frontiers | Frontiers in Tropical Diseases

REVIEW
published: 07 July 2022
doi: 10.3389/fitd.2022.893653

Advances in the Diagnosis of Leprosy

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Leprosy is a public health issue, and early detection is critical to avert disability. Despite the global attempt to eradicate this disease as a public health problem, it remains an important cause of global neurological disability. India, Brazil and Indonesia share more than 70% of the cases. The reduction of new cases is a priority in the WHO global strategy 2021-2030 which aims to reduce disease transmission in the community by diagnosing cases and identifying subclinical infection. The clinical manifestations of leprosy range from a few to several lesions. The identification remains difficult due to the limited sensitivity of traditional approaches based on bacillary counts of skin smears and histology. To aid in the diagnosis of this disease, molecular biology, and biotechnological technologies have been applied, each with its own set of benefits and downsides despite providing an essential tool to validate the clinical diagnosis of leprosy. Because of this, it is strongly recognized that specific, inexpensive point of care technologies should be developed, particularly to identify asymptomatic *M. leprae* infections or leprosy nearer to the suspected cases seeking medical attention. Thus, this review will provide an overview of the advancements in leprosy diagnosis over the world. The purpose of this review is to improve our understanding of the outcomes of current tests and technologies used in leprosy diagnosis and to emphasize critical aspects concerning the detection of leprosy bacilli.

Keywords: leprosy, diagnostics, PCR, emerging techniques, nucleic acid tests (NAT), serological tests

OPEN ACCESS

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Specialty section:
This article was submitted to Neglected Tropical Diseases, a section of the journal Frontiers in Tropical Diseases

Received: *10 March 2022*
Accepted: *11 April 2022*
Published: *07 July 2022*

Citation:
Sharma M and Singh P (2022) Advances in the Diagnosis of Leprosy. Front. Trop. Dis. 3:893653. doi: 10.3389/fitd.2022.893653

INTRODUCTION

Leprosy is caused by an uncultivated pathogen, *Mycobacterium leprae* and *Mycobacterium lepromatosis* which primarily affect the skin, mucosal surface of the upper respiratory tract, the peripheral nerves, eyes, and internal organs (1, 2). It has a long incubation period which can take as long as 20 years or more. Leprosy occurs in several clinical manifestations that extend from the spectrum that extends from tuberculoid leprosy (TT), through borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), to the poorly resistant lepromatous leprosy (LL) (3). The clinical diagnosis of pure neural leprosy (PNL) also remains a public health concern, owing to the absence of skin lesions, which are the cardinal symptoms of leprosy. Operationally leprosy is classified into Paucibacillary and Multibacillary leprosy (4). A broad range of intermediate types also exists commonly known as borderline leprosy. Even though leprosy is claimed to be eliminated in most countries, according to WHO, 213535 new leprosy cases were detected globally in 2020, with ~85% of those detected mainly in India, Brazil, and Indonesia (WHO, 2020) (5).

With over 1.25 lack new leprosy cases detected in 2019, India accounts for >60% of the total cases reported globally indicating an active transmission. Leprosy diagnosis is mostly based on clinical presentations, Acid-fast bacilli (AFB) staining methods for slit skin in smears, and lymph or histological examination of biopsy samples are useful for confirming the diagnosis of leprosy. However, these tests are less productive in the early diagnosis when clinical symptoms are often not clearly defined, for example, in some paucibacillary cases (PB) (6). Moreover, since expertise is important in the practical application of histological or molecular biological assays, these methods of detection are mostly used in reference labs and not in peripheral labs or field settings. It is the same for testing the medication sensitivity with higher labor and time use in mouse footpads since *M. leprae* is not yet successfully cultivated despite several decades of attempts for *in-vitro* cultivation. Therefore, the phenotypic drug susceptibility must be done in animals, for example, mouse footpad (MFP) testing. However, a one-year-long experiment in the MFP experiments for phenotypic sensitivity testing is not very popular (7). Moreover, the low capacity to distinguish *M. leprae* from other mycobacteria by microscopy is an important concern due to the poor precision and sensitivity of these experiments (8). In the last twenty years, many experiments in clinical and molecular settings have been conducted which have advanced the area of leprosy diagnostics (9–11). However, a variety of diagnostics tests have been proposed based on the detection of pathogen's DNA, RNA, protein or host antibodies, cytokines and other host biomarkers such as gene expression profiles (12). Based on the principle, these diagnostic tests can be broadly divided into three types: Immunological tests (Based on antigen/antibody detection), Nucleic acid-based tests, and tests based on host biomarkers (13).

Diagnostic Tests Based on Antigen-Antibody Detection

These rapid diagnostic assays detect the presence of leprosy bacilli proteins or host antibodies against them. If adequate quantities of the target antigen are present in the sample, it will bind to specific antibodies attached to a paper strip which will produce a visually detectable signal within 30 minutes. The observed antigen(s)/antibodies, which can only be expressed if bacteria are present in the host and have a high BI index, are thus usually used for acute or early infection detection (14, 15). Antibody detection is a more general form of leprosy diagnosis test, which measures the presence of antibodies in the blood of people suspected to be afflicted with *Mycobacterium leprae*. Specific antibodies are made within days or weeks after initial exposure. The intensity of the antibody response relies on a variety of factors such as bacillary load, type of immune response as well as other host factors such as age, nutritional status, disease severity and certain medications or infections such as HIV suppresses the immune response (16). Though antibody levels provide some insight into the status of infection, but they are insufficient to distinguish between recent and past infections. However, finding the evidence of infection would suggest a recent transmission (17).

Diagnostic Tests Based on Nucleic Acid Amplification Tests (NAAT)

M. leprae can be specifically detected by PCR or qPCR techniques in a very sensitive manner i.e. for detecting a very small number of genomic templates in a clinical sample. Several specific genomic regions have been targeted such as 16S rRNA (7) *rpoT* and *sodA* (18, 19) 36-kDa antigen (20), Complex 85 (21) and the repetitive sequences (LEPRPT, LEPREP, REPLEP and RLEP) (22) among other *M. leprae* genes (23–25) of which the most sensitive and specific sequence was found to be RLEP region (26). Other repeated loci can also be targeted that are present in fewer copies. Leprosy is often diagnosed by molecular testing either from PCR or qPCR (7, 8, 27). NAAT evaluations are focused on amplifying the *M. leprae's* targeted sequence of the genetic material (DNA or RNA) with PCR. NAATs are more rapid than conventional mycobacterial detection technologies and are also available at different healthcare levels, therefore allowing them to perform drug susceptibility testing, such as rifampicin (RIF), for the detection of leprosy (28). In this way, they simplify leprosy diagnostics and help improve the quality of leprosy treatment.

Diagnostics Tests Based on Host Biomarkers

The immunological spectrum of leprosy, which ranges from widespread infection to a self-limited type of disease, often complicates the diagnostic procedure. The intricate interaction between innate and adaptive immune responses, which is controlled by host genetic background and environmental variables, determines *M. leprae* infection and subsequent disease development. Biomarkers based on the host immune response to *M. leprae* are thus a topic of interest as detecting *M. leprae* in the preclinical phases of the disease is challenging. Many research groups have recognized the potential of *M. leprae* specific antigens in serologic and T cell tests since the disclosure of the *M. leprae* genome. Despite the screening process to identify the early candidate that triggers *M. leprae* specific T lymphocytes, suitable candidate antigens are yet to be identified that would cover the entire immunological leprosy spectrum.

In this review, we have tried to compile all the information available in the literature regarding the recent developments for leprosy diagnosis (**Table 1**). We refer readers to other reviews to know about the recent advancements in biomarker-based research for leprosy detection (65–68) and meta-analysis for sensitivity and specificity studies of laboratory tests (10, 29–31).

DIFFERENT DIAGNOSTICS APPROACHES FOR THE DETECTION OF *M. leprae*

Clark-Curtiss and Docherty provided the first paper of 1989 on the use of nuclear acid-based identification of *M. leprae* (69). A 2.2-kb *M. leprae* DNA fragment was used to facilitate the precise identification of bacilli in a hybridization-based technique in materials of multibacillary (MB) patients. During the same year,

TABLE 1 | Emerging molecular tests for the diagnosis of leprosy and drug resistance.

Name of Method	Name of the technique	Study Published	References
Molecular Methods	PCR, qPCR,	2019, 2021, 2022	(10, 29–31)
Serological Assays	NDO-LID	2019, 2022	(29, 30)
	PGL-1	2019, 2022	(29, 30)
	35Kda Test Card	2019, 2022	(29, 30)
	CRP, CCL4, IP-10	2019, 2022	(29, 30)
Commercial Kits	NDO-LID® test	2019, 2022, 2018	(29, 30, 32)
	NAT-HANS test	2021	(33, 34)
	HLAssureTM SE SBT Kit	2019-2021	(35–37)
	GenoType LepraeDR	2019, 2013,2015	(38–40)
	Genesig Kits for Leprosy	2018	(41)
	Biomeme	–	(https://biomeme.com/)
Mobile Applications	SkinApp	2021, 2018	(42, 43)
	LEARNS	–	https://www.novartis.com/ph-en/news/media-releases/learns-countrys-first-mobile-phone-based-leprosy-teleconsultation-system
	NIKUSHTH	–	https://hispindia.org/Pages/Leprosy%20ICMR/Leprosy%20ICMR.html
Artificial Intelligence	AI for leprosy	2021-2022	(44–46)
Other Techniques	Fluorescent Hybridization	2016-2018	(47–49)
	Sonography	2016,2021	(50–52)
	Electrochemical biosensors	2021	(53)
	MRI	2017-2019	(54–56)
Techniques can be Adapted for leprosy PoCTs	Palm PCR	2014	(57)
	Portable PCR	2019	(58)
	Backpack PCR	2018	(59)
	iiPCR	2015	(60)
	LAMP	2021	(61–63)
	CRISPR based diagnosis	2021	(64)

Woods and Cole described the use of PCR to selectively identify the *M. leprae* specific repetitive sequence RLEP in *M. leprae*, explaining agarose gel-based visualization of approximately 100 *M. leprae* cells in the armadillo liver, mouse footpads and human biopsies (70). Several other PCR systems were subsequently developed for the detection of *M. leprae* (6, 24, 71, 72), which showed the detection limits of 1 to 1000 bacilli. Observations of amplicons dependent on agarose gel were mainly reported. Later, with the advent of new sensitive techniques like NGS, various new targets were identified (27, 73–79). Several electrophysiological, ultrasonographic, Electroneuromyography (ENMG) and histological techniques are also developed and adapted to diagnose PNL (80, 81). Subsequently, many other sensitive techniques have been used previously or in development that can be updated and further used as a point of care tools in the laboratory as well as field settings as diagnostic tools such as variants of PCR (8, 24, 71, 72, 82), colorimetric assays (83), Biomarkers (66), Filter paper (84), peptide-nucleic-acid-ELISA (85, 86), reverse line probe (87), synthetic peptide reagents (88–90), Ultrasonography (50–52), Fingerstick test (91), Electrochemical biosensors (53), LAMP (61–63), MRI (54–56), LF Assays (92, 93), Fluorescent staining (47–49) and Artificial Intelligence to combat leprosy (44, 45). Here we will be discussing budding techniques that have the potential to be used in the field settings easily.

Polymerase Chain Reaction

Following the publication of the genome sequence of the *M. leprae* in 2001, species-specific genetic sequences have been sought to standardize the diagnostic procedures based on DNA analysis. These sequences were amplified using the PCR method, allowing the identification of bacillus DNA from modest numbers of *M. leprae* cells. PCR and quantitative PCR (qPCR) are extremely informative and responsive and guarantee to be diagnosed and treated early enough to guarantee the timely medication required to avoid disability and to mitigate leprosy spread (7, 71). The PCR-based approaches used to detect pathogenic DNA and RNA can also assess the viability of leprosy bacilli and be used in touch screening and monitoring programs. Several tissue origins, including skin biopsy tests, swabs of the nasal or dental, and entire blood, can be extended with PCR for *M. leprae* DNA. However, the use of skin biopsies instead of conveniently obtained samples (blood, skin scrapings, saliva etc) provides maximal performance (24).

Although *M. leprae* was considered the exclusive causative agent of leprosy, recently, *M. lepromatosis* was discovered and found in an unusual type of leprosy known as diffuse lepromatous leprosy (DLL) (94). A unique repetitive factor, RLPM, was defined by genomic analysis of *M. lepromatosis* strain (NHDP-385) on which a real-time quantitative PCR (qPCR) assay was established and validated in compliance with the guidelines for Clinical Laboratory Improvement Amendments as clinical diagnostic assays (27). Similarly, a new assay was developed that can simultaneously detect both the leprosy bacilli through PCR in a single reaction by amplifying a part of *rpoT* gene (19). Therefore, in clinical diagnosis and monitoring of leprosy in the field settings, the RLPM (*M. leprae)*, RLEP (*M. lepromatosis*) and *rpoT* based PCR, 16SrRNA, *sodA*, RLEP based PCR (18) and qPCR assays can support healthcare providers. The availability of a rapid, specific and sensitive, and field-deployable PCR assay would support local decision-making during the surveillance and screening period (95). Few PCR examples are already reported such as Portable PCR (58), Palm PCR (57), insulated isothermal PCR (iiPCR) (60), RT-insulated isothermal PCR (96), Backpack PCR (59), etc. These assays have been successfully used for several pathogens detection and can also be adapted for the detection of leprosy bacilli.

Upconverting Reporter Particle-Lateral Flow Assay (UCP-LFA)

Endemic areas of leprosy are often lacking specialized labs that highlight the need for low-complexity diagnostics (97–99). A test was developed and field-assessed known as the lateral flow test (100). Recently, Upconverting phosphor (UCP) reporter technology was used in all LFAs to increase sensitivity and quantitation (101). The test consists of the UCP technology in conjunction with low-cost immune chromatography (i.e. lateral flow). It performs exceptionally well for the identification of cytokines and anti-*M. leprae* PGL-I IgM Ab (αPGL-I) in serum. A quick test using the visual detection of immunogold particles has been used to detect antibodies against *M. leprae* (93). No sophisticated analytical laboratory equipment is required for the user-friendly UCP-LFAs. This low-cost, lightweight portable reader offers complete, instrument-assisted analysis and prevents operator interference. Using UCP-LFA, the quantity of every biomarker type present in biological samples may also be quantified, which will enable the measurement of variations in the quantities of biomarkers and adjustable cut-off values to fulfill exposure and specificity criteria for areas where the leprosy is endemic (92, 102).

Loop-Mediated Isothermal Amplification LAMP

Isothermal amplification is a simple process that rapidly amplifies nucleic acid sequences at constant temperature (103). It is favored over typical PCR approaches to get rid of the specifications/complications of a costly thermocycler system and is cost-effective. The loop-mediated isothermal amplification (LAMP) technique has many applications in the area of point-of-care (POC) research across many isothermal techniques (104). It uses four to six primers to determine particular temperature regions which can be multiplied at a constant temperature using high strand displacement DNA polymerase. Gene amplification and detection can be achieved in one step, using the basic heat block or water bath, by the incubation of the polymerase primer and template mix in a reaction buffer. It can obtain outcomes in less than 30 minutes at a single temperature with a high yield and specificity. LAMP is considered to be useful for many molecular diagnostic applications i.e. from laboratory infectious agents to different pathogens, food processing, environmental examinations etc. (105, 106). Several LAMP-based diagnostic kits and approaches have been developed to identify a variety of challenging pathogenic agents for highly infectious illnesses, including SARS-CoV-2 (107). More importantly, the advent of LAMP has led to improved diagnosis of neglected tropical diseases all over the world. Esmatabadi et Al checked different methods for identification that can be paired with the LAMP system to interpret results quickly, such as colorimetry, turbidometry, hybridization samples, lateral flow dipsticks, ELISA, gold nano-particles (108). *M. leprae* is uncultivated *in vitro* and has a highly reduced genome that has a high degree of identification with the MTB genome. Considering its unique advantages that provide a rapid and low-cost diagnostic tool for disease detection in poor and remote parts of the world, recently LAMP technology for *M. leprae* detection has been developed using RLEP sequence as a molecular target for this assay (61–63). However, no isothermal amplification assay has been developed for *M. lepromatosis* yet.

DETECTION OF *M. leprae OR* ASSOCIATED BIOMARKERS BY COMMERCIALLY AVAILABLE PRODUCTS

NDO-LID® Test

The NDO-LID® test, a new ELISA-based diagnostic test, was developed for the diagnosis of MB leprosy. The test is based on the detection of antibodies against the unique protein-glycolipid complex. The NDO-LID® immunochromatographic test takes only a tiny sample of serum or whole blood. This test includes both the LID-1 and PGL-I antigens. These antigens are immobilised on nitrocellulose membranes, which allow for the transfer and detection of the specific antibodies against these antigens in patient sera. This test, when combined with a new mobile based platform (Smart Reader® application), can give measurable and reliable data to aid in leprosy diagnosis. It detects MB patients and, perhaps, HHC at a higher risk of acquiring MB leprosy, much as the other leprosy serological tests (32, 109–112).

NAT-HANS Test

It is the first PCR-based diagnostic test for leprosy that follows Good Manufacturing Practice (GMP). This multiplex real-time PCR test has been developed to detect *M. leprae* 16S rRNA and

RLEP genes, as well as one mammalian target (18S rRNA gene) that acts as a control. This detection occurs by the increase, at each reaction cycle, of the fluorescence signal emitted by two molecular probes specific when the target DNA is present in the sample. The success of the reaction is monitored through a fluorescence signal, emitted by a third probe in the same reaction, which increases the amplification of DNA. The Brazilian Health Regulatory Agency (Anvisa) approved the NAT-HANS test for people suspected of leprosy in 2021. The test showed high sensitivity and specificity values comparable to Lateral Flow Assays with a limit of detection of 2.29 copies of the *M. leprae* genome (33, 34).

HLAssure™ SE SBT Kit (Personalised Medicine)

Dapsone is an anti-inflammatory and antibacterial medication used in dermatology to prevent and treat infectious and chronic inflammatory conditions (113). Dapsone is used to treat leprosy, malaria, and diseases as an antibiotic and as an anti-inflammatory medication but is most commonly utilised for its anti-leprotic action. However, there have been reports of Dapsone Hypersensitivity Syndrome (DHS) which poses serious concern in certain populations. HLA-B*13:01 is associated with DHS and prior screening is essential to significantly minimize the chances of occurrence of DHS. A single DNA polymorphism termed HLA-B*13:01 located in the human leukocyte antigen locus is more common in those who developed DHS (35–37). Individuals with one copy of HLA-B*13:01 risk variant are 34 times more likely to develop DHS than individuals without it. Two copies of HLA-B*13:01 make individuals times more susceptible to the syndrome (75). Using TBG Biotechnology Corp's HLA-B*13:01 assay kit, Clinicians and researchers are finally able to screen the patients for DHS. Recently, HLA-B*13:01 is validated as a biomarker for DHS in leprosy patients and Screening for HLA-B*13:01 has shown promising results in reducing DHS incidence significantly in the Indonesian population (114). The kit also provides the typing results of HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQB1 and DPB1 with high resolution by a DNA-based method (http://www.tbgbio.com/en/product/product_detail/12).

GenoType LepraeDR (Drug Resistance)

A test was developed in 2012 as a GenoType LepraeDR kit using DNA strip technology (detection of amplified DNA or RNA sample *via* hybridization and alkaline phosphatase reaction on a membrane strip) to overcome this and extended to the molecular diagnosis of antibiotic resistance in leprosy (https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/lepra/genotype-lepraedr.html). The GenoType LepraeDR enables the simultaneous identification of *M. leprae* and its susceptibility to first and second-line drugs within 5 hours. Initially, 120 *M. leprae* strains analyzed for genotypic and phenotypic characters of resistance were routinely screened for evaluation. The assay findings were found to be 100% compatible with those of the *in vivo* susceptibility test, while the PCR sequencing results were 98.3% for rifampicin, 100% for Dapsone, and 100% for fluoroquinolones (28). Later many publications have described this assay to identify the drug resistance in *M. leprae* (29, 38–40).

Genesig Kits for Leprosy (qPCR and Whole Genome Sequencing)

The Primerdesign genesig Kit® for Leprosy is intended for quantification of *M. leprae* genomes *in vitro*. The kit is intended to provide a wide detection profile. Their primers show 100 percent homology with more than 95 percent of the NCBI database reference sequences available at the time of creation. Because of the dynamic nature of genetic diversity, additional sequence information may become accessible after the initial design. The method employs a TaqMan probe to perform real-time PCR of the non-coding repetitive element RLEP, which is unique to the *M. leprae* genome. The excellent test specificity was demonstrated utilizing pathogenic and opportunistic pathogenic mycobacterium reference DNA samples, as well as PCR detection of single-copy genes of *M. leprae* such as fbp, MntH and rrs. The application of the developed approach increased the sensitivity of a commercially available test system based on single-copy rpoB gene detection to 96.8%, while the use of a commercially available test system based on single-copy rpoB gene detection provided 59.4 percent sensitivity to the detection of *M. leprae* in clinical material (41).

Biomeme

American Leprosy Mission and its network of research partners have collaborated with Biomeme, Inc. to develop and commercialize hand-held point-of-need PCR testing solutions for combatting leprosy diagnostics for use in distant locations. Their FranklinTM mobile qPCR thermocycler and field-prepared, shelf-stable reagents are easy-to-use reagents for field-friendly operations (https://biomeme.com/). Biomeme uses a polymerase chain reaction (PCR) methodology that turns a smartphone into a powerful mobile lab capable of performing PCR, RT-PCR, qPCR, and isothermal assays in real-time. A Biomeme mobile qPCR thermocycler, which is smaller than a box of tissues, is at the core of the solution. The lightweight, internally battery-powered gadget produces results in 30-45 minutes without the need for electricity, centrifugation, or cold chain logistics (https://www.ccih.org/technology-in-global-health-an-instrument-of-impact/). The use of the Biomeme platform as a field-friendly PCR-based early diagnostic tool to detect subclinical infection among household contacts and the general population has been rolled out in some parts of the world (India, Nepal and Ghana).

DIAGNOSIS OF LEPROSY THROUGH DIGITAL APPLICATION

SkinApp

Leprosy in endemic regions has been diagnosed based on the presence of one of 3 cardinal signs: skin lesion with loss of sensation associated or not with thickened peripheral nerves and microscopic detection of acid-fast bacilli in skin lesions/slit skin

smears. Keeping in mind, WHO released a manual on neglected tropical diseases by skin changes in 2018 (115). This serves as a training guide for the front-line health professionals, a detailed guide to recognizing skin NTDs employing signs and skin symptoms offering guidance about how to detect and cope with serious skin conditions that are faced by unspecialized front-line health care personnel. This pictorial training guide on neglected tropical diseases of the skin (including leprosy) has been recently converted into an interactive mobile phone application. The program allows easy access to the knowledge about skin conditions – such as its clinical symptoms, management and geographical distribution – which provides a list of possible indications to health care professionals and the general public. The software also facilitates rapid knowledge exchanging through a chatbox, which will promptly answer general questions. In collaboration with national dermatological federations, the SkinApp has been evaluated in field settings in Ethiopia, Mozambique and Tanzania under the PEP4LEP initiative in 2019. During their daily clinical activity, the primary health staff found it very helpful (42, 43).

Leprosy Alert and Response Network System (LEARNS)

LEARNS is a mobile-based leprosy identification system that enables primary health providers to send an SMS to a specialist with photos of suspected leprosy and symptoms. This helps in a dramatic reduction in the time required for testing and treatment times. Beginning in the Philippines, where nearly 2,000 new leprosy patients have been identified in leprosy annually, LEARNS was initiated with the support of a Leprosy Task Force as part of the Philippines Department of Health (DOH)-Novartis Foundation. In 2015, A diagnostic compatibility between LEARNS and the clinician-guided diagnosis was determined. LEARNS has proven to be a successful screening method for the proper detection of suspected lesions as 83 percent leprosy and 77 percent leprosy except for specifics, where the suspected lesions have been presented in the picture. About 3,500 healthcare providers have been qualified nationally in LEARNS to date (https://www.novartisfoundation.org/past-programs/accelerating-leprosy-and-malaria-elimination/leprosy-alert-and-response-network-system-learns). In line with LEARNS, similar computer-based software application like NIKUSHTHA which is developed to report leprosy in India has been started working. (https://dghs.gov.in/content/1349_3_NationalLeprosyEradicationProgramme.aspx) whereas a Portuguese mobile application system is in development for detecting leprosy suspects (116).

FUTURE DIRECTIONS

Artificial Intelligence (AI) Powered Diagnostic Tool

Artificial intelligence (AI) has become a hot topic among doctors in recent years with the evolution of information technology and interdisciplinary studies have become more common. Accurate identification of leprosy lesions is often a challenge, especially for clinicians with little experience such as those in low-burden settings. To address this problem, the convolutional neural networks (CNN) project was developed using DermnetNz datasets in 2016, achieving 91.6 percent accuracy (117). In 2018, researchers used the Kohonen Self-Organizing Maps algorithm to evaluate data from patients and their household connections using Artificial Intelligence approaches to study the epidemiology of leprosy (118). The results show a significant frequency of late diagnosis, as well as Anti PGL-1 levels in clusters, indicating a higher bacillary load and consequently a high risk of disease progression. Microsoft and Novartis Foundation have also teamed up to develop an AI-powered digital tool for the early detection of leprosy (https://www.novartisfoundation.org/news/ai-powered-diagnostic-tool-aid-early-detection-leprosy). They have named it AI4Leprosy project which aims to develop an easy-to-use AI-powered solution to accelerate the diagnosis of leprosy with skin images. Recently, a cross-platform app for leprosy screening based on artificial intelligence has been developed which can recognize patterns of leprosy cases in a database with ~94% sensitivity and 87% specificity (45). Recently, Barbieri et al. described that AI models have the potential to become a complementary diagnosis tool, especially for the remote areas where very few clinicians are available known as AI4Leprosy (46). It is an AI-enabled image-based diagnosis tool for leprosy that is based on a mix of skin images and clinical data collected using a defined method. For leprosy diagnosis, it had a high classification accuracy (90%) and an area under curve (AUC) of 96.46 percent.

DISCUSSION

The current COVID-19 pandemic has raised the awareness and preparedness levels towards the rapid and in-time diagnosis of infectious agents to reduce their transmission. It has been anticipated that financial markets will remain volatile as the virus continues to disrupt economic activity which is associated with worse infectious diseases outcomes. According to expert opinion and some guidelines, COVID-19 may influence the occurrence and severity of lepra reactions as an economic crisis may contribute to the increase in the incidence of leprosy (119). The epidemic threatened to undermine any progress that had been made in eliminating leprosy. For example, loss of financial and human resources from national leprosy programs when leprosy funding and employees are redirected to the fight against Covid-19, resulting in a significant drop in leprosy-related projects and experts. However, amid the challenging condition of lockdown, very precise and effective diagnostics for SARS-CoV-2 were developed in a record time. To this date, several commercially available tests such as CBNAAT and TrueNat were adapted for POCs for SARS-CoV-2 detection addressing several challenges such as instability of RNA targets and Biohazard issues. However, a similar focus on leprosy and other NTDs has yet to be attempted or prioritized. Real time PCR and CBNAAT/TrueNat machines/kits are more available at district levels in many places. The

development of suitable primers and probes compatible with CBNAAT/TrueNat and other systems would give an additional advantage towards the rapid detection of leprosy bacilli. These assays have the high potential to be used as POC for leprosy diagnostics. With the decline in leprosy cases, clinical expertise is bound to further decrease which may result in diagnostic delays. As a result, the period between clinical symptoms, diagnoses and treatment of patients is longer and the transmission of stays almost constant. Hence, efforts to get rid of this disease are then hindered. An optimal diagnostic test will recognize *M. leprae* infected persons at risk of developing diseases and/or leading to their spread, hence there is a need to also switch from leprosy management to prevention of infection. A screening test for verifying leprosy disease will be appropriate and probably effective in the early stages for patients and are asymptomatic. At the same time, stakeholders must strive to support the idea that zero transmission is feasible only when the assessment of *M. leprae* infection is feasible, and that biomarkers to detect asymptomatic infection, and risk of contracting the disease, are sufficiently invested in clinical studies.

AUTHOR CONTRIBUTIONS

PS and MS contributed to the conceptualization and design. Review of literature, data mining and analysis were performed by MS. The first draft of the manuscript was written by MS. Both authors read and approved the final manuscript.

FUNDING

Research work of PS is funded in part by R2STOP (effect:hope TLM) Canada, Turing Foundation and Leprosy Research Initiative (LRI) Netherlands, (Grant No. 704.16.59) the Department of Biotechnology (Government of India, Grant No. DBTRLF/Re-entry/10/2014) and Indian Council of Medical Research (ICMR). MS is the recipient of ICMR-Research Associateship. The manuscript has been approved by the Publication Screening Committee of ICMR-NIRTH, Jabalpur and assigned with the number ICMR-NIRTH/PSC/09/2022.

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