Investigating the microcosm to illuminate the macrocosm: Creating an integrated pathway to investigate chronic kidney disease of unknown aetiology in Sri Lanka

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"*Life is a paradox, and that is precisely why it is worth living*." – Paulo Coelho (Brazilian novelist)

Introduction

Chronic kidney disease (CKD) is a widespread health issue, accounting for at least 10% of the world's death toll.[1] Notably, the contribution of each risk factor for CKD varies across regions. High blood pressure has been identified as the major contributor to CKD in East Asia, Eastern Europe, tropical Latin America, and Western sub-Saharan Africa, while impaired glucose tolerance dominates in the rest of the world. Meanwhile, certain risk factors for CKD are limited to specific communities, emphasizing the relevance of a customized approach to each disease sub-group.[2]

In HIV-related nephropathy, snake bites, and infection-associated nephropathies the causative factors are obvious. However, in Balkan Nephropathy, the identification of the underlying remained as an enigma for over half a century. Similarly, two other endemic nephropathies, Itai-Itai disease in Japan and lead nephropathy in Australia, have been attributed to environmental contaminants.[3]

In Sri Lanka, the emergence of a mysterious disease was first reported in the North Central Province, among young farmers who presented with acute uremic emergencies.[4,5] In parallel, an identical disease was reported in El Salvador.[6] Soon afterward, there was growing evidence for the emergence of chronic kidney disease of unknown aetiology (CKDu) in comparable communities in Central America, India, Taiwan, Egypt, Tunisia, and Morocco[7,8] and more recently, in the central valleys of California. In general, these patients exhibited minimal symptoms, normal blood pressure, grossly abnormal renal functions, and small echogenic kidneys on imaging.[9,10] Histological evidence was compatible with chronic interstitial nephritis, most probably secondary to an environmental toxin.

Faith or fate: Multiple risk factors or multifactorial

There was a series of medical catastrophes such as Malaria, Cholera, animal attacks, fluorosis, goiter, and kidney stones among these populations. Obviously, associations between genetic, epigenetic, environmental, and behavioural risk factors with a higher prevalence of CKDu in endemic areas have been reported over the years.[12] Nanayakkara et al. reported a significantly higher prevalence of SLC13 and several other gene variants in individuals affected by CKDu compared to those unaffected in endemic areas. Selenium deficiency, a risk factor for CKD, has been identified among endemic populations (63%).[13] Also, associations between CKDu and dehydration, heat stress, betel chewing, smoking, consuming illicit liquor ("moonshine"), unsafe use of agrochemicals, *Leptospira*, Hantavirus, and inadvertent use of analgesics have been reported in some communities. Contaminants in the water, either with natural elements or agrochemical related, is one of the most compelling explanations for the mosaic pattern in the geographical distribution of CKDu. Indeed, natural contaminants, such as fluoride, calcium, magnesium and sodium have been reported in higher concentrations in these areas.[14] Also, agrochemical contaminants with

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possible nephrotoxic effects have been reported in several studies.[15] Additionally, undetected hyperglycaemia and hypertension, in addition to the aging population may have increased the risk of developing CKDu.[16] Therefore, at least according to some authors, CKDu is an inevitable outcome in endemic communities due to consequential exposure to multiple risk factors.[17]

Nevertheless, there is convincing evidence for CKDu to stand alone as a unique multifactorial disease. Developing a hypothetical model for the occurrence of CKDu is crucial in understanding the pathophysiological pathways of the disease (Figure 1).

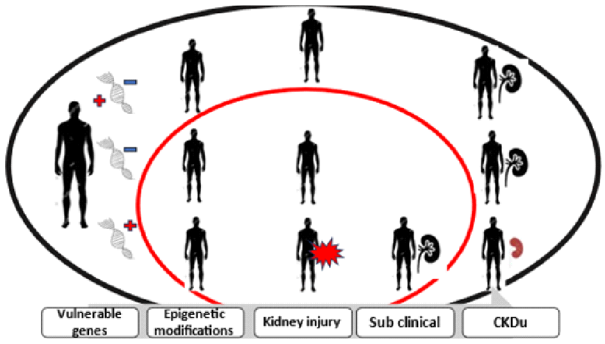

Figure 1. Hypothetical model illustrating people with susceptible genes, following years of epigenetic modifications, develop kidney injury as a result of exposure to a specific toxin in 'at-risk' environments.

Case definition of CKDu

CKDu was initially reported as a proteinuric disease, hence, urine protein-based methods have been recommended for screening.[18,19] On the contrary, as proteinuria is not characteristic in interstitial nephropathies, it is unlikely to be characteristic in CKDu. Therefore, we evaluated the performance of urine albumin creatinine ratio (ACR) against two functional markers for the identification of CKDu. Cystatin C, serum creatinine and ACR were 95%, 88% and 32% capable of identifying CKDu, respectively.[20] Subsequently, we reported the superiority of a combination of either Cystatin C or serum creatinine with ACR (>10mg/g) to enhance the detection rate of CKDu in endemic populations.[21,22]

The long-awaited, refined, multi-level case definition based on the consensus opinion of experts was published in 2018.[23] However, complexity, unknown specificity, and sensitivity were major concerns in real-world settings. Soon afterward, we hypothesized that common clinical characteristics could predict the presence of tubular interstitial disease. Interestingly, our prospective biopsy-based study confirmed that the negative albuminuria and normal serum albumin in individuals aged between 35 to 60 with impaired kidney functions could predict the presence of primary interstitial disease with 80% specificity and sensitivity. Hence, it is almost diagnostic of CKDu in endemic areas. This prediction remained accurate even in the presence of other comorbidities such as diabetes or hypertension.[24]

Early diagnostic marker for CKDu

A specific marker of a disease must be either the etiological agent itself or a biomarker directly originating from the specific pathogenic pathways of the disease in interest. Therefore, identifying a specific marker is equal to moving one step towards identifying the aetiology. The performance of tubular markers has to be superior to creatinine in the diagnosis of tubular interstitial diseases. Urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM1), cystatin C (CST3), beta 2 microglobulin (B2M), Osteopontin (OPN), alpha 1 microglobulin (A1M), tissue inhibitor of metalloproteinase 1 (TIMP1), and retinol-binding protein 4 (RBP4) have been identified in related to various pathogenic pathways in kidney diseases. We evaluated the performance of these markers in CKDu against multiple control groups. According to our results, the efficiency of a four-marker panel consisting of A1M, KIM1, RBP4, and OPN was superior to serum creatinine for early identification. The panel has the added advantage of demarcating CKDu from other CKDs.[25]

Clinical characteristics of CKDu

It has been commonly believed that the CKDu is minimally symptomatic and clinical characteristics are identical to those already described for CKD. However, even though nonspecific, we were able to report a significant symptom burden among patients with CKDu.[26] Anaemia, CKD-bone mineral disease (CKD-MBD), characteristic biochemical profile, and cardiovascular disease are among typical manifestations of CKD. Indeed, the mainstay in the clinical management of CKD is prompt identification and treatment of these manifestations. We reported a higher prevalence of anaemia (72%) and iron deficiency across all stages of CKDu. Interestingly, there has been a negative correlation between inflammatory status and haemoglobin concentration, irrespective of the disease severity.[27] In contrast to the reduced bone density and increased fracture risk in CKD, there was an increase of bone density in CKDu with the

advancement of the disease. Environmental exposure to fluoride and a low phosphate diet could be implicated in the lower prevalence of typical MBD in CKDu.[28] More importantly, both cardiovascular events (28.6%): which contribute to 50% of deaths in CKD[29], and the predicted risk, were remarkably lower (<10% in 97% of the population) in CKDu. Age (>50 years), Troponin I, and hyperuricaemia were associated with the CVD in CKDu.[30] There were no striking abnormalities in the biochemical profile other than a higher prevalence of vitamin D deficiency (65%), hyperuricaemia (40%), and hypokalaemia (20%). More importantly, it has been observed that at least 50% of the population exhibited features of concealed dehydration, an increase in serum osmolality, which positively correlated with serum sodium levels. It is noteworthy to emphasize the decisive role played by noncommunicable diseases even in rural communities; 55%, 15.7% and 59.8% of the study population developed hypertension, diabetes mellitus, and impaired glucose tolerance after the diagnosis of CKDu.[31]

Symptomatic subgroup of CKDu

CKDu is commonly believed to be a slow progressive chronic disease. Besides, we reported an acute form of the disease in endemic areas of Sri Lanka in 2016, followed by Fisher et al in Central America in 2017.[32] Fever, back pain, dysuria, and general ill health were identified as characteristic symptoms of Sym-CKDu. Patients initially had systemic evidence of inflammation, kidney dysfunction, and normal size kidneys with biopsy evidence of various combinations of acute and chronic lesions. Over time, the symptoms subsided with a reduction in activity in biopsy and diminishing the sizes of the kidneys. At the end of the three years, a substantial number of patients reached stage 4 CKD.[32]

Pathological transformation of healthy individuals to established CKDu

We selected two biomarkers, serum transforming growth factor-beta 1 (TGF-b1) and retinol-binding protein 4 (RBP4) to study the transformation process in CKDu. It has been reported, that TGF-b1 is activated in acute kidney injury, while positively or negatively regulating the inflammatory responses based on the nature of the toxin. Therefore, TGF-b1 is an anti-inflammatory as well as profibrotic biomarker that positively regulates the glomerular and tubulointerstitial fibrosis in CKD/CKDu. According to our results, TGF-b1 is closely associated with the fibrotic process in CKDu, and could be a promising biomarker to evaluate the disease progression.[32] Tranilast, losartan, glitazones and imatinib mesylate, block the production, activation, or biological activity of TGF-β which may have therapeutic implications in CKDu.

RBP4, a small protein of 21kDa, is freely filtered through the glomeruli upon the release of retinol. It is then readily reabsorbed by the proximal tubule, the most likely site of initial injury in CKDu, and finally catabolized. Interestingly, the serum RBP4 levels were expressed differentially and in an independent manner in CKDu. Indeed. the mean RBP4 levels were significantly high in CKDu in comparison to both, CKD and healthy controls.[33] Hence, it can be proposed that the elevated levels of RBP4 in CKDu were more likely due to failure of catabolism: a proximal tubular functional failure, rather than structural damage.

Ultrastructural lesions in CKDu

Interstitial fibrosis, periglomerular fibrosis, tubular atrophy, and glomerular sclerosis have been described as typical histological features of CKDu. Characteristically, immunofluorescent studies showed negative results for IgA, IgG, IgM, and compliments.[9,10] Effacement of foot processes, mild wrinkling of the basement membrane, and subendothelial oedema have been demonstrated in electron microscopic studies. There were partial tubular atrophy and loss of brush border in tubular interstitial compartment of the kidney. Interestingly, EM features of toxin mediated nephropathies, such as metal deposits, dysmorphic mitochondria (Cd) and accumulation of metallothionein (As, Cd) have not been demonstrated in CKDu.[34,35] More recently, characteristic large, dysmorphic type of lysosomes were described in relation to CKDu.[36] Almost all of these studies were on late and established CKDu. For the first time, we reported the EM features of early disease i.e. Sym-CKDu. In this study, the partial loss of brush border in proximal tubules, detachment of tubular cells, enlarged vacuoles, mitochondrial swelling associated with loss of cristae, and dysmorphic lysosomes with electron-dense aggregates were identified as early lesions. However, none of these lesions, including dysmorphic lysosomes were pathognomonic for the histological diagnosis of CKDu.

Rate of progression and outcome of CKDu

In general, decline of GFR in CKD is predictable and usually homogeneous at least in the early stages of the disease. Hypertension has been identified as a risk factor for faster progression of CKDu.[16] Also, the treatment with enalapril, the first line antihypertensive in CKD was proven to be effective in reducing proteinuria in CKDu.[37] According to our analysis of an observational cohort, it was found that the progression of CKDu

was faster (>3ml/min/1.73 m^2/year) in the majority (56%). However, a younger group of people who presented with early disease had an accelerated progression (>5ml/min/1.73 m^2/year). Further, male sex and smoking have been identified as risk factors for faster progression of CKDu.[38]

Mortality in CKDu

Investigating the mortality of a chronic disease in a disorganized system is challenging. As a result, the mortality pattern of CKDu has not been properly reported. However, a total of 2491 (25.8%) deaths have been reported in 9653 /CKD/CKDu patients in a cross-sectional study.[39] Identifying the gap, we conducted the first mortality analysis on an observational cohort (2006 to 2014) of about 3000 patients in Girandurukotte. Unfortunately, the medical records were traceable only for 153 deaths out of the total of 203 deaths (mean age 58). Of these, 94% of the patients (80% farmers) succumbed to death due to ESRD, never receiving renal replacement therapy.[40] Our findings emphasize the need for urgent and early interventions to mitigate the outcomes of this serious condition.

Interventions for changing the outcome

Screening of the total endemic population was the response from the Ministry of Health to mitigate the grave outcome of CKDu. Also, updated clinical management guidelines were published for the management of CKD/CKDu. In parallel, clinic books were redesigned according to the revised management protocol. Surprisingly, a significant proportion of patients did not require any medications. Thus, patients with hypertension, hypokalaemia, hypernatremia, acidosis, MBD, anaemia, hyperuricaemia, dyslipidaemia, and patients with unexplained faster progression were methodically streamlined and intervened. In a subsequent study (2018), we reported a significant reduction in annual eGFR decline (less than 2mL/min/1.73m^2, p=0.0184) which supports the strategic approach to retard the burden.[41] Delaying renal replacement therapy by one year for one patient, saves at least one million Sri Lankan rupees!

Place of immunomodulatory treatments in Sym-CKDu

Acute interstitial cell infiltrate which heals with fibrosis, and tubulitis indicates dysregulated immunological pathways in CKDu. In similar scenarios, immunosuppressants have been successful in changing the outcome. Therefore, a 'double-blind' randomized control clinical trial was conducted to evaluate the effectiveness of prednisolone or doxycycline in the treatment of Sym-CKDu (SLCTR/2014/00). Obviously, in prednisolone-treated group, none reached stage 4 during the follow up, while 17% of the not treated participants reached the CKD stage 4 (P 0.02). Although the sample size was small, our results support the immunomodulatory treatment at least in selected cases.[42]

The mystery continues

A recent review on CKDu concluded thus- *"Previous efforts have had a limited impact on addressing CKDu"*.[43] Nevertheless, discerning the underlying causes of a chronic disease is a complex and demanding endeavour, particularly in settings with limited resources.

CKDu revisited

An educated guess, also known as an informed hypothesis, is an effective and time-tested strategy for developing a hypothesis. However, it is important to note that research is much more than just making educated guesses. Therefore, once a hypothesis is formulated, it needs to be rigorously tested through robust experiments.

Real links versus mere correlations

Informed by past failures and guided by current CKDu insights, we devised multiple studies aligned with our hypothesis, all aimed at the overarching goal of pinpointing the aetiology. In the big picture, identifying the aetiology of CKDu looks straightforward as the disease is limited to defined geographical locations. Therefore, demarcating risk factors has to be straightforward in comparison to unaffected areas. Nevertheless, the cross-sectional study design, the most popular study design among researchers during the last two decades was unable to reveal the aetiology of CKDu with certainty. It is more likely due to the failure in differentiating disease-specific risk factors from general unhealthy exposures confined to endemic areas for CKDu.

Hantavirus and CKDu

We carried out a cross-sectional study, followed by an unmatched case-control comparison in two geographically distinct areas of Sri Lanka, to determine whether exposure to hantaviruses is a potential risk factor in patients with kidney disease. The study indicated that 50% and 17% of kidney patients and controls were seropositive for Hantavirus in the endemic area compared to 18% and 7% in the non-endemic area. The odds of exposure to hantaviruses were higher for kidney disease patients than for controls in both

areas.[44,45] Although we were delighted to observe this association, it may be spurious and could only be reflecting the seroprevalence of Hantavirus in two different communities.

Geographical dispersion of CKDu

Besides the geographical bias of CKDu, limited studies have been carried out on differentiative factors of an endemic area from a non-endemic area.[39] We critically evaluated the anthropological, biochemical, and geo-climatic factors of an endemic area (Wilgamuwa) against a non-endemic area (Mathurata) for CKDu in Sri Lanka. It was obvious that Wilgamuwa was abruptly populated for agricultural purposes, with large-scale irrigation projects. In comparison, Mathurata population is a result of natural migration. As expected, geo-environmental features are typical of dry zone lowlands in the endemic area, in comparison to wet zone hill country features in the non-endemic area. The landscape of paddy fields, types of crops, water sources, and individual engagement in farming were significantly different between the two areas but not the sociodemographic characteristics, agrochemical usage, and biological characteristics.[46]

The mosaic pattern of spatial distribution of CKDu stipulate the significance of geo-environmental factors for the occurrence of CKDu.[47] On the contrary, the majority of households affected by CKDu remain unaffected, even though the exposures are almost identical. Interestingly, a carefully selected very small sample (30 Males of 30 - 65 years of age) could predict the endemicity of CKDu. In comparison to nonendemic areas, people with high normal range creatinine (>90) were significantly higher in the endemic area which strongly supports the existance of subclinical CKDu. Additionally, there were discernible areas within the endemic region for CKDu that exhibited characteristics similar to non-endemic areas.[48]

Microenvironmental exposures in CKDu

In this study, groundwater sources consumed by biopsy-proven CKDu patients were analysed to identify causative factors of affected individuals. Groundwater used by CKDu patients is predominantly of the Ca-Mg-HCO3 type. Over 88% of the samples showed excess hardness, while 44% showed high fluoride content. Principal component analysis (PCA) indicated that the dissolution of aquifer minerals and ion exchange processes are most likely responsible for the groundwater geochemistry in the study terrain.[49]

Outcome evaluation in observational cohorts

CKDu is a slowly progressive disease. According to the available evidence, children are not at risk of developing the disease. The mean age of CKDu patients is around 50 years. Considering the reported rate of progression, the exposure may have happened in the second or third decade of life. Therefore, due to the long incubation period, it may take a couple of decades to observe the outcome changes in prospective studies. To shorten the timeline, serum creatine, a nonspecific and late marker, has to be replaced by an early diagnostic marker.[50]

Hypothesizing that continuous exposure to the risk factor causes a faster decline of eGFR, we conducted a community-based study to identify associated socio-behavioural, environmental, and biological risk factors of CKDu.[51] In this study, 300 confirmed cases of CKDu were enrolled and four monthly evaluations were performed for three years. At the end of three years, a majority of them (68%) had changed their drinking water source to RO-Water. Interestingly, there was a statistically significant lower eGFR in historical well water users in comparison to non-users. It is also noteworthy that slower CKDu progressors were located at higher elevations in the drainage basin and relatively faster CKDu progressors were located at lower elevations. Alarmingly, 68% of the sampled household wells had detectable agrochemical compounds with concentrations above global water quality standards.[41]

Surveillance and exposure evaluation in Sym-CKDu

Trace elements

Our findings on Sym-CKDu indicate the importance of surveillance followed by focus evaluations to identify the evidences of recent exposures.[32] Trace elements have been postulated for the occurrence of CKDu due to many reasons.[52] Hypothesising the Sym-CKDu resulted from recent exposure to a toxic trace element, we evaluated trace element levels in rice samples of patients. Of the 32 patients, 26 were histologically confirmed tubulointerstitial disease in biopsies. The results revealed that the mean values of Cd, As, and Pb were 0.18, 0.055, and 0.135 mg/kg, respectively. These values were reasonably below the recommended toxic levels by Codex and WHO. However, 31% of Sym-CKDu patients were consuming rice contaminated with at least one of the nephrotoxic elements Pb, As, and Cd.[53]

Leptospirosis

Leptospirosis, a spirochaetal infection that is common among agricultural communities, has been proposed as a potential etiology for CKDu.[54] In this investigation, 59 clinically diagnosed Sym-CKDu patients, 72 and 71 healthy controls from CKDu endemic and non-endemic regions, were evaluated to identify the association between *Leptospira* and Sym-CKDu. The seroprevalence of 18.6%, 6.9%, and 7.0% was observed in Sym-CKDu, endemic, and nonendemic groups, respectively, from the rapid IgM test. Among 19 serovars tested, the highest seroprevalence was observed at 72.9, 38.9, and 21.1% in Sym-CKDu, endemic and nonendemic for serovar Leptospira Santarosai and serovar Shermani.[55]

Epigenetic changes of CKDu

CKDu is exceptionally limited to adults, which indicates either adult behaviour, epigenetic modifications or, both are required to acquire the disease. Heritable changes in gene expression without changing the DNA sequence are defined as epigenetics. MicroRNAs epigenetically regulate gene expression by modulating the expression of DNA methyltransferases, histone deacetylases, and other epigenetic regulator genes. Interestingly, in our study on CKDu, low levels of urinary miRNAs were observed in CKDu patients, and in the control samples collected from the endemic region. These results suggest an association of an environmental factor causing epigenetic changes may predispose for CKDu.[56]

Conclusion

It is obvious that most of the knowledge generated through CKDu is applicable to CKDu in Sri Lanka, and other affected countries, as well as in the management of CKDs. However, addressing such intricate medical challenges necessitates dynamic study models that integrate existing evidence with advanced technology and novel analytical tools.

CKDu, the microcosm exploration provides an opportunity to identify commonalities, test innovative research approaches, and develop targeted interventions that can improve the overall management and prevention of CKD, the macrocosm.

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