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CHRONIC KIDNEY DISEASE OF MULTIFUNCTIONAL ORIGIN (CKDMFO) PREVAILING IN SRI LANKA REEVALUATED

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ABSTRACT

Chronic Kidney Disease of Uncertain Aetiology seems to be caused by several factors and hence it could be best described as Chronic Kidney Disease of Multifunctional origin (CKDmfo). CKDmfo is confined to a particular geographical location of Sri Lanka, viz., the North-Central Province and some nearby Areas where vulnerable people are mostly rice paddy farmers. The CKDmfo distinguishes from common CKDs in the sense that the former patients lack the common symptoms of CKDs. As it has been well established, the modes of initial attack are different in the two cases where the tubuli are the parts of kidneys that are first affected by the CKDmfo while in the latter cases either the contraction or expansion of the glomerular basement membrane results in, respectively, retention of simple and unwanted molecules such as creatinine in blood or filtration of even large molecules like albumin to result in albumin present in urine. Therefore, glomerular filtration rate (GFR), blood creatinine level (BCL) and albumin to creatinine ratio (ACR) in urine are accurate indicators for the diagnosis of common CKDs but these indicators are not suitable to detect early stage CKDmfo patients. This fact is highlighted in this review and suitable

tubuli specific tests recommended for the early stage diagnosis of CKDmfo. In order to understand the disease in detail, a brief account of the kidneys with its functions are reviewed and the common CKDs are highlighted. Attention is then focused on the CKDmfo and facts of CKDmfo are revealed. There are several existing theories as to the possible causative factors and their pros and cons will then be evaluated. As the disease is propagated by the initiation through tubular damage, the environmental factors pertinent to tubular reabsorption are discussed. From the points of view of interferences to the tubular reabsorption, the disease seems to correlate well with environmental factors typical of the region, i.e, excess fluoride ions in the drinking water and high hardness, both temporary and permanent hardness. While the temporary hardness is due to soluble bicarbonates of both calcium and magnesium ions, which can easily be removed by boiling water, the permanent hardness that is due to soluble ions which are not removed by boiling are mainly excess of sodium, potassium and ammonium cations and their soluble anions such as nitrate and chloride. As such, as a preventive measure, the need to supply good-quality drinking water is suggested. In providing drinkable water, it is of utmost importance to provide them with treated pipe-borne water though the difficulty in providing such waters to houses scattered in a vast area of the region is recognized. At least as a temporary measure, it is suggested to provide good quality water from reverse osmosis (RO) plants or through rain water harvesting. However, it is stressed that these solutions are only temporary measures to control the disease and eventually a proper supply of pipe-borne water has to be supplied to every human being of the country. In the case of CKDmfo, it is suggested to avoid chlorination to purify water because chlorine residues that are present in the treated water may be highly harmful to the vulnerable people. Most recent evidence converge towards fluoride to be the most likely causative factor of CKDmfo and all other ions and dehydration are also responsible for this disease.

KEYWORDS: *Chronic Kidney Disease of Unknown Origin (CKDU), multi-functional origin (mfo), tubules, causative factors, preventive measures.*

1.0 INTRODUCTION

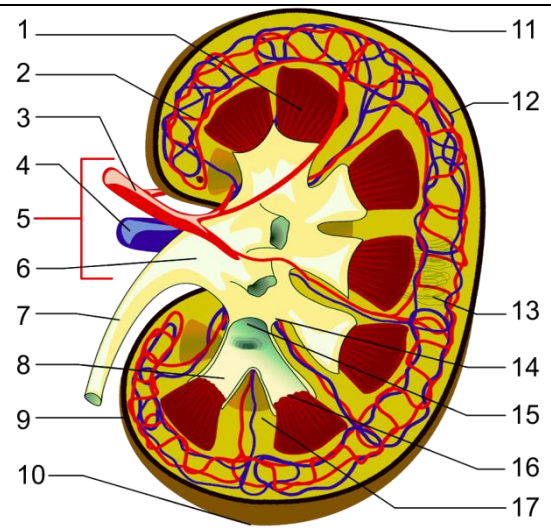
1.1 Kidneys and Their Functions: Vertebrate animals have two kidneys located in the back of the abdomen, of which the anatomy is illustrated in the Figure 1. Kidneys serve several essential regulatory functions; which include, (a) removal of excess water, salt and toxins as urine, (b) removal of metabolic waste products such as urea and creatinine, (c) serving

homeostatic functions which include the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure, (d) re-absorption of ions such as K^+, Na^+, Cl^-, and molecules such as water, glucose and amino acids, (e) production of hormones such as calcitrol and erythropoietin, and (f) production of the enzyme rennin.[1-4] Waste excretion is essential to maintain physiology and thus the health of a body. Kidneys are highly metabolically active organs and function 24 hours a day, to regulate water and electrolyte balance. In case of insufficient water intake, kidneys reduce the water excretion to maintain balances of the body.

Kidneys need a constant arterial blood pressure to filter. Thus and when the blood pressure decreases, kidneys liberate enzyme renin to adjust circulatory ions. Renin cleaves the prohormone, angiotensinogen into angeotensin, a potent vasoconstrictor, which intern increases the arterial blood pressure. In addition, kidneys regulate the production of red blood cells *via* generation of the hormone erythropoietin; a hormone that stimulates the bone marrow to produce red blood cells. Moreover, kidneys regulate acid-base balance in the blood. Acids are produced due to cell metabolism and from the foods, and kidneys help maintain constant pH of blood which is vital for the life *via* modifying re-absorption of HCO_3^- ions. The hormone, calcitriol [1,25- dihydroxyvitamin D], is the hormonally-active metabolite of vitamin D. Its' key function is to regulating the ionized Ca^{2+} levels in the blood and tissues. This is facilitated by enhancing calcium uptake to the circulatory system from the intestine, regulating the release of calcium into the blood from bones and reabsorption of calcium from the renal tubules.

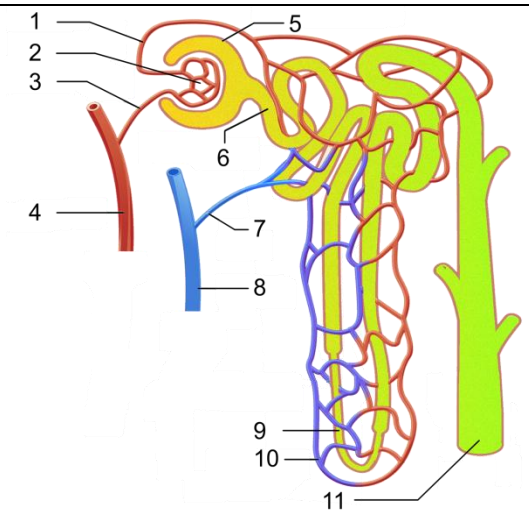
Filtration occurs in the glomerulus of the kidney microscopic structures confined to the cortical area of the kidney with very large surface area. This is a part of the nephron (Figure 1-a, 13; Figure 1-b, 2); the functional unit of the kidneys, which consists of glomerulus, Bowman's (Glomerular) capsule, proximal tubule, loop of Henle, distal tubule, collecting duct, and the latter is shared by many nephrons. Glomerular filtration takes place due to hydrostatic pressure which filters both essential nutrients, salt and water, and unwanted materials such as urea, creatinine, uric acid, NH_4^+ ions *etc.* Proximal convoluted tubules re-absorb most of the nutrients and ions, leading to excretion of unwanted and toxic materials. Re-absorption of water and salt takes place in the Loop of Henle which utilizes a counter-current mechanism.[5] Distal convoluted tubule's function is to selectively reabsorb or secrete materials based on hormonal control and physiological needs. Finally, collecting duct

reabsorbs water to concentrate urine with the help of Antidiuretic hormone and also secretes and reabsorbs stuff based on hormonal control.[6-8]



(a)

Figure. 1: (a) Anatomy of a Kidney: 1. Renal pyramid, 2. Interlobular artery, 3. Renal artery, 4. Renal vein, 5. Renal hilum, 6. Renal pelvis, 7. Ureter, 8. Minor calyx, 9. Renal capsule, 10. Inferior renal capsule, 11. Superior renal capsule, 12. Interlobular vein, 13. Nephron, 14. Minor calyx, 15. Major calyx, 16. Renal papilla, 17. Renal column.



(b)

Figure. 1: (b) Structure of a Nephron: 1. Efferent arteriole, 2. Glomerulus 3. Afferent arteriole, 4. Interlobular artery, 5. Bowman's (Glomerular) capsule, 6. Proximal convoluted tubule, 7. Venule, 8. Interlobular vein 9. Loop of the nephron, 10. Peritubular capillary network and 11. Urine flow into renal papilla.

1.2 Chronic Kidney Diseases (CKDs)

Chronic Kidney Disease of multifunctional origin (CKDmfo) prevailing in Sri Lanka is a serious kidney disease leading to irreversible kidney damage in humans. This disease is predominantly affecting certain geographically demarcated areas, such as Girandurukotte in the Badulla District, Medawachchiya and Padaviya in the Anuradhapura District, Medirigiriya and Hingurakgoda in the Pollonnaruwa District and Nickawewa and Polpithigama in the Kurunagala District of Sri Lanka.[9] However, recent findings indicate that this disease is spreading over to other Agricultural regions of the country including other regions of the North-central Province, some areas of Uva, Southern and Eastern Provinces and to Vavunia and Jaffna Districts.[9] Old CKDmfo (CKDU) map of Sri Lanka is available in many references and we do not intend to reproduce it here[10] but these maps need updating since the total number of patients is now exceeding 100,000. Although most of the affected persons were of the ages over 45 years, the age category is now shifting towards younger people.[11] Unique features of CKDmfo are that they have no common or known cause(s) for the development of Chronic Kidney Diseases (CKD); i.e. they have no past history of

diabetes mellitus, [glycosylated haemoglobin levels (HbA1c) are less than 6.5%], chronic hypertension [less than 140/90 for treated patients], snake bites or urological or renal stoma diseases, or any other known diseases including glomerulonephritis leading to CKD.[9,12] As such, these factors are used in the case definition of Sri Lankan CKDmfo. However, this is somewhat misleading as both the known causes leading to CKD and CKDmfo can coexist.[13]

1.3 Issues Related to the Glomerular Filtration

As explained above, kidneys are a complicated organ responsible for several biochemical and biophysical activities. The tiny filters that are present in kidneys, which are called glomeruli, filter off waste products from the blood. In normal circumstances, albumin is not present in urine, since albumin molecules are too large to be filtered by the pores of glomerular basement whereas creatinine is to be filtered freely. When the patient's glomerular filtration rate (GFR) remains below 60 mL min^{-1}, the kidneys have failed to filter waste products, thus those produced toxins including urea and creatinine would accumulate in the body.

The glomerular basement membrane (GBM) is the basal laminar layer of the glomerulus, i.e. the filtering unit. It consists of three layers; namely, lamina rara externa, lamina densa and lamina rara interna. The first layer, lamina rara externa, is located adjacent to podocyte processes and is made of heparin sulphate. Since in this linear polysaccharide, each monosaccharide unit contains a sulphate group the polymer is highly negatively charged. Therefore, it repels anions and thereby blocks the filtration of anions due to electrostatic repulsions. The lamina densa is located at the dark central zone and it is made of type 4 collagen and laminin. The function of this layer is to block the filtration by size, i.e., any molecule with molar mass exceeding 5800 kDa is blocked by this layer. The lamina rara interna is located adjacent to endothelial cells and is made of heparin sulphate and hence as in the case of lamina rara externa, it also blocks the filtration of anions by electrostatic repulsions. The GBM consists of messaginal cells which are smooth muscle cells that function to regulate blood flow through the capillaries and modified pericytes. Adjoining them are podocytes which have filtration slits of diameter 25 nm that are formed by the pseudopodia arising from them. The filtration slits are covered by a diaphragm that includes the transmembrane protein nephrin. When the glomerular filtration takes place, anything larger than 25 nm in size are retained and only those less than the sizes of slits, i.e., 25 nm, are filtered. As such, simple molecules like ammonia, urea and creatinine are completely removed by the glomerular filtration. Large proteins such as albumin are too large to be

filtered through 25 nm slits and are therefore retained in the blood. There are several pathological conditions that can affect glomerular filtration. In the Goodpasture's syndrome which is also known as "anti-glomerular basement membrane disease" the capillaries become inflamed as a result of damage to the basement membrane by antibodies to alpha 3 NC1 domain of type IV collagen. This is a rare autoimmune disease in which antibodies attack the basement membrane in lungs and kidneys, leading to bleeding from the lungs and to kidney failure and this can be acute rather than chronic and may result in death unless antibodies in blood are removed by immunosuppressant chemotherapy. In the Nephrotic syndrome, the structure of the glomerular filtration mechanism is changed usually due to the changes in the GBM. Some symptoms of the Nephrotic syndrome include proteinuria, hypoalbuminaemia, oedema and hyperlipidemia. It is characterized by an increase in permeability of the capillary walls of the glomerulus allowing large proteins to pass from the blood into the urine (proteinuria at least 3.5 grams per day per 1.73 m^2 body surface area). However, these pores are not large enough to allow blood cells pass through (hence no haematuria. In nephritic syndrome red blood cells also pass through the pores, causing haematuria.). A normal red blood cell is 6-8 µm in diameter and the pores in this case have been enlarged by at least 200 times. Diabetic glomerulosclerosis is yet another pathological condition in which thickening of the basement membrane takes place, which can become up to 4-5 times thicker than normal. This can be caused by insulin deficiency or resultant hyperglycemia. Alport syndrome is an X-linked hereditary nephritis which is caused by mutations in type IV collagen, leading to a split lamina densa of the glomerular basement membrane. It is a hereditary nephritis and is a genetic disorder characterized by glomerulonephritis, end-stage kidney disease, and hearing loss. As such, all these types of kidney diseases are caused by the changes that occur at the GBM. Since all of the creatinine should be filtered and all of the albumin should be retained in the blood, the measurement of albumin to creatinine ratio (ACR) in urine and the measurement of creatinine in blood together with glomerular filtration rate (GFR) are reliable indicators for CKD and 30 mg of albumin per each gram of creatinine is the upper limit of healthy glomerular filtration.

Creatinine is a waste product produced from creatinine through a direct route *via* the loss of one water molecule per creatine zwitter ionic species or by the biochemical path which utilizes creatine kinase enzyme for the phosphorilation through the conversion of ATP to ADP and subsequent dephosphorilation and dehydration. Figure 2 illustrates these processes. Creatinine does not undergo re-absorption at tubules and hence creatinine is filtered through

glomerular filtration and also by proximal tubular secretion. If the pores of the glomeruli basement membrane become two small, they are then unable to filter creatinine and hence creatinine would accumulate in blood. On the other hand, expansion of these glomerular pores would allow filtering of albumin. Thus the ratio of albumin-to-creatinine in urine is an acceptable measure of kidney function. As such, 30 mg of albumin per each gram of creatinine in urine is the upper limit for a healthily working kidney. As such, in order to diagnose common CKDs, GFR less than 60 mL/min, blood creatinine level and urine albumin-to-creatinine ratio (ACR) are reliable indicators since most of the CKDs affect the glomerular filtration by altering the pore sizes of the glomerular basement membrane which are the pores of size 25 nm in size in a healthy membrane. However, these indicators are not suitable for the diagnosis of CKDmfo since the mode of initial attack in the latter case is different to that in ordinary CKDs.

1.4 Initial attack in CKDmfo and Proper Diagnosis

Physiopathology of CKDmfo is very different to those of CKDs and the part of the kidneys that are first affected by CKDmfo is the tubules. When the tubules are attacked their reabsorption mechanism is adversely affected. Therefore, in order to understand CKDmfo, the physiology of tubules should be thoroughly studied. Redman et al. (12) have published an excellent paper titled "Additional Perspectives on Chronic Kidney Disease of Unknown Aetiology (CKDu) in Sri Lanka – Lessons Learned from the WHO CKDu Population Prevalence Study" in which they have been able to distinguish between CKD and CKDU (CKDmfo). In CKD the glomeruli is the part of the kidneys that is affected by either enlarging or blocking the pores of the GBM. However, in the case of CKDmfo the parts of the kidneys that are affected are tubules and the condition is thus named renal tubulo-interstitium. They have clearly indicated that the elevated ACR is not the biomarker for the case definition of CKDmfo. When it comes to ACR of over 30 mg/g, most of the tubules have already been damaged and therefore the CKDmfo patients are at their end-stage kidney failure. This may account for the fact that the number of CKDmfo patients diagnosed is much less than the actual number of CKDmfo patients. Redman et al. (12) have suggested that better markers of kidney damage that are sensitive to tubular injury, such as urinary neutrophil gelatinase-associated lipocalin (NGAL)-to-creatinine ratio, may be more suitable for early diagnosis of CKDmfo than ACR. It is recommended that this test be used immediately in the disease diagnosis of CKDmfo together with usual ACR. Ratnayake et al. (14) have recently compared the diagnosis tests for CKDmfo and out of dipstick proteinuria,

serum creatinine and serum cystatin C (cys. C) tests, they have suggested that S.Cys is the best functional marker to distinguish CKDmfo cases from healthy subjects in mass screening programmes. However, the high cost of S.Sys could be problematic in large scale use over entire population of the country and, therefore, as an appreciably accurate and a cost-efficient functional marker: S.Cr along with ACR, the latter is a renal damage marker, could be used for successful detection of CKDU cases in mass screenings. They have also suggested that due to the inferior sensitivity against endemic population, ACR does not seem to be favourable as an individual substitute marker and the use of cut off values derived from general population may not be accurate for an endemic population. More importantly, they have stressed the fact that given the minimally proteinuric nature of CKDmfo, lowering the current ACR cut off limit below 30 mg A/g-C may be a viable option to improve detection of CKDmfo cases.

2.0 CHRONIC KIDNEY DISEASE OF MULTIFUNCTIONAL ORIGIN (CKDmfo)

2.1 Facts of CKDmfo

Among many other factors, the most important five factors to consider are, (i) the patients are confined to a unique geographical area of the country, at least at the beginning, (ii) the disease was not found beyond 30 years ago, or may have not been diagnosed, (iii) the number of patients is continuously increasing rapidly, (iv) the affected people are mostly farmers and (v) the patients are mainly males[9,11,13,17] although, one of the NSF-funded, World Health Organization (WHO) reports[18] indicates that there are a significant number of female CKDmfo patients. However, no explanation is given for this discrepancy. Plausible causative factors suggest that there should not be any gender basis for CKDmfo if both males and females are exposed to the same toxins/conditions that would cause this disease. The gender imbalance shown previously may be due to the fact that mostly the farmers who do hard labour are males and they are the people who spray agrochemicals to farmlands and are the people mostly engaged in drinking illegal alcohol and smoking tobacco.[9] However, when the father of the family has become a victim of the unfortunate disease, the trend is for mother to do what father is used to do in farming, to continue generating family income for survival this making mother is also vulnerable for the disease.

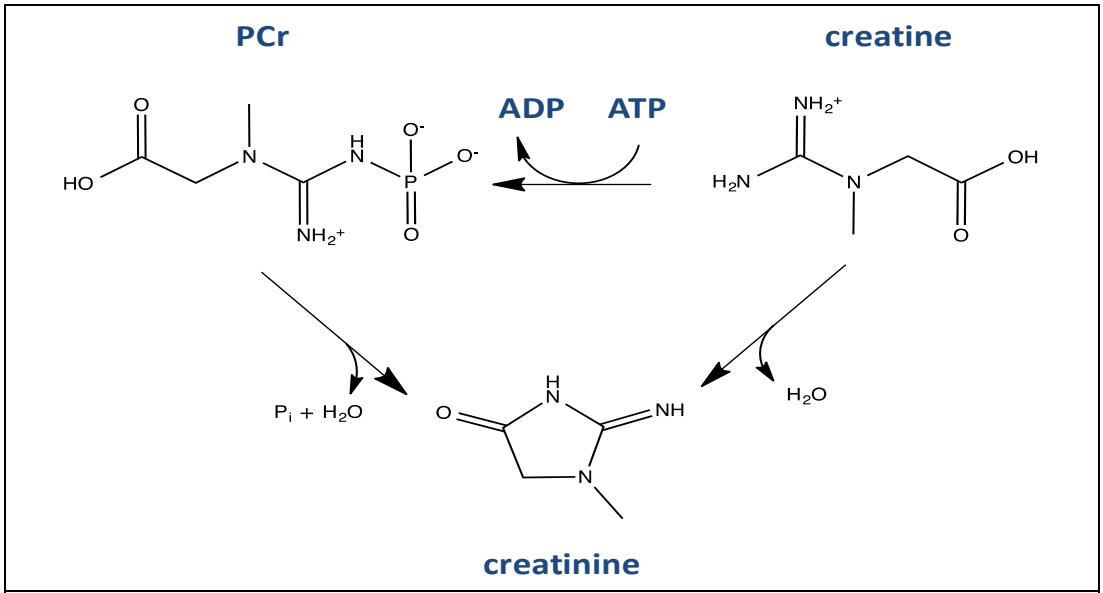


Figure. 2: Biochemical pathways of creatinine production in human body.

2.2 Implications of the facts

The disease is prevalent only in a particular geographical areas and that most of the patients are paddy farmers. The disease is caused by chronic exposure to an environmental hazard over several years; to whatever the chemicals or toxins, the aetiology of the disease could well be associated with local environmental conditions, factors associated with paddy farming, food habits, or nutrition. It would, therefore, be of utmost importance to look for unique environmental conditions of the area, the factors associated with farming such as the usage of fertilizes and agrochemicals and quality of drinking waters, foods they consume, and even air quality parameters and radiation levels in the environment.

Poor quality of waters in this particular area is well known and do contain high concentrations of various ions where the presence of excessive amounts of fluoride, phosphate and ammonium, calcium *etc.* ions which are far above the permissible levels stipulated in the WHO guidelines.[19]

As for food habits of farmers in these villages, their main staple food is rice and curries. Curries usually include vegetables, grains, green leaves, other local food stuffs such as young jack (“*polos”*), matured jack (“*kos”*), yams, local inland fish, dry fish, sprats, salmon, coconut “sambal” etc. cooked in typical Sri Lankan style using chilli powder, spices, onion, garlic, salt and sour and consumed together with comparatively large amount of boiled rice. Usually, they take three meals a day and during hard work, they usually take two cups of tea; one between breakfast and lunch and the other between lunch and dinner. Occasionally, they

consume typical Sri Lankan foods such as hoppers, string hoppers, "pittu" etc., together with tea, bread, other varieties of bread-equivalent foods, milk rice, grains, and so on. Majority of farmers belongs to the low-income level whose income is mainly derived from yields of their crops with a little more income generated through other labour work. Tea that they consume is bought from local shops and there is no quality assurance of the kind of tea that they buy and very often, this tea may be of very low-quality produced through dried and dyed used tea powder.[6]

3. HYPHOTHESES FOR ROOT CAUSES OF CKDmfo

3.1 Fluoride hypothesis

The fluoride hypothesis is based on the first such study conducted by Dassanayake and co-workers in 2005[21,25-27] who hypothesized that the excessive fluoride ions in drinking water in these areas may have an effect on this disease. They have further observed that both dental and skeletal fluorosis and CKDmfo are common in this certain geographical areas. According to their studies, water quality in this particular area is affected predominating due to increasing draught conditions and consequent building up of salts.

Subsequently, Ileperuma et al. reported that the villagers in these areas frequently use low-quality aluminium cooking utensils and the possible reactions of aluminium with fluoride ions making complex anions such as tetrafluoroaluminates and hexafluoroaluminate, under acidic conditions. They further postulated that fluoride could be contributed to this disease[28,29] although, Chandrajith et al.[21] later pointed out that the fluoride contents of drinking water does not coincide with the CKD affected villages. For example, higher fluoride contents in water have been reported in villages in and around Puttalam, Monaragala, Ampara and Hambantota, but the disease prevalence is much less. However, Chandrajith et al. have failed to realize that fluorosis, which is caused by the replacement of hydroxyl groups of hydroxyapatite in bones and teeth by fluoride ions, is prevalent mostly in these North-Western and North-Central areas of the Island where the CKDmfo is also prevalent. However, recent collaborative research of Chandrajith together with the principal author of this article on CKDmfo in Moneragala district has revealed that there is a direct correlation between CKDmfo and fluoride in drinking water of the susceptible people. Further, there is high levels of cations such as Na^{+}, Mg^{2+} etc. in these waters. As such, the most recent findings bring us to the point that fluoride would be the most likely causative factor for CKDmfo though other ions such as sodium, calcium, magnesium etc. also could have some

effect. In fact, most of the data on water quality parameters in various parts of the country do not match with each other. Some researchers claim extremely high values whereas some claim unacceptably lower values. Several factors may influence these discrepancies which include improper sampling techniques, inadequate statistical analysis, and low sensitivity of the methods used to produce data, seasonal variations of environmental water quality and so on.

The fluoride hypothesis cannot be ruled out since the ground water in these areas is relatively high in fluoride ions exceeding the WHO safe limits of 1.5 mg/L in many drinking water sources, prominently in tube wells. There are several hypotheses regarding why fluoride levels are high in certain villages. These includes, weathering of fluoride containing minerals such as fluorohydroxyapatite that are found in the local area and the large number of tube wells that have been dug in the region over the last 30 years, and so on.

Fluoride is the anion at the top of the Hofmeister series; the series showing the ability to denature proteins by various ions. Therefore, in theory, the involvement of fluoride in denaturing glomeruli basement protein cannot be ruled out (*vide infra*). However, damaging the kidneys directly by fluoride ions or hexafluoroaluminate ions alone is quite unlikely though there is a possibility for the involvement of fluoride in causing this CKDmfo disease through interfering the tubular reabsorption of water and ions as will be explained later.

Fluoride in drinking waters at a low level of ~ 0.5 ppm is essential for healthy life and dental health. WHO noted that mottling of teeth (i.e. dental fluorosis) is sometimes associated with fluoride levels in drinking-water above 1.5 mg L^{-1} and crippling skeletal fluorosis can ensue when fluoride levels exceed 10 mg L^{-1}, or even somewhat lower levels of fluoride in water consumed for a long duration. A guideline value of 1.5 mg L^{-1} was, therefore, recommended by WHO. The 1.5 mg L^{-1} fluoride guideline value that was set in 1984 was subsequently re-evaluated and concluded that there was no evidence to suggest that it should be revised (WHO, 1996, 2004). The 1.5 mg L^{-1} guideline value of WHO is not a “fixed” value but is intended to be adapted to take into account of local conditions (e.g. diet, water consumption, etc.).[30]

Ingestion of excess fluoride is detrimental to human health. Approximately 75–90% of ingested fluoride is absorbed. In an acidic stomach, fluoride is converted into hydrogen fluoride (HF) and up to about 40% of the ingested fluoride is absorbed from the stomach as

HF. HF is the most corrosive substance known. Therefore, if exposed to, HF can easily damage living cells. Fluoride not absorbed in the stomach (60%) is absorbed in the intestine and is unaffected by pH. Relative to the amount of fluoride ingested, high concentrations of cations that form insoluble complexes with fluoride (e.g. calcium, magnesium and aluminium) can markedly decrease gastrointestinal fluoride absorption.[31,32] Once absorbed into the bloodstream, fluoride is distributed throughout the body. Approximately 99% of the fluoride in the body is retained in calcium-rich zones, such as bones and teeth (i.e, dentine and enamel).[33] Fluoride is then incorporated into the hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] lattice through ion-exchange mechanism.

Porous $Ca_{10}(PO_4)_6(OH)_2(s) + xF^-(blood) \rightleftharpoons$ Denser $Ca_{10}(PO_4)_6(OH)_{2-x}F_x(s) + xOH^-(blood)$ (1)
Co-ordinating anions in the blood, including toxic ones, if any, can be adsorbed by the hydroxyl groups of hydroxyapatite present in bone and thereby the supply to other organs is suppressed. These anions are then released to the kidneys for safe discharge from the body. When the OH groups are substituted with F^- anions, this mechanism is hindered, and hence toxic substances become available to harm cells. Ca^{2+} can also be exchanged for Cd^{2+} in Cd^{2+} toxicity causing weak bones (osteoporosis).

3.2 Cadmium ion hypothesis

In 2007, Bandara et al.[22,34-36] reported that Cd^{2+} levels in the inland fish in the CKDmfo affected areas are high. Since inland fish is a popular food among the people living in these areas, they suggested a possible connection of Cd^{2+} with the CKDmfo. Being a toxic metal ion, Cd^{2+} can damage cells including renal tubules, and hence could well be responsible for this disease. Cadmium ions enter into the food chain from both water and food and through the route of inhalation.

Pesticides used by farmers are known to contain toxic substances such as Cd^{2+}, As(III), Pb^{2+} etc. that are designed to kill pests. However, this finding has not been followed up adequately to find out connections between Cd^{2+} ions and CKDmfo. Others have reported that the levels of Cd^{2+} ions in drinking water in the NCP region is lower[20] than those determined by Bandara et al.[22, 34-36] In fact, our recent studies have shown that the Cd^{2+} levels in NCP waters cannot be measured by atomic absorption spectrometry since the levels are far below 1 ppm (Unpublished Results).

It was not clear why these results are conflicting for similar water samples from the same region, and out of the two sets of results, which results are the correct. The fact that Bandara et al. have used atomic absorption spectroscopy with lower sensitivity compared to Chandrajith et al.'s Inductively-coupled Plasma Mass Spectroscopy may suggest that latter results could be more reliable than the former ones. Chandrajith et al. have studied Cd^{2+}, Pb^{2+}, Al^{3+}, Ni(II), Cu(II), Zn^{2+}, As(III) and U in various water bodies of Nikaweva and Giorandurukotte (CKDmfo endemic areas) and compared these results with those obtained from Huruluwewa (an area in the same province but CKDmfo is not found) and reported that the mean concentrations are much lower than WHO permissible levels. They have also measured Cd^{2+}, Pb^{2+}, Al^{3+}, Mn(II), Cu(II), Zn^{2+}, As(III) Se^{2-} levels in rice (*Oryza sativa* L.) that were collected from CKDmfo endemic regions and also concluded that the levels are insignificant to cause CKDmfo.

However, Chandrajith et al. have not considered the possibility of bioaccumulation of cadmium ions, even in the presence of lesser levels of Cd^{2+}, which has long retention times in the human body.[37] This may explain the bioaccumulated, high levels of Cd^{2+} in fish. Whether those who eat larger quantities of freshwater fish in this area contributing to the CKDmfo is not known and the data available to-date are insufficient to suggest Cd^{2+} as a root cause for this disease. In fact, there is no correlation between Cd^{2+} and CKDmfo in Moneragala district since Cd^{2+} levels in drinking waters are very low and are much lower than permissible levels and there is no difference between Cd^{2+} levels in drinking waters of CKDmfo susceptible people and healthy people in this area.

Correlation between renal failure and cadmium toxicity has been well established in the case of *Itai Itai* disease where this disease was found in the cadmium-polluted Jinsu river basin of the Toyama Prefecture of Japan.[38] *Itai Itai* disease was found among middle-aged and elderly females who had a higher number of pregnancies, and living in cadmium-polluted endemic areas for more than 30 years. This disease is characterized by renal tubular dysfunction and osteomalacia as observed by increased excretion of β2-microglubulin, glucose, protein and amino acids in urine. It has also been reported that the percentage tubular reabsorption of phosphate (% TRP) level is low and high levels of serum Cl, and in severe cases, increased levels of serum creatinine, showing impairment of renal function.[39]

Cadmium is taken up by the body via various roots, including inhalation via lungs, and through food and drinks to gastrointestinal track, and tobacco smoking.[9] Cadmium in blood

is bound to albumin and other high molecular weight proteins. Protein-bound cadmium ions are taken up by the liver and then coupled with a low molecular weight protein, metallothionein that containing functional SH groups. Metallothionein is produced in the liver and is involved with Zn^{2+} transportation to the kidneys. Therefore, when Cd^{2+} is ingested, it utilizes Zn^{2+} transportation mechanism to deliver to the kidneys from the liver. The concentration of metallothionein-bound cadmium is low in plasma due to continuous renal clearance. Cadmium ions that are released in the renal tubules have been found to stimulate the synthesis of metallothionein in kidneys and thereby they are rebound with metallothionein. Catabolizing and this rebounding prevent the excretion of cadmium ions from kidneys and the free cadmium ions generated through release from cadmium-metallothinein complexes cause the renal failure.[40]

WHO-study group have analysed arsenic, cadmium, lead, selenium, pesticides and some pesticides in biological samples from individuals with CKDmfo and compared with age- and sex-matched controls in the endemic and non-endemic areas.[14] Food, water, soil and agrochemicals from both areas have also been analysed for heavy metals. Their results indicate that people living in endemic areas are possibly exposed chronically to higher levels of cadmium through food chain and/or from pesticides and they may have higher urinary excretion of cadmium ions. Thus, they suggested that cadmium exposure is a risk factor for the pathogenesis of CKDmfo.[14] They have postulated, on theoretical grounds, that deficiency of selenium could be a predisposing factor for the development of CKDmfo. However, majority of results in water analysis in these areas contradict with their suggestion since the drinking waters in this area do not contain more than permissible levels of cadmium.

3.3 Cyanobacteria Hypothesis

Although Dissanayake et al. suggested that the cyanobacterial toxins could cause this disease, a year later, others have questioned the validity of this hypothesis.[13] They have reported that waters in the dug wells and tube wells, the cyanobacterial toxin levels are either undetectable or low. Additional studies are been conducted at the Institute of Fundamental Studies, Kandy, to test this hypothesis. They have pointed out that CKDmfo in Sri Lanka is connected to water resources.[9] Histopathological evidence supports the hypothesis that the disease is of tubulointerstial nephritis indicating a high possibility of toxic aetiology. The associated toxins are hepatotoxic and nephrotoxic, and hence, cyanobacterial toxins produced by algal blooms

in reservoir waters should be further explored. Cyanobacterial toxins are water-soluble and heat-stable substances with a good stability at room temperature. The WHO monograph on cyanobacteria describes 18 different types of cyanobacteria capable of producing toxins under certain conditions. These, researchers have identified 15 such toxin-producible cyanobacteria in reservoirs and canals in the CKDmfo affected areas.

They have also studied the biodiversity of cyanobacteria in selected locations of reservoirs and canals in this area. Cyanobacterial toxins have been studied at the National Research Centre for Environmental Toxicology (EnTox) at Queensland using Cylindrospermopsin (CYN) and Deoxy Cylindrospermopsin (DCYN) by LC/MS/MS reporting limit > 0.2 µg/L, Microcystins, expressed as total microcystin, done by LC/PDA, reporting limit > 0.5 µg/L.[41] Their study shows that there is an ability of cyanobacterial toxin to cause acute tubular necrosis of mice. They have also noted the regeneration of the necrosed tubules two weeks after the removal of the toxin from the drinking water. Thus, it was postulated that, if the same mechanism operates in humans, we can prevent further damage or the damage done already could be repaired, by preventing the patients/individuals of exposure to further toxins.

The answers to these fundamental questions of why in Sri Lanka and why in this particular are also seem warranted. Sri Lanka is a country with ancient hydraulic civilization and the dry-zone of the country has over 22,000 man-made, mostly ancient reservoirs; out of which 18,000 are small village tanks. Surprisingly, nowhere else in the world has this number of man-made water reservoirs and most of them have been built during ancient kingdoms. Since the retention time of water in these reservoirs is over 9 months, these water bodies are ideal grounds for cyanobacterial growth, blooming, toxin production and concentration in the prolong dry warm weather. As to the question of why in the NCP, they have put forward two possibilities, the highest number of water reservoirs are in the North Central Region and the incidence of high solar radiation for several months of the year both favouring cyanobacteria production by algal blooms in these water reservoirs. However, not all reservoirs in this area contain cyanobacteria. It is because, most of the affected reservoirs have a longer retention time for water than the unaffected reservoirs and those affected ones are particularly shallow ones. They have also noted that reservoirs fed with Mahaweli water are less affected, with an only exception of the Ulhitiya reservoir which has a significant seasonal cyanobacteria production. They have pointed out that the stagnation of water in this reservoir is quite likely so that cyanobacteial production is favourable even though the reservoir is fed with the water

from Mahaweli River. The increase of environmental temperature by of the 0.5 °C over the past 20 years, perhaps due to global warming and the possibility to more N, P, K coming from the agricultural runoff in water from excessive use of chemical fertilizers, may contribute to the cyanobacterial algal blooming and toxin production. Although, all these facts and explanations seem very logical, there is no hypothesis or a mechanism to explain how these toxins get into the affected individuals.

3.4 Arsenic Hypothesis

Jayasumana and coworkers have hypothesised that arsenic species present in pesticides could be responsible for this disease.[22,42-53] No convincing data available, but this hypothesis may have originated by considering the fact that affected people are predominantly farmers who use excessive amounts of pesticides containing toxic metal ions such as Cd^{2+}, As(III) and Pb^{2+}. Their reports indicate that the amounts of arsenic is higher than the recommended maximum amounts of arsenic species in certain samples. However, others have failed to reproduce these results making this hypothesis highly unlikely.

Even though they have tried to postulate that the arsenic levels in Sri Lankan rice is high, but reported other data suggested otherwise. In fact, the arsenic contents in Sri Lankan rice is one of the lowest among the rice producing countries in South Asia.[7] The researchers of arsenic hypothesis have put forward several contradictory arguments and exaggerated their claims without scientific evidence.

Jayasumana and co-workers suggestion of the relationship between hardness of water and the bioaccumulation of arsenic species is implausible. The soluble arsenite and arsenate compounds in pesticides become insoluble in water due to the formation of calcium arsenite/ arsenate. Arsenic ions can have two oxidation states; As(III) and As(V). Among these two oxidation states As(III) compounds of alkali metal ions are soluble in water, at room temperature, and As(V) compounds are quite insoluble. Pesticides contain water-soluble forms of As(III) more abundantly and some water soluble As(V) compounds in lesser amounts. However, pesticides containing inorganic arsenic species have been banned and those containing organic arsenic are not that toxic. The alkaline earth metal compounds of arsenic in both oxidation states are mostly insoluble in water. For example, $Ca_3(AsO_4)_2$ with varying amounts of crystalline water, $Ca_4(OH)_2(AsO_4)_2$ with varying amounts of crystalline water and $Ca_5(H_2AsO_4)_4$ are highly insoluble in water and the pK values for their dissociation equilibria are over 20 at 25 °C, and as such the equilibrium constants are lesser than 10^{-20} in

magnitude with respective mol dm^{-3} type units.[54] As such, if formed, these compounds would remove most of the arsenic ions from water through precipitation; i.e., arsenic becomes unavailable. It is likely that some may retain as free ions due to the amounts determined by saturated solubility of these substances but these concentrations are in amounts less than what has been determined by Jayasumana et al.[55] It is also possible that some of these substances may be present in water as suspended solid particles, but owing to their water insolubility, bioaccumulation of arsenic from insoluble suspended particles taken into the body through drinking water are minimal, as these particles can be excreted from the body.

They have suggested (without any evidence) that calcium arsenate crystals bound to arsenic transporters in the liver are then transported to the kidneys to result in the deposition of calcium arsenate crystallites in the kidney. Nevertheless, no such 'arsenic transporters' have been identified nor a mechanism of releasing these arsenate ions in kidneys. Owing to the low solubility of calcium arsenate, the release of arsenate groups within the kidneys by insoluble calcium arsenate crystallites is impossible unless the crystallites are "melted" within the kidney. However, the melting of calcium arsenate requires over 1,000 °C, and hence this process is simply an impossibility.

Postulating thermodynamically impossible process of replacing the phosphate groups in DNA by arsenate groups is another myth of these authors. The "life molecule", DNA, cannot have arsenic as an element. It is composed of only C, H, N, O and P as elements. The replacement of PO_4^{3-} group by AsO_4^{3-} is thermodynamically uphill process with a positive ΔG value at the body temperature. There are other erroneous claims including the replacement of phosphate groups in DNA molecules by arsenate groups in "arsenic loving" bacterium species living in 'California' lake where the water is containing very high concentrations of arsenic levels, by Wolfe-Simon. et al. in 2010.[56] This claim is also impossible from the thermodynamic point of view and as such it has been criticized by the scientists.[57] Moreover, it has been revealed that the arsenate ions that have been detected in the DNA of this bacterium species is not due to arsenate present in their DNA but that has come from the contamination in the process of careless purification of DNA thus confirming that it does not contain arsenic. This makes the entire pathophysiological process that has been put forwarded by Jayasumana and co-workers incorrect on scientific grounds.

Despite the fact that this process and the assumptions are incorrect, the involvement of toxic heavy metal ions, such as cadmium and lead ions contributing to CKDmfo have not been ruled out yet. In fact, although other researcher including the WHO study have failed to confirm (REF), Jayasumana et al, claimed that arsenic is present in urine, hair and nail samples of patients, and in water samples of their paddy fields, none of these claims were substantiated nor published in reputed scientific journals.

If As(III) forms are accumulated in a particular organ, they can easily reduce oxygen molecules supplied to the organ's cells through blood, generating reactive oxygen species such as hydrogen peroxide, hydroxyl radicals and so on, as shown in Equations (2) and (3).

$$AsO_2^-(aq) + 2H_2O(l) + O_2(aq) \rightarrow AsO_4^{3-}(aq) + H_2O_2(aq) + 2H^+(aq) \quad (2)$$

$$H_2O_2 \rightarrow 2OH \quad (3)$$

These reactive oxygen species are so reactive that they are capable of oxidizing the proteins present in the DNA molecules of cells of those organs. By this way, DNA molecules are damaged due to oxidative stress imposed by reactive oxygen species present in the cells of those organs. Since, kidney cells are very vulnerable to excessive amounts of reactive oxygen species, particularly produced by oxidizing metal ions, people who are regularly exposed to toxic metal ions are become vulnerable to renal damage. Although the standard electrode potential data provide negative ΔG for the reduction of oxygen by As(III) species thus making the process spontaneous, the standard electrode potential data on oxygen reduction by Cd^{2+} ions is not a spontaneous process and hence this reaction is impossible under the physiological conditions. As such, Cd^{2+} ions on their own are incapable of damaging DNA molecules through oxidative stress. However, Cd^{2+} ions can facilitate the generation of reactive oxygen species in indirect pathways thus becoming a possible factor for the damaged kidneys of CKDmfo patients. It is possible that at a higher concentration, As(III) species could damage kidneys of patients suffering from fluorosis.[58] This explains in part, the root cause(s) of the disease when considering the effects of fluoride, cadmium and arsenic ions separately. What is important here is the "Grand Unification Hypothesis" of all distinctly possible root causes. This is a classic example to show the importance of putting data together. Since toxic metal ions can damage organs, among other mechanisms, through oxidative stress caused by reactive oxygen species, in the presence of adequate antioxidants [such as ascorbic acid (vitamin C from citrus plant fruits), polyphenols (tea) and anthocyanins

(extracts of various flowers including "wada") etc.] would prevent harmful effects of reactive oxygen species on vulnerable cells.

3.5 Metal ion-arsenate-glyphosate hypothesis

This is a hypothesis put forward by Jayasumana et al. in a non-refereed, paid, open access journal.[44] They have quite correctly pointed out that many research groups have studied about CKDmfo but none of the hypotheses put forward so far could explain coherently the totality of clinical, biochemical, histopathological findings, and the unique geographical distribution of the disease.

They suggested a relationship between hard water and CKDmfo and glyphosate causing CKDmfo without any scientific data. They suggested that glyphosate, the most commonly used herbicide, chelate metal ions such as Ca^{2+}, Mg^{2+}, Fe(II), Sr^{2+}, donated in tridentate fashion by glyphosate, and other tridentate fashion by arsenate to form a complex polymer to carry arsenic species to the kidneys and miraculously to break this polymer within kidneys to free arsenate ions for them to damage kidneys.

Chemistry in this hypothesis is flawed and the assumptions are incorrect. They have simply assumed that the acidic environments break this polymeric complex to release metals ions, glyphosate and arsenic species. As such, the acidity generated by ammonium ions present in the kidneys has made responsible for this process. They failed to realize that once this compound is ingested, it should first enter the liver before reaching the kidneys (i.e., the first pass effect). The environment in the liver containing concentrated hydrochloric acid is much more acidic than that is prevailing in the kidneys where the acidity is due to ammonium ions. As such, this polymeric compound, if ingested, should breakdown completely in the liver before reaching kidneys. Therefore, the entire mechanism put forward by these researchers is meaningless and of no merit. In part, this seems to be due to the lack of chemistry background and knowledge of these authors.

Glyphosate is chemically *N*-(phosphonomethyl)glycine) with the structural formula shown in Figure 3 (a) in acidic environments and (b) in basic environments and is a broad-spectrum systemic herbicide which is used to kill weeds, especially, annual broadleaf weeds and grasses which compete with commercial crops grown around the globe. Glyphosate in acidic environments exists in its protonated form (Figure 3-a) and therefore it is less water soluble, but it is commonly used as a salt, most commonly as isopropylammonium salt,

which is highly water soluble. However, in highly acidic solutions the NH group can also get protonated in the structure (a) to result in -NH_2^+- while in strongly basic solution carboxylic acid group can also get deprotonated in structure (b) to result in -COO^- group.

It's herbicidal actions were identified in 1970 by John E. Franz, a chemist in Monsanto. Subsequently, the product was brought to market by Monsanto under the trade name Roundup. Since its United States patent expired in 2000, and hundreds of other companies worldwide, particularly from China have started to manufacture glyphosate in global scale. This herbicide is used worldwide in agriculture, railways, dam protection and as household weed killer of flora.[59]

Glyphosate inhibits an enzyme involved in the synthesis of three aromatic amino acids; namely, tyrosine, tryptophan, and phenylalanine; the enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyse the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (ESP) (Figure 3c). It is absorbed through foliage and translocated to growing points of plants and hence it is only effective on actively growing plants and is not effective as a pre-emergence herbicide.

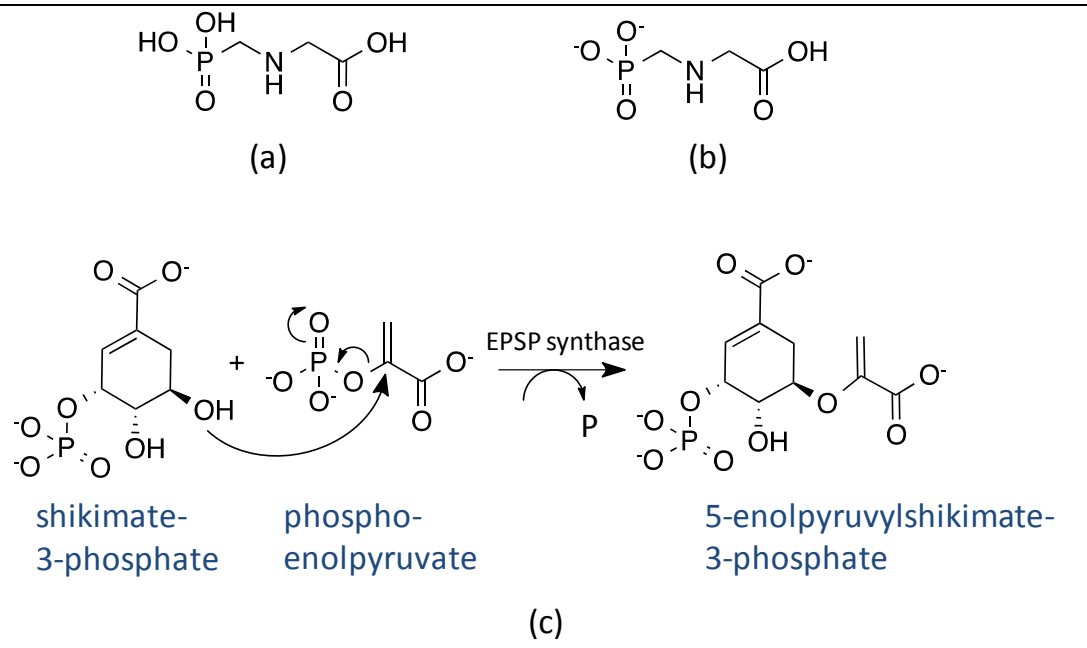


Figure 3: The structural formula of glyphosate in (a) acidic environment and (b) basic environment. Figure 3 (c): Action of glyphosate in inhibiting an enzyme involved in the synthesis of three aromatic amino acids; namely, tyrosine, tryptophan, and phenylalanine; the enzyme called 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyse the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (ESP).

The structure of glyphosate anion in strongly alkaline medium resembles that of phosphoenolpyruvate and therefore, successfully binds to the position of the binding site of phosphoenolpyruvate thus inhibiting the shikimate pathway. This has been proven by X-ray crystallographic studies.[60]

Glyphosate is used before field preparation for weed management. It kills weed and the residues are removed as bound form to cations present in the soil. Very high chelating ability of glyphosate in alkaline conditions means that any residues not absorbed by plants will be bound to any metal ions present in the soil and hence, they are removed from the aquatic environment. Usually it takes about one month after glyphosate application for the application of germinated rice seeds in the well prepared filed. The reason for this 4-weeks delay, is that by that time, glyphosate is not present in the water of the field. Therefore, there is no possibility for glyphosate to be absorbed into rice plants. If absorbed, rice plants will also be killed due to same mechanism. The fact that rice plants are not killed means that glyphosate is not getting absorbed by rice plants. As such, if the presence of glyphosate in rice produced by these paddy fields presents an interesting myth to solve. Additional pesticides are added when rice plants are growing. By the time glyphosate is not present in the water of the rice field. As such, association of arsenate-glyphosate-metal ion triple species to form the so called 'polymeric species' in the environment prevailing in soils is an impossible task since a chemical reaction cannot take place in the absence of reactants.

Research focus of CKDmfo in Sri Lanka has been diverted from serious environmental, occupational, water and soil pollution issues, related to agrochemicals, heavy metals, and fluoride or some unknown toxin, to secondary and hypothetical issues, such as algae, glyphosate, hard water, phosphate, and the poisoning of bordering villages in the NCP by former terrorist groups.[61] Rather than approaching the disease scientifically, interested parties are forcing the government to make politically motivated decisions that would have no impact on reducing the incidence of CKDmfo in the country.

3.6 Ionocity Hypothesis

This is based on the study of Dharm-wardana et al[19] where they have noted that high incidence of CKDmfo in Sri Lanka correlates with the presence of irrigation works and rivers that bring-in 'non-point source,' fertilizer runoff from intensely agricultural regions. Therefore, they have reviewed already put forward hypotheses of As, Cd and other standard toxins for the aetiology of CKDmfo. Although these studies provide reasonable quantity of

data they are inconsistent and inconclusive, and they came up with an expanded hypothesis to explain the facts pertinent to the aetiology of the disease. The first concept related to iconicity was generated by Jasekera et al in 2012.[62] Subsequently, Dhrmawardena et al expanded this ionicity theory where all the ions present in drinking waters in CKDmfo endemic areas as poetical agents that may contribute to denaturing glomeruli basement protein and thereby destroying kidneys. However, there are two major flaws in this theory. Firstly, in patents with CKDmfo, glomeruli are spared and the tubules are predominantly affected. Secondly, the ionic concentration (e.g., phosphate concentrations as they calmed) in drinking water are over 1,000-fold less than what is present in the blood, making this hypothesis also invalid.

Nevertheless, the increased ionicity of reservoir water has been explained due to fertilizer runoff into the Mahaweli River system, redox processes in the soil, and features of 'tank'-cascades and aquifers. Their study seems to match with some facts pertinent to the geology and aetiology of the disease. However, the hypothesis is physiologically not plausible; as with the glyphosate theory, no scientific data are presented.

As Dharma-wardana et al. have pointed out, there are a large number of other reports on the CKDuo in Sri Lanka. There are two conclusions that can be arrived at from these studies. These are

1. "There are elevated levels of cadmium in urine, arsenic in the hair of CKDuo patients, but "no significant difference in urine-arsenic and lead is found in CKDuo cases compared to controls. 96.5 % of the samples tested had glyphosate levels below maximum allowable limits."

2. "Levels of cadmium, lead and uranium in sources of drinking water (see Figure 4 of the report) used by individuals with CKDuo (n = 99) were within normal limits….. samples from wells, ... irrigation canals, ... , had normal arsenic, cadmium and lead levels".[63]

The quality of data depends largely on the proper sample collection and handling, and accuracy of randomisation of sample collection. The fact that lesser scattering in data of WHO reports suggests that their data collection may be better than some other studies. In this regards the inadequate statistical power, varied research methodologies used, poor sample collections techniques, non-randomised sampling, lack of standardised sample collections, etc., have question the accuracy and the validity of all research published to date with

reference to the CKDmfo issue in Sri Lanka. Moreover, for the same reason it is impossible to pool these data together to carry out a meta-analysis.[55]

However, sample collection history is also important in environmental water samples which are multi-variant and depend on the season and many other factors. None of the previous researchers including WHO team have tested so called traditionally non-nephrotoxic ions such as Cl^-, NO_3^- , CO_3^{2-} , SO_4^{2-} , PO_4^{3-} , K^+, Ca^{2+}, NH_4^+ and other commonly occurring ions. Although the relationship of low birth-weight and thus, the nephron number to kidney disease have been correlated[64]; these as well as the effects of malnutrition have not been considered in the WHO as well as in any other CKDmfo studies.

If the glomerular pores get bigger, large protein molecules such as albumin will be filtered out into urine. High osmotic pressure caused by increased iconicity may also help to damage the protein network structure although it is quite unlikely to happen in the human body. At least in vivo studies, it has been demonstrated that high concentrations of cations can denature negatively charged proteoglycans. Dharmawardana et al. have also reported an interesting correlation between ions in the intercellular fluid and in water with fertilizer runoff, i.e., K^+ and PO_4^{3-}. Increased iconicity can cause many effects which include depletion of water molecules near the glomeruli filtration membrane, changes in activities if water and ions, osmotic activity and hydrophobic interactions. Insufficient water consumption, high iconicity of drinking water and excessive sweating caused by hard labour in hot climates worsen these effects. The Hofmeister series that gives the order by which the capability of ions to denature proteins for both cations and anions are given below,

Cations: $NH_4^+ > K^+ > Na^+ > Mg^{2+} > Ca^{2+}$

Anions: $F^- > H_2PO_4^- \geq SO_4^{2-} > HCO_3^- > Cl^- > NO_3^-$

Drinking water in the CKDmfo endemic area contains excessive amounts of F^- generated from weathering of F^--containing minerals such as fluorohydroxyapatite found in the area. Agricultural runoffs and particularly those coming from accelerated Mahaweli programme where excessive amounts of fertilizer applied to tea and vegetable plantations of the upcountry of Sri Lanka invariably contains in excess of WHO recommended levels of NH_4^+, K^+ and phosphates which are high up in the Hofmeister series. Hard work, in the hot environments prevailing in paddy fields, also contributes to excessive sweating and dehydration of the farmers.[9] Because of the high salinity of drinking water and its bad taste these farmers tend to drink inadequate quantities of water thus even worsening the situation.

These factors may contribute to increased iconicity in blood that may affect the tubular reabsorption of ions and water.

3.7 Hypothesis based on Dehydration and Activation of the Fructokinase Pathway

This is another hypothesis on epidemic of CKDmfo in Nicaragua (Mesoamerican nephropathy) that has been linked to excessive recurrent dehydration of sugar cane farmers of Nicaragua who are subjected to CKDmfo.[66] These researchers have studied whether the recurrent dehydration induces renal failure due to the activation of polyol pathway resulting in the generation of endogenous fructose in the kidney that might subsequently induce renal injury *via* metabolism by fructokinase.[66] The study was carried out by using wild-type and fructokinase deficient mice types that were subjected to heat-induced recurrent dehydration. To one group of each type water was hydrated throughout the day while the other sets were subjected to dehydration in the day time followed by hydration in the night where they have ensured the same total amount of hydration to all types. Those that were hydrated only in the night were found to have renal injury with elevated serum creatinine, increased urinary NGAL, proximal tubular injury, and renal inflammation and fibrosis. They found that this renal injury is associated with the activation of the polyol pathway with increased renal cortical sorbitol and fructose levels.

Interestingly, fructokinase-knockout mice with delayed hydration were found to be protected from renal injury. As such, they have concluded that recurrent dehydration can induce renal injury via a fructokinase-dependent mechanism, likely from the generation of endogenous fructose *via* the polyol pathway. Access to sufficient water during the dehydration period can protect mice from developing renal injury. They have predicted that this as a likely cause for the Mesoamerican nephropathy and considering its similarity to Sri Lankan CKDmfo we believe that excessive dehydration may be a cause for Sri Lankan CKDmfo. Excessive dehydration can induce renal injury via activation of fructokinase pathway generating nephrotoxic species such as uralcil and uric acid and also due to increased iconicity of blood thus resulting in Hofmeister type denaturing of proteins.

4.0 Remarks on Hypotheses

Unfortunately, the research groups those who have performed studies appear to have worked independently without collaboration and the great hypotheses put forward by one group have been criticized by other group, failing to realize their importance. This has resulted in extending research studies in undesired directions rather than focusing on the right path,

based on what is learned so far. At the outset, it is clear that the confinement of the disease to a particular geographical area of the Island suggests that the factors, most likely environmental factors, such as excess fluoride in drinking water, which are typical to the region, are contributing to the disease. It is a well-known fact that pesticides contain toxic substances and these toxic substances such as Cd^{2+}, As(III) and Pb^{2+} are deadly hazardous substances to human beings also and hence the possibility of their involvement in damaging kidneys of these patients. However, these ions are virtually non-existent in these waters. Whereas, Jayasumana's hypotheses on the damage kidneys without any liver damage the kidneys, is a guess without scientific ground. It should depend on the relative kinetics such as the rate of the cadmium adsorption by the hepatic cells and the rate of the mass transport of cadmium ions to kidneys from livers. Since the adsorption of metal ions on the bone structure is much more favoured than the adsorption of metal ions on protein structures, it is quite likely that cadmium ions can also cause the CKDmfo disease.

It appears that the researchers have found the starting point and the end point of the thread of this disease but they have failed to find the intermediate parts of the thread. The fact that some of the people living in these areas having fluorosis and that the CKDmfo patients are found only in the areas of the country where the fluorosis is also prevalent mean that the fluoride hypothesis could be taken as a starting point of the disease. This may answer the question that why CKDmfo is prevalent in a particular geographical area of the country where fluorosis is also very common.

The CKDmfo is an Environmentally–induced Occupational disease.[9,11,55,61] The aetiology of the disease can well be described as multi-causative as it has been correctly pointed out by Wimalawansa in many research articles.[9,11,17,61,67-72] The fact that most of the patients are males suggest that in addition to taking these toxic metal ions with drinking water, there should be other mechanisms of toxic metal ion intake by these people because the women in these areas also drink the same drinking water. In general, male farmers spray pesticides and apply fertiliser to their paddy fields. However, due to lack of proper education, they do not take necessary precautions when applying toxic pesticides. Their feet as well as the body are unprotected, they do not use any face masks to prevent the inhalation of aerosol particles of pesticide compounds in water and also their skins are also exposed when they apply pesticides to their farmlands.

In addition, many of these farmers do not adhere to the dosage of pesticides and fertiliser recommended by the department of agriculture and the manufacturers. In fact, depending on the locality and the type of farming they are involved, they are known to use two or ten-fold excessive amounts of pesticides than the recommended amounts. There is also trend now to use pesticides in vegetables after harvesting as a postharvest technology to extend the life time of the vegetables harvested. These misuse of toxic agents further add to the uptake of excessive amounts of pesticides by these farmers thus, contributing to indirect suicide/manslaughter. It is time that the relevant authorities take a serious look at these practices and educate these farmers who produce our staple food.

Another important point is the medical treatment to those patients suffering from CKDmfo. Obviously kidney transplant is an out of question for more than 60,000 patients. One possibility is to use the regenerative medicine. This could be approached in two ways: (i) use of spider web as cell regenerative material and (ii) injection of patient's own stem cells for the repair of damaged kidneys. Both these aspects are in the research scale at present. Since CKDmfo is unique to the above geographical location of Sri Lanka, it is a responsibility of Sri Lankan learned people to get together to carry out research studies in these lines immediately to find ways and means to treat these unfortunate patients who produce our meal.

There is a trend to go for indigenous medicine but this has to be done with extreme care, as some of these agents themselves are nephrotoxic. When kidneys are incapable of purifying blood, it is hazardous to give additional substances which further burden by kidneys. Indigenous medicine relies mostly on mixtures of crude plant extracts which may contain some medicinally active components in very small amounts with an excess of unwanted materials some of which may be toxic. For example, many indigenous medical preparations use carcinogenic, mutagenic and nephrotoxic Aristolochic acids (Both Aristolochic acid I and Aristolochic acid II) containing herbal species in Sri Lankan Ayurvedic Products.

The plant used is Aristocholia indica (SAPSANDA) and is a commonest species used in Ayurvedic medicine practiced in Sri Lanka.[73,74] There are other species such as Aristalochia labiosa, Aristalochia littoralis, Arristalochia bracterolate that are grown in CKDU-prevailing areas are used in traditional medicine. In Sri Lanka, about 66 Ayurvedic Prescriptions contain Aristalochia (SAPSANDA/SASANDA) for treatment of more than twenty diseases. These species are also present in Chinese Herbs and he resulting Renal Failure has been identified

as "Chinese Herbs Nephropathy". There are reports on Chinese herbs nephropathy and its relation to Balkan endemic nephropathy is due to aristolochic acid. US F&D has issued strong warnings on these products in as early as 2001.[75], Herb Danger - Aristolochic Acid Warning.[76-80]

Preventative measures

In taking preventive measures, it is of vital importance to remove all potential (nephron) toxins including fluoride and toxic metal ions from drinking water in the CKDmfo affected region. The present technique is to use electrogenerated aluminium ions to remove these ions from drinking water and a massive plant has been fixed in the Oyamaduwa are of the Anuradhapura area. This is an expensive technique requiring day-to-day maintenance. There is a group of villagers looking after this plant. They are expected to remove the massive aluminium electrode every day and remove sludge and fix it for next use. What would be more economical and simple technique would be to design filters containing hydroxyapatite-porous carbon nanostructures to resemble the nanotechnological architecture of the partially burnt bones since it would not be a fair thing to kill animal to take their bones for human use. Besides, the extensively available fluorohydroxyapatite in the Eppawela area can be easily converted to pure hydroxyapatite nanoparticles using urea as a fuel.

We have developed this process to produce pure hydroxyapatite nanoparticles required for making prosthesis materials biocompatible, osteointegrating, non-toxic and non-corroding. We will be modifying the process to produce porous carbon-hydroxyapatite nanotechnological architectures. These novel materials derived from local minerals may well be used to produce safe drinking waters for these poor villagers. What is required now is to carry out focussed research studies to combat this deadly disease to protect farmers who produce rice for the entire population of the country. Till then the most cost-effective option is to use the reverse osmosis water purification technology[70], until the Water Board is able to provide centrally-purified, pipe borne water supplies to the entire regions.[9,17] However, we strongly believe that it would be disastrous to give chlorinated water for the vulnerable people since even the residual chlorine can dramatically affect the kidneys of the vulnerable people since chlorine is more powerful oxidant than even dichromate at least under standard conditions. We believe that UV disinfection of properly treated water to be the best option to sterilise drinking waters.

Considering all observations to-date, carefully selecting and preventing and/or regulating the use of toxic substances is a starting point. Since there is no pesticide that is non-toxic, and the modern agriculture cannot be sustained without agrochemicals, blanket banning of pesticide by the government is not a sensible, viable, or a realisation option.[61,69,72] Pesticides invariably contain toxic substances to kill pests that are damaging farmer's crops. There is no concept of "safe" pesticides and hence it will be impossible to draw a line to ban some pesticides. It is all or none concept. What is meant by "none" is that without pesticides it would be impossible to produce food for 21 million people living on this small Island. The concept of "organic farming" is useful but is not sufficient to produce food for all. As such, whether we like it or not, pesticides are a must or people of the country will quickly die due to starvation. This means that the pollution of the environment is essential for making food. It has gone a long way ahead from Chief Seattle's time in the early days of the North America to live without harming the environment. Due to exponential increase in the world population making food in amounts sufficient to feed everybody requires the pollution of the environment. When the environment is polluted, various diseases naturally evolve without control. The obvious way in would be to remove pollutant that we have added to the environment. It appears, therefore, that the pollution and de-pollution of the environment in cyclic manner are required for our survival!

There seems to be a correlation between quality of drinking water and the amount of water take up to prevent dehydration and for rehydration have direct correlation with CKDmfo. As such, it is the responsibility of the Sri Lankan Government and other responsible authorities to provide these people with good quality drinking water free of these pollutants and to educate them of the need of preventing dehydration through massive media campaigns. If these obvious measures are taken, we believe that the epidemic can be successfully controlled and the lives of our staple food producers can be secured.

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