Is an environmental nephrotoxin the primary cause of CKDu (Mesoamerican nephropathy)? CON

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During the last three decades, tens of thousands of premature deaths due to chronic kidney disease of nontraditional origin (CKDnt)\(^1\) have occurred in Mesoamerica’s Pacific coastal areas, Northern Central Province of Sri Lanka and Uddanam, India. Toxins and occupational heat stress are the two main hypotheses for primary etiology (1,2).

I present arguments in favor of a heat stress nephropathy in contrast to a toxic nephropathy. My case is based on a) the need for well-designed epidemiological studies, b) the reasons why a primary toxic etiology of CKDnt is unlikely, and c) a summary of the evidence for occupational heat stress as the prime driver of CKDnt in Mesoamerica, recognizing heat stress has not yet been explored in Sri Lanka or India.

A. Strong epidemiology needed

Finding an etiology for an epidemic is a different research scenario from establishing associations between hazards and subtle disease occurrence. An epidemic implies many affected people and a strong driver, be it a biological, chemical or physical hazard. Any presumed risk factor must be highly prevalent in the most affected subpopulations, and be specific to them. To unravel the etiology of the CKDnt epidemics, well-designed epidemiological prevalence and analytical studies are essential, revealing the distribution of CKDnt in different populations and linking adequate exposure data of any potential hazard to the disease occurrence. Epidemiology should be consistent with social science, physiology, toxicology and pathology studies, but without documented epidemiological associations no reasonable causal assertions can be drawn.

While seemingly obvious, when it comes to providing proof of CKDnt etiology such principles are often overlooked. Solid epidemiological research has been mostly developed in Mesoamerica, is largely missing in Sri Lanka, and only about to start in India. Causal assertions around CKDnt are too often based on ‘ecological’ assumptions. Although some researchers have named the regional CKDnt epidemics collectively ‘Chronic Interstitial Nephritis in Agricultural Communities (CINAC)’ (2-4), the evidence is simply inadequate to conclude that the clinical, pathological, and epidemiological features of CKDnt in Mesoamerica, Sri Lanka and India are identical or that they have a common source such as agrochemicals. The fact that agricultural work is common in the rural areas where the epidemics predominate is not evidence of agrochemical etiology. Nor is the absence of reports from hot areas of the globe evidence that the etiology is not related to excessive heat. Geographical and time trend correlations are, at best, hypothesis-generating. Even perfect correlations can be subject to the "ecological fallacy" (5).

B. The low probability of a primary toxic etiology

Pesticides and metals are the two main types of toxic agents that have been suggested causes of the CKDnt epidemics (1,2,6-8).

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Assessments of associations of ‘pesticides’ with kidney disease are abundant in the literature (9) and used in meta-analyses of CKDnt as a valid exposure indicator (10,11). However, the term ‘pesticides’ includes any of hundreds of active ingredients, each with their distinct toxicity. Their use varies markedly over regions, crops and time (9). Can we learn anything from an exposure assessment that is so general and non-specific?

Studies in non-endemic/epidemic regions have found adverse kidney effects from exposure to pesticidal active ingredients (9), including end-stage renal disease from occupational exposures to common-use herbicides like atrazine and paraquat (but not glyphosate) among licensed pesticide applicators in the USA (12) and CKDnt from environmental (dietary) exposures to organochlorine insecticides in urban Delhi (13). Such studies have been repeatedly cited in support of the pesticide hypothesis for ‘CINAC’ (3,4). But, to confirm an association to CKDnt epidemics, a relation between exposure and outcome must be demonstrated in a population at risk for CKDnt. Proper epidemiological studies are lacking (9). One exception is a community cohort in a CKDnt hot spot in Nicaragua, where baseline urinary levels to 12 pesticides, including glyphosate and pyrethroids, did not associate with rapid kidney function decline (14).

A recent pathology study hypothesized that glyphosate, paraquat and pyrethroids are calcineurin inhibitors (CNIs) underlying ‘CINAC’, based on the finding of aberrant lysosomal structures in proximal tubular cells of ‘CINAC’ patients identical to those observed in kidney transplanted patients treated with high doses of CNI immunosuppressors (4). However, such lysosomal structures are not pathognomonic (15). Additionally, experimental data on calcineurin inhibition from pesticides are nearly inexistent, and the overall assumption is based on similar ‘ecological’ coincidences as commented above. In Sri Lanka, a case-control study found an association between glyphosate and CKDnt (16), but glyphosate has not been linked to kidney injury in any other study (9,17). Other common-use pesticides have also been examined as potential causal agents of the epidemic in Mesoamerica without relevant associations emerging (9,17). Importantly, and often ignored, CKDnt occurs among workers in occupations such as mining, construction, brickmaking and shrimp farming without use of glyphosate or other specific pesticides (1,18). Yet, local CKDnt outbreaks affecting men and women alike and children, have been reported in Las Brisas, El Salvador (18), and Chapala, Mexico (19), and potential environmental toxic etiology must be investigated, adequately. A toxic agent producing epidemics should be identifiable with current epidemiological research methods and chemical analytical capacities for thousands of potential putative agents.

Regarding metals, the clinical and pathological characteristics of CKDnt are neither in accordance with the known renal effects of lead, cadmium, mercury, arsenic, lithium, or uranium, nor with well-established dose-effect and dose-response relationships (20). For a discussion with focus on CKDnt, I invite the readers to working papers at three international workshops on CKDnt in 2012, 2015 and 2019 examining the role of metals (17,21,22). It was repeatedly concluded that these metals are an unlikely cause of the epidemics in Mesoamerica and Sri Lanka.

With no evident toxic candidate as main etiology, part of the CKDnt research community is now moving towards examining mixtures of ubiquitous environmental toxins (17). But how likely is the existence of specific mixtures of low-dose toxins which are widespread and with an effect so strong that they cause CKDnt epidemics covering large geographic areas in different countries?
As in all chronic diseases, multiple conditions over the course of a lifetime will ultimately contribute to the risk of getting a disease, including low-dose exposures to toxic agents. However, regarding a driver of an epidemic, it is common-sense to focus on the obvious rather than speculating that unknown agents, unidentified after decades of an epidemic, are persistently killing tens of thousands of people, mainly workers.

C. Strong evidence for occupational heat stress in Mesoamerica

My research group recently performed an exhaustive review of all published epidemiological studies on CKDnT conducted in Mesoamerica up to mid-2019, approximately 30 population-based prevalence and analytical studies (18). In all nine prevalence studies that looked at high-risk areas, kidney dysfunction was 2.5 to 4 times more frequent in men than in women, indicative of an occupational and contrarian to an environmental etiology. The data collectively leave no doubt that manual laborers, not just agricultural workers, in hot climate are by far the most affected. A few studies report CKDnT among non-working women and children, and increased markers of early kidney injury in adolescents. Still, for Mesoamerica, any environmental toxin, genetic or behavioral traits considered as primary or contributing causes of CKDnT should be congruent with the observed occupational pattern of kidney dysfunction: i.e. much more frequent among male, manual workers in hot jobs.

Thirteen analytical studies were conducted among sugarcane workers, a high-risk population. These studies from El Salvador, Nicaragua, Guatemala and Costa Rica invariably pointed to kidney dysfunction being related to prolonged extreme physical workload in hot climatic conditions leading to repeated increased core temperatures and dehydration (18). Four studies examining workers in non-agricultural ‘hot’ occupations (mining, brick making, construction) also observed kidney dysfunction, which supports the evidence (18). Studies on other causes, e.g. pesticides, arsenic and other metals, and -importantly- leptospirosis or other infections, have found no evidence to sustain these hazards as primary causes (1,18).

Noteworthy is the intervention research conducted in El Salvador and Nicaragua that provides evidence that water-rest-shade programs, when correctly implemented, reduce incidence of kidney damage in high-risk workers (24-26). The findings that reduction of heat stress leads to reduction of kidney damage – i.e. reduction of a suspected exposure leads to less damage – are profoundly important when assessing causality, even when extended observation is still needed to verify less manifest CKDnT.

The early focus on recurrent dehydration leading to renal dysfunction over time has shifted to increased core temperature with or without dehydration (23,25,26) with inflammation responses playing a key role (27). The heat stress hypothesis is also supported by animal and human experiments (28-29) and physiology research in athletes (30). Hansson et al (27) constructed a theoretical framework of possible pathophysiologic pathways for heat-induced kidney injury, based on recent heat and exercise physiology literature. The theory was tested through longitudinal analyses of original data obtained from >800 sugarcane cutters followed-up in the above-mentioned intervention studies. The conclusion was that “pushing kidneys to the physiological limits daily, causing high tubular reabsorption demand while reducing blood flow,
and repeating pro-inflammatory stimuli such as uric acid, fructose, hypoxia, cytokines, and/or endotoxins may contribute to fibrotic changes rather than successful healing.”

The link between heat stress and CKDnt has not been examined outside Mesoamerica and should be addressed in other regions. The precautionary principle and known associations with AKI and heat illness are reason enough to prevent heat stress in manual workers.

Conclusions

- Epidemiological evidence must support hypotheses from any potential main driver of CKDnt epidemics. Epidemiological data are comprehensive in Mesoamerica, weak in Sri Lanka and just starting to be collected in India.
- In Mesoamerica, epidemiological data do not support toxins (or infections) as a primary cause. In Sri Lanka, ample environmental research has not pinpointed any specific toxin nor exposure routes.
- The evidence for heat stress as the main driver of kidney injury that could lead to CKDnt is strong in Mesoamerica and pathophysiologic mechanisms have been identified. Occupational heat stress has not been investigated in Sri Lanka or India.
- Heat stress interventions in Mesoamerica have been shown to reduce kidney injury.
Table 1. Arguments at a glance. Many studies referred to in this table are included in recent reviews (1-3, 9, 17, 18).

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<th><strong>A. Epidemiology</strong></th>
<th><strong>Without robust epidemiological evidence, no progress on etiologic knowledge</strong></th>
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<td>Hazard x Exposure = Risk</td>
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<td>Well-designed epidemiological studies are essential for establishing risks and etiology</td>
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<td></td>
<td>• Strong epidemiological evidence for occupational heat stress in Mesoamerica</td>
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<td>• No clear positive epidemiological findings for pesticides or metals in any of the three regions.</td>
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<td><strong>Too much etiological thinking based on 'ecological' observations and assumptions, subject to 'ecological fallacy'</strong></td>
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<td>Ecological fallacy:</td>
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<td>• Making inferences about risks in geographical areas or time periods without knowing individual exposure, outcome, and covariate distributions in study populations</td>
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<td>Hypothetical example applied to CKDnt:</td>
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<td>• Regions with high and low pesticide use have, respectively, high and low CKD mortality. Inference: CKD mortality is caused by high pesticide use, which is a meaningless conclusion in case of co-existence of high pesticide use and frequent demanding manual work in hot climate.</td>
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<th><strong>B. Primary toxic etiology</strong></th>
<th><strong>Pesticides PRO arguments (2-4)</strong></th>
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<td>'Identical' CKDnt epidemics in three agricultural regions with high pesticides use: chronic interstitial nephropathy of agricultural communities - CINAC -</td>
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<td>Certain globally ubiquitous active ingredients are of special interest, for example glyphosate, paraquat, and several organophosphates and pyrethroids</td>
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<td>Pesticide use is high in countless agricultural regions without CKDnt, around the globe.</td>
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<td>Use varies markedly over crops, regions and time, even for widely-used pesticides: the presence of any specific putative pesticide in all epidemic regions during decades of epidemic is unlikely.</td>
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<td>Prevalence of kidney dysfunction is high also in non-agricultural communities in Nicaragua: a mining region and a semi-urban area with brickmaking industry, unexposed to pesticides.</td>
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<td>Potential associations between ubiquitous pesticides and CKDnt can only be determined in the populations at risk for CKDnt. Such studies have been negative so far:</td>
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<td>• In El Salvador cane cutters at high risk of CKDnt: urinary levels of glyphosate and 2,4-D were negligible.</td>
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<td>• In Nicaragua in community cohort in CKDnt hotspot: no associations were found between baseline urinary levels of 12 common-use pesticides and rapid kidney decline.</td>
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<td>Comprehensive reviews of pesticides and CKDnt did not find relevant associations.</td>
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<td>CKDnt clinical emergence in the 1990s coincides with rapid increase in pesticide use</td>
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<td>In Central America excess CKD mortality in epidemic regions emerged already in the mid-1970s.</td>
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|                              | Higher prevalence of kidney dysfunction in men relate to occupational pesticide use |
|                              | Male workers can also be exposed to hazards other than pesticides. |
|                              | In epidemic areas of Nicaragua and El Salvador kidney dysfunction concentrates among workers in industrial agriculture, with cane cutters at highest risk the least exposed to pesticides, and subsistence farmers with higher pesticide exposures being far less affected. |
|                              | Worker populations unexposed to pesticides are also affected: mining, construction, brick makers, others |
CKDnt or kidney injury markers among non-working women, children and adolescents is explained by environmental pesticide exposure pathways of inhalation and dermal absorption from drifts and consumption of contaminated food and water

- Population-based prevalence studies in El Salvador and Nicaragua show:
  - Kidney dysfunction is infrequent in economically inactive populations, both men and women.
  - High M:F ratios in endemic areas are not in accordance with an environmental toxic etiology.
- Women and children in Mesoamerica are few studies, have weak designs, lack measurements of presumed environmental pesticide exposures, and lack assessments of child labor (early heat stress).
- Chronic diseases are all multifactorial; contributory factors do not exclude occupational heat stress as main driver.

‘Pesticides’ as a collective toxic exposure indicator

- ‘Pesticides’ are a toxicologically heterogeneous group with dozens of chemical families, hundreds of active ingredients, and thousands of commercial formulations with different ‘inert’ ingredients.

Associations between specific active ingredients and kidney dysfunction outcomes, in regions not affected by CKDnt

- Associations between nephrotoxic pesticidal active ingredients and adverse kidney effects are to be expected in epidemiological studies, but unrelated to CKDnt epidemics.
- Potential associations between nephrotoxic pesticides and CKDnt epidemics can only be determined in the populations at risk for CKDnt.

Occupational and environmental exposures to pesticides is the likely underlying cause of ‘CINAC’ through calcineurin pathway inhibition or modulation (4), analogous to kidney effects of immunosuppressive drugs in transplant patients

- The ‘CINAC’ – calcineurin inhibition (CNI) hypothesis is based on ecological thinking.
- There is no epidemiological evidence of pesticides related to CKDnt epidemics (all the above reasons).
- Direct experimental evidence of pesticides being CNIs is nearly inexistent.
- Whether common-use pesticides are important as CNIs must be tested experimentally.
- Any associations between suspected CNI pesticides and CKDnt epidemics need to be established through well-designed epidemiological studies.

Metals and toxic mixtures PRO arguments | Metals and mixtures of toxins CON arguments
---|---
A number of metals are nephrotoxic and have caused epidemics historically (e.g. cadmium in food chain and itai-itai disease in Japan) | Clinical and pathological characteristics of CKDnt are not in accordance with the known renal clinical effects of cadmium, lead, mercury, arsenic, lithium, or uranium, nor with well-established dose-effect and dose-response relationships.
Mixtures of ubiquitous environmental toxins may cause CKDnt epidemics | The existence of specific mixtures of low-dose toxins covering large geographic areas in different countries and with potent nephrotoxic effects is unlikely.

C. Occupational heat stress as primary etiology in Mesoamerica

Heat stress CON arguments (2-4) | Occupational heat stress PRO arguments
---|---
Increases in temperature due to climate change in CKDnt affected regions seem insufficient for epidemics of heat-stress nephropathy. | It is possible that etiologic factors and underlying physiopathological mechanisms are not the same for the different regions with CKDnt epidemics.
There are many hot regions in the world without CKDnt. | All CON arguments are ‘ecological’ in nature. Yet similar ‘ecological’ arguments PRO exist too:
- Excess kidney dysfunction is quite consistently found in lowlands in the equatorial belt, among young laborers in hot places with poor labor protections and hard physical work.
Heat stress has not been identified as an etiologic factor for CKDnt in Sri Lanka or India

| In Sri Lanka, the North Province with the hottest climate does not report CKDnt. |
| In Sri Lanka, at the time the CKDnt epidemic started, rice paddy farmers at highest risk mechanized the harvesting process, reducing their heat stress. |

- Occupational heat stress derives from the combination of extreme physical workload in high environmental temperatures. Hence, not all hot regions will be equally affected.
  - Excess kidney dysfunction is NOT consistently found in places with high pesticide use, which exist globally from the very north to the very south, in lowlands and highlands, in all continents
  - Climate change has coincided with the green revolution and increasing pesticide use, but also with profound economic changes and transition from subsistence farming to industrialized agriculture:
    - Less control over physical work demands, piece rate payments, loss of nutritional subsistence crops.
- Heat stress in relation to CKDnt has not been addressed in Sri Lanka or India
  - In well-designed epidemiological studies, heat stress should be measured objectively during harvest and non-harvest seasons along with markers and changes in kidney function.

**Strong epidemiological evidence of occupational heat stress underlying the CKDnt epidemic in Mesoamerica**

| Prevalence and cohort studies: |
| Sugarcane field workers, especially cutters, have been identified as populations at high risk for CKDnt, as well as workers in other hot jobs |
| Heat measurements in field among cane cutters show high internal and external heat. |
| Cross-shift studies show changes in kidney function and hydration parameters during cane cutting indicating heavy load on the kidney. |
| Cross-harvest declines in kidney function are most severe and incident kidney injury is most frequent among the manual laborers with the highest physical workload. |
| Incident kidney injury during the sugarcane harvest season associates with markers of physiopathological mechanisms related to heat-induced inflammation. |

**Intervention research:**

- Reduction in heat stress through water-rest-shade programs leads to reduction in kidney damage
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