



CaPO₄-Mediated CKD of Crystallo-Tubular-Nephropathy [CKD-CTN]—A Crystal and Nanotube-Induced Geo-Environmental Disease

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Importance: More than forty million people from certain tropical countries are at risk of developing a non-conventional form of chronic kidney disease (CKD), CKD of multifactorial etiology (CKDmfo). This is also known as CKD of unknown etiology (CKDu). Worldwide, it kills more than 20,000 people annually.

Findings: CKDmfo is a chronic tubulointerstitial renal disease caused by groundwater-induced hydroxy- and fluorapatite nano-crystals and nano-tube formation in renal tubules and cortical tissues, in conjunction with chronic intravascular volume depletion, chronic renal anaemia, and ischemia. To manifest this gradually developing renal failure, consuming hard water having, higher concentrations of calcium, phosphates, and fluoride for more than 10 years is necessary. The disease progresses when the kidneys fail to repair or due to ongoing renal tissue damage in the presence of micronutrient deficiency, chronic dehydration, renal ischemia, chronic inflammation, and oxidative stress. These prevents regenerations of renal tissues.

Conclusion: Consumption of stagnant groundwater concentrated with ions, like Ca²⁺, PO₄, and F⁻ due to prolonged annual droughts over many years, in conjunction with lesser water intake and chronic dehydration, creates a conducive internal milieu for CaPO₄ crystallisation in renal tissues. This proposed primary etiology of the crystal-tubular-nephropathy (CTN) provides an insight into a deeper understanding of the use of cost-effective strategies for prevention, early intervention, and eradication of CKD-CTN. In addition to the nano-crystal/nano-tube concept, we provide supporting scientific evidence that Mg²⁺ in water and the diet does not promote the disease; instead, it prevents crystal formation and developing CKDmfo, as with some other CKDs.

Relevance: We present novel CaPO₄ hydroxyapatite crystal formation concepts in a hyperosmolar fluid in renal tissues that causes CKDmfo. Besides, the protective (e.g., magnesium) and enhancing (e.g., dehydration, drinking stagnant hard water) mechanisms that cause CKD-CTN are explored. A new understanding of causative mechanisms paves a path for cost-effective targeted interventions to prevent and eliminate CKDmfo. These principles apply to all CKDmfo/CKDu-affected countries

to protect the renal health of farm labourers and others who regularly engage in physical work in hot and dry environments. Providing affordable potable water, increasing water consumption, and avoiding harmful behaviours are critical measures for eliminating CKDmfo.

Keywords: chronic renal failure, CKDu and CKDmfo, environment, fluoride, geochemistry, hard water, nephropathy, tropical countries

PERSPECTIVE

Question: Why do more than 20,000 people from low-income tropical countries, primarily middle-aged males from farming communities, die from an unusual chronic kidney disease originating from multiple factors and conditions, each year?

Findings: Chronic kidney disease of multifactorial etiology (CKDmfo; CKDu) is an environmentally induced, geogenic—Geological and Biological—chronic tubulointerstitial renal disease. This lethal kidney disease can be cost-effectively prevented and eradicated by implementing straightforward strategic actions, such as public education, providing potable water, and alleviating malnutrition, as described in this article and not by medications, renal dialysis, or constructing renal hospitals.

Implication: For the first time, this study identified a specific cause for the genesis of CKDmfo, designing and enabling of comprehensive, an affirmative and cost-effective public health action for its prevention, which would save livelihoods, the local economy, and thousands of lives.

INTRODUCTION

A mysterious chronic kidney disease of multifactorial origin (CKDmfo) or CKD of unknown etiology (CKDu), unrelated to diabetes, hypertension, and other known factors (Wimalawansa, 2016b), threatens the lives of millions of people in tropical countries (Levine et al., 2016; Parameswaran et al., 2020; Redmon et al., 2021). Over 40 million people in several low-income, tropical countries are at risk of developing this unusual kidney disease unrelated to diabetes, hypertension, and other known factors (Wimalawansa, 2016b). CKDmfo is a tubulointerstitial renal disease associated with significant premature deaths (Wimalawansa and Dissanayake, 2019; Wimalawansa, 2020).

The hard water consumed by this vulnerable population is rich in dissolved solids (Abeywickrama et al., 2020). In addition to Ca^{2+} and Mg^{2+} , water in the affected regions also has higher fluoride concentrations (F^-), phosphate (PO_4^{3-}) and total dissolved solids (TDS), although not consistently (Wimalawansa and Wimalawansa, 2015a). Those affected live in dry zones at higher elevations above natural and artificial canals. Data suggest that in conjunction with hard water that contains particular ratios of different elements, fluoride consumption (F^-) exacerbates this specific type of tubulointerstitial disease (Dissanayake and Chandrajith, 2017). Moreover, the environmental conditions in these regions are conducive to forming nano-mineral

calcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$; CaPO_4] in soft tissues at unique concentrations over an extended period within the renal tubular cells (Wimalawansa and Dissanayake, 2019). These conditions initiate and maintain a persistent low-grade, chronic inflammation in local interstitial tissue, over a period that causes tubular cell fibrosis (Gunawickrama et al., 2021).

Environmental Factors That Initiated CKDmfo—Groundwater, Soil Conditions, and Harsh Weather Conditions

Most CKDmfo-affected regions have low humic, gley soil, and shallow non-permeable hard bedrock underneath. The presence of predominantly clay soil with less sand, coupled with the overextraction of groundwater by agro-wells, may have altered the underground water dynamics in these regions. The natural weathering process constantly releases heavy metals and fluoride into groundwater. In affected countries, the situation aggravates due to governmental neglect and ongoing multiple harmful behaviours of the affected populations rapid progress of agriculture, and other environmental reasons described below.

In countries where CKDmfo is endemic, the affected regions, mostly new settlements, have poor soil drainage. People settled in these new regions (now affected with CKD-CTN) over several decades without proper planning, sanitation, and potable water. This disease neither exclusively affects farmers nor is caused by any agrochemical. In Sri Lanka, thousands of families resettled from the south to the north-central province (NCP), where there are no direct, natural water supply. The water table is deep (groundwater) due to the regolith structure not connecting with the surface water.

New migrants had to dig wells into tapping groundwater for drinking and other purposes. Consequently, thousands of tube wells were constructed in these regions to obtain water for drinking and agricultural purposes. A few years later, the Mahaweli River, the longest river in Sri Lanka, was diverted from the central province to the NCP to facilitate agriculture and other needs (Wimalawansa, 2014a; Weeraratne and Wimalawansa, 2015). The government promised these families supplies of adequate water from the Mahaweli River. However, the water was provided only to low-lying paddy fields and not to living locations. This mass migration-related development program in Sri Lanka was implemented in the late 1970s and accelerated in the 1980s without providing the basic

human needs, addressing environmental factors, or assessing groundwater quality (Weeraratne and Wimalawansa, 2015).

The Preamble to CKDmfo

Although the regolith aquifer from the NCP region extends to the south of the country, towards Ampara and Monaragala and partially towards the west towards the Puttalam district, the soil structures in these areas are distinctly different from the CKDmfo-affected locations. Farmers use similar suites of agrochemicals for farming, significantly with higher concentrations in areas like the upcountry. A key difference is that groundwater in CKDmfo non-endemic regions have neither very hard water nor higher fluoride contents. Therefore, in the absence of mentioned regolith formations, non-endemic regions have minimal or no CKDmfo (Levine et al., 2016; Wimalawansa, 2016a,b; Thammitiyagodage et al., 2017; Edirisinghe et al., 2018; Balasooriya et al., 2020; Gobalarajah et al., 2020; Imbulana and Oguma, 2021).

To complicate matters, while the groundwater is very hard in areas, such as Puttalam and Jaffna districts, it has very low F^- content and has a very low prevalence of CKDmfo. These data suggest that drinking hard water alone does not cause CKDmfo (Weeraratne and Wimalawansa, 2015; Wimalawansa, 2015a). Few other locations in the country have high F^- (and iron) contents in water, but water is relatively soft, and people living in these areas also have a very low prevalence of CKDmfo. These data show that consuming hard water (Ca^{2+}) or water with higher concentrations of F^- alone is insufficient to cause CKDmfo (Wimalawansa, 2016b, 2019a, 2020; Dunuweera et al., 2017; Wimalawansa and Dissanayake, 2019).

The Role of Ca^{2+} , PO_4^{3-} and F^- in Renal Nano-Crystal Formation

Besides those agents generating superoxide radicals, excess fluoride (F^-) also increases the kidneys' oxidative stress, catalases, and lipid peroxidation (Khalili and Biloklytska, 2009). In one study, rats treated with F^- reduce intrinsic antioxidative components, such as glutathione, glutathione reductase, glutathione peroxidase, and superoxide dismutase in renal tissues (Birkner et al., 2006; Bharti et al., 2014). In addition, total phospholipids, phosphatidylethanolamine, phosphatidylcholine, and ubiquinone content in renal tissues are also significantly reduced (Gao et al., 2008; Despres et al., 2019). These data suggest that higher exposure to F^- can be highly oxidative and toxic to renal tubules.

Formation of $CaPO_4$ -Hydroxyapatite and Fluorapatite

In vivo, renal $CaPO_4$ -hydroxyapatite formation is common. However, in general, these structures are unstable [$Ca_5(PO_4)_3(OH) = Ca^{2+} + 3PO_4^{3-} + OH^-$]; thus, not sustained. However, when F^- substitutes the hydroxyl group in apatite, it creates the more stable fluoroapatite [$5Ca^{2+} + 3PO_4^{3-} + F^-Ca_5(PO_4)_3-F$] due to stronger ionic and hydrogen bonds that stabilise the structures. With time, matrix proteins are also incorporated into hydroxy-fluorapatite, strengthening nano-minerals that resist enzymatic degradation (Robinson et al., 2004).

For example, F^- integrated into $CaPO_4$ apatite in dental enamel tissues produces crystals resistant to decay. A minute concentration of F^- incorporated into dental and skeletal tissues further strengthened the resultant carbonate-apatite crystals resistant to decay (Aoba, 1997). **Figure 1** illustrates the multiple mechanisms and pathways of developing nano-mineral crystallisation in renal tubular tissues. These nano-crystals cause chronic low-grade inflammation and oxidative stress, leading to tubular cell apoptosis, fibrosis, and renal failure.

Nano-Tube and Crystal Formation in Renal Tissues

Ultrastructural studies in human kidney tissue biopsies of patients with end-stage CKD reported 50- to 1,500-nm multi-lamellar mineral particles within renal tubules. However, no such observations were reported in non-tubular interstitial diseases (Wong et al., 2015). These polycrystalline $CaPO_4$ nano-mineral particles are also found in extracellular matrices and the cytoplasm of tubular cells. When the common form of CKD or CKDmfo advanced to stages beyond stage IIIB, it begins to cause glomerular damage and sclerosis causing proteinuria. Crystalline nephropathies are a unique form of kidney disease characterised by the histologic finding of intra-renal crystal deposition. When some crystals combine with ions like F^- in beneficial tubular fluid conditions, crystal precipitation occurs in tubular lumens and surroundings, promoting inflammation, cytotoxicity, and kidney injury (Perazella and Herlitz, 2021).

However, low molecular weight proteins and larger peptides are present in the glomerular filtration and tubular fluids even without glomerular damage: these are secreted or leaked from renal tubular cells. As tubular damage progresses, it increasingly fails to reabsorb larger peptides and proteins secreted from tubular cells, such as liver-type fatty acid-binding protein. These are measurable in urine as markers of renal tubular injury (Birkner et al., 2006), and could also get incorporated into $CaPO_4$ crystals.

Compared to hydroxyapatite, fluoridated nano-minerals are more resilient. Therefore, it facilitates crystal growth and nano-tube formation. This process attracts inflammatory cells and accelerates inflammation (Hodgkins and Schnaper, 2012; Gunawickrama et al., 2021). Later, growing crystals could block renal tubules, and nano-tubes could rupture tubular walls, attracting white blood cells and macrophages. Progressive low-grade chronic inflammation leads to tissue fibrosis and renal failure over a few years. These crystals and nano-tubes are foreign bodies that generate inflammatory responses, attracting inflammatory cells and fibroblasts that cause tissue fibrosis.

Immune cells attracted to tubulointerstitial tissues activate tyrosine kinase and caspase-1 and release various inflammatory cytokines fuelling the inflammation, accelerating renal tissue fibrosis (Birkner et al., 2006). Similar nano-mineral mechanisms causing renal toxicity have been described before (Buzea et al., 2007). **Table 1** illustrates supporting evidence that $CaPO_4$ nanoparticles are highly likely to cause nano-mineral formation in renal tubules and the interstitial tissues in CKDmfo.

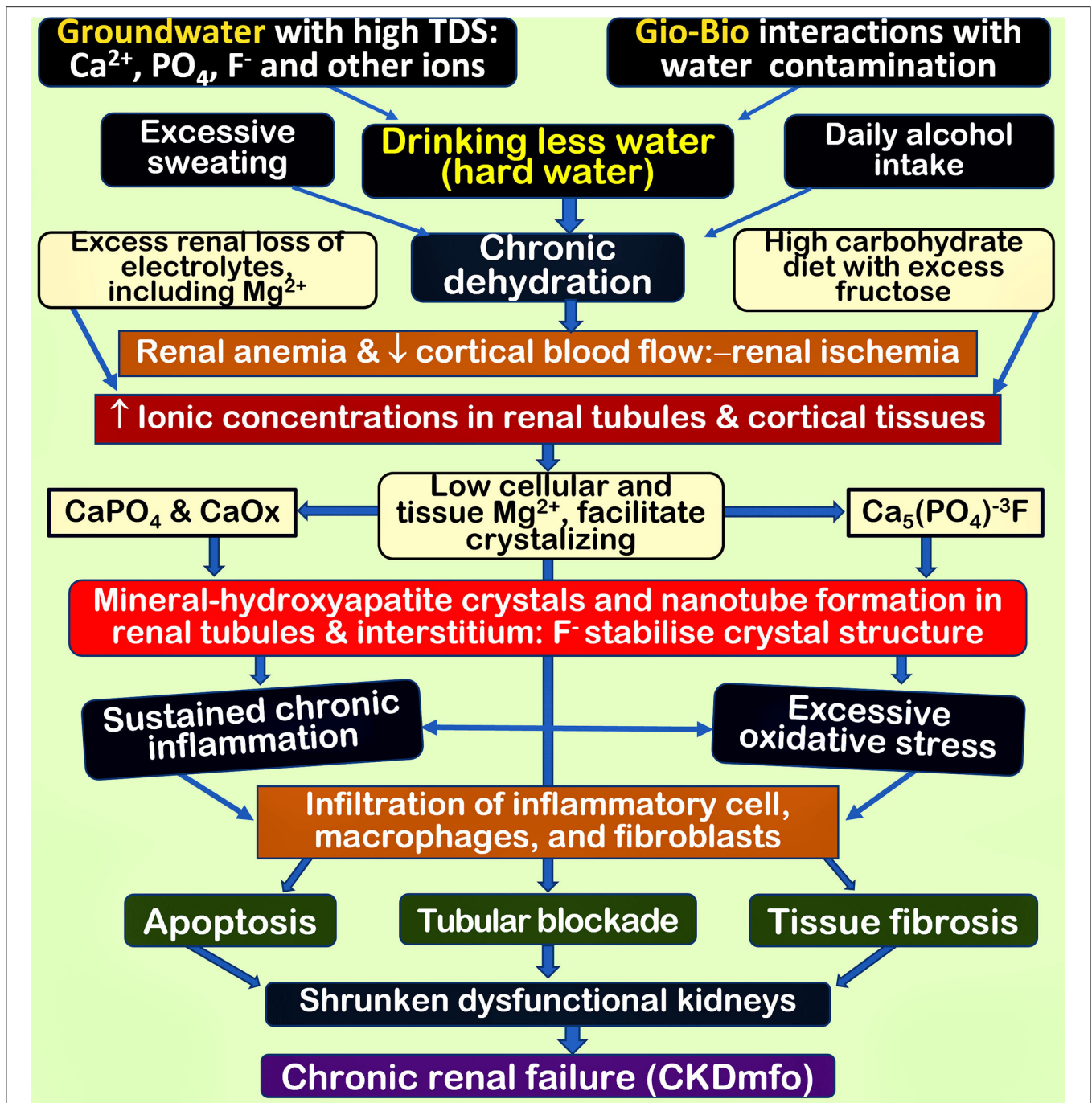


FIGURE 1 | Inter-related mechanisms and multiple pathways leading to the formation of nano-minerals in renal tubular and cortical soft tissues. The fundamental issue is high mineral concentration in the renal cortical fluid and tissues, secondary to ingestion of hard water and chronic dehydration. These multiple factors working together aggravate renal ischemia and anaemia and inflammatory cell infiltration due to nano-mineral precipitation, causing chronic inflammation and oxidative stress. Collectively, these lead to deterioration of renal functions and renal fibrosis, causing irreversible CKD-CTN (CKDmfo) [TDS, total dissolved solids; Ca^{2+} and PO_4^{3-} , Mg^{2+} , etc.]; CaPO_4 , calcium phosphate; CaOx , calcium oxalate; Mg^{2+} , magnesium; F^- , fluoride]. The figure is modified from Wimalawansa and Dissanayake (2019).

Mechanisms of Formation of Nano-Tubes in Renal Tubules

Groundwater in the CKDmfo-prone dry zonal areas in affected countries is hard (CaCO_3), containing high TDS and other

components favourable for forming apatite minerals— CaPO_4 hydroxyapatite and fluoroapatite (Dissanayake, 2016). Due to the crystal-related inflammation and micro-injuries, tubular epithelial cells undergo structural and functional changes.

TABLE 1 | Few published examples of nano-crystal formation in human renal tissues.

Published research	Findings	References
Crystalline nephropathies: These are a unique form of kidney disease characterised by the histologic finding of intra renal crystal deposition	Some crystal molecules combine with ions in favourable tubular fluid conditions leading to crystal precipitation within the tubular lumens and surroundings. It promotes kidney injury through tubular obstructions, inflammation, and cytotoxicity.	Perazella and Herlitz, 2021
Crystal nephropathies: mechanisms of crystal-induced kidney injury	Crystal-induced kidney injury (crystal nephropathies) cause different adverse outcomes based on location: deposits in the renal vasculature (type 1), the nephron (type 2), or urinary tract (type 3).	Mulay and Anders, 2017
<i>In vitro</i> and <i>in vivo</i> studies on hydroxyapatite	Dissolved hydroxyapatite [HA, Ca ₁₀ (PO ₄) ₆ (OH) ₂] with active phosphates become stable when they incorporate other ions like ions, Pb, or F ⁻	Arnich et al., 2003
Renal cell injury and formation of Ca crystal adhesion and aggregation	Nano-calcium oxalate monohydrate and calcium oxalate dihydrate crystals on injured renal epithelial cells	Gan et al., 2016
Crystal-induced inflammation and nano-mineral adhesion on tubular cell surfaces	Production of renal tubular crystals	Khan, 2004, 2010
Calcium and calcium oxalate monohydrate crystals cause renal epithelial injury	While CaOx crystals are more destructive, CaPO ₄ and CaOx crystals are formed using the same pathways causing renal tubular damage	Khaskhali et al., 2009
The formation of calcium oxalate crystals in renal tubular cells is nephron segment-specific	The internalisation of the renal nano-crystals is due to the interaction between crystals and the cell linings	Schepers et al., 2003
Calcium phosphate-induced renal epithelial injury and stone formation	Crystals are injurious to renal tubular epithelial cells and collecting ducts, where the injury is mediated by reactive oxygen species (ROS)	Aihara et al., 2003
Reduction of calcium phosphate crystals, as a target for renoprotection for CKDu	Increased tubular PO ₄ secondary to higher serum PO ₄ levels results in the formation of CaPO ₄ crystals in renal tubules that damage kidney epithelia	Wang, 2021
Surface characteristics of various substrates on fluorapatite crystal growth, alignment, and spatial orientation	Fluorapatite nano-crystal growth was demonstrated using a scanning electron microscope, which simulates prism structures	Czajka-Jakubowska et al., 2009
Hydroxyapatite composites with multiwalled carbon nano-tubes	The presence of carbon filler significantly affects the microstructure, phase composition, crystallinity, hydroxyl content of crystals, the chemical composition of the mineral, thermal properties, and electrokinetic properties of the composite	Skwarek et al., 2017
Magnesium prevents vascular calcification <i>in vitro</i> by inhibition of hydroxyapatite crystal formation	Magnesium reduces the formation of hydroxyapatite and significantly decrease Ca ²⁺ and PO ₄ fractions in crystals, especially in extracellular space in kidney tissue	Ter Braake et al., 2018

With tubular cell damage, fibrogenic cells infiltrate into renal parenchymal tissues attracting bioactive inflammatory and oxidative molecules that enhance inflammation and interstitial fibrosis.

With the above-mentioned local damage, tubular cells are subjected to progressive renal injury *via* epithelial–mesenchymal cell transition, cell-cycle arrest at G1/S and G2/M points, and ongoing oxidative and metabolic abnormalities (Liu et al., 2018). *In vivo*, these cause tubular cell inflammation, damage, dysfunction, and tubular blockade. Most people having CKD or CKDmfo are malnourished (Kalantar-Zadeh et al., 2003; Gama-Axelsson et al., 2012). Virtually all are micronutrient deficient. In addition to poor diet, impairment of intestinal and renal tubular fractional absorption of minerals, and lack of reabsorption of peptides, proteins makes the condition worse (Zha and Qian, 2017).

Hypocitraturia is a common metabolic risk factor in many persons with renal stones, as Citrate is an inhibitor in Ca stone formation. Increasing urinary citrate can act in renal tubules to reduce calcium oxalate (CaOX) and CaPO₄ saturation in tubular fluid and prevent crystal formation and growth. Urinary citrate can increase by ingesting lime juice or potassium citrate (Joshi et al., 2021), which could prevent the adhesion of calcium crystals to tubular cell membranes (**Figure 2B**) and renal epithelial cells

(Zuckerman and Assimos, 2009). A few examples of CaPO₄ crystals are illustrated in **Figure 2**.

Excess PO₄ Causing Nano-Crystal Formation in the Kidney

Hardness in groundwater is primarily due to calcium and magnesium salts: generally acceptable highest limits in drinking water (less than) are TDS 500 mg/l, calcium 75 mg/l, Mg²⁺ 50 mg/l, alkalinity 250 mg/l, SO₄ 200 mg/l, and PO₄³⁻ 0.025 mg/l, and EC 1,800 μS/cm (Unicef/WHO, 2008). However, the water hardness in the CKDmfo-affected NCP regions are often well above 500 mg/l, mostly CaCO₃; the concentration of Mg²⁺ is much less, between 15 and 30 ng/ml, well below the EPA and WHO guidelines (Unicef/WHO, 2008; Wimalawansa, 2016a). However, as described below, concerning CKDmfo, interactions (additive or synergistic effects) between different ion ratios have not been explored (Dunuweera et al., 2017; Wimalawansa, 2019a, 2020; Wimalawansa and Dissanayake, 2019). **Figure 3** illustrates a renal biopsy specimen stained for CaPO₄ crystals and soft tissues, highlighting the presence of CaPO₄ crystals within renal tubules and in surrounding tissues.

It is unclear whether any food component or malnutrition increase the susceptibility to CKDmfo or chronic disease (poverty-associated) causing malnutrition. When the

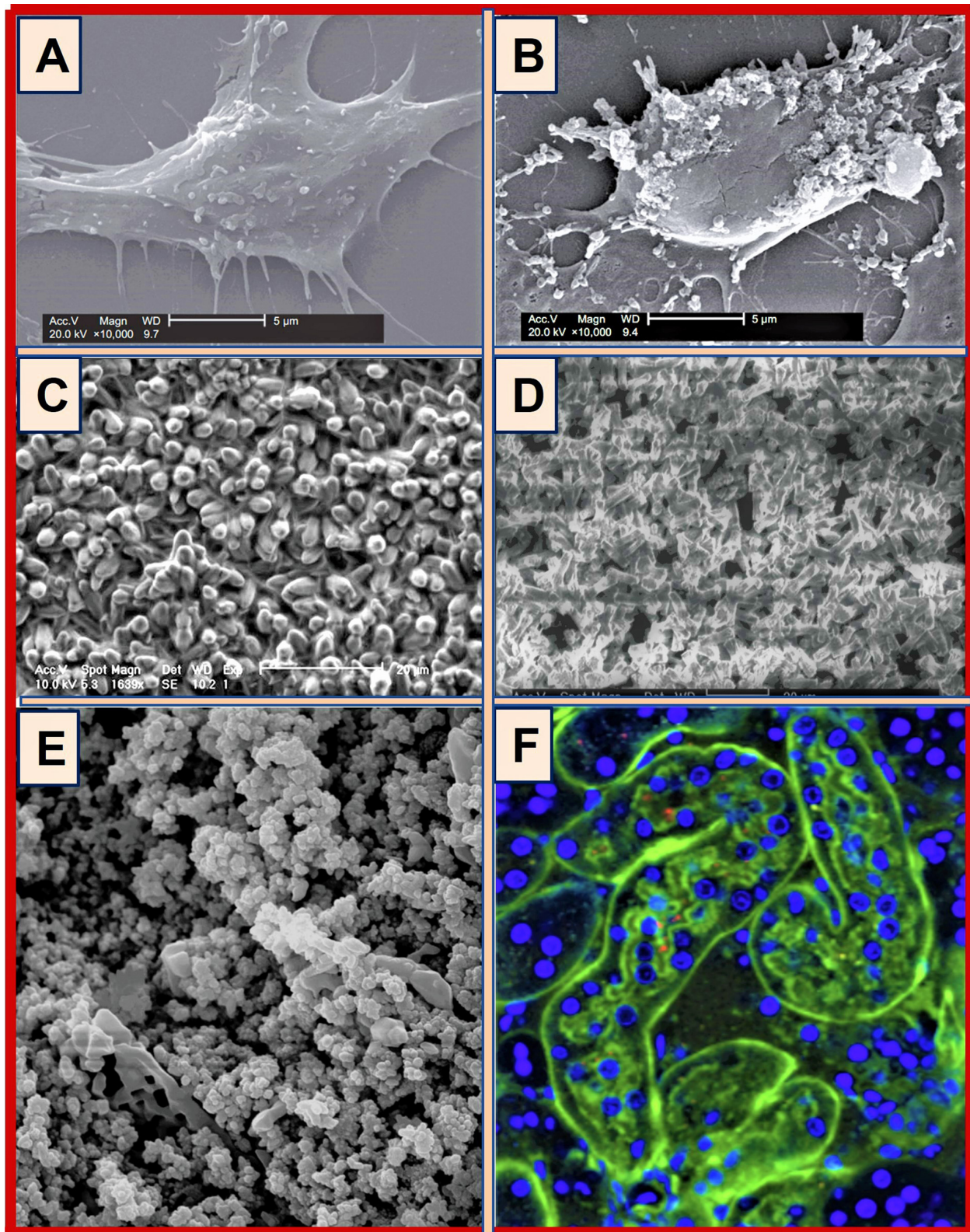


FIGURE 2 | Examples of hydroxyapatite and fluorapatite crystals: **(A)** CaPO₄-crystal formation on the surface of Vero cells treated with H₂O₂—**(A)** control; **(B)** 0.5 mmol/l, H₂O₂ treatment (scale bars: 5 μm) [COD, calcium oxalate dihydrate; SEM, scanning electron microscopy] (modeled from Gan et al., 2016); **(C)** SEM images of fluorapatite crystal growth on etched titanium surface; **(D)** Random growth of fluorapatite crystals on glass (adapted from Czajka-Jakubowska et al., 2009); **(E)** SEM image of hydroxyapatite in aqueous solution (after Arnich et al., 2003); **(F)** CaPO₄ nano-crystals in renal tissue and tubular cells in mice [CaPO₄ crystals in tube lumens (green; FITC-dextran); cell nuclei (blue; Hoechst)] (after Shiizaki et al., 2021).

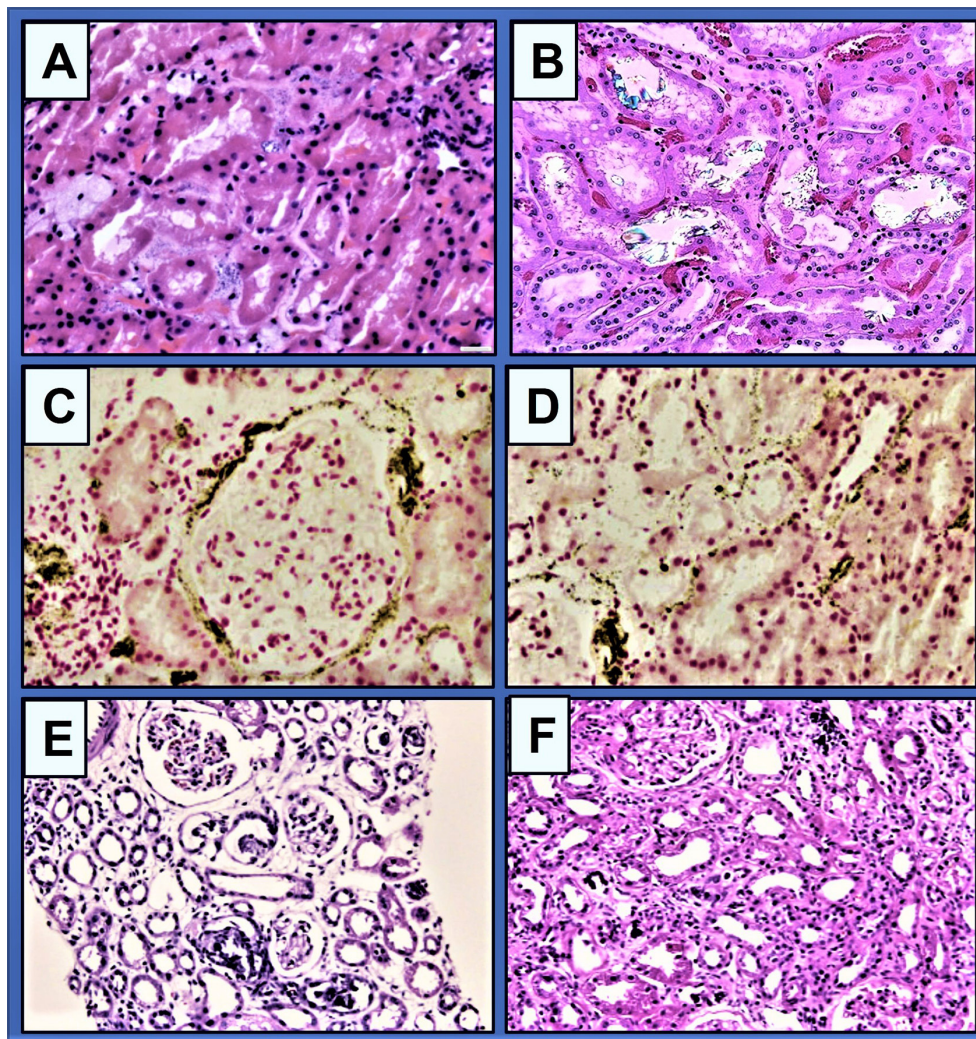


FIGURE 3 | (A) Granular and globular morphologic characteristics of CaPO_4 crystal deposition in renal biopsies. Crystals are mildly basophilic on haematoxylin-eosin stained and non-birefringent under polarised light (original magnification, $\times 400$; bar, $20\ \mu\text{m}$) (after Zhang et al., 2021); (B) Granular crystals in a renal biopsy, in inherited crystalline nephropathy (after Perazella and Herlitz, 2021); (C,D) There are granular CaPO_4 crystals in the interstitium and along the tubular basement membranes, peritubular capillaries, and Bowman capsules. Confirmation of CaPO_4 using von Kossa staining (magnification, $\times 400$; bar, $20\ \mu\text{m}$) (after Zhang et al., 2021); (E,F) An example of phosphate nephropathy illustrating intratubular CaPO_4 crystals, tubular atrophy, and interstitial fibrosis (haematoxylin and eosin stain: after Fogo, AB. AJKD) (Fogo et al., 2017).

solubility product of Ca^{2+} and PO_4^{3-} exceeds the threshold, CaPO_4 nanoparticles are formed (Ho et al., 2018). This vicious cycle is facilitated by excess PO_4^{3-} present in water (derived from waterways and reservoirs) and Ca^{2+} in hard water (Wimalawansa and Dissanayake, 2019) in the affected regions secondary to the overuse of phosphate fertiliser in hilly areas of the country (Wimalawansa, 2015a).

Chronic Dehydration Leads to Saturation of Tubular Fluid CaPO_4

Patients with tubular crystallopathy typically have hypokalaemia (potassium, $2.2\ \text{mEq/l}$), hypochloraemia (chloride, $97\ \text{mEq/l}$), and hypomagnesaemia (magnesium, $1.0\ \text{mg/dl}$; $0.41\ \text{mmol/l}$),

together with raised serum creatinine and PO_4 . Secondary to excessive sweating, lesser water intake, and regular alcohol use, maintains chronic persisting dehydration and saturation of the interstitial and tubular fluid microenvironment conducive for precipitation of CaPO_4 and CaOx . Consequently, it increases the risk of forming and propagating nano-minerals within tubules, interstitium, and tubular cells (Tiselius, 2013; Sethmann et al., 2017). This phenomenon also increases the risks of vascular calcification with all CKD types, increasing the risks of premature deaths (Birkner et al., 2006).

The key factors essential for triggering nano-tube and nano-crystal formation include chronic dehydration and associated renal ischemia, as well as regular longer-term consumption of hard water with high TDS, CaPO_4 , and fluoride cause (i.e.,

transient, or persistent higher concentration of tissue Ca^{2+} and PO_4^{3-} concentration). The combination of the mentioned multiple factors creates a vicious cycle of enhanced oxidative stress, chronic inflammation (Gunawickrama et al., 2021), and tubular cell fibrosis, causing CKDmfo. **Figure 4** summarises the breakdown pathways of the Geo-Bio barrier causing precipitation of nano-minerals in renal tubular tissues leading to chronic inflammation, tissue fibrosis, and renal failure.

It is not unusual that farm labourers in the CKDmfo-affected regions are chronically dehydrated. They regularly experience excessive sweating due to exposure to harsh, warm, and humid weather conditions, consume less fluid due to unpalatable hard water, and the daily use of illicit alcohol daily. These conditions are favourable in generating concentrated urine and tissue fluids in the renal cortical regions, driving nano-mineral formation (Wimalawansa, 2014a). Renal tissue nano-minerals are not visible by standard radiological methodologies. However, when nano-crystals grow above 1 mm (as in soft tissue and coronary calcification), they become visible with high-density imaging methods, such as computed tomography.

Local Conditions in the Kidney That Facilitate Crystallisation

There are several types of calcium-related nephropathies. Common types of renal tubular crystals formation are crystal nephropathy (type 2), damage to renal vasculature (type 1), and urinary urolithiasis (type 3); all these cause renal injuries that can lead to CKD (Mulay and Anders, 2017). Crystals formed by minerals, proteins, endogenous metabolites, or exogenous drugs and toxins also could cause tubular crystallopathies (Mulay and Anders, 2017).

Formation of crystals and other microparticles causes renal damage *via* different mechanisms. For example, the NLRP3 inflammasome causes apoptosis *via* a pathway through a speck-like protein containing CARD/caspase-1 (NLRP3/ASC/caspase-1) (Schroder and Tschoopp, 2010; Mulay and Anders, 2017) and exert cytotoxicity through the necroptosis signalling pathway (Mulay and Anders, 2017), whereas vitamin D has a profound immunomodulatory effect in suppressing NLRP3/ASC/caspase-1 and toll-like receptor-induced harm (Matias et al., 2021; Nunes et al., 2022)—therefore is a potential mode for therapeutic intervention. However, slowly growing crystal-mediated granuloma formation, as with CKDmfo, is present in chronic crystallo-nephropathies that lead to CKD and renal fibrosis.

Other Causes and Conditions That Increase the Risk of CaPO_4 Crystal Nephropathy

As described in the introduction, water hardness or F^- alone does not cause CKDmfo. For example, in Sri Lanka, in Jaffna, Ampara, and Puttalam districts, water hardness is $>1,000$ mg/l. In CKDmfo-unaffected areas like hill-country, farmers use higher agrochemical quantities without CKDmfo (Wimalawansa, 2016a). While the natural phosphorus levels are <0.03 mg/l, orthophosphate levels vary between 0.005 and

0.05 mg/l [4], but what is clinically meaningful is the total PO_4^{3-} content. In CKDmfo-affected regions, the average PO_4^{3-} concentration of reservoirs water is higher, between 0.8 and 1.4 mg/l (Dharma-Wardana et al., 2015; Wimalawansa, 2015a). Nevertheless, PO_4^{3-} concentrations alone are not too high to cause health hazards. Meanwhile, the consumption of hard water (Ca^{2+}), coupled with intake of higher concentrations of $\pm \text{F}^-$ for more than a decade in the presence of a favourable milieu increases the risks for CaPO_4 crystallisation in renal tubules.

Metallothioneins I/II (MT) have protective and anti-apoptotic effects in renal proximal tubular cells. The expression of these molecules suppresses the excessive oxidative stress, including that induced by F^- (Chmielewska et al., 2015). In addition, endogenous antioxidants and MTs induced by zinc (Zn) and selenium (Se) protect against oxidation and ischemic injuries (Birkner et al., 2006). Meanwhile, there is no credible scientific evidence that arsenic, heavy metals, or agrochemicals cause CKDmfo. Even if small quantities of any of these get absorbed into the gastrointestinal tract or absorbed, there are built-in inherent protective mechanisms, such as chelation, enzymatic reactions, and detoxication (MTs) in the gut, liver, and kidneys that would eliminate most of these (Goyer, 1989; Hma et al., 2018).

It is noteworthy that the WHO recommended water quality standards are focused on individual elements without considering potential added and synergic effects between macro and micro-trace elements (1997; Wasana et al., 2017) or changes of effects from pH. Nevertheless, there are many biological and physiological examples where such interactions can be beneficial or harmful. Based on small-scale rat studies using hard water (Ca^{2+} or Mg^{2+}), potential additive effects of F^- have been reported with Mg^{2+} and suggested as a cause for CKDmfo. However, these models neither reflect the reality of ion-ion interactions (see above) nor reflect the human behaviours in affected populations (Wasana et al., 2017; Torreggiani et al., 2020).

Mg Deficiency Contributes to Nano-Crystal Formation and CKDmfo

Mg^{2+} is an important macro-mineral that is essential for enzymatic reactions. In renal tissues, it competes with Ca^{2+} in determining the rate and the concentrations of accumulation of Ca^{2+} and homeostasis of elements. Higher concentrations of Mg^{2+} antagonise Ca^{2+} , preventing CaPO_4 and CaCO_3 nano-crystal formation. In contrast, chronic hypomagnesemia increases the risk of CaPO_4 nano-crystal formation in renal tissues. Moreover, in rats fed on an Mg^{2+} -deficient diet, the deficiency-associated complications are accentuated by low dietary potassium. Thus, the functions of these cations and anions are interlinked. Therefore, using these ions individually in experiments to assess their functions can be misleading.

While high dietary calcium intake reduces plasma Mg^{2+} (Ericsson et al., 1986), supplementation of Mg^{2+} or low-dose fluoride treatment mitigates the accumulation of unphysiological calcium levels in renal tissue. Researchers need to incorporate the mentioned critical and complex status related to dietary

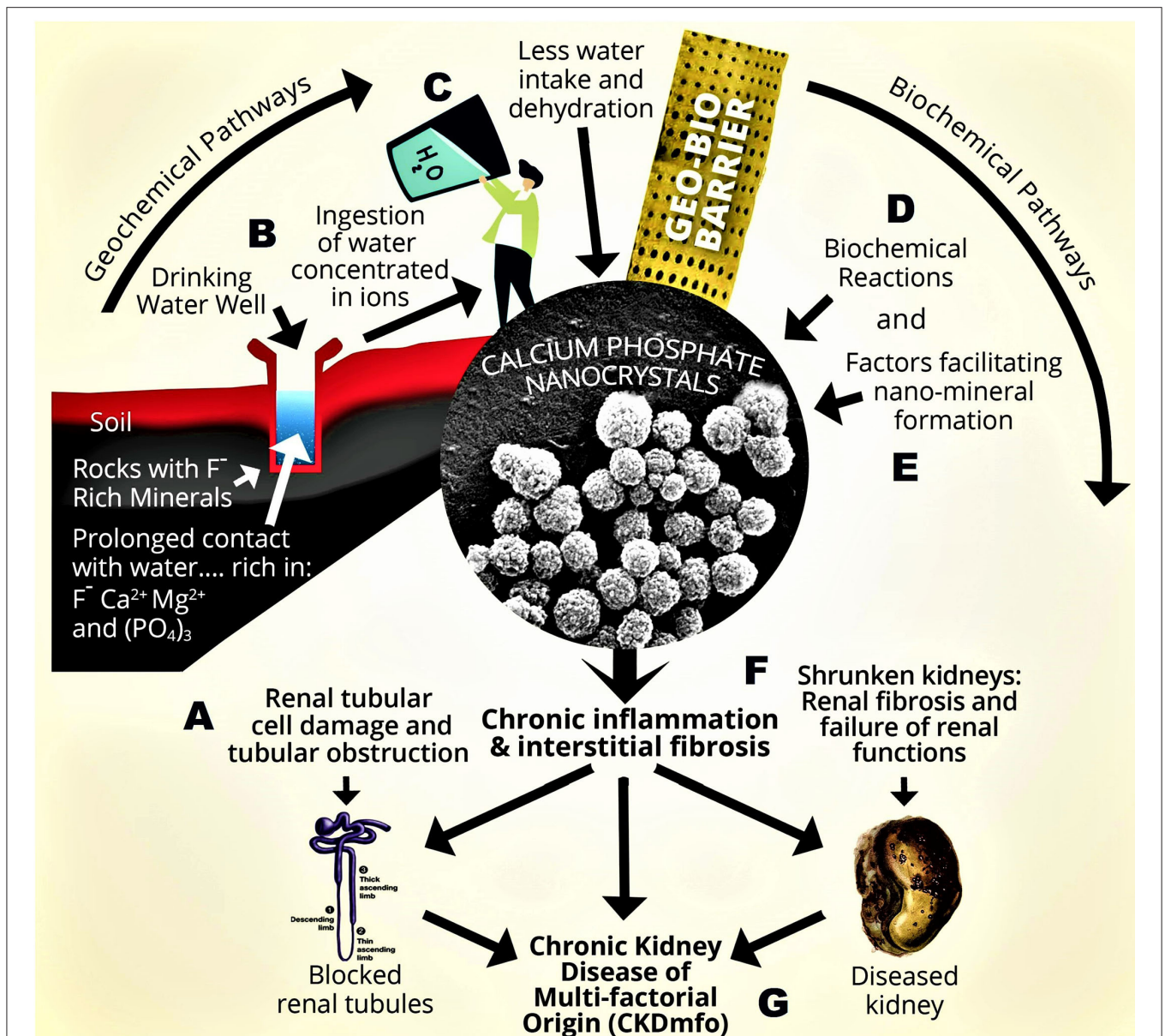


FIGURE 4 | Geological and biological (Geo-Bio) pathways for forming nano-tubes and nano-crystals in chronic kidney disease of CKD-CTN (CKDmfo). A conceptual diagram illustrating pathways for the formation of $CaPO_4$ nano-crystals in kidney tubules causing CKDmfo: **(A)** Stagnant groundwater is interacting with mineral-rich rocks, containing F^- , Ca^{2+} , Mg^{2+} , and PO_4^{3-} . **(B)** Long duration of water-rock interaction. **(C)** Consumption of hard water (lesser amounts) with high concentrations of ions. **(D)** Biochemical reactions and supersaturation, precipitating $CaPO_4$ nano-crystals and nano-tubes in kidney tubules. **(E)** Factors enhancing the nano-mineral precipitation. **(F)** Low-grade chronic inflammation, oxidative stress, and tissue fibrosis. **(G)** Onset of CKDmfo [TDS = total dissolved solids (Ca^{2+} and PO_4^{3-} , Mg^{2+} , etc.); Mg^{2+} , magnesium; F^- , fluoride; $CaPO_4$, calcium phosphate; $CaOx$, calcium oxalate].

and drinking water and ion-ion interactions. Therefore, studies should not be designed in isolation but should consider potential additive or competitions of ions *in vivo* physiological situations.

Hundreds of studies have reported the renal protective effects of Mg^{2+} , including renal electrolyte handling and maintenance of *in vivo* electrolyte balances of critical elements, such as Na^+ , K^+ , PO_4^{3-} , Mg^{2+} , and Ca^{2+} , vital for health (Wassenaar et al., 2018; Mazzaferro et al., 2021). Nevertheless, due to hormonal

abnormalities, such electrolyte imbalances (i.e., dyshomeostasis) are common in all CKD types, especially tubulointerstitial diseases (CKD-CTN) such as CKDmfo. These get worsened in the presence of malnutrition (Wang et al., 2018; Bousquet-Santos et al., 2019), which is universally present in persons with CKDmfo. While Mg^{2+} supplementation is helpful, the ideal way to manage electrolyte homeostasis is through a balanced diet (Anupama et al., 2020).

Does Magnesium Contribute to the Causation of CKDmfo?

Hypomagnesemia worsens several common disorders, such as diabetes, metabolic syndrome, parathyroid and other endocrine disorders, osteoporosis, and all of CKD types, leading to poor clinical outcomes (Al and Rodriguez, 2013). *In vitro* and *in vivo* models demonstrated that tissue hypomagnesaemia worsens several disorders, including CKD, cardiovascular diseases, vascular and soft tissue calcification, fatigue and depression, and impair bone-mineral metabolism (Al and Rodriguez, 2013; Galassi and Cozzolino, 2014; Floege, 2015; Wassenaar et al., 2018). Therefore, it is difficult to envisage how a smaller increase in the concentration of Mg^{2+} in drinking water as postulated by some (minute quantities compared to Mg^{2+} ingested *via* food (Wimalawansa, 2016a; Liyanage et al., 2022) could cause CKDmfo (Wasana et al., 2016, 2017). As illustrated in **Table 2**, one needs to be concerned with hypomagnesemia and not excess Mg^{2+} in the circulation and kidneys.

Hypomagnesemia increases the risk of $CaPO_4$ nano-crystal formation in renal tissues. Whereas Mg^{2+} , alpha-Klotho, vitamin K, and zinc prevent $CaPO_4$ -nano-crystal formation in the kidney, FGF23 and calciprotein particles modulate this process (Vervloet, 2019). Mg^{2+} deficiency can be detrimental to most organ systems, including endocrine, bone, renal, and cardiovascular (**Table 2**). It is a common and effective way to use metallic element-based binders, such as Mg^{2+} , aluminum, and Ca^{2+} , to bind PO_4^{3-} to prevent the load and reduce CKD-metabolic bone disease (CKD-MBD) (Vervloet, 2019; Pendon-Ruiz De Mier et al., 2020). In addition, Mg^{2+} supplementation reduces vascular calcification, prevents renal function deterioration, and stabilises CKD-MBD. Mg^{2+} also improves all hormonal actions, including calcitriol, and its (vitamin D) receptor functions. These are also important in CKDmfo (Galassi and Cozzolino, 2014; Pendon-Ruiz De Mier et al., 2020; Kula and Bansal, 2021).

While it may not be fully implementable due to poverty, changing dietary habits, including clean (potable) water intake, is essential to preventing deterioration CKDmfo. Such interventions improve the general condition of those affected and reverse early renal abnormalities in the initial stages of the disease. In contrast, many other studies confirmed improved general wellbeing and reversing the initial stages of CKD and CKDmfo when supplemented with magnesium or magnesium-based phosphate-binding agents (Al and Rodriguez, 2013; Floege, 2015). **Table 2** illustrates the potential causes of Mg^{2+} depletion and hypomagnesemia in CKD patients.

As illustrated in **Table 2**, in addition to acute and chronic diseases, there are specific conditions and circumstances where hypomagnesemia is manifest. Except for inherited/genetic diseases, most others are easily modifiable.

Contaminated Groundwater vs. Spring Water

Ongoing seismic and geochemical processes aid the erosion of underground bedrocks, releasing various ionic components into the water, including $CaPO_4$ and F^- , into groundwater (Mikac et al., 2011). During the construction of deep tube

wells, some tap into F^- -rich groundwater pockets, pumping out water containing high content of F^- and other contaminants (Wimalawansa, 2020). In parallel, the PO_4 concentrations in surface (e.g., reservoirs) and groundwater have increased due to the excessive application of agrochemicals in agriculture in distinct regions (Wimalawansa, 2016b).

The increasing demand for water that began in the 1960s to resettle communities and vast expansion of agriculture needed to overexploit groundwater in the CKDmfo-affected dry zonal regions (Panabokke, 2007). Besides the CKDmfo-affected regions in Sri Lanka, there are shallow regolith aquifer (between 5 and 20 m) and a deeper fracture zone aquifer (between 30 and 40 m) (Imbulana and Oguma, 2021). Most large agro-wells and domestic dug wells in this region are tapped into regolith aquifers (Pathmarajah, 2002).

The occurrence of higher hardness (the equivalent of $CaCO_3$, above 385 mg/l) and alkalinity ($CaCO_3$, above 365 mg/l) and high TDS are salient parts of groundwater in CKD-affected villages. In comparison, although present in some regions, salinity and ionicity and higher Mg^{2+} contents do not directly contribute to the causation of CKDmfo. Ongoing chemical weathering and dissolution of carbonates and silicates rocks [$Ca-Mg-HCO_3$] represent the geochemistry of groundwater. Continual weathering of carbonate and silicate components in regolith aquifers in the CKDmfo-affected regions, enhanced by overexploitation, evaporation, and cation ion exchange, all significantly influences the hardness, electrical conductivity (EC), and dissolved components in hard, groundwater, raising Ca^{2+} , Mg^{2+} , and HCO_3 concentrations (Elango and Kannan, 2007) in affected countries.

Isotopic studies suggested that CKDmfo-endemic villages are associated with groundwater sources and not fed directly with rain or surface water (Edirisinghe et al., 2018). Therefore, ions and chemical pollutants get considerably concentrated in groundwater during the dry season, increasing the risks for CKDmfo (Wimalawansa, 2020). In contrast, those who consume surface or spring water have a significantly lower incidence of CKDmfo. Besides, the prevalence of CKDmfo is higher in those who consume water from deep wells connected with the stagnant aquifer (Balasubramanya et al., 2020) but not with shallow wells.

There are dozens of natural springs amid CKDmfo-affected regions. Springwater quality is far better than groundwater and does not require processing before consumption. Residents who consume water from such natural springs are rarely affected by CKDmfo even though these villages are located adjoining those with a high incidence of CKDmfo (Jayasekara et al., 2013; Wimalawansa, 2015b; Wimalawansa and Wimalawansa, 2015b). Isotopic studies demonstrate that such natural springs are not connected to the groundwater aquifers (Wimalawansa, 2016b; Wickramarathna et al., 2017; Edirisinghe et al., 2018).

Higher total hardness in water is universal in the CKDmfo-affected regions (Weaver et al., 2015). In contrast to higher Mg^{2+} content reported in some groundwater samples (Edirisinghe et al., 2018), others have reported lower Mg^{2+} concentrations in samples from CKDu-endemic areas compared with the non-endemic areas (Dissanayake and Chandrajith, 2007; Wasana et al., 2016). Thus, having elevated Mg^{2+} in groundwater is

TABLE 2 | Causes that lower tissue and/or serum magnesium concentrations in persons with CKD.

Potential causes	Example of conditions	Reference
Chronic diseases	Many protracted diseases cause hypomagnesemia	Rodelo-Haad et al., 2020; Gautam and Khapunj, 2021
Acutely ill patients and those who receive parenteral nutrition	Severe infections, acute pancreatitis Gastric bypass surgery	Cunningham et al., 2012; Gragossian et al., 2021
Alcohol abuse	Mostly through urinary waste	Rodelo-Haad et al., 2020
Gastrointestinal and renal losses	Tubular nephropathies	Cunningham et al., 2012; Azem et al., 2020
Endocrine abnormalities	Hungry bone syndrome following parathyroidectomy, diabetes, hypertension, etc.	Greco, 2012; Rodelo-Haad et al., 2020
Secondary to nephrotoxic medications	Diuretics, proton pump inhibitors, aminoglycoside antibiotics, chemotherapeutic drugs (cisplatin, cyclosporine), etc.	Cunningham et al., 2012; Rodelo-Haad et al., 2020; Gragossian et al., 2021
Rare inherited renal tubular disorders (<i>via</i> renal loss of Mg ²⁺)	Gitelman syndrome, Bartter syndrome, etc. Familial hypomagnesemia with hypercalciuria and nephrocalcinosis Other rare genetic renal diseases	Rodelo-Haad et al., 2020; Gragossian et al., 2021

incidental, and, not common to all affected villages. In contrast, most CKDmfo-affected locations have higher F⁻ concentrations in groundwater (Wimalawansa, 2016b; Imbulana and Oguma, 2021). As described below, Mg²⁺ protects renal tissues partly from its antagonistic effects on Ca²⁺ and PO₄ (Ter Braake et al., 2017; Sakaguchi et al., 2018).

The Amount of Mg²⁺ Derived From Drinking Water

Most CKDmfo-endemic regions have regolith aquifers with higher, Ca²⁺, Mg²⁺, F⁻, and bicarbonate concentrations. Increases in ions in stagnant water are due to prolonged contact time with an aquifer, weathering of underground rocks, and evapotranspiration. Importantly, those wells connected to regolith aquifers used by residents in the affected locations are not connected to the surface agricultural water table/sources, such as water reservoirs and irrigation canals, as reported by isotopic tracer studies (Rango et al., 2015; Wickramarathna et al., 2017; Edirisinghe et al., 2018).

The finding of marginally higher Mg²⁺ in some water samples (but within the WHO-recommended upper limit) (Wasana et al., 2016, 2017; Liyanage et al., 2022) seems coincidental and non-significant on clinical grounds (Balasooriya et al., 2020) and remains a hypothesis. Reported rat studies suggested a connection between hard water Mg²⁺/F⁻ and CKDmfo (Thammitiyagodage et al., 2017; Wasana et al., 2017). One study used water from an endemic region, while the other used laboratory-made, hard water (Wasana et al., 2016, 2017; Thammitiyagodage et al., 2017). While being attractive, such data are too remote to conclude the causation of CKDmfo, especially with hundreds of other scientific studies confirming the protective nature of Mg²⁺ on renal functions.

The contribution of Mg²⁺ from drinking water to daily intake is negligible. One average-sized banana supplies more minerals and micronutrients than the total daily intake of such from water. The average amount of Mg²⁺ intake from 2–l drinking water is between 20 and 30 mg/ml (even in water from

CKDmfo-affected locations) (Balasooriya et al., 2020), while a medium-sized banana provides ~65 mg (Wimalawansa, 2016a). Therefore, it is hard to envisage how <20 mg/l in drinking water (Wickramarathna et al., 2017) would cause CKDmfo, even if interactions could be present. The tolerable upper limit of Mg²⁺ supplementation is 350. Consuming higher doses than this without Mg²⁺ losing disorders, as illustrated in **Table 2**, for a prolonged period can cause hypermagnesemia (Wyskida et al., 2012). Like other minerals, prolonged hypermagnesemia increases morbidities and all-cause mortality (Galan Carrillo et al., 2021).

The Role of Mg²⁺ in Renal Diseases

In addition to maintaining homeostatic and physiologic balances in the body, Mg²⁺ facilitates and functions as a cofactor in biochemical and enzymatic reactions, neural conduction, and the release of peptides and hormones and activating receptors (Greco, 2012). Normal serum magnesium levels are between 1.56 and 2.68 mg/dl (Gragossian et al., 2021). The kidney's pathological handling of Mg²⁺ can result in either hypomagnesemia or hypermagnesemia (Cunningham et al., 2012; Massy and Druke, 2015; Quinones et al., 2019; Van Laecke, 2019). Serum Mg²⁺ reflects poorly, the body storage of magnesium.

Hypomagnesemia is present when the serum total magnesium concentration drops below 1.50 mg/dl. It can indirectly lower serum and tissue potassium and increase serum calcium concentrations, further impairing electrolyte imbalance (Quinones et al., 2019). Clinical signs and symptoms of severe hypomagnesemia include tremors, generalised muscular weakness, cardiac arrhythmia or ischemia, dysfunction of hormones, and death (Cunningham et al., 2012). In addition, persistent hypomagnesemia significantly increases vascular calcification, endothelial dysfunction, cardiovascular diseases (Van Laecke, 2019), increase vulnerability to infections, and all-cause mortality, especially in those with CKD stages 3 and above (Quinones et al., 2019; Azem et al., 2020).

Hypomagnesemia and Hyperphosphatemia Exacerbate CKDmfo

In addition, hypomagnesemia increases the intracellular Ca^{2+} concentration through negative-regulation of calcium channels, suppressing N-methyl-D-aspartate receptors and nuclear factor-kappaB, phagocytic cells. The resultant increase of Ca^{2+} activates the renin-angiotensin hormonal system raising blood pressure and further reduce the blood supply to renal cortical regions, aggravating renal tubular ischemia and fibrosis (Rayssiguier et al., 2006). Such reactions generate an internal milieu beneficial for growth and aggregation of nano-mineral apatite causing renal tubular blockade, nano-ruptures of tubules with inflammation, tubular cell fibrosis, and death. Collectively, these escalate renal ischemia and inflammation and reduce renal functions. CaPO_4 crystals get attached to TLR4 on tubular cells and undergo endocytosis at their brush borders, triggering a vicious cycle of inflammation and fibrosis (Figure 5; Moe, 2021).

Those with CKD show raised parathyroid hormone levels (secondary or tertiary hyperparathyroidism) and FGF23, reduced PO_4 reabsorption, and increased luminal PO_4 concentration, which likely to worsen CKD (Moe, 2021). Once CaPO_4 reaches a threshold in tubular or tissue fluids, crystallisation begins; crystals bind to cell-surface brush-border TLR4 on tubular cells, causing tubular damage (Moe, 2021). Crystals are internalised, initiating inflammation fibrosis in the tubule interstitium, and reducing renal functions, as shown in Figure 5.

Irrespective of the cause, the progression of CKD is associated with worsening vascular calcification, imbalances of mineral metabolism (CKD-MBD), inflammation, increased oxidative and metabolic stress, coagulation abnormalities, endothelial dysfunction, and accumulation of uremic toxins (Van Laecke, 2019). Hypomagnesemia worsens the mentioned pathologies and associated comorbidities through multiple mechanisms that worsen underlying nephropathy (Cunningham et al., 2012; Rodelo-Haad et al., 2020). These lead to poor clinical outcomes, including premature death, as in CKD-CTN (Quinones et al., 2019).

Contrary to some beliefs, dietary Mg^{2+} and slightly elevated serum Mg^{2+} are protective (Van Laecke, 2019). Besides, Mg^{2+} supplementation was reported to reduce or reverse vascular calcifications and CKD-associated deaths. This partly occurs through inhibition of the Wnt/ β -catenin signalling pathway (Rodelo-Haad et al., 2020). Severe hypermagnesemia is rare and almost always observed in patients with end-stage renal failure who consume higher doses of magnesium supplements or magnesium-containing antacids (Van Laecke, 2019). Adverse effects of hypermagnesemia include nausea, vomiting, flushing, lethargy, and hypotension (Cunningham et al., 2012; Nishikawa et al., 2018; Rodelo-Haad et al., 2020).

Low Mg^{2+} and Klotho and High PO_4 Further Aggravate CKDmfo

The average plasma inorganic PO_4 concentration in adults is between 2.5 and 4.5 mg/dl: a level higher than 4.5 mg/dl is considered hyperphosphatemic. The average dietary phosphate intake is $\sim 1,000$ mg/day. At physiological pH of 7.4, PO_4 exists as both $\text{H}_2\text{PO}_4^{(-)}$ and $\text{HPO}_4^{(2-)}$ and acts as an

extracellular fluid buffer (Peacock, 2021). Either PO_4 overload or hypomagnesemia can aggravate renal failure. Mg^{2+} deficiency causes hypovitaminosis D, worsens kidney damage, induced by an increased tubular load of PO_4 , as in CKDmfo and other crystalloid nephropathies (Cunningham et al., 2012).

On the other hand, Mg^{2+} supplements can counteract or reverse the adverse effects from excess PO_4 on renal tubular cells and reduce vascular calcification. Besides, *in vitro* data show that adequate Mg^{2+} alleviates proximal tubular cell injury from high PO_4 (Sakaguchi et al., 2018). Therefore, in geographical regions with high PO_4 content in drinking water, as in most CKDmfo-endemic regions, one should consider using magnesium supplements to counteract adverse effects of phosphate in the kidney.

Renal α -Klotho is a co-receptor for fibroblast growth factor-23 (FGF23) synthesised in bone. FGF23 is a phosphaturic hormone that regulates $1,25(\text{OH})_2\text{D}$ and involves ageing. A high tubular PO_4 load in CKD reduces renal tubular expression of α -Klotho, a renal protective molecule. FGF23 increases renal PO_4 excretion by reducing type IIa Na^+/PO_4 co-transporter expression in proximal tubular cells (Bergwitz and Juppner, 2010). In early CKD, the circulating FGF23 rises to counteract hyperphosphatemia. Increased FGF23 enhances urinary fractional excretion of PO_4 (Sakan et al., 2014) but suppresses 1α -hydroxylase enzyme, thus reducing circulatory $1,25(\text{OH})_2\text{D}$ (calcitriol) concentration and stimulating PTH release (secondary hyperparathyroidism) (Bergwitz and Juppner, 2010).

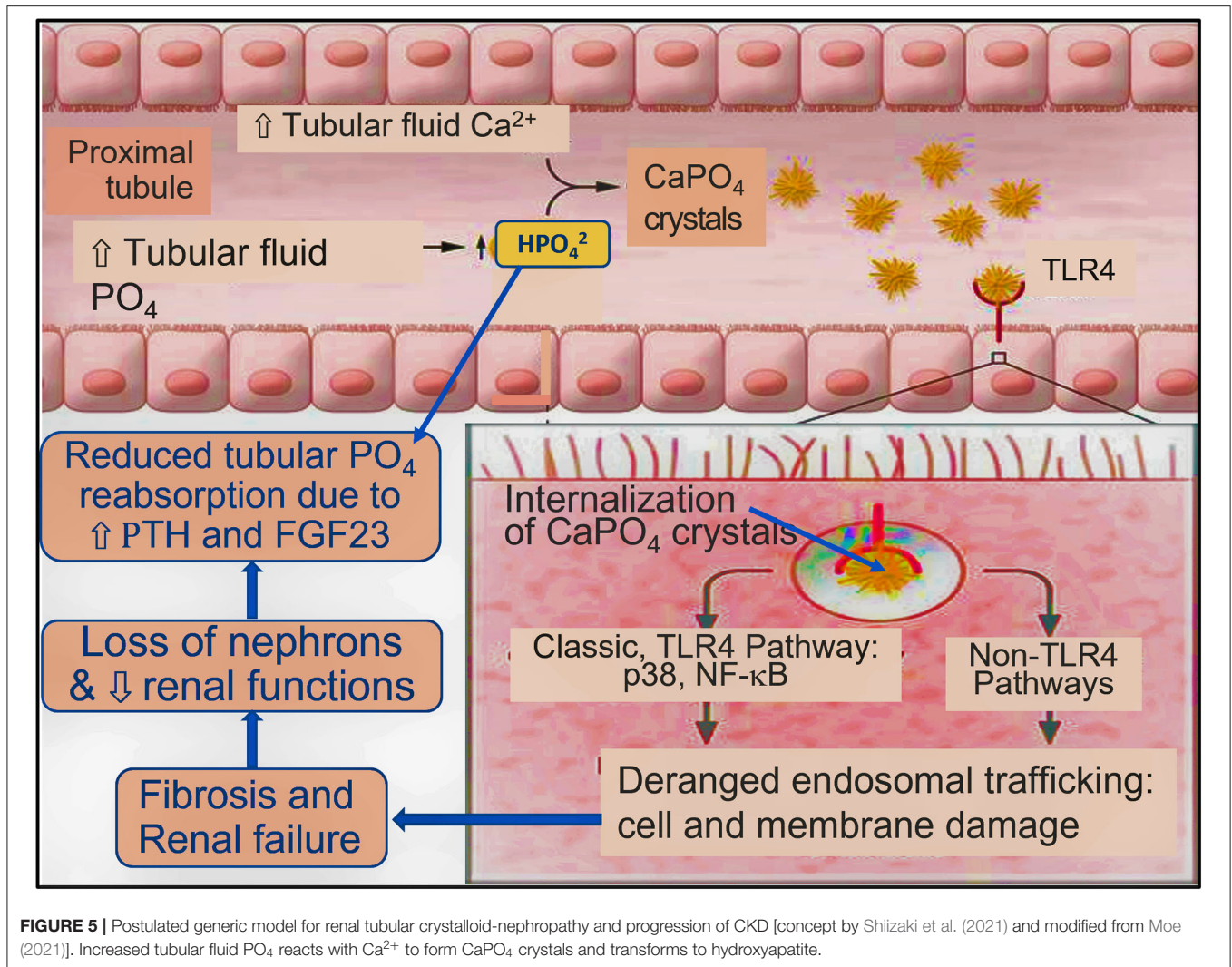
Additional Factors Contributing to the Genesis of CKDmfo

Current data suggest that the final common pathway of renal tubular cell damage and apoptosis occurs *via* oxidative stress (Figure 1). As a part of the intervention, it is necessary to modify *in vivo* conditions that favour the formation of nano-minerals. These include preventing dehydration, dietary modifications, alleviating chronic inflammation, and renal anaemia. In addition, other innovative approaches are also necessary to minimise renal tubular cell oxidative stress and mitochondrial dysfunction.

Dietetic Fructose in Renal Tubular CaPO_4 Nanotube Formation

Rats fed with an Mg-deficient diet developed hypomagnesemia, reduced renal tissue Mg^{2+} , and increased serum ionised Ca^{2+} concentrations. The condition worsens in rats fed with refined sugars, glucose, or fructose, than those with complex carbohydrates (Koh et al., 1989). In the fructose-fed group, renal Ca^{2+} content was eight times greater than in the control group.

In addition, there are negative synergistic interactions between low Mg^{2+} (high Ca^{2+}) with excess fructokinase activity in kidneys (Andres-Hernando et al., 2017; Wrighton, 2017). The risk for mineral crystallisation is increased due to chronic dehydration and associated low urinary output (i.e., concentrated urine), which worsen the clinical condition (Garcia-Arroyo et al., 2016; Roncal-Jimenez et al., 2016). The presence of chronically high-concentrated urine due to dehydration, in conjunction with hypercalciuria, low urinary Mg^{2+} -to- Ca^{2+} ratio, and renal interstitial or urine pH below 6.8 is highly



conductive to precipitating CaPO_4 crystals, even in the absence of hypercalcaemia (Koh et al., 1989). Low urinary Mg^{2+} and high-fructose diets further aid the mineral crystallisation process in kidneys.

Impairment of Mitochondrial Functions Causing Tubular Failure

Mitochondrial dysfunctions in renal tubular cells are partly due to ROS-mediated oxidative stress and chronic inflammation: these contribute to renal tubular cell dysfunction, atrophy, and fibrosis (Ye et al., 2015). Excess ROS generated *via* toxins, infections, metabolic derangements, micronutrient deficiency such as vitamin D and E, and dietary and *in vivo* synthesis of antioxidants (Wimalawansa, 2019d), reduce the generation of ATP (Bataille et al., 2011; Guan et al., 2015). The diminished availability of ATP will continue to impair renal tubular cell functions.

Multiple mechanisms, including mitochondrial chaperone prohibitin, protect tubular epithelial cells from oxidative stress-induced damage by directly inhibiting the oxidative process, preventing mitochondria dysfunction, and inhibiting cell

apoptosis (Ye et al., 2015). Therefore, increasing mitochondria prohibitin in renal tubular cells could be used to alleviate nephrotoxic agent-induced tubular nephropathies.

In parallel, the synthetic antioxidants, N-acetyl-L-cysteine (NAC), a glutathione (GSH) precursor and a stimulator, could reduce intracellular oxidative stress by working through multiple mechanisms. The latter includes attenuating c-Jun NH_2 -terminal kinase and p38 mitogen-activated protein kinase phosphorylation, scavenging ROS, and activating extracellular regulated kinase 1/2 (ERK1/2), providing cytoprotection for renal epithelial cells (Zhang et al., 2011).

PROBABLE CAUSE FOR CKDmfo

Dietary Factors Increasing the Risk of CKDmfo

The diets of individuals in CKDmfo-affected regions are high in carbohydrates, fewer fats, protein, and micronutrients. Besides, a considerable proportion of calories come from fructose, with enhanced fructokinase activity that negatively affects renal functions. High concentrations of Ca^{2+} , PO_4 , and F^- with low

Mg^{2+} are conducive to precipitating $CaPO_4$ /hydroxyapatite and fluoroapatite nano-crystals in tubular fluid and renal cortical tissue (Aihara et al., 2003; Schepers et al., 2003; Khan, 2004, 2010; Czajka-Jakubowska et al., 2009; Khaskhali et al., 2009; Roncal Jimenez et al., 2014; Gan et al., 2016; Skwarek et al., 2017; Ter Braake et al., 2018; Wang, 2021). **Table 3** illustrates the proposed causes in chronological order (earliest to present) assorted to date with related references.

Worldwide, about 30% of patients with end-stage renal disease have no specific underlying nephropathy diagnosis, and up to 15% of these cohorts carry genetic mutations such as HNF1 β mutations (Tin et al., 2015). Examples of other rare developmental abnormalities associated with nephropathies are PAX2, EYA1, and GATA3 mutations: many of these conditions are associated with hypomagnesemia, which increases the risk of developing all CKDs. However, the incidence is so rare, thus genetic screening is not warranted.

Scientists have proposed several theories to explain the mechanisms of bio-calcification (Miller et al., 2004; Yiu et al., 2015). When $CaPO_4$ crystals form in renal tubules and interstitial tissues, they activate the Juxta-Glomerular apparatus, and other hormonal mechanisms that increase blood pressure and reduce the blood supply to kidneys. They reduce the Ca and PO_4 fractional reabsorption, enhance fluid PO_4 , and modulate ionised calcium. This vicious cycle further increases the proximal tubular fluid $CaPO_4$ saturation (Robertson, 2015), desirable for crystallisation (Linnes et al., 2013).

The formation of nano-crystallisation and hydroxyapatite nanoparticle scallops in the kidney is facilitated by higher inorganic Ca^{2+} and PO_4 concentrations (Martel et al., 2014). In the presence of a high ambient F^- concentration, hydroxyapatite changes into more stable fluorapatite (Wong et al., 2015) $Ca_5(PO_4)_3F + 9F^- \rightarrow Ca_5F_{10} + 3(PO_4^{3-})$.

Fluorapatite crystals are hard but inflammatogenic, attracting white blood and other immune cells. The reported nanoparticles are composed of polycrystalline $CaPO_4$ crystals similar to bone mineral. The nano-crystals and nano-tubules are deposited within renal tubules and extracellular tissues surrounding renal tubules (Wong et al., 2015).

The absence of such data fail to provide reliable information to understand multifactorial complex interactions or generate clinically meaningful evidence to make firm conclusions (Wimalawansa, 2016b). At times, such data can be even misleading. Therefore, studying compounds in isolation in complex diseases like CKDmfo should be avoided. One example is ayurvedic medications, where effectiveness cannot be demonstrable by studying their isolated compounds. Similarly, based on relatively higher concentrations of Mg^{2+} and F^- in drinking water (Wickramarathna et al., 2017) and a couple of laboratory rat studies (Wasana et al., 2017) that have not been reproduced, it is inappropriate to make the sweeping conclusion that Mg^{2+} and F^- interactions cause CKDmfo (Wimalawansa and Dissanayake, 2019).

Novel Targets for Interventions—Effective Disease Modulators

There are highly cost-effective anti-inflammatory and anti-oxidant dietary supplements available that prevent generation

of reactive oxygen species (ROS), as well as agents targeted to eliminate the vicious cycles of oxidative stress and chronic inflammation and to correct mitochondrial dysfunction. Most of these interventions are economical and widely available but not routinely used in treating persons with CKDmfo.

Renal tubular cells are metabolically highly active, and their transmembrane receptor-based transportation functions are energy dependent, based on the availability of ATP—energy. Meanwhile, the renal tubular reabsorption of ions such as Ca^{2+} , PO_4^{3-} , and Mg^{2+} occurs in nephrons through different ion channels and transporters, which are also energy-requiring processes (Blaine et al., 2015). Because of the high energy requirements of renal tubular cells, there are abundant mitochondria present in the renal tubular cells for continuously generating ATP/energy for the ongoing active solute filtration and absorption process (Ralto et al., 2019).

Tubular cell-directed nephrotoxins and ROS, reduce mitochondrial respiration. Consequently, it continues to deplete ATP (but not Na-K-ATPase-driven increased consumption) and increase the intracellular accumulation of lactic dehydrogenase and non-esterified fatty acids, dampening the mitochondrial respiratory functions. Tubular cell hypoxia, partly due to renal anaemia, tissue fibrosis, and intrarenal hypertension, further lessens the intracellular synthesis of ATP, and GSH induce tubular epithelial cell apoptosis. However, this process is antagonised by extracellular acidosis (around pH 6.7): compounds that chelate intracellular Ca^{2+} and improve oxygenation of tubular cells could reverse the pathology (Zager et al., 2008)—another potential target for intervention.

Limitations of This Study and Why Most Published Hypotheses Are Incorrect

The primary limitation of this study is the limited confirmatory renal ultrastructural data on the formation of nano-crystals and nano-tubes in CKDu, which is currently under investigation. Meanwhile, none of the other theories or hypotheses illustrated in **Table 1** could explain the entire process of tubular dysfunction fibrosis leading to CKDmfo. There is no credible, published data that any agrochemical, heavy metals, arsenic, algae, or fungal toxins or ayurvedic medications cause CKDu/CKDmfo.

Based on the above scientific data and understanding of the biology and physiology of magnesium on renal functions, the idea that higher Mg^{2+} intake *via* drinking water (or food) (Wasana et al., 2016, 2017; Dharma-Wardana, 2018) causes CKDu is improbable. On the contrary, X-ray diffraction and energy-dispersive spectroscopy demonstrated that Mg^{2+} prevents $CaPO_4$ crystal formation in media and vascular systems associated with CKD (Ter Braake et al., 2018).

Mg^{2+} reduces hydroxyapatite formation and significantly decreases Ca^{2+} and PO_4 fractions in crystals, including extracellular space, thus reducing the risks for *in vivo* crystallisation (Ter Braake et al., 2018). In summation, all biological, physiological, *in vitro*, and *in vivo* evidence suggests that Mg^{2+} protects renal tissues from injuries, among others preventing crystal formation. CKDu/CKDmfo is a Geo-Bio-mediated, naturally induced chronic disease (**Figure 4**) that arises from multiple factors. It is 100% preventable with proactive

TABLE 3 | A summary of proposed theories for the causation of CKDmfo.

	Postulated assumptions and concepts	References
1	Arsenic, silica, and lead	Levine et al., 2016; Ananda Jayalal et al., 2020; Gobalarajah et al., 2020
2	Paranormal (divinity-causing) the disease	Multiple newspaper articles
3	Snake bites, illegal liquor, or tobacco smoking	(Wimalawansa, 2016a; Thammitiyagodage et al., 2017; Edirisinghe et al., 2018; Gobalarajah et al., 2020
4	Ayurvedic medication (e.g., Aristolochia)	Wanigasuriya et al., 2011; Wimalawansa, 2014a,b; Faleel and Jayawardena, 2020
5	Excessive use (abuse) of non-steroidal anti-inflammatory drugs (NSAIDs)	Chen et al., 2008; Bandara et al., 2010, 2011; Wanigasuriya et al., 2011; Who-CKDu-Final Report: Jayathilaka et al., 2013; Jayasumana et al., 2014; Levine et al., 2016
6	Cadmium (and other heavy metals)	Robinson et al., 2004; Birkner et al., 2006; Gao et al., 2008; Wimalawansa and Wimalawansa, 2014a; Despres et al., 2019; Wimalawansa, 2019a; Gobalarajah et al., 2020
6	Fertiliser or iatrogenic causes	Dharma-Wardana et al., 2015; Gunatilake et al., 2019; Mcdonough et al., 2020
7	Agrochemicals (organophosphates): pesticide, pesticides and metabolites, adjuvants, and herbicide	Peiris-John et al., 2006; Bandara et al., 2011; Wanigasuriya et al., 2011; Jayasumana et al., 2014; Wimalawansa, 2014a, 2015b; Rajapaksha et al., 2021
8	Glyphosate (hard water and nephrotoxic metals) chelation with metal ions	Peiris-John et al., 2006; Jayasumana et al., 2014; Gunatilake et al., 2019
9	Blue-green algae (Cyanobacterial toxins)	Wanigasuriya et al., 2008; Desalegn et al., 2011; Wimalawansa, 2014a
10	Fungal toxins—ochratoxin A	Wanigasuriya et al., 2008; Stalin et al., 2020
11	Low body mass index (low calcium and selenium intakes)	Kulathunga et al., 2020
12	Hantavirus/orthohantavirus/leptospirosis	Wimalawansa, 2014a,b, 2015b; Stalin et al., 2020; Sunil-Chandra et al., 2020; Muthusinghe et al., 2021
13	Genetic predisposition (gene–environment interactions)	Wanigasuriya et al., 2007; Wanigasuriya, 2012; Nanayakkara et al., 2014
14	Climate change: excessive sun exposure to sun, heat stress, and dehydration	Who-CKDu-Final Report: Jayathilaka et al., 2013; Wimalawansa, 2014a; Murray et al., 2015; Chang and Yang, 2021; Petropoulos et al., 2021
15	Ionicity/salinity (exposure to ionic species)	Jayasekera et al., 2012; Dharma-Wardana et al., 2015
16	Relative deficiency of zinc or selenium in the diet that counters heavy metals	Prasanthi et al., 2010; Diyabalanage et al., 2016; Branca et al., 2018
17	Alterations in the calcium-to-magnesium and sodium/potassium ratios	Dissanayake and Chandrajith, 2007; Chandrajith et al., 2011
18	Fluoride	Ileperuma et al., 2009; Chandrajith et al., 2011; Dharmaratne, 2015; Mondal et al., 2016; Wimalawansa, 2016b, 2020; Wasana et al., 2017; Dissanayake, 2019; Jia et al., 2019; Fernando et al., 2020; Nanayakkara et al., 2020
19	Agent or an ailment that is yet to be found	Wimalawansa, 2013a, 2014a, 2015c; Wimalawansm, 2015
20	Oxidative stress	Wimalawansa, 2014a; Rodrigo et al., 2021
21	Interactions between elements (Mg ²⁺ and F ⁻)	Wasana et al., 2016, 2017; Thammitiyagodage et al., 2017; Dharma-Wardana, 2018
22	Multifactorial etiology	Wimalawansa, 2013a, 2014a,b, 2015b,c, 2016a,b; Wimalawansa and Wimalawansa, 2014b, 2016
23	Chronic dehydration, hard water (Ca ²⁺), and fluoride causing hydroxy- and fluoroapatite crystallo-nephropathy	The current novel concept: Wimalawansa and Dissanayake, 2019

measures, as described here and previously (Wimalawansa, 2019a,b, 2020; Wimalawansa and Dissanayake, 2019).

DISCUSSION

CKDmfo is an environmentally induced preventable chronic disease (Wimalawansa, 2015c, 2016b). It is predominant in remotely located economically poor farming and related communities and associated with drinking groundwater, harmful behaviour, and other factors. This chronic tubulointerstitial disease is caused by consuming naturally contaminated groundwater, which aids the formation of CaPO₄ crystals in the kidneys. In the favourable micro-environmental conditions

in vivo, nano-mineral crystal formation occurs in vulnerable renal tissue. This process is likely to be modulated by additive or synergistic interactions between concentrated Ca²⁺, PO₄, F⁻, and other ions, especially when tissue and intracellular Mg²⁺ concentrations are suboptimal. These conditions induce hydroxyapatite and fluoroapatite nano-crystal formation (Wimalawansa, 2015b; Wimalawansa and Dissanayake, 2019), creating a progressive, negative vicious cycle of tubular cell dysfunction, apoptosis, fibrosis, and CKD-CTN.

Irrespective of underlying reasons, changes in rain patterns and associated extended dry seasons, inferior quality groundwater, and lack of potable water negatively affect human and animal health. Because of the close association of the

Geo-Bio pathway (Figure 4) leading to diseases, populations who live in intimate association with the immediate geo-environment are the most vulnerable.

While tropical countries are particularly affected by climate change, it is unknown whether this is related to CKD-CTN. In certain equatorial countries over the past four decades, the annual dry period has extended from 6 to 8 months, associated with an increase of $\sim 1^{\circ}\text{C}$ of average temperature. In addition, overexploitation of groundwater has adversely affected the available water supplies in the dry zonal regions, as in Sri Lanka and other CKD-CTN affected countries. The fatal disease was initiated following the migration of people into new territories (now affected with CKD-CTN), without proper environmental assessments and preparations, and acquired harmful behaviour over the past four decades. Consuming contaminated groundwater for many years derived from deep stagnant dug wells in higher elevations (i.e., away from tanks and streams) probably causes this disease (Wimalawansa, 2015b, 2016b; Wimalawansa, 2015).

As described, multiple approaches are necessary to overcome this silent, ongoing epidemic of CKD-CTN in affected countries (Wimalawansa, 2015b, 2016b). Since CKD-CTN is closely related to the environment where the affected people live for decades and consume *natural* groundwater, lifestyles, drinking water, and dietary habits must address systematically to overcome the adverse effects on the kidney and other vital organs. Providing financial assistance, renal protective medications (i.e., renal replacement therapy) (Varughese et al., 2020), and dialysis would never prevent the CKD-CTN. Governments of the affected countries must focus on disease prevention: the right approach and solutions based on the mentioned root cause to eradicate CKD-CTN (Wimalawansa, 2015).

The data described here demonstrate that (A) consumption of stagnant groundwater (hard water with higher concentrations of $\text{CaPO}_4 \pm$ fluoride over many years, (B) chronic dehydration due to multiple reasons, and (C) the biological conditions and the local micro-environment in renal tissue being conducive to form nano-crystals could cause this deadly renal disease. Besides, the provision of reverse osmosis-purified clean water (Greenlee et al., 2009; Wimalawansa, 2013b, 2018) over 3 years significantly reduced ($\sim 50\%$) the incidence of CKDmfo. Putting data together facilitated the identification of mentioned factors that fulfilled the Bradford Hill criteria as the probable cause for the CKD-CTN (CKDmfo/CKDu). Providing potable water with low concentration of Ca^{2+} , PO_4 , and F^- and other contaminants and supplementing Mg^{2+} or adding it to RO, drinking water are the most cost-effective solutions to overcome this, otherwise deadly disease.

CONCLUSION

The novel concepts described here would leap forward in understanding the etiology of CKDmfo and taking affirmative action to prevent its occurrence and save lives. We have provided evidence that nano-crystallisation of CaPO_4 -hydroxyapatite and fluorapatite in renal tubular tissues is critical for developing

CKD-CTN (CKDmfo/CKDu). In addition, we have provided overwhelming scientific evidence that higher amounts of Mg^{2+} help to prevent deterioration of CKD and CKD-CTN. Meanwhile, there is no credible evidence that excess Mg^{2+} contributes to or cause CKD-CTN.

Irrespective of the occupation, CKD-CTN affects those subjects when the internal environment in the kidney is conducive for CaPO_4 -hydroxyapatite crystal precipitation (Wimalawansa and Dissanayake, 2019). It is not a disease that exclusively affects those working in agriculture. People who have no connection with agriculture or agrochemicals also get the disease if the conditions are right. Consequently, agriculture- or agrochemical-based derogative terminology and acronyms should not be used to describe this disease (Ileperuma et al., 2018). The present findings enable respective administrations and charitable foundations to provide cost-effective modalities to prevent renal damage and reverse the early renal failure of this fatal disease.

In addition to a district- or country-wide broader chronic disease prevention program (Wimalawansa, 2015c), an area-wide cost-efficient strategy for early diagnosis and prevention of CKD-CTN is essential to rescue renal functions before irreversible harm occurs (Wimalawansa, 2015b, 2019c). Taking proper and proactive actions can remove the risks for more than forty million people and prevent an estimated 1.5 million premature deaths over the next few decades from CKD-CTN in affected countries. A proactive and cost-effective approach will save funds, opportunity costs, and lives in affected countries. The costs and opportunity costs that are associated with CKD-CTN can be further reduced by executing a broader chronic disease prevention program, in parallel with others. Such would help millions of families affected with CKD-CTN, in tropical countries.

Besides, the described concepts and findings will pave the path to develop highly effective, targeted solutions at a minimal cost to protect the health and lives of the working-age population, who regularly engage in physical work in a harsh environment. Spending 10% of the current healthcare expenditure on the above-mentioned disease prevention programs could lead to elimination of the CKD-CTN. Interventions need to encompass multiple cost-effective strategies, such as providing potable water, alleviating malnutrition, dietary habits, modifying harmful lifestyles, and educating the public and professionals on minimising chronic disease risks. These are critical for altering the current negative trend to save lives.

AUTHOR CONTRIBUTIONS

SW wrote the paper and related research contributions, while CD provided intellectual input and editing and both authors approved it for publication.

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