UK, and 14.2% in Italy (Grasselli et al., 2020; Mortality Analysis, 2020; Yang et al., 2020). Many factors may account for the difference in case fatality ratio across world regions, e.g., population density and settlement, the proportion of the elderly in the society, the affordability and accessibility of national healthcare systems, and the *ethnic background*, which implies genetic variation.

Studies on ethnic disparities of COVID-19 are challenging since there is no well-defined concept of ethnicity. On the one hand, this term refers to self-identification of people with a particular cultural group based on customs, norms, and ideologies. On the other hand, ethnicity is the cultural and genetic heritage of the person's ancestors. The country of birth may inappropriately identify ethnicity because of the global tendency towards migration (Clarke et al., 2008). Another valid biological category for medical studies is race. Hypothetically, there is an association between genes that determine race and health. This does not comply with the data that genetic variations within races are more pronounced than between them (Egede, 2006).

Several studies and systematic reviews tried to explore whether ethnicity was a risk factor for severe COVID-19 disease form. However, the relationship between ethnicity and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) infection remains uncertain (Pan et al., 2020). There are some inconsistencies in the findings on the association between ethnicity and clinical outcomes including hospitalization (Sze et al., 2020). However, a common limitation of such studies is that they focus on the West European or North American communities and consider other ethnicities as minorities. Though important for clinical risk stratification and proper patient management, such data are limited for multinational communities of the Gulf region with only few papers providing information on this issue (Almazeedi et al., 2020; Hannawi et al., 2021).

There is ambiguity regarding factors that account for the dissimilarities in COVID-19. Some authors show importance of disparities in the amount and location of adipose tissue whereas other authors point out a role of socioeconomic disparity (e.g., food insecurity and involvement in high-risk frontline jobs) (Islam et al., 2020; Krams et al., 2020). Environmental factors can also contribute to the diversity in the course of SARS-CoV-2 infection among people of different origins. The community of Dubai Emirate (United Arab Emirates; UAE) is an exceptionally interesting study sample because of its unique set of environmental, population, and economic factors. There is a pressing need to investigate these aspects of COVID-19 because this will contribute to proper risk management and foster further development of the community medicine (Habuza et al., 2021).

1.1 Studies on Ethnicity-Related Dissimilarities of COVID-19 in the Middle East

Commonly, researchers from the Middle East compare ethnicity subgroups by such aspects as migration during the pandemic and mental state. They do not explore an association between COVID-19 severity and outcomes with the patient's race or ethnicity. Some studies provide descriptive statistics on ethnicity and citizenship of the participants (Al-Rifai et al., 2021; Hannawi et al., 2021) including pediatric patients (Elghoudi et al., 2020; Al-Rifai et al., 2021). Few of them categorize patients into Arabs and Asians to analyze their racial difference in disease severity and outcomes with adjustment for comorbidities (Ali et al., 2021;

Abbreviations: ACE, angiotensin-converting enzyme; ACE-I, angiotensin-converting-enzyme inhibitors; Adm, admission to the hospital; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; ARB, angiotensin-II receptor blockers; ARDS, acute respiratory distress syndrome; AUC, area under the ROC curve; AST, aspartate aminotransferase; BAME, Blacks, Asians, and Minority Ethnic groups; BMI, body mass index; CFR, case fatality ratio; CK, creatine kinase; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; DBP, diastolic blood pressure; GCS, Glasgow coma scale; HR BPM, heart rate beats per minute; ICU, intensive care unit; IQR, interquartile range; LDH, lactate dehydrogenase; LCR, lymphocyte-to-C-reactive protein ratio; ML, machine learning; NLR, neutrophil-to-lymphocyte ratio; NN, neural network; PCR, reverse transcription-polymerase chain reaction; ROC, receiver operating characteristic; RR/min, the time elapsed between two successive R-waves on the electrocardiogram; SARS-CoV-2, severe acute respiratory syndrome-related coronavirus 2; SBP, systolic blood pressure; SD, standard deviation; SES, socioeconomic status; SOB, shortness of breath; SOFA, Sequential organ failure assessment; SpO_2, oxygen saturation; TMPRSS2, transmembrane serine protease 2; WBC, white blood cells.

Deeb et al., 2021). Unique research of antibody titers and epitope coverage is carried out predominantly on Caucasians and South Asians with few Middle Eastern patients included (Smith et al., 2021). One study in Israel compared the impact of COVID-19 on Jewish and Arab populations (Haklai et al., 2021).

1.2 Studies on Ethnic Features of COVID-19 Across the World

Table 1 and both **Figures 1** and **2** summarize study cohorts of 50 open-access papers that reported original findings on the dissimilarities of COVID-19 among ethnic groups. The papers were retrieved consecutively from the Google Scholar search engine with the query comprising the following keywords: "race, ethnicity, COVID-19". In this paper, we want to characterize research methodology traditionally used in such studies and discuss some of their limitations. The majority of the studies related to ethnicity and COVID-19 outcomes were conducted by scientists from either the USA or the UK (Bassett et al., 2020; Hsu et al., 2020; Karaca-Mandic et al., 2021; Lundon et al., 2020; Rentsch et al., 2020; Gianfrancesco et al., 2021; Kabarriti et al., 2020). The US population is traditionally divided into Hispanic, non-Hispanic Whites, non-Hispanic Blacks, Asians, and certain minorities including Alaska Natives. The above-mentioned studies did not include Arabs as a separate ethnic entity. Many studies have been devoted to an association between mortality from COVID-19 and race (Bassett et al., 2020; Hsu et al., 2020; Kabarriti et al., 2020; Lundon et al., 2020; Gold et al., 2020; Golestaneh et al., 2020; Gianfrancesco et al., 2021; Price-Haywood et al., 2020; Rentsch et al., 2020; Rossen et al., 2020), an effect of ethnicity on *hospitalization* (Hsu et al., 2020; Lundon et al., 2020; Price-Haywood et al., 2020; Gianfrancesco et al., 2021), *admission to intensive care unit* (ICU) (Hsu et al., 2020), and *the need for mechanical lung ventilation* (Hsu et al., 2020; Lundon et al., 2020). Few studies explain the influence of ethnicity on *COVID-19 outcomes* in a socio-demographic context (Lundon et al., 2020; Raifman and Raifman, 2020; Millett et al., 2020). Some scientists from North America compared the mortality rate among people of different origin before and after the COVID-19 pandemic (Golestaneh et al., 2020). Authors compared laboratory findings among ethnic subgroups of patients with COVID-19 (Price-Haywood et al., 2020). Researchers adjust the study subgroups with regard to comorbidities and background conditions (Lundon et al., 2020; Golestaneh et al., 2020; Gianfrancesco et al., 2021).

For epidemiological analysis, scientists from the UK aggregate data from the UK Biobank (Niedzwiedz et al., 2020), National

TABLE 1 | Exploratory analysis on number, age, and gender of participants in ethnic studies.

Ethnicity	Papers	Total	M, %	F, %	Age			
					From	To	Mean	Std
Whites	38	23,391,600	45.11	54.86	47.33	78.33	57.93	11.66
Other Minorities	37	1,610,802	44.22	55.75	32.90	68.65	50.97	13.09
Asians	29	1,437,685	54.09	45.91	35.67	69.67	53.46	6.46
Blacks	38	1,250,327	42.30	57.68	41.33	74.33	51.15	9.86
Hispanic	17	698,888	35.04	64.94	18.00	65.00	42.36	10.37
Jews	2	511,283						
Arabs	7	170,781	56.55	43.45	24.40	45.80	44.50	
Total	50	29,071,366	45.93	54.05	33.22	67.20	50.19	9.22

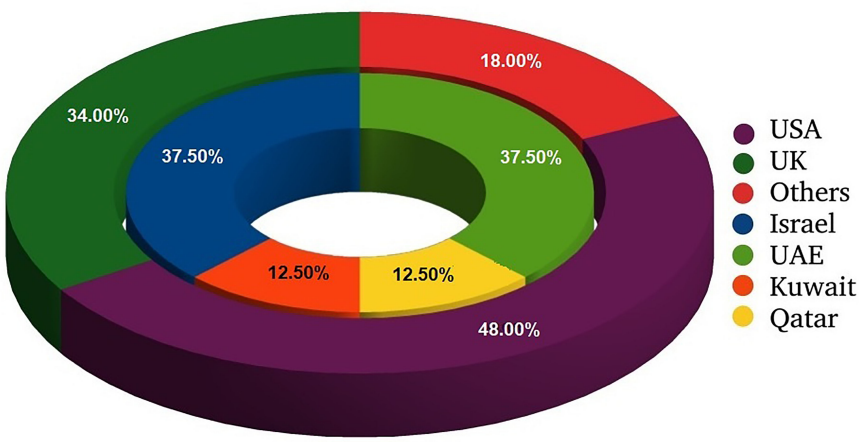

FIGURE 1 | Distribution of research papers on ethnical issues of COVID-19 with regard to location of study. External circle presents all analyzed studies; internal one shows information on studies conducted in Middle East.

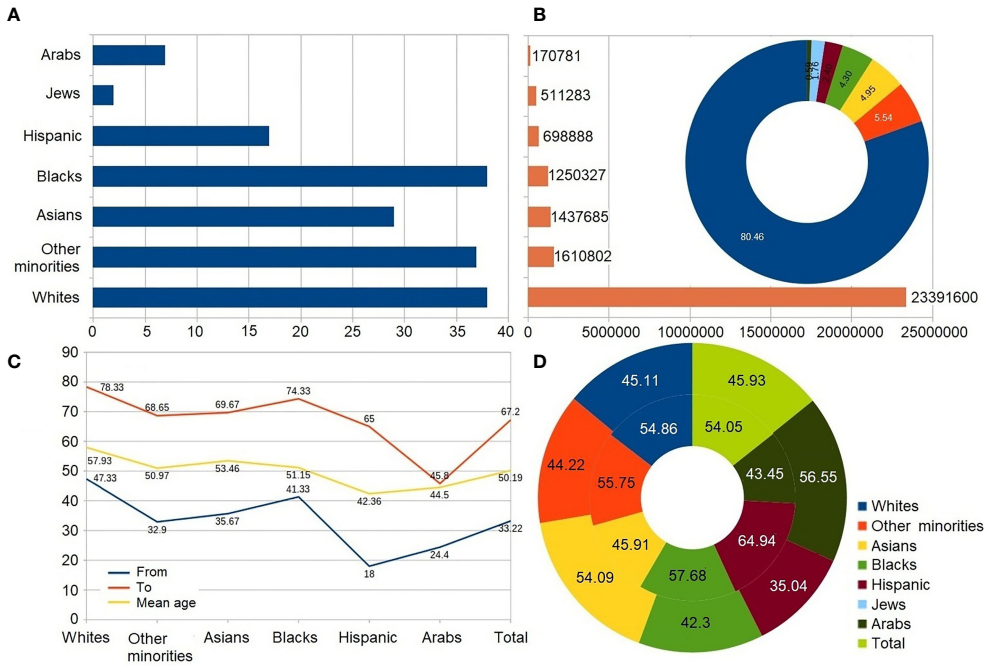

FIGURE 2 | Analysis of the ethnicity-based research papers. Circle diagrams report data in percentages. Results are calculated as average value of data reported in papers. **(A)** Number of papers. **(B)** Size of study cohort; circle diagram represents percentage out of all studied population. **(C)** Average age of participants. **(D)** Gender distribution. External/internal circle represents male/female rate.

Healthcare Service registry (Aldridge et al., 2020), Open SAFELY platform (Collaborative, 2021), and a few hospitals (Sapey et al., 2020; Apea et al., 2021). The specialists compare Blacks, Whites, South and East Asians, and minority ethnic groups, and they do not study peculiar features of COVID-19 among Arabs as a separate ethnic group (Alaa et al., 2020; Aldridge et al., 2020; Harrison et al., 2020; Hull et al., 2020; Niedzwiedz et al., 2020; Patel et al., 2020; Sapey et al., 2020; Apea et al., 2021; Collaborative, 2021; Nafilyan et al., 2021). The research objectives of the present study are as follows: investigate the relationship between race and incidence of SARS- CoV-2 infection (Niedzwiedz et al., 2020; Collaborative, 2021), disease *outcomes* (Harrison et al., 2020; Patel et al., 2020; Alaa et al., 2020), *and both hospitalization and ICU admission* (Apea et al., 2021). To adjust the supposed risk factors, various authors stratify the study samples by age, body mass index (BMI), comorbidities (Harrison et al., 2020), the number of people in the household, the accommodation type, income, and other factors (Nafilyan et al., 2021). While the majority of researchers aggregate data on epidemiology and patient management, few of them integrate ethnic-specific patterns of COVID-19 with laboratory findings, e.g., biochemical data of positively tested individuals (Apea et al., 2021).

1.3 The Timeline of the Spread of COVID-19 in the UAE

The United Arab Emirates was among the first countries to implement strict measures to control the pandemic, e.g., closing the national borders, limiting internal public movements and gatherings, shutting down schools, and the use of distant learning, and implementing work from home protocols. These measures smoothed the peak of the disease incidence and allowed the healthcare system to re-organize hospitals to effectively manage the case load and the COVID-19 outbreak, creating multiple field hospitals that can handle mild cases and converting several hotels to isolation facilities run by healthcare staff.

On January 29 2020, the UAE announced its first confirmed case of COVID-19. It was the first country in the Middle East to register a case of COVID-19. The first patient was a Chinese tourist who arrived in the UAE from Wuhan on January 16. By the end of January, there had been five confirmed cases of COVID-19 in the UAE. On January 30, WHO declared the novel coronavirus outbreak a PHEIC (Public Health Emergency of International Concern) (Tayoun et al., 2020). **Figure 3** shows the major steps taken by the UAE government to limit the spread of COVID-19.

The UAE population has increased substantially in the last 2 decades as a result of the remarkable growth in its economy. The economic growth has led to the influx of expatriate workers from all over the world. This accounts for the uneven distribution of age (more than two-thirds of the population is between 25 and 45 years of age) and the heterogeneous ethnic backgrounds. According to Dubai Statistics Center for 2019, the Dubai population was 3.3 million individuals, which constitutes 34% of the whole UAE population. Only 1.2% residents are older than 65 years of age (Wang et al., 2020; Yang et al., 2020; Zhou et al.,

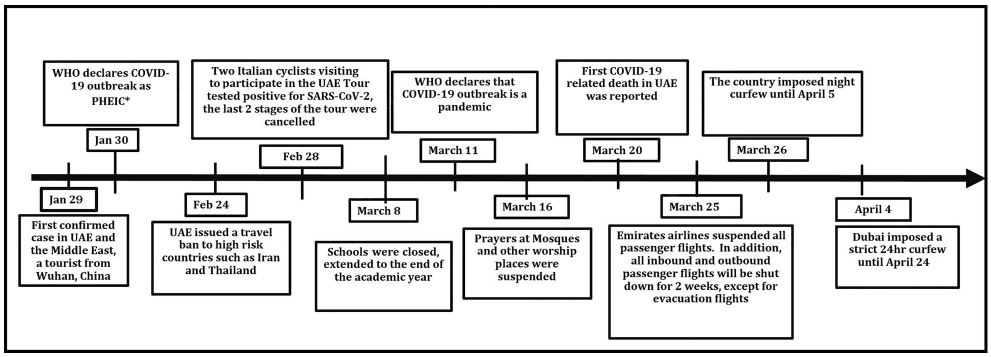

FIGURE 3 | Timeline of COVID-19 outbreak in UAE.

2020). Males represent 69.5% of the population. The vast majority of the individuals (92%) are non-UAE nationals, with 59.4% from South Asia and approximately 6% from the Philippines, which has led to a marked variation in sociodemographic structure of the UAE population.

2 OBJECTIVES

We intended to stratify the risk factors for the multinational society of Dubai. To address this objective, we had the following specific tasks:

- Explore the ethnic-specific features of COVID-19 by analyzing the disease course in people of different ethnic groups.
- Rank the most significant features that account for the ethnic-specific course of COVID-19 in the Dubai population.
- Build a model of ethnic specificity of COVID-19 based on the clinical presentation and laboratory findings on admission and evaluate its performance.

3 MATERIALS AND METHODS

3.1 Study Sample

We obtained retrospective data routinely collected as part of standard primary and secondary care. The study sample comprised all COVID-19 patients consecutively admitted to Mediclinic Parkview Hospital, Dubai (UAE), from the date of the first confirmed case, February, 26, 2020, until May, 31, 2020. At the beginning of the pandemic, all the patients with COVID-19 verified by reverse-transcriptase polymerase chain reaction (PCR) were hospitalized to Mediclinic regardless of the disease severity or medical insurance coverage. This made our study cohort representative of the entire Dubai population. The cohort included many asymptomatic and mild cases. A thorough description of the flowchart for the management of COVID-19 patients is given in our recent paper (Statsenko et al., 2021). See the patient management with a flow chart in our recent paper (Statsenko et al., 2021). Details of the current study are provided below.

The inclusion criteria were as follows (1): aged 18 years old or above (2); positive SARS-CoV-2 real-time PCR from a nasopharyngeal swab; and (3) inpatient admission. Patients meeting the inclusion criteria were followed until discharge. The national guidelines regulated inpatient management. As a part of the standard of care, baseline blood tests and inflammatory markers were obtained. Multiplex PCR assays were used to test respiratory samples for influenza and other respiratory viruses. Supportive oxygen therapy was initiated if oxygen saturation measured with pulse oximeter dropped below 94% or respiratory rate increased above 30 breaths per minute. Patients who were clinically suspected of having superimposed bacterial pneumonia were administered empirical broad-spectrum antibiotics at the discretion of the treating physician. The antiviral and antimalarial therapies were guided by "National Guidelines for Clinical Management and Treatment of COVID-19", a standardized guideline for all health sectors in the UAE (National Emergency Crisis and Disasters Management Authority, 2020).

It was a single-center study with a relatively short duration (3 months) as the UAE government standardized healthcare service for COVID-19 patients during this period in the following way. All the patients with a positive PCR test were hospitalized to either government-funded or private health facilities even if they were asymptomatic. The diagnostics and treatment of COVID-19 were provided free of charge in accordance with the National Guidelines (National Emergency Crisis and Disasters Management Authority, 2020). Because of this, the sample of the study is representative for the adult population of the country. Dubai Mediclinic is affiliated with the governmental medical school—Mohammed Bin Rashid University of Medicine and Health Sciences—and is the optimal center for medical teaching and research in Dubai.

3.2 Data Collection

We extracted the electronic health records of all consecutive patients with either admission or discharge diagnosis of COVID-19, coronavirus infection, unspecified, SARS-associated

coronavirus as the cause of diseases classified elsewhere, other coronavirus as the cause of diseases classified elsewhere, and pneumonia due to SARS-associated coronavirus (ICD10 codes U07.1, B34.2, B97.21, B97.29, and J12.81). Patients with negative SARS-CoV-2 PCR were excluded from the study. The information from electronic health records was extracted using a standardized data collection form by trained researchers. The form was adapted from ISARIC Rapid Case Record Form (COVID-19 CASE RECORD, 2020), see *Subsection 3.3*.

Variables of interest were manually extracted from electronic health records. The abstraction team of 7 physicians was trained and supervised by the principal investigator. To ensure the accuracy of the data entered, the team worked in the following manner. A team member extracted the required information to a data collection sheet. Another physician double-checked the information from the data collection sheet before entering it to an Excel spreadsheet. Any discrepancies were resolved by the supervisor.

3.3 Dataset Description

In our study, we classified the subjects into the following ethnic groups: Middle Easterns (Arabs of both the UAE and non-UAE nationality, Africans from the countries mentioned in **Table 2**), South Asians (patients from Afghanistan, Bangladesh, Bhutan, Maldives, Nepal, India, Pakistan, and Sri Lanka), East Asians (patients from China, Hong Kong, Philippines, Taiwan, Japan, Mongolia, North Korea and South Korea), Caucasians, and others.

To determine the case severity, we used the UAE National Guideline for Clinical Management and Treatment of COVID-19 (National Emergency Crisis and Disasters Management Authority, 2020):

- *Asymptomatic form* refers to a patient with no symptoms.
- *Mild form*—clinical symptoms of upper respiratory tract infection and no signs of pneumonia.
- *Moderate form*—fever and respiratory symptoms with radiological findings of pneumonia.
- *Severe form*—any one of the following criteria: respiratory distress (respiratory rate > 30/min), oxygen saturation <93% at rest, P/F ratio of less than 300.
- *Critical form*—any of the following criteria: acute respiratory distress syndrome (ARDS) with P/F ratio < 200, sepsis, multi-organ failure, altered level of consciousness (GCS<13).

The list of variables comprising the complete cohort dataset is as follows:

- **Demographics features**. Patient's age, gender, ethnicity, weight, height, body mass index (BMI), and occupation (for the patients who are healthcare or laboratory workers), travel history within 14 days prior to symptom onset, and exposure to a confirmed case of COVID-19.
- **Comorbidities**. History of chronic cardiac disease, hypertension, chronic lung disease, asthma, chronic kidney disease, diabetes, active malignant cancer, immunosuppressed state, human immunodeficiency virus infection (HIV), active smoking, and pregnancy status in female patients. Medication history: whether the patient was taking any of the following medications prior to the admission: angiotensin-converting enzyme inhibitors (ACE-I), angiotensin II receptor blockers (ARB), and/or non-steroidal anti-inflammatory drugs.
- **Symptoms at presentation**. Cough, sputum production, sore throat, chest pain, shortness of breath (SOB), fever, headache, confusion, nausea or vomiting, diarrhea, myalgia, malaise, and loss of smell or taste.
- **Vital signs**. Temperature, heart rate (HR BPM), respiratory rate, systolic (SBP) and diastolic blood pressure (DBP), SpO2, and sequential organ failure assessment (SOFA) score at the time of admission and at the time of transfer to ICU if applicable.
- **Laboratory findings**. The following parameters were collected *on admission and at the peak of illness*: white blood cell (WBC) count, lymphocyte count, platelet count,

TABLE 2 | Nationalities of Middle Eastern ethnic group.

Ethnicity	Subethnicity	Nationality	Number of cases
Middle Easterns 148 (100%)	Arabs 114 (77.03%)	UAE	73 (49.32%)
		Oman	8 (5.41%)
		Lebanon	5 (3.38%)
		Syrian	5 (3.38%)
		Jordon	5 (3.38%)
		Bahrain	3 (2.03%)
		Iran	3 (2.03%)
		Pakistan	2 (1.35%)
		Canadian Arab	2 (1.35%)
		Palestine	2 (1.35%)
		USA Arab	2 (1.35%)
		Yemen	1 (0.68%)
		Australian Arab	1 (0.68%)
		Iraq	1 (0.68%)
		French Arab	1 (0.68%)
	Africans 34 (22.97%)	Egypt	30 (20.27%)
		Sudan	3 (2.03%)
		Cameroon	1 (0.68%)

TABLE 3 | Comparison of patients of different ethnic groups.

		Total	South Asians	Middle Easterns	East Asians	Caucasians	Others	*p*-value	Missing values
		***n* = 560**	***n* = 244 (43.57%)**	***n* = 148 (26.43%)**	***n* = 94 (16.79%)**	***n* = 60 (10.71%)**	***n* = 14 (2.5%)**		
Patient age		39.0 [33.0–49.0]	39.0 ± 11.35	38.5 ± 16.66	37.0 ± 8.86	**43.5 ± 11.48***	38.5 ± 6.31	0.0933	
Patient gender	Female	189 (33.75%)	**55 (22.54%)***	55 (37.16%)	**50 (53.19%)***	22 (36.67%)	7 (50.0%)	**<0.0001**	
	Male	371 (66.25%)	**189 (77.46%)***	93 (62.84%)	**44 (46.81%)***	38 (63.33%)	7 (50.0%)		
Comorbidities									
Current smoking		36 (6.43%)	10 (4.1%)	**15 (10.14%)***	4 (4.26%)	7 (11.67%)		**0.0401**	
Chronic cardiac disease		20 (3.57%)	8 (3.28%)	**10 (6.76%)***	1 (1.06%)	1 (1.67%)		0.1214	
Hypertension		115 (20.54%)	47 (19.26%)	37 (25.0%)	21 (22.34%)	8 (13.33%)	2 (14.29%)	0.3449	
Asthma		38 (6.79%)	14 (5.74%)	9 (6.08%)	9 (9.57%)	6 (10.0%)		0.4489	
Chronic kidney disease		7 (1.25%)	2 (0.82%)	4 (2.7%)			1 (7.14%)		
Diabetes		98 (17.5%)	51 (20.9%)	**37 (25.0%)***	**8 (8.51%)***	**1 (1.67%)***	1 (7.14%)	**<0.0001**	
Active malignant cancer		6 (1.07%)	2 (0.82%)	3 (2.03%)	1 (1.06%)				
Physical examination									
BMI	adm	27.0 [23.92–30.44]	26.5 ± 4.2	**28.0 ± 7.03***	26.64 ± 5.37	26.85 ± 4.45	27.05 ± 3.72	0.2195	49.64%
Body temperature, °C	adm	37.0 [37.0–37.9]	37.0 ± 0.7	**37.0 ± 0.62***	37.2 ± 0.74	37.0 ± 0.86	37.4 ± 0.71	**0.0343**	
HR BPM	adm	85.0 [78.0–95.0]	85.0 ± 14.08	85.0 ± 12.11	**89.0 ± 13.68***	**80.0 ± 14.97***	84.5 ± 12.19	**0.0014**	
SBP	adm	124.0 [114–135]	**124.0 ± 16.02***	**120.5 ± 16.12***	124.5 ± 20.31	123.5 ± 12.73	123.5 ± 14.63	0.0786	
DBP	adm	78.0 [70.0–84.0]	**78.0 ± 10.98***	**74.0 ± 9.69***	**80.0 ± 11.46***	76.0 ± 9.88	72.0 ± 10.69	<0.0001	
RR/min	adm	18.0 [18.0–18.0]	**18.0 ± 4.53***	**18.0 ± 2.8***	18.0 ± 4.14	18.0 ± 1.48	18.0 ± 1.03	**0.0394**	
SOFA score	adm	0.0 [0.0–0.0]	0.0 ± 2.16	0.0 ± 1.09	0.0 ± 1.56	0.0 ± 0.7	0.0 ± 0.52	0.2733	0.71%
Laboratory findings									
WBC, $\times10^9$/L	adm	5.8 [4.5–7.2]	**6.1 ± 2.51***	**5.2 ± 3.78***	5.95 ± 2.94	5.65 ± 4.55	**4.2 ± 1.86***	**<0.0001**	0.54%
	min	5.5 [4.1–7.2]	**6.0 ± 10.79***	**4.7 ± 2.21***	6.0 ± 3.63	5.3 ± 5.48	**3.6 ± 1.88***	**<0.0001**	0.54%
Platelets, $\times10^9$/L	adm	224.0 [180–272]	227.0 ± 68.77	**207.0 ± 72.21***	**260.5 ± 106.96***	**206.5 ± 55.27***	222.5 ± 57.78	**<0.0001**	0.36%
	min	224.0 [178.0–272.0]	224.0 ± 85.51	211.0 ± 77.6	**260.0 ± 104.38***	**207.0 ± 60.95***	222.5 ± 79.8	**<0.0001**	0.36%
Lymphocytes, $\times10^9$/L	adm	1.55 [1.06–2.1]	1.62 ± 0.79	1.57 ± 0.71	1.52 ± 0.67	1.37 ± 0.75	1.04 ± 0.6	0.3291	0.54%
	min	1.49 [0.89–2.09]	1.54 ± 0.87	1.5 ± 0.76	1.43 ± 0.74	1.32 ± 3.91	1.04 ± 1.67	0.6228	0.54%
Neutrophils, $\times10^9$/L	adm	3.43 [2.4–4.57]	**3.72 ± 2.14***	**2.94 ± 2.43***	3.56 ± 2.4	3.48 ± 2.93	2.24 ± 1.49	**5.7e-05**	0.54%
	min	3.24 [2.13–4.49]	**3.55 ± 2.44***	**2.57 ± 1.83***	**3.6 ± 2.25***	2.89 ± 2.26	**2.14 ± 1.5***	**3.3e-07**	0.54%
Neutropenia,	adm	12 (2.14%)	2 (0.82%)	**8 (5.41%)***	1 (1.06%)	1 (1.67%)		**0.0336**	0.54%
$<1\times10^9$/L	min	20 (3.57%)	5 (2.05%)	**11 (7.43%)***	1 (1.06%)	3 (5.0%)		**0.0309**	0.54%
NLR	adm	2.07 [1.31–3.49]	2.12 ± 4.39	**1.89 ± 2.66***	2.48 ± 3.85	2.12 ± 3.17	1.9 ± 1.75	**0.0206**	0.54%
	min	2.06 [1.3–3.69]	**2.14 ± 10.34***	**1.78 ± 2.93***	**2.56 ± 8.34***	1.82 ± 4.07	1.86 ± 2.16	**0.0013**	0.54%
LCR	adm	0.26 [0.05–0.96]	0.28 ± 17.31	0.25 ± 16.73	**0.2 ± 12.6***	0.41 ± 3.04	0.33 ± 0.84	0.2146	10.89%
	peak	17.05 [1.61–61.85]	21.07 ± 80.43	15.79 ± 67.21	11.11 ± 57.17	18.64 ± 88.51	4.33 ± 692.68	0.6871	10.71%
T.bilirubin, μmol/L	adm	9.0 [6.0–12.6]	**10.0 ± 6.87***	**8.0 ± 5.06***	**8.0 ± 5.77***	8.2 ± 4.58	7.0 ± 4.9	**<0.0001**	1.96%
	peak	9.85 [6.5–14.38]	**10.4 ± 21.82***	**8.6 ± 6.09***	9.5 ± 10.81	11.4 ± 8.1	9.0 ± 5.09	**<0.0001**	1.79%
ALT, U/L	adm	28.0 [17.25–47.75]	29.0 ± 33.34	25.0 ± 40.81	34.0 ± 38.83	25.0 ± 24.5	29.0 ± 23.13	0.1533	1.79%
	peak	32.0 [19.0–67.75]	35.0 ± 465.54	**27.0 ± 5109.99***	37.0 ± 80.04	29.0 ± 58.7	29.0 ± 118.76	0.1017	1.79%
AST, U/L	adm	24.0 [18.0–36.22]	25.0 ± 25.33	**23.0 ± 34.69***	28.0 ± 21.46	24.0 ± 16.12	24.5 ± 18.35	0.0709	1.79%
	peak	25.5 [19.0–44.0]	26.0 ± 447.91	**25.0 ± 345.96***	**29.0 ± 51.14***	24.5 ± 18.3	24.5 ± 65.43	0.1035	1.79%
D-dimer, mg/L	adm	0.4 [0.2–0.6]	0.3 ± 1.59	0.4 ± 0.51	0.4 ± 2.5	0.3 ± 0.44	0.4 ± 0.35	0.6844	15.36%
	peak	0.4 [0.3–0.7]	0.4 ± 4.65	0.4 ± 2.06	0.4 ± 4.15	0.35 ± 2.31	0.6 ± 0.34	0.4907	15.36%
APTT, s	adm	37.4 [35.0–41.0]	37.55 ± 10.97	37.0 ± 12.06	**39.3 ± 4.96***	37.0 ± 4.96	35.5 ± 3.81	**<0.0373**	13.04%
	peak	38.0 [35.15–42.35]	38.0 ± 22.65	37.6 ± 13.07	**40.8 ± 25.56***	37.0 ± 5.2	36.5 ± 5.04	**<0.0116**	13.04%
Creatinine, μmol/L	adm	76.1 [67.0–89.0]	**79.8 ± 37.3***	75.3 ± 28.23	**70.0 ± 31.39***	82.0 ± 17.44	68.5 ± 42.64	**0.0023**	1.07%
	peak	78.0 [67.78–91.0]	**80.8 ± 57.55***	76.0 ± 31.03	**70.0 ± 47.76***	83.5 ± 17.86	68.5 ± 42.64	**0.0009**	1.07%
CK, U/L	adm	106.0 [66.0–173.0]	**119.5 ± 947.62***	**84.0 ± 230.14***	117.0 ± 535.75	88.5 ± 102.47	105.5 ± 54.77	**<0.0005**	22.5%
	peak	109.5 [66–199]	**127.0 ± 988.14***	**86.0 ± 243.77***	122.5 ± 10275.55	88.5 ± 136.52	105.5 ± 96.62	**<0.0005**	22.32%
CRP, mg/L	adm	5.8 [1.75–27.0]	5.3 ± 64.11	5.9 ± 50.07	6.85 ± 68.44	5.35 ± 59.98	6.3 ± 43.76	0.8459	0.89%
	peak	6.5 [1.9–50.65]	5.9 ± 86.33	7.95 ± 64.16	8.3 ± 83.66	5.75 ± 67.84	6.3 ± 93.69	0.8856	0.89%
LDH, U/L	adm	192.0 [159.0–264.0]	**195.0 ± 182.27***	**181.0 ± 86.08***	**235.0 ± 200.88***	175.0 ± 70.41	167.5 ± 123.58	**<0.0001**	16.96%
	peak	194.0 [160.0–280.0]	195.0 ± 626.18	**183.0 ± 647.29***	**238.0 ± 239.95***	175.0 ± 89.53	167.5 ± 125.35	**<0.0001**	16.96%
Troponin, ng/mL	adm	0.0 [0.0–0.0]	0.0 ± 0.83	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.03	0.0 ± 0.0	0.5822	24.11%
	peak	0.0 [0.0–0.0]	**0.0 ± 1.17***	0.0 ± 0.07	0.0 ± 0.1	0.0 ± 0.03	0.0 ± 0.0	0.2089	24.11%
Ferritin, ng/mL	adm	216.7 [84.5–475.5]	216.7 ± 1499.98	**191.9 ± 535.61***	286.0 ± 1604.47	261.0 ± 524.67	289.0 ± 1231.74	0.0743	9.46%
	peak	230.0 [89.95–595.5]	230.0 ± 5730.89	**205.0 ± 2834.62***	469.0 ± 3214.62	261.0 ± 590.38	321.6 ± 1219.98	0.0931	9.46%

(Continued)

TABLE 3 | Continued

		Total	South Asians	Middle Easterns	East Asians	Caucasians	Others	*p*-value	Missing values
		n = 560	*n* = 244 (43.57%)	*n* = 148 (26.43%)	*n* = 94 (16.79%)	*n* = 60 (10.71%)	*n* = 14 (2.5%)		
Fibrinogen, mg/dL	adm	396.0 [330.0–529.5]	390.0 ± 255.83	384.0 ± 146.48	**439.0 ± 154.57***	395.0 ± 163.35	393.0 ± 160.1	0.1837	27.32%
	peak	405.0 [331.2–554.0]	395.0 ± 499.63	386.5 ± 168.25	**459.5 ± 159.54***	404.0 ± 185.15	445.5 ± 183.97	0.0608	27.32%
Clinical severity								**<0.0005**	
Asymptomatic/Mild		343 (61.25%)	160 (65.84%)	96 (64.86%)	**44 (45.83%)***	35 (59.32%)	8 (57.14%)		
Moderate/Severe		171 (30.54%)	**54 (22.22%)***	45 (30.41%)	**43 (44.79%)***	23 (38.98%)	6 (42.86%)		
Critical		46 (8.21%)	**29 (11.93%)***	7 (4.73%)	9 (9.38%)	1 (1.69%)	0 (0.0%)		
Outcome									
Put on oxygen therapy		112 (20.0%)	50 (20.49%)	27 (18.24%)	24 (25.53%)	8 (13.33%)	3 (21.43%)	0.4331	
Transferred to ICU		72 (12.86%)	38 (15.57%)	**12 (8.11%)***	15 (15.96%)	7 (11.67%)		0.1102	
Discharged alive		545 (97.32%)	234 (95.9%)	143 (96.62%)	94 (100.0%)	60 (100.0%)	14 (100.0%)	0.1475	
Complications	count	0.0 [0.0-0.0]	0.0 ± 1.71	0.0 ± 0.97	0.0 ± 1.41	0.0 ± 0.64	0.0 ± 0.45	0.3597	
Having any complication		123 (21.96%)	56 (22.95%)	25 (16.89%)	25 (26.6%)	13 (21.67%)	4 (28.57%)	0.4202	
ARDS		76 (13.57%)	39 (15.98%)	13 (8.78%)	17 (18.09%)	7 (11.67%)		0.0873	
Liver dysfunction		54 (9.64%)	25 (10.25%)	9 (6.08%)	**16 (17.02%)***	2 (3.33%)	2 (14.29%)	0.0242	

**Statistical data are expressed as IQR, Median ± SD, or absolute number of cases and their percentage in studied sample.*
If distribution of variable differs significantly ($p < 0.05$) in ethnic cohort plotted against all other ones, its value is marked in bold and with asterisk.
Disparities in distribution of data across five ethnic groups are presented with p-values in separate column. p-value is marked in bold if difference between all ethnic groups is statistically significant ($p < 0.05$).

activated partial thromboplastin time (APTT), the activity of the enzymes—alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), and the concentration of the total bilirubin, D-dimer, creatinine, C-reactive protein (CRP), sodium ion, troponin, ferritin, and fibrinogen. Blood hemoglobin and serum sodium were recorded on admission.

- **Case management and clinical course**. Medications used: antiviral medication, azithromycin, other intravenous antibiotics, antimalarial, antifungal medication, IL-6 blocker "Tocilizumab", convalescent plasma, steroids (either intravenous or oral), low-molecular-weight heparins, supplemental oxygen, invasive ventilation, vasopressors, and extracorporeal membrane oxygenation. The length of hospital stay, the duration between symptom onset and admission (in days), the duration between the first positive SARS-CoV-2 PCR and the first negative set (first of 2 consecutive negative PCRs), need for ICU care, and the duration of stay in ICU.
- **Complications**.
- *Septic shock* was defined according to the 2016 Third International Consensus Definition for Sepsis and Septic Shock (Singer et al., 2016).
- *Bacterial pneumonia* was diagnosed when patients showed clinical symptoms or signs of pneumonia and a positive culture of a new pathogen was obtained from lower respiratory tract specimens (qualified sputum, endotracheal aspirate, or bronchoalveolar lavage fluid) after admission.
- *Bacteremia* was diagnosed when patients showed clinical symptoms or signs of systemic infection and one or more positive blood culture that was not thought to be a contaminant.
- *ARDS* was diagnosed according to the Berlin definition (Force et al., 2012).
- *Acute cardiac injury* was diagnosed if serum levels of cardiac biomarkers (troponin I) were above the 99th percentile upper reference limit, or if new abnormalities were shown in echocardiography.
- *Acute kidney injury* was defined according to Kidney Disease Improving Global Outcomes (KDIGO) (Khwaja, 2012). It is based on the highest serum creatinine level and urine output. Specifically, the diagnosis could be made if there is an increase in serum creatinine levels by 0.3 mg/dl or greater (26.5 μmol/L or greater) within 48 h.
- *Liver injury* was diagnosed if there was an increase in liver enzymes (AST or ALT) over 3 times the upper limit of normal.
- *Seizure, meningitis*, or *encephalitis* confirmed by CSF analysis and culture, cardiac arrhythmia, cardiac arrest, myocarditis (if clearly documented by a cardiologist or an intensivist), new onset cardiomyopathy (if the baseline cardiac function is unknown, assume new), critical illness myopathy or neuropathy (if documented by an attending physician or diagnosed with the electrophysiologic testing), bleeding or disseminated intravascular coagulation (DIC), the use of renal replacement therapy, the development of pressure ulcer, and other complications.
- **Primary Outcome**. Discharged alive.

3.4 Statistical Analysis

The data were checked for accuracy and then for normality using the Shapiro–Wilk test; none of the attributes were normally distributed; the non-parametric tests were used to compare each pair of independent samples. The bivariate relationships between the features were assessed with the Mann–Whitney *U* test or Kruskal–Wallis test for the continuous variables, and with Fisher's Exact test or Chi-square test for the quantitative ones.

TABLE 4 | Comparison of patients of Middle Eastern subethnicities.

N		Total	Arabs	Africans	*p*-value	Missing values
		***n* = 148**	**114 (77.03%)**	**34 (22.97%)**		
Patient age		38.5 [29.0-57.0]	41.5 ± 17.64	34.5 ± 11.69	0.08009	
Patient gender	female	93 (62.84%)	71 (62.28%)	22 (64.71%)	0.84238	
	male	55 (37.16%)	43 (37.72%)	12 (35.29%)		
Comorbidities						
Current smoking		15 (10.14%)	7 (20.59%)	8 (7.02%)	**0.04529**	
Chronic cardiac disease		10 (6.76%)	1 (2.94%)	9 (7.89%)	0.45502	
Hypertension		37 (25.0%)	2 (5.88%)	35 (30.7%)	**0.00284**	
Asthma		9 (6.08%)	1 (2.94%)	8 (7.02%)		
Chronic kidney disease		4 (2.7%)		4 (3.51%)		
Diabetes		37 (25.0%)	4 (11.76%)	33 (28.95%)	**0.04474**	
Active malignant cancer		3 (2.03%)		3 (2.63%)		
Physical examination						
BMI	adm	28.0 [23.62–32.5]	29.0 ± 7.26	27.13 ± 6.33	0.22498	
Body temperature, °C	adm	37.0 [37.0–37.5]	37.0 ± 0.63	37.0 ± 0.62	0.34961	
HR BMP	adm	85.0 [76.0–92.0]	85.0 ± 13.5	85.0 ± 11.65	0.49363	
SBP	adm	120.5 [112.0–131.25]	120.0 ± 16.63	121.0 ± 15.94	0.27736	
SOFA score	adm	0.0 [0.0–0.0]	0.0 ± 0.96	0.0 ± 1.12	0.07010	1.35%
Laboratory findings						
WBC, $\times10^9$/L	adm	5.2 [4.1–6.4]	5.4 ± 4.15	4.75 ± 2.06	0.12581	0.68%
	min	4.7 [3.6–6.3]	5.1 ± 2.18	4.3 ± 2.27	0.11972	0.68%
Platelets, $\times10^9$/L	adm	207.0 [174.0–271.0]	212.0 ± 74.88	202.0 ± 62.49	0.46522	0.68%
	min	211.0 [167.5–263.0]	209.0 ± 79.44	221.0 ± 71.14	0.28758	0.68%
Lymphocytes, $\times10^9$/L	adm	1.57 [1.14–2.2]	1.57 ± 0.73	1.64 ± 0.62	0.49267	0.68%
	min	1.5 [0.96–2.1]	1.51 ± 0.7	1.5 ± 0.78	0.31689	0.68%
Neutrophils, $\times10^9$/L	adm	2.94 [2.0–3.9]	2.29 ± 1.87	3.11 ± 2.56	0.09368	0.68%
	min	2.57 [1.78–3.7]	2.29 ± 2.06	2.78 ± 1.75	0.07491	0.68%
Neutropenia, $<1\times10^9$/L	adm	8 (5.41%)	4 (11.76%)	4 (3.51%)	–	0.68%
	min	11 (7.43%)	5 (14.71%)	6 (5.26%)	0.12709	0.68%
NLR	adm	1.89 [1.11–3.09]	1.51 ± 1.93	1.96 ± 2.84	0.20026	0.68%
	min	1.78 [1.11–3.14]	1.5 ± 1.99	1.83 ± 3.14	0.11309	0.68%
LCR	adm	0.25 [0.06–0.97]	0.33 ± 12.11	0.24 ± 17.84	0.19674	8.11%
	peak	15.79 [2.64–57.2]	21.74 ± 52.97	15.26 ± 70.99	0.15690	7.43%
T.bilirubin, μmol/L	adm	8.0 [5.62–10.17]	7.9 ± 5.49	8.05 ± 3.09	0.31502	1.35%
	peak	8.6 [5.93–11.0]	8.95 ± 6.64	8.35 ± 3.53	0.25986	1.35%
ALT, U/L	adm	25.0 [17.0–44.0]	23.5 ± 44.64	32.5 ± 24.03	0.05078	1.35%
	peak	27.0 [19.0–54.0]	25.5 ± 67.1	32.5 ± 10467.88	0.13466	1.35%
AST, U/L	adm	23.0 [18.0–31.0]	23.0 ± 38.5	23.5 ± 16.8	0.09486	1.35%
	peak	25.0 [18.0–36.75]	25.0 ± 45.91	25.5 ± 704.33	0.19697	1.35%
D-dimer, mg/L	adm	0.4 [0.3–0.6]	0.4 ± 0.55	0.3 ± 0.19	**0.01554**	10.81%
	peak	0.4 [0.3–0.7]	0.4 ± 1.34	0.3 ± 3.59	0.07021	10.81%
APTT, s	adm	37.0 [35.0–40.4]	37.3 ± 13.36	37.0 ± 4.84	0.36875	8.78%
	peak	37.6 [35.0–41.3]	37.85 ± 13.83	37.1 ± 9.8	0.37892	8.78%
Creatinine, μmol/L	adm	75.3 [67.0–87.0]	76.0 ± 31.03	75.0 ± 14.8	0.23535	0.68%
	peak	76.0 [67.0–88.0]	77.2 ± 33.37	75.15 ± 21.06	0.25633	0.68%
CK, U/L	adm	84.0 [50.0–140.0]	84.0 ± 255.0	93.0 ± 47.79	0.38742	18.24%
	peak	86.0 [51.25–153.75]	86.0 ± 267.35	100.0 ± 107.07	0.34588	17.57%
CRP, mg/L	adm	5.9 [1.92–19.77]	6.4 ± 52.34	4.0 ± 41.36	0.08961	1.35%
	peak	7.95 [2.12–39.75]	9.05 ± 60.5	5.65 ± 74.93	0.22795	1.35%
LDH, U/L	adm	181.0 [149.0–228.25]	181.0 ± 85.87	166.0 ± 86.73	0.15974	12.16%
	peak	183.0 [149.0–234.75]	186.0 ± 124.13	166.0 ± 1334.94	0.12929	12.16%
Troponin, ng/mL	adm	0.0 [0.0–0.0]	0.0 ± 0.01	0.0 ± 0.0	0.41498	22.3%
	peak	0.0 [0.0–0.0]	0.0 ± 0.06	0.0 ± 0.11	0.36905	22.3%
Ferritin, ng/mL	adm	191.9 [66.0–326.09]	192.31 ± 569.48	158.25 ± 393.37	0.44227	4.73%
	peak	205.0 [77.0–481.0]	205.0 ± 3204.1	194.35 ± 584.34	0.36707	4.73%
Fibrinogen, mg/dL	adm	384.0 [321.75–478.5]	392.0 ± 140.09	326.0 ± 160.17	**0.00758**	24.32%
	peak	386.5 [325.75–510.75]	397.0 ± 156.97	346.0 ± 198.61	**0.01501**	24.32%
Clinical severity					0.80646	
Asymptomatic/Mild		119 (80.41%)	28 (82.35%)	91 (79.82%)		
Moderate/Severe		22 (14.86%)	4 (11.76%)	18 (15.79%)		

(Continued)

TABLE 4 | Continued

N		Total	Arabs	Africans	*p*-value	Missing values
		***n* = 148**	**114 (77.03%)**	**34 (22.97%)**		
Critical		7 (4.73%)	2 (5.88%)	5 (4.39%)		
Outcome						
Put on oxygen therapy		27 (18.24%)	5 (14.71%)	22 (19.3%)	0.62210	
Patients in ICU		12 (8.11%)	3 (8.82%)	9 (7.89%)	1	
Discharged alive		143 (96.62%)	32 (94.12%)	111 (97.37%)	0.32389	
Complications	count	0.0 [0.0–0.0]	0.0 ± 1.88	0.0 ± 1.19	0.37253	
Having any complications		25 (16.89%)	5 (14.71%)	20 (17.54%)	0.79936	
ARDS		13 (8.78%)	3 (8.82%)	10 (8.77%)	1	
Liver dysfunction		9 (6.08%)	3 (8.82%)	6 (5.26%)		

Significant differences between cohorts ($p < 0.05$) are marked in bold.

As we intended to find features inherent to the specific ethnic group, we also evaluated the differences between each group versus the others.

Machine learning (ML) classification model. We utilized ML algorithms to check if there were unique patterns within the data that can unambiguously identify the ethnic group (Middle Eastern, South Asian, East Asian, Caucasians, and Others). In our dataset, the ethnic group "Others" was in the minority (14 patients, 2.5%), so we excluded it from the analysis.

The list of variables used to build the model was as follows:

- *Physical examination on admission*: temperature, HR BPM, SBP, DBP, the time elapsed between two successive R-waves on the electrocardiogram (RR/min), oxygen saturation (SpO_2), SpO_2 on room air *vs*. oxygen therapy, Glasgow coma scale (GCS), and SOFA score.
- *Symptoms on admission*: cough, sputum, sore throat, chest pain, SOB, fever, headache, confusion, having any gastrointestinal symptom (e.g., nausea, vomiting, diarrhea), myalgia, malaise, and loss of smell or taste.
- *Laboratory findings on admission*: the count of platelets, WBC, and fractions of leukocytes; the concentration of hemoglobin, total bilirubin, D-dimer, creatinine, sodium, CRP, troponin, ferritin, and fibrinogen; the activity of ALT, AST, CK, and LDH; and the length of APTT.

Feature selection. To assess the importance of the features fed to the ML models as classifiers by ethnicity, we employed four ensemble tree-based estimators such as AdaBoost, Gradient Boosting, Random Forest, and Extra Trees. These models were trained on the whole dataset and used to rank the features in ascending order concerning their predictive potential.

4 RESULTS

4.1 The Cross-Ethnic Groups

Out of 560 patients, 43.8% were South Asians, 26.4% were from the Middle East, 16.8% were East Asians, 10.7% were Caucasians, and 2.50% are under Others (see **Tables 2**, **3**). The UAE nationals represented half of the Middle Eastern patients, i.e., 13% of the entire cohort. Overall, males accounted for two-thirds of the study population, which remained true across different ethnic groups except for the East Asians where the gender distribution was almost equal. **Table 2** lists nationalities in the Middle Eastern ethnic group. The comparison of the patients of the Middle Eastern subethnicities—Arabs and Africans—is given in **Table 4**. There were marked differences in the SOFA score, the level of D-dimer ($p = 0.015$), and fibrinogen ($p = 0.007$) between the subethnic groups on admission. The pronounced disparity between the Arabs and Africans in the background diseases (hypertension - $p = 0.003$; diabetes and current smoking - $p = 0.015$) may account for the mentioned differences. We did not find a noticeable difference between the subethnic groups in disease severity ($p = 0.81$).

Comorbidities. Hypertension is the most common comorbidity, present in 20.54% of the study cohort, with no remarkable differences between groups ($p = 0.345$). Diabetes was present in 17.50% of patients, and its incidence differed significantly among ethnic cohorts ($p = 0.0001$). The Middle Eastern population had the highest proportion (25%) of patients with diabetes and the prevalence was higher than in the other ethnic groups ($p = 0.005$). In comparison, East Asians and Caucasians had a substantially lower proportion of patients with diabetes (8.51%, $p = 0.01$ and 1.67%, $p = 0.006$ consecutively). Active smoking was present in 6.4% cases and almost half of them were Middle Easterns.

Symptoms. Each patient had between two and four symptoms on admission. The most common symptoms were fever (58.04%), followed by cough (53.93%), myalgia (38.93%), sore throat (30%), and shortness of breath (26.96%). The frequency of the symptoms and the values of the body temperature ($p = 0.034$), pulse ($p = 0.001$), and respiratory rate ($p = 0.039$) varied among the ethnic groups. However, the distinction in the major results of the physical examination did not have a clear clinical value. On average, the SOFA score was approximately equal in the ethnic groups on admission ($p = 0.273$).

Physical examination. Middle Eastern patients had a considerably higher average BMI compared to other ethnicities ($p = 0.033$).

Laboratory findings. APTT was longer in East Asians and reached almost the upper limit of the reference range on admission (39.3 ± 4.96 s; $p = 0.015$). Fibrinogen concentration

was also the highest in this ethnic group (p = 0.0045). East Asians had the highest group level of the LDH activity (p = 6.77e 05), which is a non-specific biomarker of a massive tissue breakdown and a predictor of mortality in COVID-19 patients (Yan et al., 2020). Besides this, East Asians had the highest thrombocyte count (p = 4.42e – 06) and a minimal lymphocyte-to-C-reactive protein ratio (LCR) (p = 0.04) both serving as laboratory high-risk complication markers.

On admission, the mean count of leucocytes, neutrophils, lymphocytes, and thrombocytes of the ethnic groups were within the reference range. However, the maximal numbers of WBC and neutrophils were noted in the group of South Asian patients (p < 0.011). Patients of Middle Eastern ethnicity had considerably lower count of thrombocytes (207.0 ± 72.21, p = 0.046), WBC (5.2 ± 3.78, 4.58e – 05), and neutrophils on admission (2.94 ± 2.43, p = 1.88e – 05). In this group, the percentage of people with moderate and severe neutropenia (<1.0×10^9/L) was distinctly higher than in the other groups (5.41%, p = 0.008). This accounted for the lowest neutrophil-to-lymphocyte ratio (NLR) in the Middle Eastern patients (1.89 ± 2.66, p = 0.001). The tendency remained the same at the peak of the disease.

Disease severity. Almost two-thirds of our cohort (61.25%) were asymptomatic or had mild symptoms. Moderate-to-severe disease was seen in 30.54% of the cohort, and 8.21% were critical. There was a marked disparity in the distribution of patients from distinct severity levels in the ethnic groups (p < 0.0005). In Caucasians, the portion of patients diagnosed with moderate-to-severe disease was higher than in South Asians and Middle Easterns (38.98% vs. 22.22% and 30.41%) despite the least number of comorbidities in the Caucasian group. This was also noticeable in East Asian patients. On the contrary, patients from the Middle East had a higher number of comorbidities (chronic cardiac disease 7%, diabetes 25%, smoking 10%), and essentially higher mean BMI (see above), yet they had a much lower proportion of patients with critical disease course—4.73% vs. 11.93% in South Asians, and 9.38% in East Asians.

Disease outcome. There was no marked difference between ethnic groups in primary outcome of COVID-19 (p = 0.147). The overall mortality was 2.68%. Twenty percent of the total cohort required oxygen supplementation and a lower proportion of patients from the Middle East required ICU admission compared to the other groups (8.11% vs. 12.86% in the overall sample; p = 0.044). The rate of complications was similar in different ethnic groups except for liver dysfunction which was observed in a higher proportion of East Asian patients (17.02% vs. 9.64% in the total cohort; p = 0.008).

4.2 Ranking the Most Important Features

Table 5 and **Figure 4** display the values of impurity-based attribute ranked averaged across four tree-based ML classifiers (Random Forest, AdaBoost, Gradient Boosting, and ExtraTrees).

4.3 Classification Concerning Ethnicity With Neural Network

To evaluate the classifier output quality, we trained several ML classification models using a stratified 10-fold cross-validation technique to generalize the models to the true rate error. For each fold, we used 90% of the data to train the model and then tested it on the remaining 10%. The decision matrices built on the test dataset for all folds were combined and used to calculate the performance metrics. The best performance measures were obtained with a three-layer fully connected neural network (NN).

Figure 5 depicts receiver operating characteristics (ROC) for multi-class classification model. To generalize the area under the ROC curve (AUC) for the multi-classification problem, the average AUC of all possible pairwise combinations of groups was computed, and then unweighted mean was considered as a metric. In the figure, we also present micro-average (aggregates the contributions of all classes to calculate the metric) and macro-average (computes the metric independently for each class and then takes the average) AUCs.

Table 6 lists the confusion matrix of the trained model for each group, indicating true-positive, true-negative, false-positive, and false-negative numbers. Each row of the error matrix represents the actual class, while each column shows the instances in a predicted class. Precision, recall, harmonic score, accuracy, macro average (unweighted mean per class), and weighted average (support-weighted mean per group) of the classification performance are specified in **Table 7**.

5 DISCUSSION

5.1 The Comparison of the Ethnic Groups

An ethnic group is a group of people whose members identify with one another through common cultural heritage. This term

TABLE 5 | Ranking scores of variables selected for ML model.

Score	Feature	Score	Feature	Score	Feature	Score	Feature
0.06883	Platelets	0.04361	Hemoglobin	0.02051	SpO_2	0.00644	Headache
0.06522	Total bilirubin	0.04336	Lymphocytes	0.01256	Cough	0.00634	SOB
0.05150	DBP	0.04148	CRP	0.01248	Sore throat	0.00634	RR/min
0.05104	WBC	0.03849	CK	0.01215	Troponin	0.00446	Sputum
0.04947	LDH	0.03812	ALT	0.00988	Myalgia	0.00300	Chest pain
0.04722	HR BPM	0.02838	D-dimer	0.00942	Fever	0.00287	GCS
0.04681	APTT	0.02825	Na	0.00931	SOFA score	0.00187	SpO_2 on RA vs.
0.04594	Creatinine	0.02825	AST	0.00925	GI symptoms		O_2 therapy
0.04503	Ferritin	0.02822	Fibrinogen	0.00761	Smell/taste loss	0.00046	Confusion
0.04428	SBP	0.02483	Temperature	0.00672	Malaise		

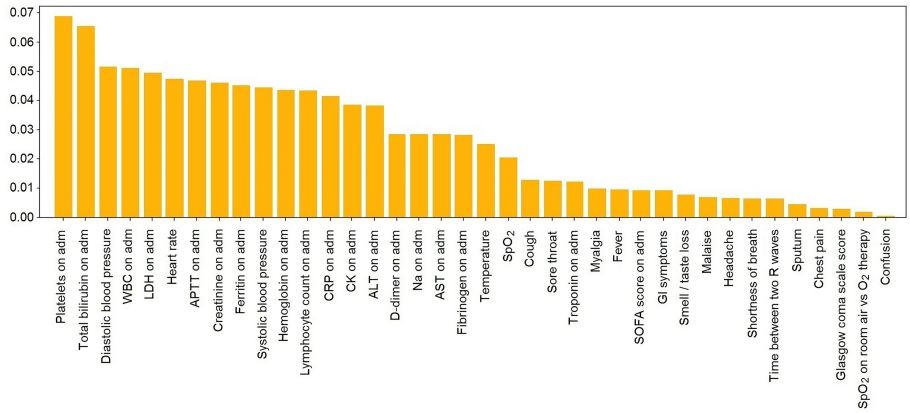

FIGURE 4 | Performance of employed NN classification method.

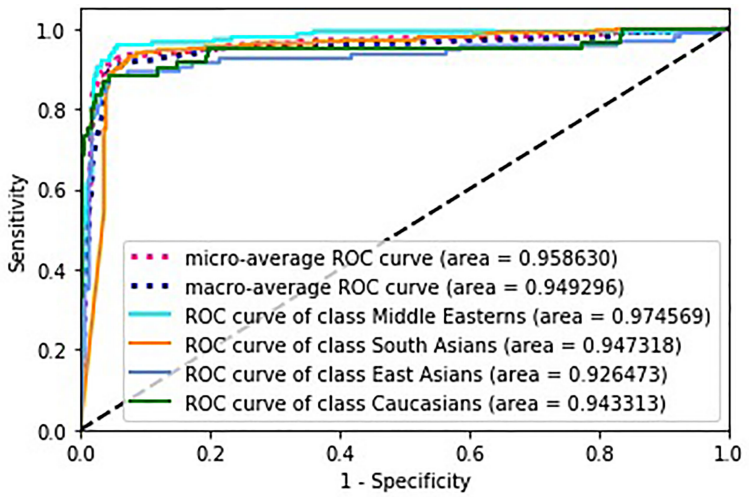

FIGURE 5 | Performance of neural network classification model.

usually reflects a shared culture and social behavior; however, it can also be used to imply variations in genetic makeup between different groups. Several studies showed remarkable ethnicity-related differences in clinical features of various diseases. For example, deaths for Hepatitis C were higher among Native Americans and Blacks compared to Caucasians. Studies demonstrated that an immunologic basis can explain this difference (Sugimoto et al., 2003). Moreover, during the 2009/2010 influenza pandemic, differences in mortality rates were observed, where non-Caucasians had a markedly higher mortality rate compared to Caucasians (Zhao et al., 2015).

5.1.1 Genetic Factors

Genetic factors may account for ethnic disparities. However, the variation in genes within ethnic groups can also be high, especially in Arabs, unified by the Arabic language rather than a common origin. The ethnic group primarily inhabits 22 member states of the Arab League in Western Asia and North Africa (Frishkopf, 2010). Though the majority of the North Africans speak Arabic as their native language, they have a Berber (not Arab) origin. Reasonably, there are genetic disparities among Arab nationalities despite cultural, geographic, and linguistic similarities among them. To study Arabian genealogy, geneticists analyze Y-DNA haplogroup tree (Mahal and Matsoukas, 2018). With this method, they showed that the haplotype of Jordanian bedouins had its traces in Palestinians, Yemeni, Moroccan, Libyans, and Tunisians. There was a low genetic diversity among these subethnicities (Almahasneh et al., 2018). Another study justified genealogical relatedness between Iraqi and Kuwaiti individuals. A non-significant genetic distance was shown for the following ethnic pairs: Northern Iraqi and Lebanese, Kurdish and Iranian, Iraqi and Iranian (Dogan et al., 2017).

TABLE 6 | Confusion matrix to assess accuracy of prediction with three-layer dense NN model classifying cases by ethnic group.

		Predicted			
		Middle Easterns	**South Asians**	**East Asians**	**Caucasians**
Actual	Middle Easterns	121	22	2	3
	South Asians	4	232	5	3
	East Asians	5	6	82	1
	Caucasians	2	3	1	54

TABLE 7 | Classification metrics of neural network model.

	Precision	**Recall**	**F1 score**	**Support**
South Asians	0.88	0.95	0.92	244
Middle Easterns	0.92	0.82	0.86	148
East Asians	0.91	0.87	0.89	94
Caucasians	0.89	0.90	0.89	60
Accuracy			0.90	546
Macro average	0.90	0.89	0.89	546
Weighted average	0.90	0.90	0.89	546

Analogously, the genetic background of the UAE citizens was influenced by the neighboring countries and remote geographic regions. In males, Y haplogroups had similarities with the Middle Eastern, Central, and South Asian genes. Fifty-two percent of Emirati men had the Middle Eastern haplogroup J, 21% of them inherited the E haplogroup common in West and East Africa, and 14% of the individuals had the R haplogroup originated from Central and South Asia and Eastern Europe (Daw Elbait et al., 2021). A close genetic distance between inhabitants of distinct Arab countries denoted that they had a common genetic background. This enabled us to analyze data for Arabs without dividing them into subethnicities in the current study. To identify a genetic background of each individual accurately, we would require expensive genetic testing.

There has been an abundance of publications on COVID-19; however, data on ethnicity and COVID-19 remain limited. Observations from the UK and the USA highlighted increased disease severity and mortality among Blacks, Asians, and Minority Ethnic groups (BAME) (Pan et al., 2020; Lab, 2020). Some authors analyzed peer-reviewed literature to study the effect of ethnicity on COVID-19 outcomes and found no differences (Pan et al., 2020). The same scientific group inspected preprint articles, some of which suggested poorer outcomes in BAME compared to White patients. These publications compared Caucasians to non-Caucasians, mostly Asians, Blacks, and Hispanics.

No studies ascertained specifically the morbidity and mortality of Middle Eastern patients during the COVID-19 pandemic. We believe our findings are of particular interest as this ethnic group displays a higher risk factor profile, yet fewer patients had a critical disease course compared to other groups. We also identified differences between South Asian and East Asian ethnic groups. These two distinct ethnic groups are often considered as one common group in most publications.

Literature on systemic hypertension and cardiovascular disease reflected considerable variation in disease manifestation, outcome, and response to different pharmacological agents among different ethnic groups. A relevant example is that ACE-I and ARB medications were less effective in reducing blood pressure in patients of African ethnicity. In fact, these patients had worse cardiovascular outcomes when started on ARB monotherapy (Brewster et al., 2016).

Angiotensin-converting enzyme 2 (ACE2) receptor is thought to play a critical role in the pathogenesis of COVID-19 as SARS-CoV-2 uses the ACE2 receptor for cell entry (Hoffmann et al., 2020). The virus also uses transmembrane serine protease (TMPRSS2) and Furin peptidase to invade human cells (Al-Mulla et al., 2021). Different studies finished up with inconsistent findings on the genetic predisposal and protection against SARS-CoV-2 among ethnic group in multinational countries. For example, in the USA, African Americans at a higher risk of COVID-19 compared to other ethnicities that live in the country (Whites, Asians, American Indians, Alaskan Natives, and other minorities). This might be explained by the increased gene expression of ACE2 and TMPRSS2 genes in this ethnic group. Additionally, African Americans with asthma are at a greater risk of suffering from the severe form of COVID-19 (Peters et al., 2020). African Americans and Whites have a lower ACE2 expression cell ratio than Asians (Cao et al., 2020). The distribution of ACE1 and ACE2 genotype rates matches CFR in various countries (Gupta and Misra, 2020). The highest CFR (9.6%) is in Europe, followed by North America (5.9%) and Asia (3.5%) (Dongarwar and Salihu, 2020). In a multi-ethnic society, the highest CFR is registered in Blacks (Goldstein and Atherwood, 2020). This suggests gene–environment interactions and ethnic disparities in immune response to COVID-19 (Goldstein and Atherwood, 2020; Nepomuceno et al., 2020).

Factors that influence ACE2 receptor expression, such as the use of ACE-I and ARB, are supposed to affect disease course and severity (Madjid et al., 2020). Gene variation can explain variance of susceptibility to SARS-CoV-2 among ethnicities (Cao et al., 2020).

A thought-provoking question that presents itself is whether patients from the Middle East have an ACE2 receptor morphology that is protective against developing a more severe COVID-19 disease. Researchers investigated whole-exome sequences of individuals from Middle Eastern populations to explore natural variations in the ACE2. They identified two activating variants in the ACE2 gene: K26R and N720D. The variants are more common in Europeans and rare in the Middle Eastern, East Asian, and African populations. The variants change ACE2 gene expression and make people more vulnerable to SARS-CoV-2 infection. Previous studies suggest that K26R can activate ACE2 and facilitate binding to the receptor binding domain while N720D enhances TMPRSS2 protease cutting (Al-Mulla et al., 2021). K26R variant occurs in European people with a frequency of about 0.5%, which predisposes them to more severe SARS-CoV-2 disease. Another single-nucleotide polymorphism of ACE2 that may genetically protect from SARS-CoV-2 disease is more common in African people with a frequency of about 0.3% (Calcagnile et al., 2020). In contrast, deleterious variants that suggest a possible decrease in Furin protease function are detected more frequently among Middle Easterns than Europeans (Al-Mulla et al., 2021).

5.1.2 Socioeconomic Factors

Disparities in SES can also account for ethnic and race disparities in COVID-19. Previous research highlighted a strong association between SES and disease outcomes. The ethnic groups with the lower SES are at risk of contracting COVID-19 (Garg, 2020; Kopel et al., 2020). It remains unclear whether this can be explained by a host genetic interaction (e.g., higher prevalence of underlying chronic disease) or non-genetic behavioral factors such as higher-density living, the use of public transportation, and possibly lower health literacy (Singu et al., 2020). Data related to SES in the UAE (e.g., the level of education and the monthly income) are not routinely collected in hospital medical records so it remains indistinguishable whether SES affected disease severity in our study or not. However, this seems unlikely as the South Asian patients (who represent 43% of our cohort) were younger and had no considerable comorbidities, but had a similar disease outcome to other ethnic groups.

The UAE is a high-income country that has a high rate of young people and a disproportion between men and women due to the recruitment of male workforce (Paulo et al., 2017; Paulo et al., 2018). Such distribution of males and females can explain the prevalence of men admitted to the Mediclinic Parkview hospital, which was used as a research center for our study. Apart from gender and age disparities, the UAE has an uneven distribution of residing nationalities. Emirati citizens make up 11.48% of the population whereas most residents come from India (27.49%) and Pakistan (12.69%), and Egyptians constitute the largest diaspora among non-Emirati Arabs (4.23%) (UAE Population Statistics, 2021).

Although health insurance is mandatory, there is a wide range of insurance providers, and continuous care of expatriates is not well maintained (Paulo et al., 2017; Paulo et al., 2018). With a new place of affiliation, an employee gets a new insurance plan (How to Get Health Insurance in the UAE? News, 2021) which depends on a job role and official monthly income associated with it. The lower the job grade is, the narrower is the insurance coverage. To improve the situation, some companies unified insurance plan for all their employees.

5.1.3 Hematological Abnormalities

COVID-19 can manifest with a profound inflammatory response, which may cause severe immune damage to the lungs. Coronaviruses are able to infect bone marrow cells, which can result in abnormal hematopoiesis (Desai et al., 2021). That is why SARS-CoV2 infection can cause several hematological abnormalities (Mank et al., 2021). The most common abnormalities in COVID-19 include neutrophilia, lymphopenia, and thrombocytopenia. WBC count can be normal or decreased upon admission, and it increases with disease progression. Also, an elevation in the WBC count can be caused by co-infections or medications (e.g., prednisone) (Khartabil et al., 2020).

Lymphopenia leads to the dysfunction of immune system in severe COVID-19 and makes the patients vulnerable to bacterial infections (Chen et al., 2020; Sun et al., 2020). Pronounced lymphopenia and thrombocytopenia carry poor prognosis especially if accompanied by the elevated D-dimer level (Desai et al., 2021). Both neutrophilia and neutropenia are predictive of poor outcomes and severe respiratory failure in this category of patients (López-Pereira et al., 2020). However, neutropenia is less common in COVID-19. There were only a few reports of the decreased neutrophil count in these patients (Ai et al., 2020; Ahnach et al., 2020; Yarali et al., 2020). The exact reason for neutropenia in the disease remains unknown. The suggested mechanisms of neutropenia development include bone marrow suppression and accelerated peripheral destruction of neutrophils. These mechanisms have been well described in other viral infections including HIV, cytomegalovirus, Epstein–Barr virus, viral hepatitis, and influenza (Munshi and Montgomery, 2000). Both the moderate (<1,000 cells/μl) and especially the severe neutropenia, which is also called agranulocytosis (<500 cells/μl), are conditions with an extraordinary risk of infections. The conditions require patient monitoring and empirical antibiotic therapy along with the administration of granulocyte colony-stimulating factor in some cases (Devi et al., 2021).

The neutrophil-to-lymphocyte and lymphocyte-to-C-reactive protein ratios are well-established inflammation markers that reflect systemic inflammatory response (Lagunas-Rangel, 2020). NLR is a widely used biomarker for assessing the severity of bacterial infections (Naess et al., 2017; Sun et al., 2020). The increase in neutrophil count indicates the disease aggravation. The decrease in lymphocyte count denotes impairment in immune functioning (Celikbilek et al., 2013; Huang et al., 2019). NLR is shown to be an independent risk factor of severe COVID-19 (Borges et al., 2020). The ratio increases dramatically

in patients with the severe disease form (Lagunas-Rangel, 2020). The lymphocytopenia and the increase in the NLR are the most obvious hematological abnormalities associated with the disease.

The low LCR along with the high NLR suggest a poor prognosis in COVID-19 patients (Lagunas-Rangel, 2020). The LCR can capture the early part of the inflammatory cascade more sensitively than the NLR as the CRP levels have been shown to rise earlier than either neutrophilia or lymphopenia is seen in the course of disease. The low LCR and the high NLR observed at different time frames can be regarded as independent predictive markers for in-hospital complications and mortality in COVID-19 patients (Liu et al., 2020).

In our study, the minimal neutrophil count and the maximal percentage of cases with neutropenia ($<1.0\times10^9$/L) were observed in the group of Middle Eastern patients. Among 10 patients with neutropenia, 2 presented with the severe disease and died, 7 patients had comorbidities, and 3 of them developed complications. The NLR was also minimal in the Middle Eastern group. A rise in NLR across the disease as well as high initial levels of the NLR are the markers of poor disease outcomes and high mortality. This finding is aligned with the fact that the Middle Eastern group had the lowest number of patients who required intensive care and developed the critical disease.

In the group of East Asians, we observed the minimal values of LCR and the tendency toward the highest NLR. This correlates with the high proportion of patients with the moderate and severe disease and the maximal number of patients who developed liver dysfunction in this ethnic group.

Both LCR and NLR should be interpreted in conjunction with the clinical data to identify patients at risk of poor prognosis of COVID-19. Neutropenic conditions should be followed up to prevent concomitant infections worsening the disease severity.

5.2 The Top-Ranked Features of the Model for Classification Concerning Ethnicity

The top-ranked features listed in **Table 5** may represent the ethnic-specific response to the disease. Notably, the count of platelets was the top ranked variable in the model that reflects ethnic-specific features of COVID-19. Because of the disturbed coagulation in COVID-19 patients, there are considerations for the potential role of platelet function and/or platelet activation in the disease severity (Larsen et al., 2020). Furthermore, APTT is also a valuable feature of the classification model (the 7th one out of a total number of 38).

The WBC count and the level of lymphocytes on admission are also among the top-ranked attributes. The facts support the hypothesis that some mechanisms of the immune response to COVID-19 are specific to the ethnicity of the patient. Meanwhile, lymphopenia is known to be an essential clinical feature in patients with severe SARS-CoV-2 infection (Zheng et al., 2020).

The activity of LDH enzyme ranks 5th among the most valuable predictors. The biochemical constants (e.g., total bilirubin and creatinine concentration) may account for genetic-based differences in the enzyme regulations and metabolism. The presented symptoms, SOFA, and GCS scores are at the bottom of the list of the valuable features; i.e., the clinical appearance of COVID-19 is not specific to the patient's ethnicity.

5.3 The Classification Model and Its Performance

To check the quality of the outcome of the supervised ML model, we employed several algorithms and compared their performance. The NN outperformed all the other methods. We tuned parameters of the model in terms of the number of hidden layers and neurons, optimizer, and hyperparameters and built the three-layer fully connected NN. It showed up to 90% averaged accuracy in the classification by the ethnic group.

The high accuracy of the model supports our hypothesis of the occurrence of ethnic-specific features and patterns in the dataset. As seen from the error matrix (**Table 6**) and performance matrix (**Table 7**), the best performance is shown for the most numerous class of South Asians. The highest rate of false-positive values was obtained for the Middle Eastern class, which comes second in terms of the number of patients. The misclassification can be explained by some similarities between the two classes rather than overfitting of the ML algorithm.

To assess the performance of the model, we built the ROC curves for each class separately and calculated the appropriate AUCs for micro and macro average. **Figure 5** clearly indicates the high performance of the model with regard to an ethnic group. Micro averaged curve and its AUC indicate high performance for each group as it is calculated globally.

6 CONCLUSION

- In our cohort, Caucasian or East-Asian COVID-19 patients tended to have a more severe disease despite a lower risk profile. In contrast to this, Middle Eastern COVID-19 patients have a higher risk factor profile but they did not differ markedly in disease severity from the other ethnic groups.
- The accurate ethnicity classification model, which is based on the laboratory, physical, and clinical findings, reveals the presence of ethnic-specific features of COVID-19.
- The high performance of the ML NN method applied to the classification by the ethnic group from the laboratory and clinical findings supports the occurrence of features and patterns that are specific to ethnicity. This may impact the development of medical treatment and protocols based on ethnic background.
- Larger studies are needed to explore the role of ethnicity in COVID-19 disease features.

7 LIMITATIONS

One of the major strengths of the study was the recruitment of a cohort reflective of all adult age groups. This enabled us to calculate actual risk estimates. The *second* positive is that all the patients diagnosed with COVID-19 were hospitalized regardless

of their disease severity. The diagnostics was performed in full accordance with the common "National Guidelines for Clinical Management and Treatment of COVID-19" (National Emergency Crisis and Disasters Management Authority, 2020), which provided us with the unique study cohort representative of the adult population.

The current study has several limitations. *First*, it is a single-center study in the Emirate of Dubai, which is the most populated city in the UAE with the highest percentage of expatriates (91%), and it does not cover other cities such as Ras-Al-Khaimah where expatriates make up 69% of the population. Thus, UAE nationals might be underrepresented in this cohort.

Second, we were unable to assess the possible impact of socioeconomic factors. The relationship between ethnic background and socioeconomic status with health outcome is complex and multidimensional. Data on socioeconomic status in the UAE (e.g., the level of education and the monthly income) are not routinely collected in hospital medical records. Although we consider its influence on the health state of people with different ethnicity, it is impossible to estimate the effect of the aspect within the society of Dubai. The information on personal income does not reflect the spectrum of expenditures by an individual. Thus, the above-mentioned factors should be the focus of a separate study on economics and public health. Information on the socioeconomic status is missing in the dataset analyzed. The health insurance plan is not a valid marker of socioeconomic status in the UAE (see the *Discussion* section) and the UAE government provided free medical care to all COVID patients during the study period.

Third, there is no reliable and affordable tool for segregating examinees into ethnic groups and subethnicities. Apart from geographic and cultural similarities, ethnicities have common genes that were not analyzed in the current study. Since the data on the patients' Y haplogroups were not available, we divided the study cohort by geographic location. Large ethnic groups were used in this study. This allowed us to build accurate classification models that justified an association between the disease course and ethnicity. However, we were unable to analyze statistics on COVID-19 in distinct nationalities as the correspondent subgroups were low in numbers and unbalanced.

Fourth, although scientists pay much attention to the association of genetic (e.g., ACE2) factors with the COVID-19 severity and outcomes, the settings of our study did not allow us to focus on this aspect. Genetic tests are quite expensive procedures and are not covered by health insurance. During the first wave of the COVID-19 outbreak, genetic factors were not the focus of research activities. As the pandemic evolves, the analysis of such factors may be helpful for the healthcare sector in multinational countries including the UAE.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request at the site of Big Data Analytics Center (https://bi-dac.com/covid19-dubai-dataset/).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Mediclinic Middle East Research and Ethics Committee (MCME REC) (reference number MCME.CR.104.MPAR.2020), Dubai Scientific Research Ethics Committee (DSREC), and Dubai Health Authority (protocol number DSREC-05/2020_25). Written informed consent for participation was not required for this study using secondary deidentified data in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ML, NS, RS, DK, and SN collected the dataset. FA and YS wrote the manuscript. TH performed the statistical analysis, prepared the figures and tables for data presentation and illustration. TT analyzed the hematological findings and contributed to writing Results and Discussion sections. NZ, TL, and DS contributed to the literature review and data analysis. All authors contributed to the article and approved the submitted version.

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