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Enigma of an endemic chronic kidney disease: the quest for the Holy Grail of etiology

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Recognition of a new form of chronic kidney disease (CKD) in the paddy-farming heartland of Sri Lanka coincided remarkably with the emergence of a similar CKD from sugar cane-growing communities of Central America.[1,2,3] In both regions, the gradual recognition that the disease is of epidemic proportions has led to this being highlighted in mass media as well as medical literature, the first published reports appearing in early 21st century,[1-4] but this was recognized at least in the early nineteen-nineties, and may have been present earlier.

There were many features common to the CKD in both Central America and Sri Lanka; almost all patients came from agricultural communities, many were involved with farming activity, but some non-farming individuals living in the same community too were affected, and the common traditional causes of CKD such as diabetes and hypertension were not causative. In Central America, the disease was termed Mesoamerican Nephropathy (MEN) and the Sri Lankan nephropathy was commonly called CKDu (Chronic Kidney Disease of Uncertain cause). Later, similar reports of CKD emerged from the Uddanam District of Andhra Pradesh in South India which is now known as Uddanam Nephropathy,[5] and more recently from Tamil Nadu state of India.[6] The name CKDu is highly misleading as it confusingly mixes up all CKD of unknown cause together, which includes those presenting with small kidneys with uncertain diagnosis, those labelled as of unknown cause when diagnostic facilities are poor, and those with other idiopathic chronic tubulo-interstitial nephropathies.[7,8] It has been proposed that the name "chronic interstitial nephropathy of agricultural communities" (CINAC) is a more appropriate common term for this CKD,[9] as the two factors, namely, that it is a chronic tubulo-interstitial nephropathy (CTIN), and that almost all affected are from areas of high agricultural activity, are two of the identified common "signatures" of the disease.

For a disease with devastating consequences, the innocuous early features of presentation often hamper early diagnosis. Absence of proteinuria even in stage 3 CKD has made a single assay for micro-albuminuria unreliable as a screening measure. The multi-level case definition criteria proposed by the Sri Lanka Society of Nephrology[10] excludes diabetics and those with more than mild hypertension from the "suspected" level of diagnosis as the prevalence of diabetes and hypertension in most areas of Sri Lanka is more than 10%. Diagnosing all patients with diabetes and moderate-to-severe hypertension who have proteinuria as "suspected" CKDu patients was thought to impose an unrealistic number of cases on the CKDu case registries and exaggerate the magnitude of the problem to policy makers.[10] While it is understood that diabetics and hypertensives can and do get CINAC,[11] this decision was considered to be justified, as all diagnosed CKD cases are routinely referred to specialist clinics, and patients with diabetes and hypertension who have CKDu would be distinguished in these better-equipped clinics with facilities for retinal screening and renal biopsies, and eventually counted back on the CKDu databases. Nevertheless, diagnostic criteria proposed from both Sri Lankan and Mesoamerican researchers can only narrow the diagnosis down to a primary chronic tubulo-interstitial disease of any cause. Most pathological analyses agree that this is a primary chronic tubulo-interstitial disease with secondary glomerulosclerosis,[12,13] although some have noted that the glomerulosclerosis is disproportionate to the amount of interstitial fibrosis and tubular atrophy, hence there may be some degree

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of primary glomerular involvement, speculated to be an ischemic glomerulopathy, supported by the presence of vascular hyalinosis.[14] Since many other causes of CTIN can exist in the community, which includes diabetes, hypertension and other ischemic and toxic nephropathies,[8] a specific diagnostic feature for CINAC is sorely needed. A recent detection of such a pathological lesion is possibly a "signature" for CINAC which could differentiate it from other common CTIN.

An international consortium of researchers from USA, Sri Lanka, El Salvador, France, Slovenia and India, led by University of Antwerp in Belgium has reported a distinctive constellation of lesions in the proximal tubules of CINAC patients.[11] Modified Jones staining of renal biopsy specimens from 3 endemic agricultural areas in Sri Lanka, El Salvador and India and from a "non-endemic" agricultural area in France revealed silver positive granules in the cytoplasm of cortical proximal tubular cells (PTC) under light microscopy (LM) and electron microscopy (EM). These granular inclusions were seen inside dysmorphic bodies and only seen in PTC and not distal tubular cells (DTC). The dysmorphic structures were proven to be lysosomes by staining with lysosomal associated membrane protein 1 (LAMP1), cathepsin B and lysosomal acid phosphatase. On EM nearly all enlarged and dysmorphic lysosomes contained homogenous, nonmembrane bound, dark electron dense rounded and irregular "aggregates" dispersed throughout the lysosomal matrix. The PTC were demonstrated to be non-proliferative with little apoptosis and had fragments shedding into the lumen suggesting senescence. In contrast, the DTC had prominent proliferative activity and frequent apoptosis, indicating well-functioning tubules, probably compensating for the dysfunctional PTC. This phenotype of the senescent PTC and hyperfunctioning DTC is highly suggestive of a toxic nephropathy.

LM/EM examination of healthy and pathological controls revealed that the same lysosomal phenotype is seen in some other toxic nephropathies; namely, kidney transplant patients on calcineurin inhibitors (CNI), those on lithium, lomustine, clomiphene and tenofovir and in light chain nephropathy. It is of note that all above drugs (and light chains) have the ability to modify the calcium and calmodulin dependent serine/threonine protein phosphatase enzyme calcineurin.[11] While it was also seen in a small number of implantation kidney donor biopsies (9%), the frequency of the lesion increased remarkably with exposure to CNI after 6 months (50%) and 12 months (67%) as shown in protocol biopsies at these intervals and in 76% of indication biopsies where CNI exposure is expected to be more. The same lesion was reproduced in rats given cyclosporin- a CNI- but was not seen in dehydrated rats. This dysmorphic, aberrant lysosomal structure was not seen in many other chronic renal conditions, including several types of chronic glomerulonephritides and pyelonephritis.

So, what is the significance of this hitherto unreported finding? Recurrent heat stress and an environmental toxin are the two main proposed etiologies to CINAC with possible contributions from genetics and infections. Heat stress with recurrent acute kidney injury has been proposed as the main driving factor behind global CINAC epidemics but there is much evidence against this hypothesis which has been reviewed recently.[15] The morphological similarity of the lysosomal inclusion bodies to that seen in some other toxic nephropathies and lack of these in dehydrated rats gives strong credence to the second theory of a possible toxic etiology to CINAC, together with prior evidence.[16] Secondly, presence of these aberrant lysosome – along with the history and other clinical features – can be used as a signature diagnostic feature for CINAC in endemic areas as well as in areas hitherto considered non-endemic. Thirdly, the histopathological analogy with nephropathies caused by CNI toxicity suggests chronic calcineurin inhibition as a possible toxic pathway in pathogenesis of CINAC. Fourthly, there are some environmental toxins – namely pesticides such as paraquat, glyphosate and pyrethroids – which have known calcineurin inhibitor properties. Pesticides have been proposed as possible etiological agents for CINAC,[17,18] but epidemiological evidence up till now has been inconsistent,[19] perhaps because most epidemiological studies on pesticide use have been poorly designed.[20] The proposition of CINAC as a lysosomal tubulopathy possibly with an implication of chronic low-dose CNI toxicity in its pathogenesis opens up new avenues for research on pesticides as possible causative agents. Finally, the presence of these aberrant lysosomes in transplant patients on CNI suggests a new diagnostic approach to toxicity with tacrolimus and cyclosporin, drugs commonly used in organ transplantation.

There are many questions which require answers. The number of CINAC patients studied has been relatively small (34 patients). A double blind, controlled study is needed with a larger number of patients and controls to determine the utility of this pathological feature as a specific diagnostic test for CINAC, especially differentiating this from CTIN of other causes. CNI activity and other features of calcineurin inhibition needs to be investigated in those with CINAC, with controls. Further research should be undertaken on the lysosomal bodies themselves which should include a systematic morphometric analysis of these.

Transcriptomic and proteomic studies of renal and animal tissue will give clues to a putative toxin. Are these lysosomes responsible for the observed tubular injury or are they just an epiphenomenon? Can they be reproduced by animal studies and human cell culture studies with an array of toxins including pesticides, heavy metals, fluorides or a combination of these? In investigating the effects of toxins on humans and animals it is often forgotten that the published reference "toxic levels" may mean little in long-term, low-dose toxicity, and that acute and chronic toxic effects are not substitutable in interpretation of effects of toxins. The study by Vervaet, et al does not prove a toxic etiology but gives strong evidence for it, nor does it confirm chronic CNI inhibition as the main pathway of pathogenesis of CINAC nor has it made the role of pesticides in etiology of CINAC a certainty, but opens our eyes to exciting new avenues for research on the enigma of the etiology of a devastating kidney disease.

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