Kidney360

Is an Environmental Nephrotoxin the Primary Cause of CKDu (Mesoamerican Nephropathy)? PRO

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Chronic interstitial nephritis in agricultural communities (CINAC), also named CKD of unknown origin (CKDu) or Mesoamerican nephropathy (MeN), is defined as a form of CKD affecting young men and—less often—women. Its etiology is unrelated to diabetes, GN, hypertension, or other known causes of CKD. Patients with CINAC live and work mainly in poor agricultural communities, often in hot tropical regions, and are exposed to potentially toxic agrochemicals through work by ingestion of contaminated food and water, and/or by inhalation (1,2). The epidemic dimension of CINAC was first observed in the 1990s in Sri Lanka and Central America and has since been found to be an important cause of CKD-related deaths in an increasing number of countries (3,4).

Patients with CINAC have bilateral, small, irregularly contoured kidneys with mutual size discrepancy of <1.5 cm on ultrasound, as observed in advanced cases of analgesic nephropathy and aristolochic nephropathy (5,6). Almost all patients with CINAC exhibit a proximal tubular lesion: tubular cell atrophy, basement membrane thickening, deficient proximal tubular cell (PTC) regeneration, loss of function, distal tubular proliferation/hypertrophy, and variable extents of interstitial fibrosis and cellular infiltration. Overt glomerular injury is rare in the early stages, whereas secondary glomerulosclerosis develops in later CKD stages. By electron microscopy, PTCs demonstrate enlarged dysmorphic lysosomes ($\geq$1.2 μm) containing homogenous nonmembrane-bound, electron-dense, rounded/irregular "aggregates" dispersed throughout the light-to-medium, uniform, electron-dense lysosomal matrix (7) (Figure 1). These features are also observed in a number of toxin-induced nephropathies.

Despite many similarities, a toxin-induced etiology has mainly been considered in Sri Lanka, whereas recurrent heat stress–induced AKI has been hypothesized to be the predominant cause in Central America.

There are arguments disputing a major causal role of heat stress and dehydration in CINAC. The epidemic upsurge of CINAC was first observed in the 1990s in Sri Lanka and Central America shortly after the introduction and rapid increase in the usage of agrochemicals and the replacement of labor-intensive buffaloes by mechanization. During this sudden increase

in prevalence, there were no major changes in ambient temperature and rainfall that could foster an epidemic-scale increase in heat stress. The dehydration hypothesis is incompatible with the mosaic geographic distribution of CINAC-endemic provinces in Sri Lanka, in view of a homogenous distribution of the agricultural population with the same climate, equal hours of sunshine and rainfall, as well as type of agriculture (1). Furthermore, in contrast to El Salvador and Nicaragua, CINAC epidemics are not reported in the well organized national public health registry of Cuba, despite being a sugarcane-cultivating country with similar geo-climatic factors as the Central American region. There are also many individuals globally (*e.g.*, those working in blast furnaces, miners working deep underground) who are exposed to the same harsh conditions as sugarcane workers but who have never developed rapidly progressive CINAC, despite regular screening programs during active working periods and retirement.

In Sri Lanka and El Salvador, a number of studies show a chronic interstitial nephritis in women comparable to the disease observed in male agricultural workers (8,9). Women, who stay at home, are less or negligibly exposed to heat stress and the harsh working conditions in the fields. Nevertheless, they develop CINAC, with a slower natural course as compared with their farming husbands (9). This can only be explained by their ingestion or inhalation of the same toxins present in the environment they share with their partners.

In an endemic area in Nicaragua, it has been shown that school children aged 12–18 years with no prior employment history have elevated urinary concentrations of the tubular injury markers neutrophil gelatinase-associated lipocalin and N-acetyl-D-glucosaminidase, indicating early tubular damage (10). Likewise, a high prevalence of CKD in children and adolescents has been reported in three agricultural CINAC-endemic regions in El Salvador (11). This suggests the possibility of established early kidney damage before future occupational exposure to heat stress, dehydration, or agrochemicals. In addition, the increased CKD-related mortality pattern among women, children, and adolescents in El Salvador and Nicaragua

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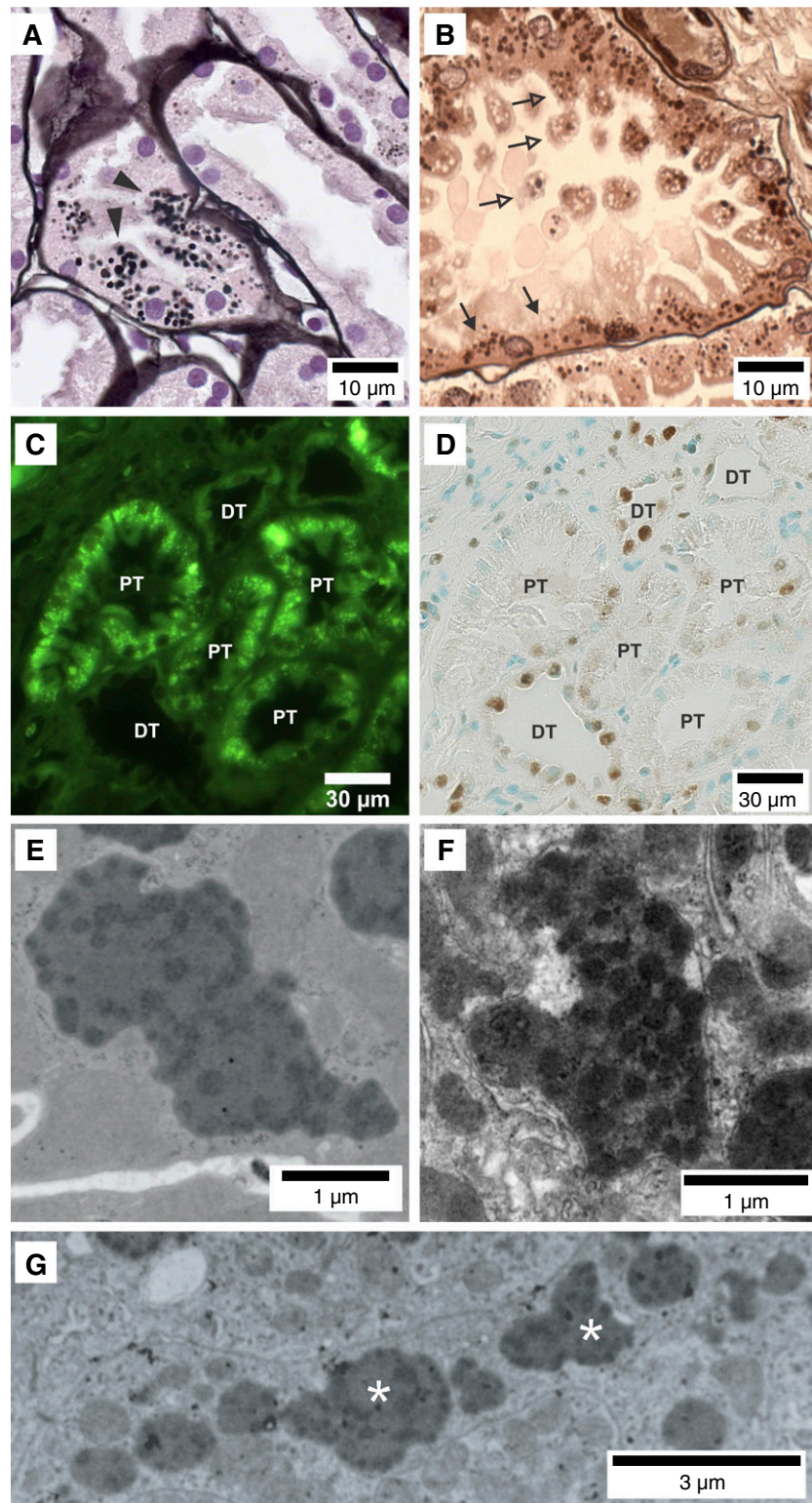
Figure 1. | Overview of the constellation of proximal tubular lesions observed in patients with chronic interstitial nephritis in agricultural communities/CKD of unknown origin. (A) Proximal tubular cells containing many enlarged argyrophilic granules, demonstrated to be lysosomes (arrowheads) (7) (Periodic acid–Schiff methenamine [PASM] staining). (B) Affected proximal tubule demonstrating enlarged lysosomes, flattened atrophic epithelial cells with loss of brush border (solid arrow) and apical blebbing and cell fragment shedding (open arrows; PASM staining). (C) Affected proximal tubules (PT) display autofluorescent granules, a subset of which were positive for lysosomal markers (7). (D) The same section as in (C) was immunohistochemically stained for the proliferation marker proliferating cell nuclear antigen. There were very few scattered proliferating epithelial cells in the affected proximal tubules. In contrast, unaffected distal nephron cells demonstrated prominent proliferative activity. (E–G) Proximal tubular cells with enlarged dysmorphic lysosomes ($\geq$1.2 μm) containing homogenous, nonmembrane-bound, electron-dense, rounded/irregular "aggregates" dispersed throughout the light-to-medium, uniform, electron-dense lysosomal matrix. (G) Two hallmark lysosomes (white asterisks), accompanied by several smaller ones. DT, distal tubule.

suggests there are additional factors, beyond the hypothesized heat stress–dehydration mechanism, that point to the broader environmental context surrounding this epidemic (3). From the perspective of animal experiments, clear dehydration or heat stress alone over 4 weeks does not lead to the constellation of proximal tubular lesions as observed in patients with CINAC (7).

An alternate theory asserts that increased uric acid is a causal factor. Although elevated serum levels of uric acid have been reported in 55%–75% of patients with CINAC, this is probably secondary to the reduced GFR because hyperuricemia is a prevalent finding in patients with CKD (12). Observed (slight) increases in serum uric acid are therefore unlikely to be a primary CINAC cause, and are not considered a criterion for diagnosis (5).

There are strong epidemiologic data supporting toxin(s) as the cause of CINAC, although it is beyond the scope of this manuscript to discuss them all. A comprehensive study of the drinking water in Sri Lanka and two recent systematic reviews summarize the acquired knowledge of the last 30 years.

First, a recent study in Sri Lanka assessed the relationship between potential nephrotoxic elements (arsenic, cadmium, lead, uranium, silica, strontium, and fluoride) in drinking water and urine samples collected from individuals with and without CKDu in endemic areas, as well as from individuals without CKDu in nonendemic areas (13). All water samples—from a variety of water sources (*i.e.*, shallow and deep wells, springs, piped and surface water)—contained extremely low concentrations of potential nephrotoxic elements, and all were well below international drinking water guideline values.

Second, a systematic review of epidemiologic studies that addressed associations between any indicator of pesticide exposure and any outcome measure of CKD came to the following conclusions (14). Four studies, out of 21, with stronger designs and better exposure assessment (from Sri Lanka, India, and the United States) showed exposure responses or clear associations with different pesticides (glyphosate, organochlorine, alachlor, atrazine, metolachlor, pendimethalin, paraquat).

Third, a United States cohort study of licensed applicators observed associations between ESKD and a considerable number of specific pesticides. Most interesting is the association with paraquat, among other pesticides, which also was implicated in ESKD among the wives of the pesticide applicators (8,15). Paraquat is one of the few pesticides with established acute nephrotoxicity after administration of high doses over a short time. The increased risk of ESKD related to intermitted paraquat use associated with other agrochemicals could be a consequence of episodes of clinical or subclinical AKI caused by nephrotoxic pesticides as suggested by others (16).

Fourth, a study in a CKDu-endemic area in Sri Lanka found a significant association with overall pesticide application (odds ratio [OR], 2.3; 95% CI, 1.0 to 5.6) and use of glyphosate (OR, 5.1; 95% CI, 2.3 to 11.3), adjusted for age, sex, education, family CKD, and exposure modifiers (17). It was the only study conducted in CKDu-endemic areas that investigated a potential exposure-response relationship by combining questions on water intake from different sources in relation to water hardness and levels of the herbicide glyphosate detected in water. With drinking pipe water or reservoir water with soft water and with trace or no detection of glyphosate as the reference, drinking from serving wells with hard water and intermediate concentrations of glyphosate (median, 0.6 μg/L) yielded an adjusted OR of 2.5 (95% CI, 1.1 to 5.7). Drinking from abandoned wells with very hard water and highest concentrations of glyphosate (median, 3.2 μg/L) yielded an adjusted OR of 5.5 (95% CI, 2.9 to 10.3).

Fifth, a methodologically sound review concluded there was no consistent evidence to support the association between CKD and heat stress and dehydration, whereas this was the case for agrochemicals (18). Although physiologic/pathophysiologic and mainly epidemiologic reasoning as well as some experimental animal studies support the concept of heat stress and dehydration as causes of chronic kidney damage, no solid evidence of this is available in humans, nor are there studies that indisputably show that they are the single or preponderant cause of the onset of CKDu. However, Chapman *et al.* (18) found consistent evidence for the adverse effect of agrochemicals on CKD and, in some studies, an association with ESKD. In this meta-analysis, which included 13 studies from different regional areas, the overall effect was positive, and became significant when cross-sectional studies were removed.

Next, regarding epidemiologic evidence, several PTC changes further corroborate a toxin-induced etiology and suggest involvement of a particular pathway. Lysosomal morphology varies greatly in PTCs depending on different factors such as proteinuria and causes of tubular injury. The CINAC lysosomes have a specific morphology, requiring electron microscopic examination at high magnification for accurate identification. The striking morphologic similarities (PTC lysosomal lesions, tubular atrophy, and fibrosis) observed in patients with CINAC, calcineurin inhibitor (CNI)–treated patients, and some toxic nephropathies (such as clomiphene, lomustine, and lithium) suggest a common tubulotoxic etiology. Many of these nephrotoxic drugs exert direct or indirect modulatory effects on calcineurin, a phosphatase regulating activity of NF of activated T cells (involved in immunosuppression), and—interestingly—transcription factor EB (involved in autophagy, lysosomal biogenesis, cargo, and exocytosis) (19–21). Although the involvement of NF of activated T cells in CNI toxicity has not been unequivocally proven and other pathways (*e.g.*, TNF-related weak inducer of apoptosis/fibroblast growth factor-inducible 14) may be involved, it remains clear that calcineurin-mediated immunosuppression and nephrotoxicity are intimately linked (22–24). Finally, it is important to note that other nephrotoxic models such as analgesic nephropathy, aristolochic acid nephropathy (*i.e.*, rats receiving only this compound), cisplatinum, and almost all (seven out of eight) tenofovir cases do not show the CINAC and CNI-associated lysosomal lesions, supporting the idea that a specific pathway or set of pathways is involved.

Evaluation of indication, implantation, and protocol renal transplant biopsy specimens revealed that the lysosomal CINAC lesion is acquired in association with sustained CNI exposure. The lysosomal lesion was found in <10% at implantation and up to 76% of indication biopsy specimens, whereas protocol biopsy specimens at 6 and 12 months after transplantation showed a prevalence of 50% and 67%, respectively.

The hypermetabolic PTCs, with their pronounced oxygen consumption/delivery ratio of 79%, are highly susceptible to a repeated toxic/hypoxic insult (cadmium, aminoglycosides, cisplatinum, tenofovir, aristolochic acid, *etc.*), particularly when there is increasing intracellular concentration of reabsorbed and secreted potential toxin(s) (*e.g.*, aminoglycosides, cisplatinum, paraquat). A substantial number of hydrophilic pesticides (*e.g.*, paraquat, 2,4-dichlorophenoxyacetic acid, pyrethroids) are eliminated by the kidney through glomerular filtration and proximal tubular reabsorption/ secretion, and hence may concentrate in the PTCs (25). It is well documented that these toxins generate reactive oxygen species, inactivate calcineurin, and induce cellular damage (26,27).

The PTC damage and interstitial expansion/fibrosis in patients with CINAC in the absence of overt glomerulosclerosis fits with the insights developed by Grgic *et al.* (28) that selective, targeted, and repeated nonlethal injury of the PTC is sufficient to initiate maladaptive repair and drive the formation of interstitial fibrosis, loss of peritubular capillaries, and secondary glomerulosclerosis. This is consistent with López-Marín *et al.*'s (29) study reporting chronic tubulointerstitial nephropathy (interstitial fibrosis, tubular atrophy) with secondary glomerular and vascular damage in Salvadorian agricultural communities. In addition, a prospective histopathologic study by Fischer *et al.* (16) of 11 Nicaraguan patients with MeN, biopsied at their earliest clinical appearance, identified patchy tubular cell injury/ atrophy and interstitial inflammation in the cortex and corticomedullary junction, without involvement of glomeruli, indicating that an infectious or toxic agent is a likely cause of renal injury.

Tubular type proteinuria (*i.e.*, low mol wt proteinuria) and increased proximal tubular markers in the urine are observed in most cases of patients with CINAC, further corroborating proximal tubular injury/damage (1,30). However, moderate to overt proteinuria can be seen in some cases, particularly in association with advanced CKD (31).

Summary

CINAC, MeN, and CKDu from different regions (Sri Lanka, El Salvador, India, and France) express the same morphologic lesions, epidemiologic profiles, and clinical manifestations, indicating a comparable renal disease around the globe (7). A CINAC histopathologic constellation of lesions has been identified to be useful in routine investigation of renal biopsies of patients clinically suspected of CINAC, provided they meet the specific morphologic criteria (7).

Renal biopsies demonstrating an increased prevalence of this renal CINAC lysosomal lesions are (up to now) associated with toxin-induced nephropathies, although the nature of the toxin is not yet determined. However, epidemiologic, experimental, and pathologic arguments point toward agrochemicals/pesticides. Heat stress or dehydration, when present, may contribute to the development and progression of CINAC toward ESKD.

The striking parallel between renal biopsy specimens of patients with CINAC and patients treated with CNIs suggest calcineurin pathway inhibition as a putative mechanism, although involvement of other pathways cannot be excluded. Interestingly, some pesticides have (direct or indirect) CNI activity and hence may have several biologic effects in common with the classic CNI (cyclosporine, tacrolimus) such as CNI, sodium ion/potassium ion-ATPase inhibition, electrolyte disturbances, immunosuppression (susceptibility to infections), and nephrotoxicity.

According to M. Haas on our recent study (7,32): "Although multiple questions remain, related to the pathways involved in this toxic nephropathy, to the possible treatment/reversibility of CINAC as well as its prevention, the study from Vervaet and coworkers represents an important step forward in our understanding of this devastating condition, and one that will undoubtedly stimulate additional investigation" and animate debates.

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See related commentary, "Is an Environmental Nephrotoxin the Primary Cause of CKDu (Mesoamerican Nephropathy)? Commentary" and debate, "Is an Environmental Nephrotoxin the Primary Cause of CKDu (Mesoamerican Nephropathy)? CON," on pages 602–603 and 596–601, respectively.