EPIDEMIC OF CHRONIC KIDNEY DISEASE OF UNKNOWN CAUSES IN NICARAGUA: epidemiology, causal hypotheses, and public health interventions

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TESIS DOCTORAL
Mención Internacional

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You must be the change you wish to see in the world.
-Mahatma Gandhi

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"Las huellas de las personas que caminaron juntas nunca se borran."

-Proverbio africano.

Me gustaría dedicar este esfuerzo a toda la gente que he conocido durante el proceso de la investigación que aquí se presenta, porque me han enseñado grandes cosas, en particular a Donald Cortez (1972-2013), por rebosar generosidad, integridad y liderazgo.

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Madrid, Agosto 2013;

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PROLOGUE: of how my involvement in this research began

Every story has a starting point. During the 2007-2008 academic years, I studied a Master in Public Health (MPH), with an International Health concentration, at Harvard School of Public Health. While living in Brookline (Massachusetts), I collaborated with a small NGO called Brookline-Quezalguaque Sister City (BQSC) Project. Quezalguaque is a rural municipality of around 8,000 inhabitants located in the department of Leon (Nicaragua), and was designated Brookline's Sister City in 1987. This collaboration has allowed assisting the city in infrastructure construction, emergency relief (e.g. hurricane Mitch), and education and health projects, as well as building a volunteerism's network and providing a stimulus for social change. As part of this collaborative effort, the Health Committee of the project (where Daniel Brooks, an epidemiologist from Boston University participated) decided to address an apparent Chronic Kidney Disease (CKD) epidemic in the region, moved by the high mortality due to CKD, as well as the increasing concern of the disease among health workers and the general population. This project was planning to estimate the prevalence of the disease at this small community and to shed light on the possible risk factors causing the disease in Quezalguaque, adding up to the ongoing research in the region. This study was carried out during June-August 2008, in cooperation with the Universidad Nacional de Nicaragua, in Leon (UNAN-Leon), particularly with CISTA (Research Center on Health, Work and Environment), the regional Ministry of Health department (SILAIS-Leon), and the Quezalguaque Health Center.

As part of these efforts, I traveled to Costa Rica during the winter session and met with members of SALTRA (a regional program on work and health in Central America funded by the Swedish international development agency) at Universidad Nacional de Costa Rica (UNA), to understand the work being done on CKD in the region. These meetings were useful in framing this public health problem as a regional epidemic of CKD of non-traditional and unknown causes. I then traveled to Nicaragua in March 2008 with the BQSC project and spent one week working with government officials and UNAN-Leon with the objective of assessing the feasibility of the prevalence study, including selecting a local IRB (ethical committee), that could review this research. During my stay there, it became clear to me that very few patients had access to a full range of treatment including end stage renal disease care such as dialysis and kidney transplants in Quezalguaque. Concerns regarding how to perform a risk assessment, manage positive screened patients, how to define and classify renal insufficiency cases, what is the most efficient way to improve early detection and prevention of progressive loss of renal function, and how to prevent or attenuate complications and comorbidities in such a context, became the core elements of my master's thesis, entitled "Evidence-based

guidelines to design a Community Renal Health Program in Nicaragua".

My relationship with Boston University School of Public Health (BUSPH) began in 2009 when, invited by Daniel Brooks, I became a core member of a team based at BUSPH that was submitting a proposal to investigate the causes of a two long unexplained epidemic of chronic kidney disease in Nicaragua. During 2009-2011 I combined my participation in this research with the training residency program on "Preventive Medicine and Public Health" in Madrid. From May 2011 on, I have been engaged full time with this project, as part of collaboration between the Preventive Medicine and Public Health Department of the Universidad Autónoma de Madrid (UAM) and BUSPH, with stays as an international scholar at BUSPH and extended time spent in the field research activities carried out in Nicaragua. This constitutes the core of my PhD thesis with international mention.

SUMMARY/RESUMEN

Introduction

There is a long time neglected epidemic of CKD of unknown causes (CKDu), recently named Mesoamerican Nephropathy (MeN), which probably extends from South Mexico to Panama. The author of this thesis document has been studying with researchers from Boston University this disease since 2009, as part of a mediation process between a sugar cane company and sick ex-workers and their families.

Research Objectives

1. Describe the epidemiology of CKDu/MeN, and causal hypotheses proposed to date.

2. Gain a better understanding of the CKDu/MeN causal web, based on Nicaraguan health professionals´ perceptions.

3. Assess kidney damage through urine biomarkers among Nicaraguan adolescents from different schools.

Methods

In order to address the 3 research objectives proposed, a systematic review of the literature published to date, a qualitative study of semi-structured in person interviews with Nicaraguan physicians and pharmacists (n=19), and a cross-sectional study of 245 school children from different regions of Nicaragua, were conducted.

Results

Regarding Objective 1:

-Mortality rates and prevalence studies have confirmed that communities where agriculture, mining and port-related occupations are prevalent, and particularly those in lowlands of the Pacific coast are the ones most affected. Contrary to the typical distribution of a chronic [renal] disease, it affects primarily men in their 20-40s (relatively young). Women, although less affected, follow also the same distribution as men (e.g. higher prevalence's in low altitude).

-Risk factors (susceptibility, initiation and/or progression) associated with CKDu in the different prevalence and case control studies to date, as well as well known environmental causes of CKD thought to play a role in this epidemic, have brought researchers to think that etiology is presumably multifactorial. The variety of hypothesized causes include: heat and heavy physical work load (chronic volume depletion), exposure to heavy metals such as arsenic or cadmium, agrichemicals, systemic infectious diseases such as leptospirosis, and chronic intake of potential nephrotoxic medications such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics or aminoglycoside antibiotics.

Regarding Objective 2:

-Physicians and pharmacists interviewed working in the front line of the disease described it in a way consistent with results from the studies conducted so far in the region.

-Our analysis articulated perceptions of physicians and pharmacists in a causal framework where heat stress and subsequent volume depletion experienced by manual laborers may play a role in the frequent occurrence of dysuria-like symptoms (locally called “chistata”), which are often treated with non-steroidal anti-inflammatory drugs, diuretics and antibiotics that may be further nephrotoxic.

Regarding Objective 3:

- Adolescents from four schools in three different regions of Nicaragua showed biomarkers of kidney tubulo-interstitial damage ranked by mortality rates in the general population by region. For example, the results for NAG were most consistent in this regard, with the relative concentrations rank ordered by school according to the mortality rate risk profile in both boys and girls. On the other hand, Albumin-Creatinine Ratio, which is primarily associated with glomerular damage, did not demonstrate these patterns, and macroalbuminuria measured with dipstick, was virtually absent.

-An unusual finding is that girls had higher levels than boys for all biomarkers, with particularly large differences for ACR and IL-18.

Conclusions

The epidemiological information on MeN/CKDu is still very narrow and presents several limitations, but has helped to describe the distribution of the disease and propose some risks factors.

Dehydration during hard work, and nephrotoxic medications (such as NSAIDs and antibiotics) appear as a plausible causal hypothesis that needs to be the focus of further research. Also, early risk factors for CKD in the region may be present even during teenage, prior to other additionally exposures.

Multi-level public health responses to the epidemic cannot wait until efforts to identify the causes are completed, especially because treatment is not widely available, and poverty and premature death are key elements that have turned this disease into a real public health crisis of regional dimensions.

Introducción

Nos encontramos ante una epidemia olvidada de enfermedad renal crónica de causas desconocidas, denominada recientemente Nefropatía Mesoamericana (NeM), que se extiende probablemente desde el Sur de México a Panamá. El autor de esta tesis doctoral ha estudiado, junto con investigadores de la Universidad de Boston, esta enfermedad desde 2009 en el contexto de un proceso de mediación entre una compañía de la caña de azúcar y ex-trabajadores y sus familiares.

Objetivos de investigación

1. Describir la epidemiología de NeM y las hipótesis causales propuestas hasta la fecha.

2. Profundizar en el conocimiento sobre la red causal de NeM, basándose en las percepciones de profesionales de la salud Nicaragüenses.

3. Evaluar el daño renal en adolescentes nicaragüenses de cuatro colegios a través de biomarcadores urinarios.

Métodos

Con la finalidad de abordar los 3 objetivos de investigación propuestos, se realizó una revisión sistemática de la literatura publicada hasta la fecha sobre NeM, se llevó a cabo un estudio cualitativo de entrevistas personales semi-estructuradas en Nicaragua con médicos y farmacéuticos (n=19), y un estudio transversal de 245 escolares en diferentes regiones de Nicaragua.

Resultados

Respecto al Objetivo 1:

-La información basada en las tasas de mortalidad y en estudios de prevalencias confirman que ciertas comunidades dedicadas a la agricultura, minería, y trabajadores portuarios presentan prevalencias de ERC elevadas, particularmente aquellas comunidades localizadas a nivel del mar en la Costa del Pacífico. Al contrario de la típica distribución de enfermedad renal crónica, NeM afecta principalmente a hombres jóvenes de 20-40 años (relativamente jóvenes). Las mujeres, aunque se encuentran menos afectadas, también presentan una distribución similar a los hombres (ej. mayor prevalencias en comunidades localizadas a menor altitud).

-Los factores de riesgo (susceptibilidad, iniciación y/o progresión) asociados a NeM en los diferentes estudios de prevalencia y de casos y controles hasta la fecha, así como aquellas causas ambientales conocidas de ERC que pudieran tener un papel en esta epidemia, han llevado a pensar que la etiología podría ser multifactorial. La variedad de las hipótesis causales incluye: exposición al calor y trabajo manual intenso (depleción de volumen crónica), exposición a metales pesados como cadmio, o a arsénico, agroquímicos, enfermedades infecciones sistémicas como la leptospirosis, el uso crónico de medicamentos potencialmente

nefrotóxicas, tales como antiinflamatorias no esteroideos (AINEs), analgésicos o antibióticos aminoglucósidos.

Respecto al Objetivo 2:

-Los médicos y farmacéuticos entrevistados que trabajan en primera línea de la epidemia describen la epidemia de manera consistente con los resultados de los estudios llevados a cabo en la región.

-Nuestro análisis articuló las percepciones de médicos y farmacéuticos en un esquema de red causal donde el estrés por calor y la depleción de volumen subsecuente que experimentan trabajadores manuales podría jugar un papel en la aparición frecuente de síntomas similares a la disuria (llamados localmente "chistata"), que son normalmente tratados con antiinflamatorios no esteroideos, diuréticos y antibióticos que pueden ser también nefrotóxicos.

Respecto al Objetivo 3:

-Los adolescentes de cuatro colegios en tres regiones diferentes de Nicaragua mostraron biomarcadores de daño renal túbulo-intersticial en un orden creciente igual al que muestran las tasas de mortalidad por ERC en la población general de las mismas regiones. Por ejemplo, los resultados para NAG fueron las más consistentes en este sentido, con concentraciones medias relativas por colegio ordenadas según el perfil de riesgo basado en las tasas de mortalidad por ERC en su región, tanto para chicos como para chicas. Por otra parte, la razón albúmina-creatinina urinaria (RAC), asociada principalmente con daño glomerular, no demostró estos patrones, y no hubo evidencia de macroalbuminuria medida con tira reactiva de orina.

-Un hallazgo inusual fue que las chicas presentaron niveles superiores a los chicos para todos los marcadores, con diferencias particularmente notorias para IL-18 y RAC.

Conclusiones

La información sobre la epidemiología de NeM es aún escasa y presenta múltiples limitaciones, pero ha sido esencial para describir la distribución de la enfermedad y proponer algunos factores de riesgo.

La deshidratación durante el trabajo y los medicamentos nefrotóxicos (como AINEs y antibióticos) aparecen como una hipótesis causales plausibles que ameritan más investigación. Asimismo, algunos factores de riesgo para NeM tempranos podrían estar presentes durante la adolescencia, previamente a otras exposiciones adicionales, y requerirían de más investigación.

Las respuestas multinivel de salud pública no pueden esperar a que se completen los esfuerzos para identificar las causas de NeM, especialmente porque el tratamiento no es de acceso universal y la pobreza y muerte prematura son elementos clave que han convertido esta enfermedad en una crisis de salud pública de dimensiones regionales.

CHAPTER I: GENERAL INTRODUCTION

Es pobre mi país [Nicaragua]

pero brilla como un cielo caído al descuido sobre la tierra,

un cielo como un tapete mullido

dulce, juguetón, como el abrazo de un niño.

Gioconda Belli, Nicaragua 2007.

1.1 Chronic Kidney Disease: a global public health problem

1.2.1 Global burden of Chronic Kidney Disease

Chronic kidney disease (CKD) is becoming a major public health issue worldwide and an important contributor to the overall non-communicable disease burden. CKD is associated with major serious consequences including increased risk of premature mortality, end-stage renal disease (ESRD), accelerated cardiovascular disease (CVD), mineral and bone disease, adverse metabolic and nutritional consequences, infections, reduced cognitive function and increased risk of acute kidney injury (AKI) (Levey et al, 2007).

Estimates of the global burden of diseases report that genitourinary diseases were responsible annually for 928,000 deaths or 1.6% of the total number of worldwide deaths (12th cause of death), and 14,754 000 DALYs, 1% of all global DALYs (17th cause of disability) (WHO, 2004).

In the United States, as many as 26 million adults may have CKD, an increase from approximately 10% of the US adult population between 1988 and 1994 to over 13% just one decade later (Coresh et al, 2003; Coresh et al, 2007). This rise in the prevalence of CKD likely reflects similar increases in obesity, diabetes, hypertension, and cardiovascular disease (Coresh et al, 2007), which are expected to continue to increase in coming years (Levey et al, 2007). Similar rates are seen worldwide, with CKD prevalence ranging from 6% to 16% in sample populations of Spain (Otero et al, 2010), Japan (Kimura, 2007), and Australia (Chadban et al, 2003). Systematic review of CKD prevalence studies is arduous because of significant variations in methodologies and a lack of a consistent definition for CKD until 2002 (McCullough et al, 2012).

A considerable proportion of these CKD patients will experience the progression of the disease to ESRD, and will need Renal Replacement Therapy (RTT,) such as dialysis or kidney transplant. Not surprisingly, 90% of those receiving RRT in the world are in developed countries, and they constitute the tip of the iceberg, usually reflected in national registries of ESRD or patients on RRT. The cost of treating patients with ESRD is substantial and poses a great challenge to provision of care. It is estimated that currently 1,800,000 patients are receiving dialysis treatment worldwide at a global cost of US $72 billion and this trend is increasing. In Europe, dialysis alone takes up about 2% of health care budgets, with only a small proportion (<0.1%) of the population needing treatment (Lameire et al, 2005).

1.1.2 Definitions and classifications of CKD, AKI, AKD

Defining CKD and classifying the stages of severity permits a common language for communication among health care workers, patients and their families, researchers and policy-makers as well as a framework for developing a public health approach to affect care and improve outcomes of CKD.

The National Kidney Foundation (NFK) Kidney Disease Outcome Quality Initiative (K/DOQI) defined Chronic Kidney Disease for the first time in 2002 as “the presence of kidney damage or

decreased level of kidney function maintained for 3 months or more, irrespective of a primary diagnosis" (K/DOQI, 2002). This definition is intended to account for all conditions that affect the kidney and have the potential to cause either progressive loss of kidney function or complications resulting from a decreased level of kidney function.

Kidney function is usually measured through the glomerular filtration rate (GFR), which describes the flow rate of filtered fluid through the kidney, or the creatinine clearance rate (CCr or CrCl), which is the volume of blood plasma that is cleared of creatinine per unit time, a useful measure for approximating the GFR that usually involves 24-hour urine collection. Both GFR and CCr may be accurately calculated by comparative measurements of substances in the blood and urine (eg. inulin), or estimated by formulas using just a blood test result (eGFR and eCCr). eGFR is estimated using formulas (such as MDRD, CKD-EPI, Cockcroft-Gault, etc) that account for age, weight, serum creatinine and other corrective factors such as gender and race (which may influence muscular mass). CKD definition is based on the NHANES III study in USA (1988-1994) and consists of 5 stages (see table 1.1). In stages 3-5, the level of glomerular filtration rate (GFR) is below the "normal" cut-off (60ml/min/1.73m2). Renal disease is often progressive once glomerular filtration rate falls below 25% of normal. Early stages of CKD (1-2) manifest with only kidney damage in the setting of overtly intact or slightly decreased GFR, defined by pathological abnormalities or markers of damage such as proteinuria, including abnormalities in blood (serum creatinine), urine tests (urine sediment) biopsies or imaging studies.

Table 1.1 -Stages of CKD

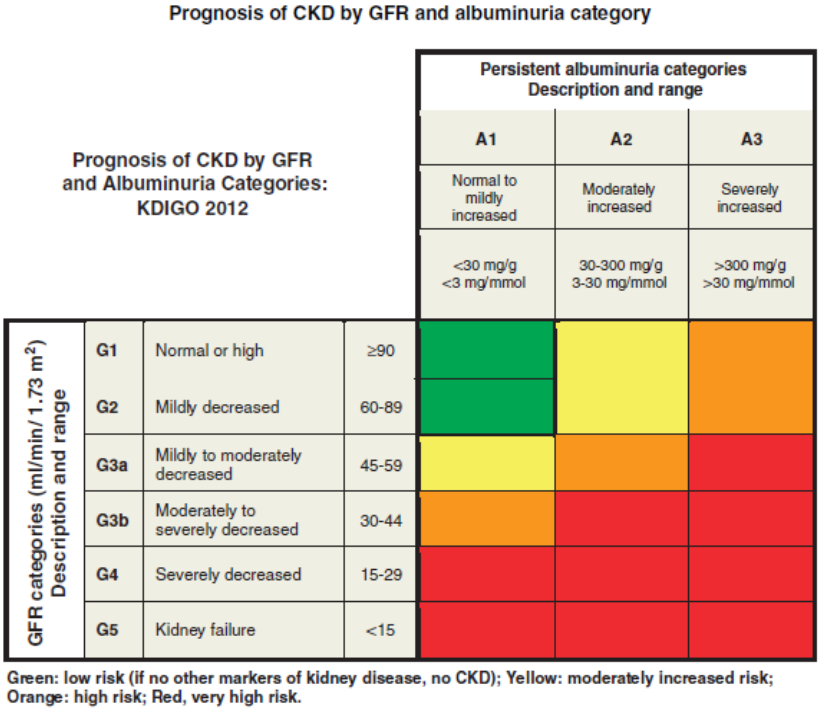
Stage	**Description**	**Creatinine clearance (≈GFR) (ml/min/1.73 m2)**	**Action**
1	Kidney damage with normal or raised GFR	>90	Screening CKD risk reduction
2	Kidney damage with mildly impaired GFR	60-89	Diagnosis and treatment Treatment of comorbidities Slowing progression
3	Moderately impaired GFR (chronic renal failure)	30-59	Evaluating and treating complications
4	Severely impaired GFR	15-29	Preparation for kidney replacement therapy
5	End stage renal disease (uraemia)	15	Replacement

Source: adapted from National Kidney Foundation—K/DOQI, 2002.

The "Kidney Disease: Improving Global Outcomes" (KDIGO) initiative was born in 2004 (Eknoyan et al, 2004), trying to integrate and develop different clinical guidelines globally. This project has launched new guidelines in 2012 (KDIGO, 2013). The CKD guideline retains the 2002 definition of CKD but presents an enhanced classification framework for CKD, elaborating on the prognosis of the disease (based on results from meta-analyses), and including a worldwide used marker of kidney damage: albuminuria (Levey et al, 2011). It also includes "heat maps" based on a composite ranking of relative risks of all-causes and cardio-vascular mortality, ESRD, AKI, etc. Colors from green to red indicate groups of patients at progressively higher risk for the major outcomes, and constitutes a communication tool for clinicians,

researchers and the public health agencies to use these risk categories to describe and prioritize efforts for patients and populations (see figure 1.1).

Figure 1.1- Prognosis of CKD by GFR and albuminuria categories.



Source: KDIGO/2012.

CKD definition arbitrarily uses a duration of >3 months (>90 days) as delineating ''chronic'' kidney disease. Nevertheless, in epidemiological studies, kidney damage is usually ascertained as albuminuria estimated from the albumin-to-creatinine ratio (ACR) in a random ('spot') urine sample (or, if not available, sometimes dipstick urine protein and total urine protein-to-creatinine ratio), and GFR is estimated from serum creatinine. These measurements are made at a single point in time, and usually clinical diagnosis is not ascertained. Less than 3 months would fall under the category of Acute Kidney Disease (AKD), a very ambiguous term criticized by some authors (Palevsky et al, 2013). On the other extreme, acute kidney injury or impairment (AKI) is a broad clinical syndrome defined, irrespective of the etiology, as an abrupt, within hours to days, decrease in kidney function (see table 1.2). AKI is a predictor of immediate and long-term adverse outcomes; it is more prevalent, as well as a significant risk factor, for patients with CKD (KDIGO, 2012).

Table 1.2 -AKI definition

-Increase in SCr by ≥0.3 mg/dl (≥26.5 μmol/l) within 48 hours; or -Increase in SCr to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or -Urine volume <0.5 ml/kg/h for 6 hours

Source: KDIGO, 2012

AKI severity grades (3 stages) are based on the RIFLE/AKIN criteria (see figure 1.2), which rely on Serum Creatinine (SCr) and urine output (Bellomo et al, 2004; Mehta et al, 2007), as

surrogates of kidney damage. More sensitive and specific biomarkers of kidney damage are arising (NGAL, KIM-1, IL-18, etc.), but still are not widely used in clinical settings. Some of them will be introduced in chapter VI.

Figure 1.2 -Comparison of RIFLE and AKIN criteria for diagnosis and classification of AKI

AKI staging	Urine output	RIFLE	
Serum creatinine	(common to both)	Class	Serum creatinine or GFR
Stage 1 Increase of more than or equal to 0.3 mg/dl (≥26.5 μmol/l) or increase to more than or equal to 150% to 200% (1.5- to 2-fold) from baseline	Less than 0.5 ml/kg/h for more than 6 hours	Risk	Increase in serum creatinine ×1.5 or GFR decrease >25%
Stage 2 Increased to more than 200% to 300% (>2- to 3-fold) from baseline	Less than 0.5 ml/kg per hour for more than 12 hours	Injury	Serum creatinine ×2 or GFR decreased >50%
Stage 3 Increased to more than 300% (>3-fold) from baseline, or more than or equal to 4.0 mg/dl (≥354 μmol/l) with an acute increase of at least 0.5 mg/dl (44 μmol/l) or on RRT	Less than 0.3 ml/kg/h for 24 hours or anuria for 12 hours	Failure	Serum creatinine ×3, or serum creatinine >4 mg/dl (>354 μmol/l) with an acute rise >0.5 mg/dl (>44 μmol/l) or GFR decreased >75%
		Loss	Persistent acute renal failure=complete loss of kidney function >4 weeks
		End-stage kidney disease	ESRD >3 months

Source: KDIGO, 2012

1.1.3 Etiology of CKD

The causes of CKD are numerous (table 1.3) and typically are divided into vascular, glomerular, tubulointerstitial and obstructive etiologies. This classification not only provides a convenient pathophysiologic framework, but also may relate to clinical manifestations. Vascular and obstructive causes are relatively limited in number and more readily diagnosed, while glomerular and tubulointerstitial diseases have a far broader spectrum of causes. Glomerular diseases often present with typical urine findings, including proteinuria (consisting primarily of albumin), as well as the presence of red blood cells or red blood cell casts in the urine sediment. Tubulointerstitial diseases may also have proteinuria; however, this urinary protein is often not albumin, but rather includes proteins of tubular cell origin and the total amount excreted is usually much lower than that seen in glomerular diseases. White blood cells, renal tubular cells, and white blood cell casts may be present in the urine sediment in tubulointerstitial diseases, but often the sediment is unrevealing. Clinical features distinguishing the different etiologies are more likely to be present early in the course of CKD. As CKD progresses to advanced stages, the specific etiology is often not apparent clinically, and even biopsies may be of limited yield because late stages of most kidney diseases, regardless of etiology, manifest with extensive scarring and fibrosis (Brooks, 2009).

Remarkably, hypertension and diabetes are the major causes of CKD in developed nations, and increasingly in developing countries. In developing nations, chronic viral infections may also contribute substantially to the burden of CKD from glomerular diseases (Levey et al, 2007). Exposure to nephrotoxic drugs and environmental toxins usually affects the kidney's tubulo-interstitial compartment.

Table 1.3- Etiopathology differential approach to CKD

Classification	Condition	Associated Disease States		Manifestations
VASCULAR	Main Renal Arteries	Renal artery stenosis Fibromuscular dysplasia		Often accompanied by hypertension
	Intrarenal vasculature	Thrombotic microangiopathies Benign and malignant nephrosclerosis Atheroembolic disease		Minimal albuminuria, often with bland urine sediment
	Renal vein	Renal vein thrombosis		May be a cause of or be caused by nephrotic syndrome
GLOMERULAR	Focal GN	Age <15 yo	Post-infectious, IgA, TMBD, Hereditary nephritis, HSP, mesangial proliferative GN	Active sediment without reduced GFR or nephrotic syndrome
		Age 15-40 yo	IgA, TBMD, SLE, Hereditary nephritis, mesangial proliferative GN	
		Age >40 yo	IgA	
	Diffuse GN	Age <15 yo	Post-infectious, MPGN	Active sediment with reduced GFR and variable proteinuria
		Age 15-40 yo	Post-infectious, SLE, RPGNs, Fibrillary GN, MPGN	
		Age >40 yo	RPGNs, vasculitides, fibrillary GN, Post-infectious	
	Nephrotic Syndromes	Age <15 yo	MCD, FGS, mesangial proliferative GN	Heavy proteinuria, usually bland sediment
		Age 15-40 yo	FGS, MCD, Membranous, Diabetes, Pre-eclampsia, Late stage post-infectious	
		Age >40 yo	FGS, Membranous, diabetes, MCD, IgA, Amyloid/LCDD, HTN/nephrosclerosis, Late stage post-infectious	
TUBULOINTERSTITIAL	Drugs and toxins	Analgesic nephropathy (non-steroidal anti-inflammatory drugs) Aminoglycoside associated nephrotoxicity Aristilochic Acid Calcineurin inhibitors Chemotherapeutic agents (Cisplatin, nitrosureas) Contrast induced nephropathy Lithium Metals (Lead, cadmium, arsenic, uranium, mercury) Ochratoxin Pigment nephropathy (rhabdomyolysis)		Minimal albuminuria, usually bland sediment, even in presence of reduced GFR, tubular proteinuria
	Hereditary	Fabry Disease Hereditary nephritis (Alport syndrome) Medullary Cystic Disease Polycystic Kidney Disease		Fabry Disease is rare and may have glomerular or tubular manifestations Hereditary nephritis is often accompanied by hearing impairment; Medullary cystic disease may be indolent and is difficult to diagnose PKD manifests with massively enlarged cystic kidneys

	Autoimmune	Sarcoid Scleroderma Sjogren's syndrome SLE Other vasculitides	Highly variable presentations, usually with other systemic manifestations and typically (except sarcoid) accompanied by marked hypertension
	Metabolic	Cystinosis Hyperuricemia Hyperoxaluria Hypokalemia Nephrocalcinosis	Minimal albuminuria, usually bland sediment, even in presence of reduced GFR, tubular proteinuria
	Other	Chronic infections, ureteral reflux Chronic ischemia, acute tubular necrosis Hypertension Lymphoproliferative diseases and myeloma Sickle cell disease	Minimal albuminuria, usually bland sediment, even in presence of reduced GFR, tubular proteinuria
OBSTRUCTION	Ureteral	Bilateral obstructing stones (or unilateral if single functional kidney) Malignancy Strictures Retroperitoneal fibrosis	May have hematuria, but otherwise bland sediment
	Bladder	Benign or malignant prostate enlargement Urethral stricture Neurogenic bladder Infections Medications	

FGS, focal glomerulosclerosis; TMBD, thin basement membrane disease; HSP, Henoch Schonlein purpura; GN, glomerulonephritis; SLE, Systemic lupus erythematosus; MPGN, membranoproliferative glomerulonephritis; RPGN, rapidly progressive glomerulonephritis; MCD, minimal change disease; LCDD, light chain deposition disease; HTN, hypertension

Source: Brooks, 2009

There are likely multiple risk factors involved in CKD each stage (Levey et al, 2007). These can broadly be conceptualized as development factors and progression factors (table 1.4). Development factors include susceptibility factors (which increase vulnerability to kidney damage, with examples including older age, family history/genetic predisposition, and even elements like volume depletion), and initiation factors (which actually cause kidney damage, with examples including diabetes, autoimmune diseases and toxins). Progression factors cause worsening damage and progressive decline in GFR, with examples including higher blood pressure, smoking and poor diabetes control. Certain factors, with sustained exposure, may even fall into more than one of these categories. For example, low birth weight and CKD may be related with a defective nephrogenesis provoked by intrauterine malnutrition. Early post-natal malnutrition is also linked to overweight in adulthood and ultimately develops into diabetes and diabetic nephropathy (Codreanu et al, 2006).

Table 1.4 - Risk factors for CKD

Factor Category	Definition	Examples
Development		
Susceptibility	Increase susceptibility to kidney damage	Older age, family history of CKD, low kidney mass/low birth weight, minority status, low SES, volume depletion
Initiation	Directly cause kidney damage	Diabetes, hypertension, autoimmune diseases, systemic infections, recurrent urinary tract infections, nephrolithiasis, obstruction, drug toxicity
Progression	Cause worsening kidney damage and more rapid decline in GFR	Higher levels of proteinuria, higher blood pressure, poor diabetes control, smoking, cardiovascular disease

Source: Brooks, 2009.

1.2 Clusters of CKD around the globe

As mentioned previously, CKD is considered a key contributor to the global burden of non-communicable diseases, being a key determinant of the poor health outcomes of chronic diseases, but also a consequence of diabetes and hypertension, highly prevalent currently across the general population.

However, the causes of CKD are multiple (see table 1.3), and its distribution sometimes follows a geographical cluster, affecting particular segments of the population. For example, an increased prevalence in Pima Indians and Australian Aborigines has been associated with a high prevalence of diabetes and possibly low birth weight linked to a decrease in renal mass and nephrones (Hoy, 2000).

In other populations, environmental toxins, particularly heavy metals, have been identified as a cause of CKD (Soderland et al, 2010). Although clusters of CKD induced by heavy metals usually occur among industrial workers in occupational settings, whole populations could be exposed through food or water. Examples include the appearance of a cadmium nephropathy along the Jinzu river (Japan), associated with contaminated rice, or a nephropathy due to organic mercury poisoning along the Minamata Bay in Japan, associated with the ingestion of mercury contaminated fish (Iesato et al, 1977; Jarup, 2002).

Other environmental toxins have been identified as causes of CKD. In Tunisia, contamination of the food supply with ochratoxin A, a nephrotoxic mycotoxin, has been linked to an increased prevalence of CKD (Abid et al, 2003). But certainly, a striking case (because of its regional multi-country scope and the amount of time it has taken to elucidate its causes) is the Balkan endemic nephropathy (BEN) (Bamias e tal, 2008; Debelle et al, 2008). BEN is a form of interstitial nephritis, first identified in the 1920s (although first published in the 1950s) among several small communities along the Danube River and its major tributaries, in the modern countries of Croatia, Bosnia and Herzegovina, Serbia, Romania and Bulgaria. Although the

etiology for BEN is not fully certain, chronic exposure to dietary aristolochic acid is known to be the major risk so far. At the beginning, heavy metals and ochratoxin A were hypothesized as causes, but an outbreak in 1993 of CKD among users of a Chinese herbal medicine for weight loss at a clinic in Brussels restarted the investigation on aristolochic acid, a toxic product produced from the seeds of Aristolochia clematitis (birthwort). In 1967, this theory had been proposed, that Balkan nephropathy was caused by the contamination of the baking flour in endemic areas by seeds of Aristolochia clematitis, but this hypothesis had not garnered much attention. However, the clinical manifestations of the disease in these patients (mostly women) were remarkably similar to those seen in the Balkans, with similar morphologic characteristics and the occurrence of uroepithelial tumors. The subsequent identification of aristolochic-specific DNA adducts in urinary tract tissue from patients with Balkan nephropathy and the development of an animal model (Debelle et al, 2002), has pointed out aristolochic acid as BEN's most probable cause.

Other clusters of CKD with unknown causes remain still unsolved. An apparently new form of chronic kidney disease of unknown etiology (CKDu) has emerged in several areas of Sri Lanka, comprised in the North Central Region of the island (Athuraliya et al, 2011). This Sri Lankan CKDu is not related to diabetes, hypertension, or any other traditional causes of chronic kidney disease. The disease is characterized by a slow, progressive, asymptomatic development, frequently starting at a young age group. Hospital statistics suggest that as many as 8,000 people are currently undergoing treatment for this condition. The disease is more prevalent among men engaged in agriculture, usually from a low socio-economic status. CKDu in this country has become a major public health problem, with a big social and economic impact, that has brought the Ministry of Health and the World Health Organization to conduct research and design public health interventions (WHO, 2010). One of the major tasks undertaken is the establishment of a National Registry of CKDu in Sri Lanka. Recent research conducted by the Sri Lankan MoH and WHO included prevalence studies, biological and environmental sampling. Results point exposure to arsenic, cadmium and agrichemicals as possible risk factors associated (WHO, 2013).

There are anecdotic reports of similar CKD epidemics in the South-East coast of India (Machiraju et al, 2009), or in Egypt (Kamel et al, 2010), but much less information is available on them. Whether or not these clusters of CKD of not yet known causes are similar to that occurring in the Central American region remains unknown.

1.3 A new epidemic cluster: Mesoamerican Nephropathy (MeN) or CKD of unknown causes (CKDu) in the Central American region

A new epidemic of chronic kidney disease in several areas of the Mesoamerican region, defined as a nephropathy of non-traditional etiology that usually affects young adult males from rural communities is currently being detected in the lowlands of the Pacific coast (Wesseling et al, 2013). The causes of the disease remain basically unknown. However, it is now known with certainty that this epidemic constitutes a public health problem of substantial

magnitude and severity. For example, in El Salvador, CKD appears as the second cause of mortality in adult males (El Salvador MoH, 2007).

Main milestones around this novel disease concentrate in the last 2 decades. Although some internal medicine physicians and nephrologists of reference national hospitals (e.g. personal communication with Dr. Norman Jirón, Managua, 2009) date back to as far as the 70's, this unusual increase of cases among young males coming from rural and coastal areas of Honduras, El Salvador, Nicaragua and Costa Rica, it is not until the mid-2000 decade that series of cases and small studies started to appear in peer-review journals or formal reports (Garcia-Trabanino et al, 2002; Dominguez et al, 2003; Cerdas, 2005; Garcia-Trabanino et al, 2005; Cuadra et al, 2006). And it is not until the current decade that community based prevalence studies (some with case control nested sub-analysis) are finally undertaken and published, with government figures being also shared to the broader community.

Recently, the epidemic has also gained attention from the regional and international public health agenda. Governments of Central America and the Pan American Health Organization (PAHO) have recognized that this epidemic (named the Mesoamerican Nephropathy, MEN, or Chronic Kidney Disease of unknown or non-traditional causes, CKDu) is a real threat to public health in the region. The Commission of National Ministries of Health of Central America (COMISCA) signed a declaration in 2012 prioritizing this epidemic and calling governments into action, and in 2013 a high level meeting on "CKD of unconventional causes in Central America" was convened in El Salvador[1]. Also, researchers from more than ten countries got together in San José, Costa Rica, in November 2012, under the umbrella of SALTRA (Program in Health, Work, and Environment in Central America), coordinated from the Central American Institute for Studies on Toxic Substances (IRET) of the Universidad Nacional (UNA) in Costa Rica. As part of that workshop, a Technical Report (Wesseling et al, 2013), that contains reviews of different topics by many researchers, was released in 2013[2]. This report calls for urgent and collaborative actions to elucidate the causes and find solutions for prevention and mitigation. Also, as a result from this meeting, a Research Consortium with a Board of Directors has been created with its main role to advocate in favor of CKDu/MeN, and coordinate research efforts.

Finally, very recently (June 17th, 2013), PAHO dedicated its 152nd Executive Committee Session to the topic "Chronic Renal Disease in Agricultural Communities of Central America", delineating the main strategic objectives for interventions at national and regional levels and proposing a permanent technical group of experts to assess national governments on this public health issue (PAHO, 2013).

Along with this prioritization in the public health agenda of the region, mass media has also echoed the epidemic, particularly the implications of this tragedy (focusing on its human faces) that devastates communities. Examples are BBC[3], The Guardian[4], El Pais[5], and many national newspapers[6].

[1] San Salvador declaration can be found at:
http://new.paho.org/els/index.php?option=com_content&task=view&id=778
[2] The technical report is publicly available at http://www.regionalnephropathy.org
[3] http://www.bbc.co.uk/news/magazine-16007129
[4] http://www.theguardian.com/world/2012/oct/14/kidney-disease-killing-sugar-cane-workers-central-america

Chapter 4 of this thesis will attempt to document and describe the epidemiology (where, who, when) of this epidemic based on mortality data, and the outcomes of the peer-reviewed manuscripts published to date.

1.4 Investigating MeN/CKDu in Nicaragua

The research work presented in this document took place in a unique context full of complexities and stakeholders that played an important role in the research process, and that it is worth mentioning here.

Boston University School of Public Health (BUSPH) was contracted in 2009 by the Compliance Advisor/Ombudsman's office (CAO) of the World Bank to investigate the cause(s) of this two decade-long unexplained excess occurrence of CKD in Central America. Such a process was initiated by a complaint filed by the NGO: Center for International Environmental Law (CIEL), on behalf of the Asociación de Chichigalpa por la Vida (ASOCHIVIDA), a community-based organization of individuals suffering from CKD- or their widows- who formerly worked at the sugar cane fields of Ingenio San Antonio (ISA), which is owned by National Sugar Estates Limited (NSEL). The complaint alleged that the International Finance Corporation (IFC), and Multilateral Investment Guarantee Agency (MIGA), of the World Bank Group failed to address the health and well-being of workers or the environment when delivering a substantial loan to NSEL, the primary example of harm being the CKD epidemic.

In response to the initial complaint, CAO proposed a mediation (or "Dialogue") process between representatives of ASOCHIVIDA and NSEL. Dialogue meetings led (among other activities such as exploring options for improved care or alternative income generation projects for affected community members) to a request for applications to prepare a scoping study to consider the questions of what are the causes of CKD in the Western region of Nicaragua, and, whether there is any relationship between the practices of the ISA and the causes of CKD.

BUSPH was selected by the Dialogue participants to conduct this scoping study, and assembled a multidisciplinary and multi-country team of researchers with expertise in epidemiology, occupational and environmental health, nephrology, and public health experts. During the second half of 2009, we reviewed the existing information available on CKD in Nicaragua and Central America, identified data gaps, evaluated the feasibility and usefulness of additional studies and design options that could yield the necessary information to answer the Dialogue

[5] http://sociedad.elpais.com/sociedad/2011/12/25/actualidad/1324846356_918435.html, http://internacional.elpais.com/internacional/2013/05/14/actualidad/1368496548_623955.html, http://internacional.elpais.com/internacional/2012/02/11/actualidad/1328991118_487033.html?rel=rosEP

[6] http://www.confidencial.com.ni/articulo/5638/miles-de-caneros-mueren-por-enfermedad-renal http://www.nacion.com/2012-08-15/AldeaGlobal/fallas-renales-en-guanacastecos-triplican-las-del-promedio-del-pais.aspx

members' questions. Some of those studies[7] we carried them out in a second phase contract during 2010-2012, and included:

- An **industrial hygiene/occupational health assessment** (August 2010), which evaluated the potential hazards associated with chemicals and work practices at ISA;
- An **investigation of water quality** (August 2010), which included the analysis of a large number of contaminants in water samples collected from locations selected by ASOCHIVIDA;
- A **qualitative analysis of interviews with physicians and pharmacists** (September 2011), which summarized data from semi-structured interviews with health professionals in Nicaragua to assess their perceptions regarding renal disease in the region;
- A **pilot cohort study** (February 2012), which assessed the feasibility of conducting a complete retrospective cohort study to evaluate the relationship between work practices at ISA and CKD;
- An **investigation of biomarkers in workers** (April 2012), which evaluated biological markers of kidney injury and CKD in ISA workers, miners, construction workers, and port workers;
- An **investigation of urinary biomarkers in adolescents** (June 2012), which evaluated evidence of subclinical kidney damage among adolescents in different areas of Nicaragua.

Outcomes from these research activities have not yielded the silver bullet answer to those initial research questions, but have served to gather a lot of important information, better described the epidemiology of the disease, and provided clues regarding concrete risk factors. Finally, they have contributed to raise awareness and create momentum (along with efforts by many other researchers) to study this serious public health problem. Some of these results, where I have had a leading role, are presented in the upcoming chapters of this PhD thesis.

[7] Reports for all studies conducted can be found at: http://www.cao-ombudsman.org/cases/document-links/links-82.aspx

CHAPTER II: THEORETICAL FRAMEWORK

"When elephants fight, it is the grass that suffers."

-African proverb.

Causation of CKDu/MeN is not well established yet, but the evidence available points towards a multicausal model, where the influence of diverse groups of factors operating at various levels, from the molecules up to the social and economic determinants, and environmental aspects (including the relationships between humans and the natural ecosystem) should be considered. Furthermore, theoretical models that embrace different levels could help envisioning the environmental, biological and social factors that contribute to and/or influence the development of CKD, how they relate to each other, and the embedded complexity of the causal framework ("Working with an ecosystem perspective" Mergler D, Jakobsson K, Ramírez-Rubio O. In: Wesseling et al, 2013, pp 163-15). Socio-ecological models of health described in this chapter include multiple levels of causation to explain health and disease, and are helpful as a starting point to explain and draw the lines, in which CKDu/MeN and its context are circumscribed, as well as to frame and structure the recommendations that emerge from this work.

2.1 Socio-ecological models of health

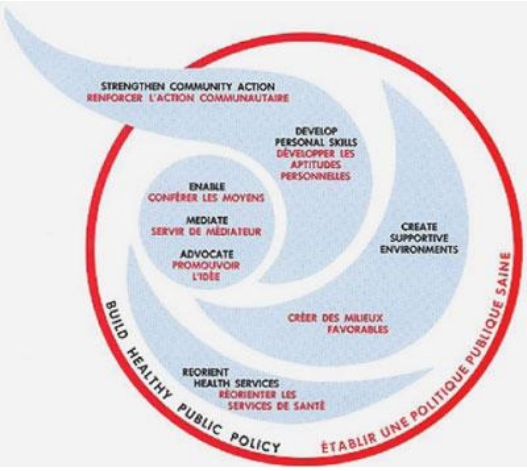
Several models have emerged in the last decades that consider a systemic perspective of health problems (see table 2.1). These models analyze the multiple levels of factors (psychological, social, and organizational) that influence health behaviors, and how those factors interact across those levels (e.g. individual, organizations, communities, policy environments, etc). This analysis is particularly relevant when the intention behind is designing effective multi-level interventions aimed at changing behavior (e.g. smoking cessation). These models, strongly based on the behavioral sciences and public health paradigms, try to explain the nature of people's transactions with their environments.

Table 2.1- Examples of models designed to explain behavior

Model	Author (year)
Ecological psychology	Kurt Lewin (1951)
Environmental Psychology	Roger Barker (1968)
Social Ecology	Rudolph Moos (1980)
Systems Theory	Urie Bronfenbrenner (1979)
Ecosocial Model	Thomas Glass and Matthew McAtee (2006)

Many authoritative documents that guide public health programs nationally and internationally are based on these models and principles. For example, the Ottawa Charter (WHO, 1986) took a step forward understanding health as a responsibility beyond the health care system, building on the discussions generated by the Declaration on Primary Health Care at Alma-Ata (WHO, 1978), and the World Health Organization's "Targets for Health for All" document signed in 1984 by the member states of the European region (WHO, 1984).

Figure 2.1- Ottawa Charter logo.



Source: WHO, 1986.

However, the word "ecological" in all these frameworks refers to the inclusion of multiple levels of factors affecting human health (including the environment), rather than the preservation and sustainability of our ecosystem or the natural environment itself (i.e. the interaction of living organisms such as plants, animals and microbes, and the nonliving components of their environment like air, water and mineral soil), which also has radical effects on humans health (e.g. access to safe water, food, energy, etc).

Despite the converging interest in linking the upstream social and environmental determinants of health, the fields of "environmental health" and "social determinants of health" have tended to remain conceptually and operationally distinct (Parkes et al., 2003). Slowly, the significant link among ecosystem change, social inequalities and health has also started to influence those whose primary focus is the social determinants of health. Research around these social determinants have tended to focus on health inequities in relation to social gradient, stress, early life, social exclusion, work, unemployment, social support, addiction, food and transport (Wilkinson et al, 2003). While at first glance these themes may seem unrelated to the themes of ecosystems and health, connections are beginning to be made.

For example, the WHO Commissioner on the Social Determinants of Health clearly identified the links between the social determinants of health and the effect of environmental change on health inequities:

"Putting all these levels in context is the natural environment, and the macro-level to micro-level effects of environmental change. Risks to health include heat waves and other extreme weather events, changes in infectious disease patterns, effects on local food yields and freshwater supplies, impaired vitality of ecosystems, and loss of livelihoods. If present trends continue the adverse health effects from human induced environmental changes will be distributed unequally. The poor, the geographically vulnerable, the politically weak, and other disadvantaged groups will be most affected…Addressing the intersection between social determinants of environmental change and the effect of environmental change on health inequities will benefit sustainable ecological and population health alike." (Marmot, 2007, p.1156)

Those connections have also occurred at the "policy level". For example, "Health in All Policies"(HiAP) is an incipient approach to policy making first used by the Finnish Presidency of the European Union (EU) in 2006, in which decision-makers in other sectors (e.g. urban planning and environment protection) routinely consider health outcomes, including benefits, harms, and health related-costs, strengthening the link between health and other policies.

2.2 Eco-health, a new comprehensive model

It is in that conceptual battle, that EcoHealth emerges at the interface between ecological and health sciences, with the purpose of studying the interactions between environment, development, sustainability, and health across local, regional, and global scales. This ecosystem approach to public health issues acknowledge the complex, systemic nature of public health and environmental issues, and the inadequacy, to some extent, of conventional methodologies for dealing with them. Thus, this approach is inherently transdisciplinary, recognizing complex biophysical, social, cultural, political and economic relationships between the ecosystem and human health, and embracing concepts such as social justice, gender equity, multi-stakeholder participation and sustainability. Practitioners of social and political disciplines; biology, epidemiology, veterinary and human medicine, and public health; are key to understand these drivers of social and ecosystem change, and their ultimately influence in human health and well-being. This integrative thinking aims to better understand processes such as climate change, loss of biodiversity, antibiotic resistance, health inequalities, degradation and pollution of air, land, water resources, etc.; and how they are affecting our health (Wilcox et al, 2004).

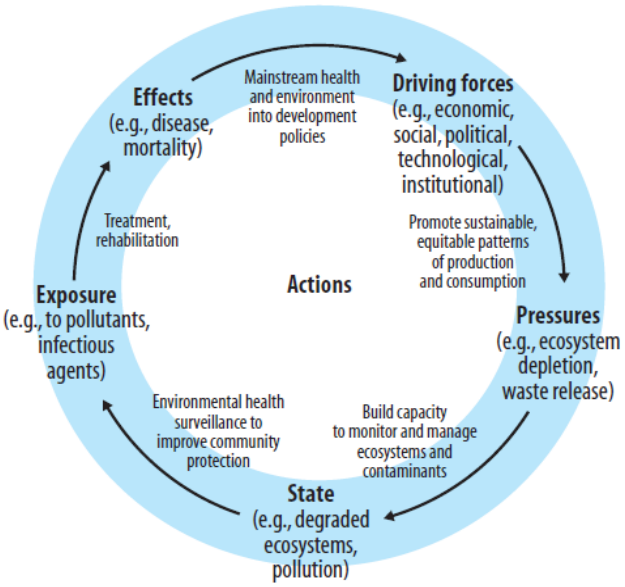
Ecosystem management offers a strategy for what McMichael et al (2008) describe as "true primary prevention" by reducing or eliminating the human pressures on the environment that create disease and health inequities, as well as lessening existing health risks for vulnerable communities.

Ecosystem approaches to health arose in the 1990s in Canada from a group of experts dealing with diverse problems ranging from Great Lakes contamination to zoonotic diseases. Canada's International Development Research Centre (IDRC) played a lead role in supporting an international community of scientists and scholars who advanced ecosystem approaches to health (Webb et al, 2010). The launch of the journal EcoHealth (Wilcox et al, 2004), the official publication of the "International Association for Ecology and Health" since its formation in 2006, gave further impetus to the emerging field, providing an integrated, international platform for dissemination, peer-review and scholarly development. Ecohealth has also been cultivated through recognition of the common ground with the field of "conservation medicine" (Aguirre et al., 2002), and what is sometimes described as "One Health"—linking human and animal health with increased attention to ecosystem context (Zinsstag et al., 2008).

There are several conceptual models that seek to frame the relationships between environmental and social determinants of human health (VanLeeuwen et al, 1998; Parkes et al, 2003; Millennium Ecosystem Assessment Board, 2005). One of them, is the DPSEEA framework

(see figure 2.2), pronounced “deep sea”, that stands for Driving forces, Pressures, State, Exposure, Effects and Actions; and was developed as part of the HEADLAMP (Health and Environment Analysis for Decision Making) Project, a collaboration of the World Health Organization, the United Nations Environment Program and the United Nations Environmental Protection Agency in the early and mid-1990s (Corvalan et al, 2000). Although simplistic and formulated only thinking on negative exposures and health effects (thus, not taking into account positive health promotion through the environment), it emphasizes action. It makes the point that intervention in environmental contexts to improve human health can be targeted at a variety of scales, and by different actors. This multi-step action model addressing upstream and downstream factors has guided also the exercise of outlining public health interventions for MeN/CKDu (see Chapter “Recommendations”).

Figure 2.2- The DPSEEA model



Source: Carneiro et al., 2006, adapted from Corvalan et al., 2000.

2.3 Epidemic of CKDu/MeN in Central America with an Eco-Health perspective

While one can pinpoint specific causal pathways for each 21st century epidemic (both re/emerging infectious and chronic diseases), they probably reflect more general systemic and cultural changes, including population growth and mobility, a huge shift in agriculture to economies of scale and mass distribution, land use changes, physical activity patterns; and ultimately, better detection methods and a more public alert.

Along these lines, CKDu/MeN is a two decade-long unexplained excess occurrence of chronic kidney disease, that has been repeatedly neglected by many institutions because of the uncertainty regarding its causes, and the complex social, ecological, and economic drivers that seem to surround the disease. Although the causes of the disease are still unknown, most

seem to relate to environmental and/or occupational exposures. It can be argued that climate change and non sustainable environmentally development may be behind some of the causes and/or drivers of the epidemic (see table 2.2).

Table 2.2- Eco-health dimensions of CKDu/MeN

	Downstream- -Upstream		
	Micro level (e.g. individuals and families)	**Meso level (e.g. communities)**	**Macro level (e.g. Pacific Coast Mesoamerica)**
Ecological dimension	-Subsistence farming -Individual unsafe water wells	-Monoculture (e.g. sugar cane, cotton) -Use of agrichemicals and safety issues -Extreme weather events (e.g. flooding) -Volcanic area -Contamination of community water sources	-Degraded ecosystems -Deforested lands -Loss of biodiversity -Tropical climate (high temperatures and humidity) -Increasing temperatures (global warming)
Socio-economic dimension	-Lack of economic gain possibilities -Poor households -No land owning -Limited access to social security and pension benefits -Lack of access to secondary and higher education	-Movement of populations and human settlements (e.g. civil wars in Nicaragua and El Salvador, migrant workers, etc) -Gender differences at work-force -Mistrust on large companies, that have distanced from workers and their communities through subcontracts	-International political economy -Agribusiness -Free trade zones and working places -Weak labor norms' supervision and enforcement
Health care dimension	-Limited access to health care, particularly specialized care.	-Mandatory screenings of creatinine prior to work (renal health as a pre-condition to work) -At health care settings: limited resources, burnt out personnel	-Susceptible populations to multiple infections and disease (inc. CKD) -Health care and Public Health regional institutions lack of action
Emotional dimension	-Widowhood and orphanage -Stigma	-Affected communities with hundreds of lost members (mostly young males), and "no space at cemeteries"	-Lack of trust in public institutions

Source: Ramirez-Rubio, O (2013), adapted from Ali (2004).

From an ethnographic point of view, the profile of a "typical" CKDu / MeN affected person[8] is a young man (although women are also known to suffer from CKDu) of 20-40 years, usually a farmer or field worker (but not always) clearly trapped in a poverty cycle. The dynamics are different in each community, but usually most studied areas, located on the Pacific coast of El Salvador, Nicaragua and Costa Rica, are similar: large deforested coastal areas of land devoted to cultivate single crops (sugar cane, peanuts, cotton, etc), and separated from the inner land by a volcanic range. The cycle of poverty is increased with the burden of this disease, very debilitating and marked by a silent and chronic progression in which the only treatment is renal replacement therapy (dialysis or renal transplantation), barely accessible in these

[8] For a portrait of these situations, watch videos: http://www.youtube.com/watch?v=4c3kNNqfBDQ, http://www.youtube.com/watch?v=I1ki-mL_rv4

countries. Health systems are overwhelmed by the lack of resources, and trained and motivated health workers. Large companies become the main screening centers, testing their employment seekers and discovering the iceberg of the epidemic.

Affected families and communities are being overwhelmed by the disease, finding no responses from local or national authorities. The burden of stigma, widowhood and orphanage is normally borne by women. Although probably anecdotic, affected men have abandoned their families and home after a diagnosis of a terminal CKD (personal communication with one of the psychologists of Hospital España, Chinandega, specialized in HIV/Aids and CKD, 2009). Also, the fact that the cause is unknown puts pressure on "individual" behaviors that are questioned (alcohol, drugs and tobacco consumption, nutrition habits, hygiene and home conditions) as possible initiation or progression factors of the disease.

This is the crude context in which advocacy, companies' social responsibility actions, mediation, public policy planning, and even research occurs.

Figure 2.3- Members of the same family affected by CKD

Source: La Isla Foundation

CHAPTER III: RESEARCH HYPOTHESES AND OBJECTIVES

"The real voyage of discovery consists not in seeking new landscapes, but in having new eyes".

- Marcel Proust

3.1 Research Plan

As outlined in Chapter I, we started to investigate this epidemic of CKD of unknown causes in 2009, in the context of an occupational dispute among sick sugar cane ex-workers and the company where they used to work. One of the first research questions posed by the dialogue members was when, where and who was affected by this disease. Back that year the information to this respect was limited and mainly based on non-published national statistics, reports, and bachelor and master's theses.

In 2013, there are already 7 community prevalence studies (some with case-control studies embedded) with some sort of random sampling, which will be reviewed in **Chapter IV**, along with mortality rates by region, year and sex from some of the Central American countries. Also, key results from a follow-up study of sugar cane workers during a harvest season that has not yet been published (McClean et al, 2012) will be included. Main causal hypotheses argued to date by researchers, as well as the main risk factors of susceptibility, initiation and/or progression being explored on the published studies will be outlined. This review exercise describing the epidemiology of the disease and its key aspects has been undertaken in two commissioned editorials in peer-reviewed journals (Brooks et al, 2012; Ramírez-Rubio et al, 2013a) annexed in this document, and as a chapter of a technical report (Brooks & Ramirez-Rubio, in Wesseling, 2013).

Additionally, during the process of conducting field research, we learned from members of the mediation process, and from informal discussions with area physicians and workers, of the frequent occurrence of a set of symptoms seemly related to dehydration, referred to locally as "chistata," characterized by painful urination and often accompanied by "kidney" and/or back pain, and the common diagnosis of urinary tract infections (UTIs) among young men. Since the list of CKD potential causes is extensive but includes the use of nephrotoxic medications as an additional risk factor, we decided to conduct a qualitative research based on interviews in Chinandega and Leon (regions with the highest CKD mortality rates in Nicaragua) both with physicians who are likely to diagnose or treat chistata, UTI, and/or CKD and with retail pharmacists who are likely to fill prescriptions or sell medications to treat these conditions. This piece of research is presented in **Chapter V**, along with its publication in a peer-reviewed journal (Ramirez-Rubio et al, 2013b).

Studies to date have focused on the general adult population or working population at occupational settings. However, since most of these affected workers are diagnosed with CKD, which is a chronic and progressive loss in renal function over a period of months or years, at very young ages; and because most of the hypothesized environmental/occupational exposures could already be happening (perhaps with less intensity) earlier in life; we were interested in studying whether initial damage may begin in childhood. After our experience using new biomarkers of kidney damage among workers (detecting significant differences depending of job task undertaken and showing increases along the harvest season in certain jobs) (McClean et al, 2012), we decided to use those in adolescents before they start working in very different regions of Nicaragua (with presumed different environmental exposures and family history of CKD). The results of this pilot study are presented in **Chapter VI**, along with its manuscript that it is in the process of submission to a peer-reviewed journal.

Finally, public health responses to the epidemic cannot wait until efforts to identify the causes are completed, especially because treatment is not widely available, and poverty, stigma, and premature death are key elements that have turned this disease into a real public health crisis. Thus, key public health interventions will be outlined in a **Recommendations Chapter**, along with a Brief Policy Paper that was commissioned by Instituto de Salud Carlos III, as a request by the Ministry of Health of Costa Rica in the context of bilateral cooperation projects that both institutions share (see Annexes).

3.2 Objectives

Concrete objectives of the activities outlined in the research plan are:

Objective 1

Despite its scientific and public health importance and its potential global reach, most health researchers and public health practitioners are unaware of this epidemic. The goal of Chapter IV is to describe the epidemiology of MeN/CKDu (frequency of mortality, morbidity, geographical, sex and age distribution), and establishing the causal hypotheses and relationship between development and progression risk factors, by conducting a review of the literature published to date on MeN/CKDu in Central America.

Objective 2

While there has been a great deal of media and activism focused on CKD and potential causes in Nicaragua, general knowledge, opinion or practice of physicians and pharmacists in the region are not known. The aim of the qualitative semi-structured interviews presented in Chapter V is to increase our understanding of health professionals´ perceptions regarding CKD in the region (characteristics of the affected population, causal hypotheses, symptoms, diagnostic tools, treatment and prognosis); in order to get a deeper understanding of MeN/CKDu causal web, and determine whether further study of the relationship among hydration practices, diagnosis of UTI/chistata and use of medications is warranted.

Objective 3

The aim of the pilot study presented in Chapter VI with adolescents in different schools of Nicaragua (selected based on a priori risks) is to assess kidney damage through urine markers of tubule damage; as well as urine albumin, a traditional marker of increased glomerular permeability and damage, and urine dipsticks; and note differences between schools, if any.

CHAPTER IV: EPIDEMIOLOGY, RISK FACTORS, AND CAUSAL HYPOTHESES FOR MeN/CKDu

"The art of epidemiological thinking is to draw conclusions from imperfect data" -
George W. Comstock-

“But not too strong conclusions and not too imperfect data…” -
… adds Daniel Brooks-

4.1 Introduction

For almost two decades, large areas of Central America have been impacted by an under-recognized epidemic of chronic kidney disease of unknown causes (CKDu) whose victims are disproportionately young and male (Ramirez-Rubio et al, 2013a). Exact figures are unavailable, but El Salvador, for example, has the highest overall mortality from kidney disease in the world (with Nicaragua also included in the ten highest countries) (WHO, 2008), and CKD is the second leading cause of mortality among young males in the country (El Salvador MoH, 2009). Very recently COMISCA (Central American Ministries of Health) and PAHO (PAHO, 2013), signed the "El Salvador Declaration", declaring this epidemic of CKDu or CKD of non-traditional causes, a regional public health threat. Also, researchers working in this topic in the region named the CKDu, the "Mesoamerican Nephropathy" or MeN (Wesseling et al, 2013).

Despite its scientific and public health importance and its potential global reach, very little has been published around this epidemic. The goal of this review is to provide an overview of the epidemiologic, etiologic, and public health dimensions of the epidemic, by summarizing the evidence published to date and identifying gaps in knowledge to support further research efforts.

This review exercise describing the epidemiology of the disease and its key aspects has been undertaken in two commissioned editorials in peer-reviewed journals (Brooks et al, 2012; Ramirez-Rubio et al, 2013a) annexed in this document, and as a chapter of a technical report (Brooks & Ramirez-Rubio, in Wesseling, 2013). Extracts and paragraphs of those documents can be found in this chapter.

4.2 Methods

A comprehensive literature search in PubMed and Google scholar was conducted in order to identify articles related to MeN/CKDu in Central America. Inclusion criteria included:

- Papers with an explicit mention to an epidemic of CKD of unknown causes or non-traditional causes;
- All study designs and/or methods;
- All Mesoamerican/Central American countries/contexts;
- Papers published in any year, until June 2013.
- Papers published in English, Spanish or Portuguese.

Also, results include official government data (such as mortality rates due to CKD in Central American countries) to further illustrate and describe this epidemic. Generally, data from non-online grey literature was not systematically searched for extraction this time, but has been part of our efforts in the past (Brooks et al, 2009), and information from specific reports have been included.

Some of the key words used included chronic kidney disease, chronic renal failure, kidney disease, end stage renal disease, proteinuria, glomerular filtration rate, creatinine, AND

prevalence, mortality, screening, prevention, treatment, complications, risk factors, AND developing countries, Latin America, Central America, in relation to CKD.

4.3 Results

4.3.1 Extent of the problem

Reports (sometimes anecdotic; i.e. an article in a newspaper) of excess cases of CKDu are concentrated in the Pacific coast lowlands to the west of the mountain range that extends throughout Southern Mexico and Central American countries (see figure 4.1). El Salvador, Nicaragua and north-west Costa Rica are the regions with far more information, including prevalence studies published in peer-reviewed journals and mortality data disaggregated by sex, age or geographical divisions (departments, provinces, etc). Incidence data is lacking in the region.

Figure 4.1- Map of reported unusual number of cases of CKD.

Source: Ramírez-Rubio,O & Amador, J.J. 2012

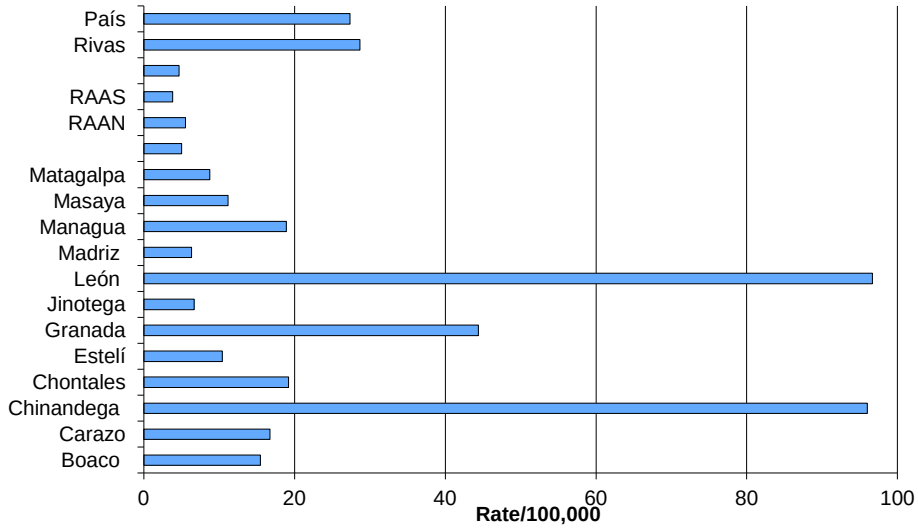
Since cases seem to concentrate in certain geographical areas (eg. CKD prevalence is high in communities of the Northwest departments of Nicaragua but not in Managua, despite having being actively studied in both places), CKDu would be described in this document as an epidemic or a cluster, since cases seem to aggregate at least in place (not known in time), and they are suspected to be greater than the number expected, even though the expected number, and the population's baseline is not known (CDC, 2006).

4.3.2 Mortality statistics

Mortality data is the only nationally based system of surveillance for CKD. Furthermore, to the extent that the case fatality rate for CKD is high, it serves as a lagging indicator for incidence. For example, Tonelli et al (2006) in a meta-analysis that included 39 cohort studies or survival analyses of randomized, controlled trials with patients stages I-IV, calculated the predicted risk for death during the median follow-up of 4.9 years in 25% (95% CI 17 to 35) for a GFR = 40 ml/min. The overall number of deaths due to MeN/CKDu in Mesoamerica as a whole or in any of the individual countries is uncertain, primarily because nobody has attempted to estimate what percentage of deaths from CKD could be classified as CKDu, a figure that likely varies substantially by country. However, El Salvador has the highest overall mortality rate from kidney disease in the world with 51.8 deaths per 100,000. Nicaragua and Honduras are also included in the top 10 countries with highest kidney mortality worldwide (25.5 and 24.6 deaths per 100,000 respectively) (WHO, 2008), and CKD appears as the second leading cause of mortality among men of working age in El Salvador (El Salvador MoH, 2009).

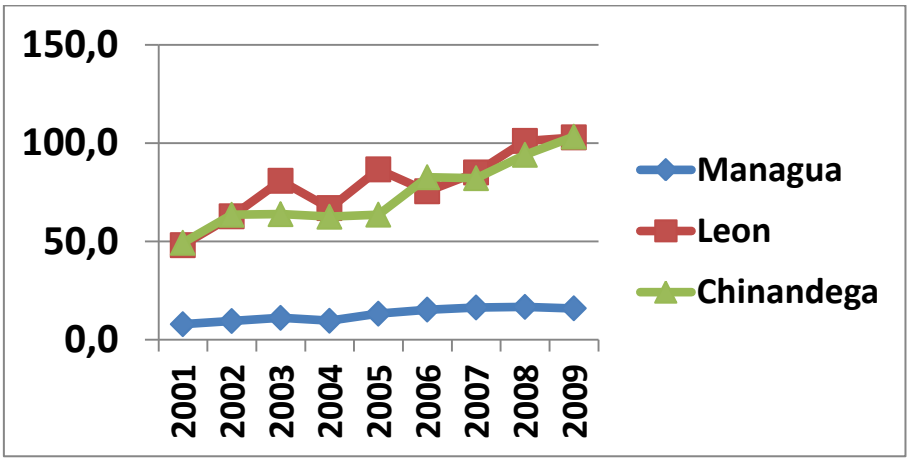
Mortality figures also show how the epidemic seems to concentrate in certain regions within countries. In Costa Rica, the Northwest province of Guanacaste appears to be the main region affected (Cerdas, 2005). In El Salvador, most CKD cases in the end stage of the disease referred to a national hospital in the capital city came from coastal areas of the country (Garcia-Trabanino, 2002). In Nicaragua, mortality due to kidney disease during 2009-2011 was almost 9 times as high in León and Chinandega as the median mortality rate in the country (11 deaths per 100,000), and more than twice as high as the rate in Granada, the next highest department (Figure 4.2). On the other hand, other regions of the country, such as in the east, do not appear to be affected. Mortality due to kidney disease in León and Chinandega has also increased over time, increasing twofold between 2000-2009 (Figure 4.3). Mortality data at the municipal level indicates that Chichigalpa (Chinandega department, sugar cane area) and Larreynaga (León department, mining area) have similar rates of mortality and are the highest in Nicaragua. In Chichigalpa, one out of every four deaths was due to CKD (personal communication with Torres C, 2009).

Figure 4.2. CKD mortality rate (x 100,000), Nicaragua, 2009-2011



Source: Nicaragua Ministry of Health. Dirección General de Planificación y Desarrollo. Oficina Nacional de Estadísticas.

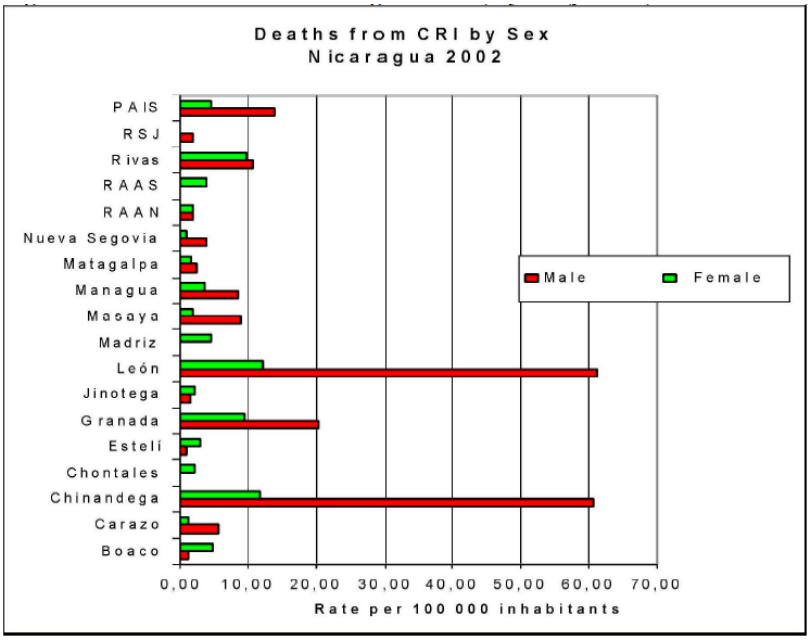
Figure 4.3- CKD mortality rate (x100,000), Nicaragua 2001 - 2009



Source: Nicaragua Ministry of Health. Dirección General de Planificación y Desarrollo. Oficina Nacional de Estadísticas.

The mortality rate among men is much higher than among women. In 2002, the male:female ratio across the whole country was approximately 4:1 (Figure 4.4). In León and Chinandega, the male:female ratio was approximately 6:1, while there was little or no male excess in many other departments with low mortality rates from CKD. Departments with a male excess tend to be in the Pacific region. Although mortality rates among women were much lower than in men, in 2002 rates for women were still highest in León and Chinandega, and were also elevated in Granada and Rivas, all departments in the Pacific region.

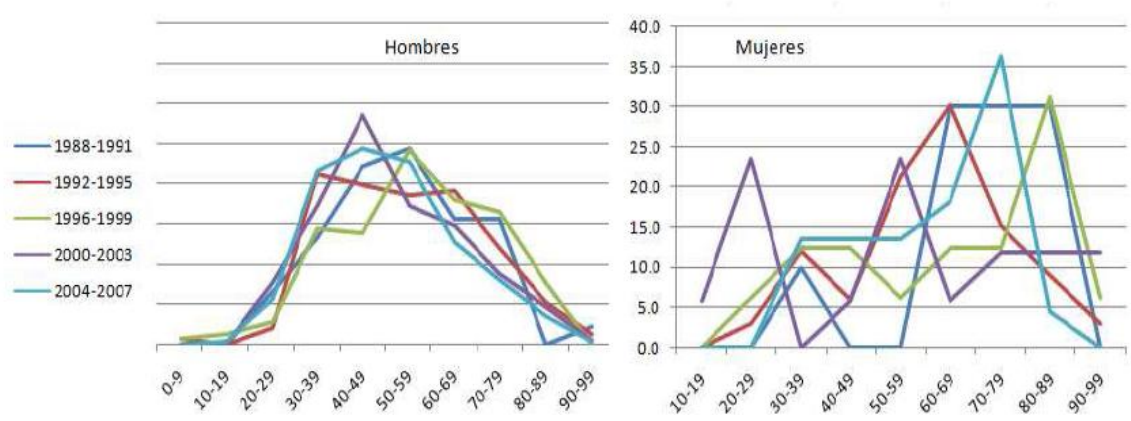
Figure 4.4- CKD mortality rates by sex and departments in Nicaragua (2002).



Source: Nicaragua Ministry of Health. Dirección General de Planificación y Desarrollo. Oficina Nacional de Estadísticas.

Another key demographic characteristic of the epidemic is the young age at which victims are affected. In Chichigalpa, for example, deaths were concentrated in young-middle age groups among men, while female deaths did not show a particular pattern (Figure 4.5).

Figure 4.5 Distribution of CKD deaths (1988-2007) in Chichigalpa (Nicaragua) by sex and age.



Source: Narváez-Caballero & Morales-Mairena, 2008.

4.3.3 Prevalence data and key risk factors investigated

There have been a number of studies (most of them unpublished) conducted in Nicaragua and other Central American countries in the past ten years. For a more exhaustive list, one can access reviews by Cuadra et al (2006) and Brooks et al (2009). Some of these studies were carried out using proteinuria measured by dipstick as a measure of kidney damage. However,

as diabetes and hypertension are unlikely to be important causes of CKDu in Mesoamerica, measures of glomerular proteinuria may be less informative. Nevertheless, virtually all of these studies detected elevated rates of proteinuria (mostly in the microalbuminuria range). The study conducted by Dominguez and colleagues (2003) is of particular interest because it is the only study to our knowledge that has produced prevalence estimates in certain communities of Mexico, Guatemala, and Honduras (El Salvador was also included). 40 % of the men in the coastal areas showed some degree of proteinuria, compared to 14% of women in the coastal areas and 10% of men living at >500 masl. 28% of men with proteinuria living in the coastal zone were <45 years old, and only 12% of them reported pre-existing hypertension or diabetes.

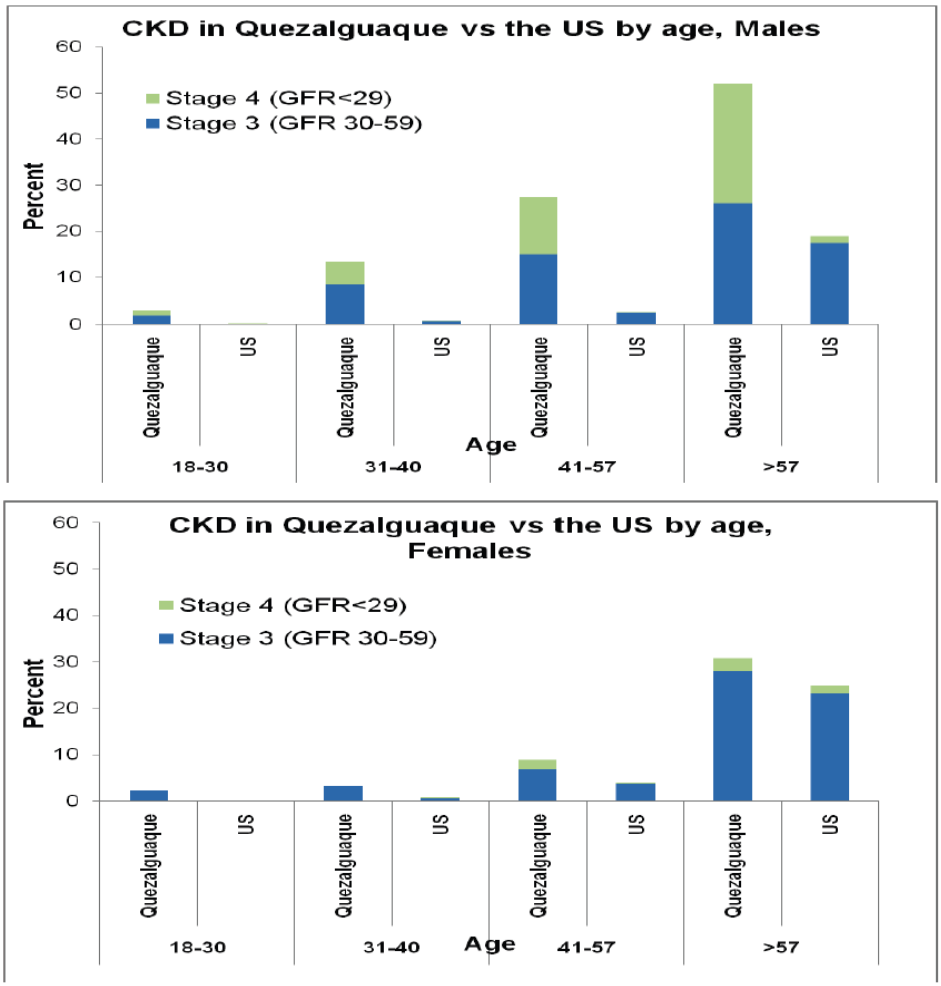
As mentioned before, CKDu/MeN seems to follow a cluster pattern. For example, the Pan-American Health Organization (PAHO) sponsored a study that was conducted in the city of Managua (only 125 km away of Chichigalpa, one of the hotspots of the epidemic in Nicaragua) in 2003 to assess prevalence of and risk factors for chronic disease, particularly diabetes and hypertension, but included a serum creatinine test at the request of the Ministry of Health. A total of 1,704 respondents (85% response rate) were randomly selected using a stratified neighborhood sampling plan. Less than 1% of subjects between the ages of 20-59 had eGFR values <60ml/min per 1.73m2, and there were no differences in prevalence between males and females [J Amador, unpublished data].

Table 4.1 summarizes prevalence studies published in the peer-reviewed literature. All studies analyzed serum creatinine and reported the GFR. In addition, they all assessed medical, occupational, environmental and behavioral risk factors by questionnaire. Four main conclusions have emerged from these studies (Brooks et al, 2012):

1. There are clear differences in risk of CKD according to type of industry and occupation. Workers in the sugarcane cultivation, mining, and fishing or shipping industries have higher prevalence rates, while areas in which coffee growing and services dominated show no evidence of excess disease.
2. Persons living at low altitudes are more likely to have CKD than those living at higher elevations. Peraza et al (2012) have demonstrated how this pattern persisted even when comparing two sugar cane communities at different sea levels.
3. Males appear to be much more affected than women. Women, although less affected, follow also the same distribution as men (i.e higher prevalence's in low altitude communities and higher risk with long term exposure to coastal sugar or cotton plantation work). According to Peraza et al (2012) adjusted OR for 10 years increments of time performing this work was 3.1 (95% CI 2.0-5.0) and 2.3 (95% CI 1.4-3.7) for men and women, respectively.
4. Age groups affected by CKD are much younger than the age distribution that one would expect for a chronic disease. To illustrate this point, O´Donnell et al (2011) compared the distribution of eGFR among age and sex groups in Quezalguaque (a municipality located in León, Nicaragua) with the same groups in the USA based on data from NHANES (Figure 4.6). The overall prevalence of decreased eGFR was 1.8 times greater in Quezalguaque. However, the relative differences were much greater at younger ages: men ages 30–41 in Quezalguaque had rates 16 times as high and also

were much more likely to have Stage 4 disease compared to their counterparts in the USA. Women in Quezalguaque also had elevated age-specific prevalence compared to women in the USA, but the differences were much more modest.

Figure 4.6- Prevalence of kidney disease in Quezalguaque compared with the USA using the NHANES 1999–2006 data



Source: Adapted from O´Donnell et al. (2011).

Table 4.1- Summary of regional community-based studies of chronic kidney disease that used serum creatinine and eGFR as disease estimates

Locale(s) Studied	Occupation*	N	Altitude†	Prevalence and Population	Risk Factors
El Salvador: 2 Communities Jiquilisco, Usulután and Sesori, San Miguel (García-Trabanino, 2005)	Agriculture	291	Low	• Study included men only • Sampling pattern only tested serum creatinine in male coastline residents with 1+ or greater proteinuria	• Dipstick proteinuria (1+ or greater) noted in 46% in low altitude coastal community vs. 13% in high-altitude inland community • 37 of 80 men with lab measurements had creatinine≥1.5 mg/dL; of these 37, 81% had 1+ proteinuria and only 19% 2+ or greater proteinuria
		62	High		
Nicaragua: 9 Communities of León (Telica, Malpaisillo, León), and Chinandega (El Viejo, Corinto, Posoltega, El Realejo, Chichigalpa, Somotillo) (Sanoff, 2010)	Varied	997	Not stated in article but likely varied elevations	• Only in men (after too few cases found in women) • 12.4% of population with eGFR <60 ml/min/1.73 m2	• Agricultural field labor, consumption of “lija” (alcohol available in a form susceptible to contamination by toxins), and consumption of >5L of water per day independently associated with presence of reduced eGFR • Hypertension and diabetes not more common in CKD
Nicaragua: 5 Communities León and Chinandega (Torres, 2010)	Subsistence Farming + Mining	445	Low	• High prevalence of reduced eGFR <60 ml/min/1.73 m2), with 14% of men but only 3% of women • Abnormal creatinine levels in 31% and 24% of male and female agricultural workers, respectively, at 100-300 m above sea level, but not occurring at higher altitudes	• Abnormal creatinine levels not common in higher altitude village (i.e coffee) and services village • Banana/sugarcane independently associated with higher creatinine in men and mining/subsistence farming independently associated in both men and women • High self reported use of NSAIDs and history of UTI, not associated with decreased kidney function in the multivariate analysis
	Banana/Sugar	384	Low		
	Fishing	216	Low		

	Services	140	Low		
	Coffee	92	High		
El Salvador: BajoLempa region (Orantes, 2011)	Agriculture	775	Low	• eGFR<60 ml/min/1.73 m2 in 17% of men vs 4% of women	• Non-steroidal anti-inflammatory drug use common • 44% respondents reported renal disease, but 40% of those cited UTIs • Most affected individuals with neither diabetes nor hypertension
Nicaragua: Quezalguaque, León (O´Donnell 2011)	Inland Varied, agriculture	771	Mostly Low	• High prevalence of reduced eGFR, (<60 ml/min per 1.73m2), 20% of men vs 8% of women • 13.4% of men age 30-41 with low eGFR	• Residence at lower altitude (<500 m) associated with lower eGFR
Nicaragua: Matagalpa (Laux, 2012)	Coffee	267	Very High	• Very low prevalence (0,7%)of reduced eGFR, with no individuals below 40 years old having reduced eGFR	
El Salvador: 5 Communities (Peraza, 2012)	Sugar	129	Low	• High overall prevalence of reduced eGFR	• Discrepant findings in sugar communities with eGFR below 60 ml/min per 1.73m2 common (18% of men) at low altitude sugar communities but rare (0-2%) in the high altitude sugar community; • Women less affected than men but following the same pattern (8% in low altitude sugar cane vs 1-3% in other communities) • Proteinuria uncommon and low-grade
	Sugar/Services	159	Low		
	Sugar	120	High		
	Coffee	124	Very High		
	Urban Services	132	High		

Source: adapted from Weiner et al, 2012.

4.3.4 Follow-up studies

There has been only one follow-up study in the region that investigates MeN/CKDu. This study, yet unpublished and conducted by our group (McClean et al, 2012), assessed the renal function during the 6-months harvest period of 2010-2011 of 284 sugar cane workers contracted for different job tasks (cane cutting, seed cutting, seed planting, irrigating, pesticide application, drivers and factory workers). Miners, port and construction workers kidney function was also assessed with only one point in time measures. Kidney damage markers, such as serum creatinine and glucose, urinary albumin, NGAL, NAG and IL-18, were measured. This study also assessed heavy metals exposure in a subsample of these pre-post harvest paired samples (blood lead concentrations, and urinary cadmium, total arsenic and uranium).

Results from linear regressions showed a significant decrease during the harvest of the estimated Glomerular Filtration Rate (eGFR) in those job categories that involve more strenuous tasks and are most exposed to heat, namely cane ($p = 0.006$), and seed cutters ($p = 0, 03$), along with significant increases in the levels of NAG, NGAL and IL-18. Decreased kidney function was prevalent in the non-sugar cane workers when compared to US prevalence. Albuminuria and glycosuria, however, were minimal in all the job categories.

On the other hand, overall concentrations of lead, cadmium, arsenic and uranium levels did not exceed international exposure standards, did not increase significantly during the harvest and there were no significant differences between the different job categories. Furthermore, levels of lead, cadmium and uranium were not associated with any biomarker of kidney damage or kidney function. Concentrations of total arsenic in urine exceeded WHO's guideline of 100 µg/L for 3 workers out of 99 sampled. Though there were not significant differences in urinary arsenic by job category, workers with the highest arsenic exposures (above the 90^{th} percentile) were found to have significantly higher serum creatinine ($p=0.04$) and significantly lower eGFR ($p=0.01$), while controlling for age and sex. The fact that the inorganic fraction of arsenic, which is the most nephrotoxic, was not measured makes it difficult to conclude whether arsenic may be associated with kidney damage in this context.

4.3.5 Biopsies

There is only one published report of a series of biopsies on CKDu/MeN patients. Wijkström and colleagues (2013, on press) performed light microscopy, immunofluorescence, and electron microscopy examinations on 8 male patients that used to work on plantations in EL Salvador with estimated glomerular filtration rates from 27 to 79 mL/min/1.73 m2. They found a similar pattern in all 8 biopsy specimens, consisting on extensive glomerulosclerosis (29%-78%) and signs of chronic glomerular ischemia in combination with tubular atrophy and interstitial fibrosis, but only mild vascular lesions. The electron microscopy indicated podocytic injury, and among other findings were no or low-grade albuminuria, normal blood pressure, no diabetes, frequent use of NSAIDs, increased levels of tubular injury biomarkers, and hypokalemia (in 6 of 8 patients). The authors were surprised by the presence of relatively extensive glomerular sclerosis, but preserved glomeruli and no vascular changes; and argue

whether those changes might be a sequelae of dehydration and NSAIDs intake, with subsequent activation of the renin-angiotensin-aldosterone system.

4.3.6 Causal hypotheses

Risk factors emerging from the mentioned studies, and well known environmental causes of CKD thought to play a role in this epidemic, have served researchers to build up a list of potential causes of CKDu/MeN. Researchers at the SALTRA workshop in Costa Rica (November 2012), convened to prioritize the different hypotheses (see table 4.2) based on the level of evidence and plausibility gathered to date, as well as their feasibility for being studied (Wesseling et al, 2013).

Table 4.2-Proposed Priorities for Exploring Hypotheses for Causes of MeN

Highly Likely, High Priority to Investigate Further
Heat stress and dehydration (including electrolyte imbalances)
Non-steroidal anti-inflammatory drugs (NSAIDS)
Possible, High Priority to Investigate Further
Arsenic
Fructose intake
Nephrotoxic medications, including homeopathic medications
Leptospirosis and other endemic infections
Possible, High Priority but Logistically Difficult at this Time
Genetic susceptibility and epigenetics
Low birth weight and other prenatal, perinatal, and childhood exposures that increase susceptibility
Unlikely but strongly believed, Medium Priority to Investigate Further
Pesticides
Urinary tract diseases and sexually transmitted diseases (STDs)
Little Information, Medium Priority to Investigate Further
Calcium in drinking water, or water 'hardness'
Medication contamination and use of homeopathic medicines and non-approved drugs
Unlikely, Low Priority for Further Investigation
Lead
Mercury
Cadmium
Uranium
Aristolochic acid

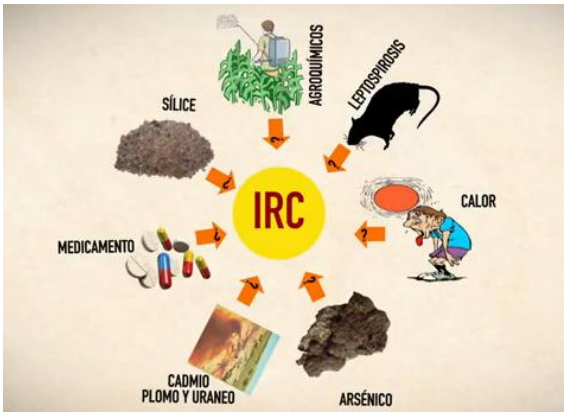
Source: Wesseling et al, 2013.

This section does not intend to dig into each of the causal hypotheses proposed to date, but to provide an overview of the main ones being considered to date. Brief concept papers written by several researchers for each of the causal hypotheses have been included in the SALTRA report (Wesseling et al, 2013). While some factors are well-known causes of CKD (e.g. heavy metals), others still require the development of new biological pathways to be tested in experimental and observational studies (e.g. heat exposure). All together, they constitute a complex map of causal hypotheses. A proposed causal web by Ramirez-Rubio et al. (2013b) can be found in the next chapter. Furthermore, CKD development in this context may require that two or more factors are present (e.g. heat exposure + NSAIDs, fructose intake or agrichemical use). Indeed, connections between the different risk factors (susceptibility, initiation and/or progression), proposed because of its biological plausibility and/or its association with CKDu in the different prevalence and case control studies to date, have brought researchers to think that etiology is presumably multifactorial, with multiple insults to

kidney damage being responsible of kidney function deterioration over time. Even more, susceptibility factors may be distributed along the life-course including childhood, with initiation and/or progression factors playing a role during adulthood, particularly during occupational exposures (Ramirez-Rubio et al, 2013a; Wesseling et al, 2013).

In any case, most speculated scenarios would involve the same mechanism: repeated subclinical insults to the kidney or overt acute kidney injury (AKI) episodes leading to CKD. Although it generally had been held that AKI leads to CKD only in the absence of functional recovery from the initial injury, recent evidence suggests that residual subclinical damage may increase the risk of CKD (Coca et al, 2007; Venkatachalam et al, 2010; Coca et al, 2012).

Figure 4.7 Causal hypotheses of CKDu/MeN



Source: Image from a YouTube video titled "CAO Case in Nicaragua: Collaborative research on Chronic Kidney Disease by Boston University". Available at: http://www.youtube.com/watch?v=DAll0C9Ax_E

Given the tropical climate and challenging working conditions in the region, investigators have proposed volume depletion and heat stress as one of the main potential mechanisms for CKD in the region (Brooks et al, 2012; Wesseling et al, 2013). Altitude appeared in the different prevalent studies as an important factor to take into account (i.e. higher prevalence rates have been found at communities laying in lower altitudes). It is unclear whether the association between altitude and decreased kidney function represents a direct effect of altitude, as suggested elsewhere due to the hypoxia mechanisms damaging the renal tubules (Ghahramani et al, 2011), or to the particular distribution of environmental (e.g. water quality, heavy metals, infectious diseases) and/or occupational exposures (e.g. heat, agrichemicals). So far, altitude has been mainly considered a proxy to detect differences in temperature and weather conditions between the lowlands (hot and humid) and highlands (a little bit cooler) in this region, although this fact needs confirmation. Also, it is unclear whether the ambient temperature in Mesoamerica has increased over time due to global warming. Given the nature of the work (strenuous physical activity under very hot temperatures), witnessed by our group during an industrial-hygiene and occupational health assessment in 2010 (McClean et al, 2011), as well as other investigators (Crowe et al 2009; Delgado Cortez, 2009), performed by workers that showed an increased risk of kidney damage, investigators have postulated that a chronic state of dehydration plus muscle damage leaves individuals susceptible to accumulation of damage to the kidneys from repeated subclinical insults, which eventually develops into CKD. Along these lines, hydration and re-hydration practices deserve also further attention. For example, Johnson and colleagues are studying whether the combination of

dehydration eventually coupled with hydration with fructose enriched fluids, may result in increased fructose levels in the proximal tubules which may further damage proximal tubular cells (Wesseling et al, 2013, pp117-118).

With regard to heavy metals, there are documented chronic renal effects in humans from exposure to arsenic, lead, cadmium and mercury. In the context of CKDu/MeN, arsenic accumulates more evidence than the rest so far. As Laws & Amador explain in the SALTRA report (Wesseling et al, 2013) many of wells sampled in Telica, a municipality of Leon (Nicaragua) had arsenic levels exceeding the WHO exposure guidelines, and there is evidence of human exposure of arsenic-induced skin lesions in near-by communities. Finally, as noted above, high arsenic levels were also found to be associated with decreased kidney function among workers sampled by Boston University (McClean et al, 2012).

NSAIDS and other nephrotoxic medication intake are a key factor that may play an important role in a multifactorial causality web. They are widely available without prescription, but also commonly prescribed for urinary symptoms that may have a relationship with dehydration, as will be described in the next chapter (Ramirez-Rubio et al, 2013b).

Agrochemicals are extensively used in the region. Particularly, organophosphate and organochloride (highly used in the 80s) exposure has been found in the general population in breast-milk and blood (CIRA, 1997; Azaroff, 1999). Some of the current used agrichemicals (such as 2, 4-D, glyphosate, etc) have shown some evidence of kidney damage (but not CKD) in the literature (McClean et al, 2011). Evidence against this hypothesis are that the facts that CKD does not appear as prevalent in high altitude agricultural communities when compared to low altitude ones (although similar cultural practice and agrichemicals are used), and that high frequency of reduced eGFR has been found as well in occupations with no exposure to agrichemicals (miners, stevedores and construction workers). Nevertheless, this evidence is not enough to conclude that agrochemicals are not playing a role in this epidemic, being a hypothesis that deserves further attention. Furthermore, pesticides exposure is believed to be the primary cause of CKDu/MeN by most of the affected population, as well as many public health officials and researchers (as per an interview with El Salvador Minister of Health; Conner Gorry, 2013), and its use in the region is surrounded by safety issues concerns, that probably deserve immediate actions (whether or not they are related to CKDu/MeN).

Finally, infection by *leptospira*, is another interesting hypotheses to be considered. Leptospirosis is an emerging infectious disease and an occupational hazard for people who work outdoors, including sugar-cane field workers (WHO, 2003); plus high seroprevalence of leptospirosis has been described in northwestern Nicaragua (Ashford et al, 2000). Manifestations of leptospirosis range from subclinical infection to severe disease accompanied by acute kidney failure. To date, there has been little research regarding the potential for leptospirosis infection to cause chronic tubular dysfunction or CKD in the absence of severe and hemorrhagic acute illness (Daher et al, 2004; Cerqueira et al, 2008; Ganoza, et al 2010). Presently, we are studying in collaboration with the Centers of Disease Control (CDC, USA) *leptospira* infection (acute and chronic) in the urine and blood samples provided by the longitudinal workers study mentioned above (McClean et al, 2012).

The following table 4.3 provides the strengths and weaknesses of the different causal hypotheses based on the general knowledge and scientific evidence generated to date (Weiner et al, 2012).

Table 4.3- Potential Causes of Endemic CKD in Central America

Possible Causes	Supporting Data	Opposing Data
Toxins		
Agrichemicals	• Extensively used in the region • High rates of morbidity and mortality documented in areas with high proportion of agricultural workers • With high volume of water intake, low concentrations of toxins still may yield high kidney exposure • High organophosphate exposure in El Salvador agricultural communities	• No single agrichemical linked to tubulointerstitial kidney disease without significant other organ involvement • Similar agrichemicals used at higher altitudes but CKD does not appear highly prevalent • High frequency of reduced eGFR found in occupations with no exposure to agrichemicals: miners, stevedores, construction workers
Heavy Metals	• Typically manifests with indolent tubulointerstitial nephritis • Lack of sophisticated water delivery systems • Active volcanic region • Arsenic common in water and soil in the region • With high volume of water intake, low concentrations of toxins still may yield high kidney exposure • Association between high urinary arsenic levels and low eGFR levels among tested workers	• Limited evidence of high water levels of heavy metals, including cadmium, lead, arsenic, uranium and 10 others in water samples tested once in Chichigalpa, 2010 • No evidence of high urinary levels of cadmium, lead, or uranium or any association between these metals and reduced eGFR among tested workers • Male predilection
Aristolochic Acid	• Clinical course and manifestations consistent with Balkan endemic nephropathy • Multiple species of Aristolochia present in Nicaragua and used for herbal remedies	• Male predilection • No reported increased risk of genitourinary tract malignancies • No data regarding use of herbal remedies containing Aristolochia in epidemic areas
Medications	• Wide, poorly regulated availability of antibiotics, including aminoglycosides • Extensive use of non-steroidal anti-inflammatory drugs • May predispose to kidney failure in the setting of other toxins	• Unusual cause of chronic kidney disease at the population level in the absence of other factors, particularly in otherwise healthy individuals
Infections		

Leptospirosis	• Extremely common in this region • Associated with acute kidney injury • High seroprevalence • Agricultural workers known to be a high-risk group	• Insufficient number of diagnosed cases to account for epidemic; prevalence of a chronic carrier state unknown • Acute, severe disease not subtle • Uncertain link to CKD unless via acute kidney injury
Pyelonephritis	• Pyuria and genitourinary symptoms common in local population, including men	• Urine cultures in the setting of leukocyte esterase positivity or symptoms showed no growth in all 50 male workers tested, whereas 3 of 11 female workers had positive cultures
EpiGenetics/Developmental susceptibility	• Low birth weight (and therefore low nephron mass) fairly common • Recognized CKD anecdotally more common in persons with greater indigenous ancestry • Genes associated with protection against tropical diseases seem to increase the risk of CKD among persons with African ancestry allelic variation, including Hispanic populations	• On clinically performed ultrasonography, no evidence of systemic developmental or anatomic abnormalities • Nothing is known about the genetic admixture of the local population nor the prevalence of alleles that have been shown to be associated with an increased risk of CKD • Greater indigenous ancestry associated with lower socioeconomic status, which is a risk factor for CKD in other populations
Volume depletion, heat-related	• Low altitude Western Nicaragua is hot and humid, and is considered the hottest region in Nicaragua • CKD more common among workers engaged in heavy manual labor, typically consisting of men • CKD more common at lower altitudes	• Heat-associated acute kidney injury is not common in high income countries and, when present, tends to manifest coincident with multi-organ organ injury • Requires that acute kidney injury, either recognized or subclinical, be the cause of CKD • No documentation of frequent episodes of clinically recognized AKI caused by heat-related causes

Source: adapted from Weiner, 2012

4.4 Discussion

Mortality and prevalence data available indicates that CKD is among the most common causes of death in some Central American countries, and most important, CKDu/MeN cases are not distributed equally along sex, age, geographic regions, altitude and occupational groups.

As described above, epidemiological information about CKDu is still very limited. There are as yet no official or unofficial estimates of the numbers of new cases of CKDu, people living with the disease, or deaths attributable specifically to CKDu in Mesoamerica. The available information is based mainly on records of deaths attributable to CKD and a few community prevalence studies. For the most part, these studies have been conducted in areas where large numbers of cases of CKD have been reported; the extent of disease in other areas is not well understood. The fact that big companies in the agriculture or mining sector are conducting active screening programs prior to hire workers is contributing to identify cases and hotspots of the epidemic, facilitating to identify the causes attributed to a particular death, especially in poor and rural areas, or simply CKD cases that otherwise would go undiagnosed. It is not know whether in places where there has not been such an active screening linked to research projects or occupational health programs, cases may stay unrecognized, due to the lack of symptoms in early stages of CKD, the slow progression and the few clinical manifestations until kidney failure and uremia is present. Even more, these reasons also apply to explain why this epidemic is either occurring now or, if it has been going on for some time, just being recognized now. Critically, several Central American countries underwent conflicts and wars until a couple of decades ago, with life expectancy, sanitation and available health care increasing in the past years dramatically, which would have set up the conditions for a chronic disease to show as a prevalent one.

Nevertheless, there is variation in the extent of ascertainment of mortality data at national Ministries of Health by country within Mesoamerica. One study in the municipality of Chichigalpa (Nicaragua) compared the availability of mortality data from the national ministry of health register with the civil registration at the municipality, and found that 21% of deaths in the latter were not recorded in the national register (Narváez-Caballero et al, 2008). The benefit of relying on mortality data depends on the absence of substantial bias by geography, occupation, sex, and age in extent of reporting of death certificates or attribution bias in cause of death. However, to our knowledge, no studies have assessed the potential for bias.

The community- prevalence studies listed also present a number of limitations that result primarily from a lack of resources and have been described in earlier reviews (Cuadra et al, 2006; Brooks et al, 2010). One limitation common to all studies was that creatinine was tested only at a single point in time. Because the case definition for CKD requires two consecutive GFR values <60ml/min per 1.73m2, a single measurement overestimates the prevalence, but there is little information on its magnitude (Bottomley et al, 2011; de Lusignan et al, 2011). It should be noted that the U.S. National Health and Nutrition Examination Survey (NHANES), which serves as the primary source for prevalence of reduced GFR in the U.S. population, likewise uses only a single measurement; therefore, comparisons of prevalence in Mesoamerica with those in the United States are based on the same testing frequency. All

studies listed in the table used the Modification of Diet in Renal Disease (MDRD) study equation. However, in a community-based study where the great majority of subjects will have eGFR≥60ml/min per 1.73m2, the CKD-EPI equation appears to be more accurate (Stevens et al, 2010). Limitations also include the cross-sectional design (i.e. reliance on prevalent rather than incident cases), and the strong questionnaire-based assessments. With few exceptions, data on risk factors have been collected exclusively by questionnaire instead of environmental or biological testing, with potential for exposure misclassification in general and recall bias in particular (e.g. agrichemical use). Furthermore, questions have not been validated, nor have they been standardized across research groups and there is a lack of information on quality control measures.

Nevertheless, prevalence studies to date have served an important role in moving beyond mortality data, providing additional quantification to the magnitude and regional variation of MEN, and adding evidence for certain hypotheses about the causes of MEN/CKDu.

Further efforts should prioritize active surveillance of CKD incidence, its social and demographic determinants at the population level. Strengthened surveillance systems capable of monitor new cases arising from the clinical practice and current active surveillance systems in workplaces and research interventions are needed. As part of this effort, a consensus around a case definition of this particular presentation of CKD is also needed. This would benefit efforts to determine cause(s) of MEN, as well as provide information on whether the rate of cases is increasing, stable, or decreasing, which would be important for public health planning and evaluation of interventions. To date, data on the incidence of CKD are lacking since it is not usually included in the set of diseases included in surveillance systems in the region, which are focused almost exclusively on infectious diseases. Also, systematic data on the natural history of the disease are also lacking, such as time between early injury and occurrence of disease, latency time from occurrence to diagnosis, and survival time from diagnosis to death. A better understanding of these components might help focus on certain hypotheses by providing a framework for timing of exposure, along with an understanding of factors that might slow the progression of the disease.

In addition, research priorities to address gaps in knowledge include: 1) Prevalence studies in additional areas selected on a systematic basis, 2) Longitudinal studies capable of assessing incidence and progression, 3) Burden of disease studies, quality of life, and social determinants of the disease (social inequalities and poverty).

Taken together, strong advances have occurred in the past few years with Central American Ministries of Health and the Pan American Health Organization recognizing MeN/CKDu as a regional public health priority, and research projects taking place; but there is still a significant task to identify the causes of this puzzling epidemic and devoting enough resources to prevent, diagnose and treat the disease in a time manner.

CHAPTER V: HEALTH PROFESSIONALS' PERCEPTIONS AND BELIEFS REGARDING ETIOLOGY OF MeN/CKDu AND RELATED CONDITIONS, AND THEIR TREATMENT APPROACHES

"I want to understand the world from your point of view. I want to know what you know in the way you know it. I want to understand the meaning of your experience, to walk in your shoes, to feel things as you feel them, to explain things as you explain them. Will you become my teacher and help me understand?"

— James P. Spradley

5.1. Introduction

Chronic kidney disease (CKD) is a serious and increasing global health problem. Treatment for its most severe form, end stage renal disease, with dialysis or transplant is currently not available in many parts of the world (Levey et al, 2007; Moeller et al, 2002). Major known risk factors for CKD include diabetes and hypertension (Lea et al, 2002; Ejerblad et al, 2006). However, in lower income countries CKD may be associated with chronic glomerulonephritis and interstitial nephritis, which are generally ascribed to infectious and parasitic agents (Soderland et al, 2010). In Central America, case reports and government statistics document high mortality due to CKD, particularly among younger men and in certain regions of the Pacific coast (Garcia-Trabanino, 2002; Cerdas, 2005; Cuadra et al, 2006). Community prevalence studies in Nicaragua and El Salvador are consistent with these mortality data and have attempted to assess associations with pharmaceutical, behavioral, environmental and occupational exposures (Garcia-Trabanino, 2005; Torres et al, 2010; Sanoff et al, 2010; Laux, 2011 et al; O'Donnell et al, 2011; Orantes et al, 2011; Peraza et al, 2012). However, the causes of the high prevalence of CKD remain unknown.

Our team has been working in northwestern Nicaragua since 2009 as part of a mediation process (termed the "Dialogue Table") including the management of Nicaragua Sugar Estates Limited (NSEL), a major sugar producer in northwestern Nicaragua that operates the Ingenio San Antonio located in the town of Chichigalpa, and the Association of Chichigalpans for Life (ASOCHIVIDA), a group of approximately 2000 former NSEL workers and community members who are affected by CKD. The Dialogue Table was established in 2008 after ASOCHIVIDA filed a complaint with the Compliance Advisor Ombudsman (CAO), the independent office that handles complaints from communities against the World Bank Group's private sector arm which had provided funding to NSEL. CAO formed the Dialogue Table in response to the complaint, and the participants determined that they wanted an outside scientific group to make an independent assessment of the epidemic. Our team led by the Boston University School of Public Health was selected by Dialogue Table participants.

Our research occurred in stages, beginning with a "scoping study" summarizing the available information on CKD in the region, identifying data gaps, and recommending research activities to address those gaps (Brooks et al, 2009). During the process of conducting the scoping study we learned from members of the Dialogue Table and from informal discussions with area physicians of the frequent occurrence of a set of symptoms referred to locally as "chistata," characterized by painful urination and often accompanied by "kidney" and/or back pain, and the common diagnosis of urinary tract infections (UTIs) among young men. Our final list of potential causes was extensive. We identified several feasible activities that would provide more information to evaluate these hypotheses (Brooks et al, 2009, McClean et al, 2010).

Among the subsequent research activities, we conducted qualitative interviews in Chinandega and Leon (regions with the highest CKD mortality rates in Nicaragua) both with physicians who are likely to diagnose or treat chistata, UTI, and/or CKD and with retail pharmacists who are likely to fill prescriptions or sell medications to treat these conditions. Medication use was on our list of hypotheses because

members of the Dialogue Table reported that use of analgesics, and potentially nephrotoxic antibiotics that would require prescriptions in many higher income countries, were common in Nicaragua and could be obtained without a prescription. Medications are a common cause of *acute* kidney injury and may be associated with CKD (Sandler et al, 1989). For example, non-steroidal anti-inflammatory drugs (NSAIDs) are a frequent cause or contributor to acute kidney failure in the setting of severe volume depletion or other nephrotoxins. Aminoglycosides (a class of broad spectrum antibiotics) also are common causes of acute kidney failure, with risk factors including preexisting kidney disease, concomitant nephrotoxic medication use, and dehydration/volume depletion.

While there had been a great deal of media and activism focused on CKD and potential causes in Nicaragua, we did not know the general knowledge, opinion or practice of physicians and pharmacists in the region. Our aims in this study were to: (1) increase our understanding of health professionals´ perceptions regarding CKD in the region (characteristics of the affected population, causal hypotheses, symptoms, diagnostic tools, treatment and prognosis); (2) determine whether further study of the relationship among hydration practices, diagnosis of UTI/chistata and use of medications is warranted; and (3) explore potential opportunities for public health interventions related to the CKD epidemic aimed at physicians and pharmacists. This was the first effort known of by our team to engage physicians and pharmacists in Nicaragua in a formal assessment of the CKD epidemic.

5.2. Methods

Recruitment and interviews were conducted in November 2010. In order to select interviewees, we acquired a list of public or government health care institutions from the Ministry of Health and a registry of pharmacies in Chinandega and León. We also acquired a list of private health institutions or clinics where CKD patients may seek care from the Social Security System. Each of the two regions (Chinandega and Leon) contained one main referral hospital, 13 and 16 public health centers, 6 and 4 social security or private clinics, and 145 and 212 pharmacies, respectively. We selected physicians from the health care institutions in each region so as to achieve a diverse sample with regard to sex, location, type of institution, and specialty of physician, and then selected pharmacies based on proximity to the sampled health care facilities. We estimated that 10 physicians and 10 pharmacists would be a feasible number to interview given time and budget constraints, would be sufficient to reach data saturation (i.e., redundancy in results to the point where interviews are not providing new information), and would enable us to identify trends and themes in the data.

We prepared an interview guide that consisted of 34 and 24 open-ended questions asked of physicians and pharmacists, respectively (available upon request). Open-ended questions asked in qualitative studies are typically designed to learn the opinion, beliefs, experience and reported behavior of the interviewee without prescribing response options or leading interviewees to a particular answer. We developed the interview guide with our hypotheses in mind, to learn from interviewees their opinion about the strength of various hypotheses based on their experience, and also to learn from physicians

and pharmacists about their own hypotheses, if any, regarding the causes of CKD in the region. We began with broad questions such as, “What can you tell me about CKD in Nicaragua?” following up with more specific questions such as: “Do you think CKD is a problem in Nicaragua?”, “Has the number of cases you see each year changed in the time you have been in practice?” and “How would you describe the affected population?” For each major topic, we started with a broad question, “What do you think are the causes of CKD in Nicaragua?” and then followed up with specific questions that might indicate their opinion of hypothesized causes. Questions asked of both groups addressed the following topics: 1) prevalence and causes of CKD in Nicaragua; 2) population most affected; 3) beliefs regarding dysuria-related symptoms, prevalence of UTI and sexually transmitted infections; and 4) knowledge regarding indications and risks associated with use of potentially nephrotoxic medications. Additionally we asked physicians about standards and criteria for diagnosis and treatment of CKD and the availability and cost of diagnostic tests and medications. Interviews included a series of follow-up questions or probes to elicit additional information on these topics if not volunteered. A draft of the physician interview guide was tested with a physician in Nicaragua associated with our study team to determine if the length, organization and Spanish translation were appropriate.

Interviews were conducted individually and in-person by a native Spanish speaker (ORR). Informed consent was obtained at the time of the interview. Ethics approval was obtained both from the Institutional Review Board of Boston University Medical Campus and the Nicaraguan Ministry of Health. All interviews were electronically recorded, transcribed and translated into English by a professional transcription agency and cross-checked for accuracy by the interviewer. All identifiers (i.e., names of individuals, locations and names of institutions) were removed from English and Spanish versions of the transcripts prior to analysis. To protect anonymity, all research participants are referred to by a masculine pronoun regardless of sex.

Interviews were analyzed using standard social science methods for analyzing qualitative data (Patton, 2002). An initial list of 33 codes (words and phrases used to tag portions of the transcript) was created based on the interview questions, which were based on existing hypotheses (Bogdan, 1982). Coding the transcripts enables analysts to retrieve codes and associated data and to assign values of frequency, presence/absence and relationship with other codes (MacQueen et al, 1998). For example, the code “CKDOCC” allowed us to tag and retrieve all text, including positive and negative examples, in which the occupation of CKD patients is referred to either in response to the question about the populations that are most affected, or in a different part of the interview. Text associated with each code can then subsequently be analyzed, and additional codes developed (e.g., OCCAG, to tag more specific mentions of agricultural occupation) (Bogdan et al, 1982). Two analysts (MKS, ORR) separately coded the text and then together reviewed the codes. After agreement was reached on coded text, each analyst prepared summaries of the findings and met again to test agreement. The last step was to organize codes under thematic or conceptual headings and to assign meaning to the codes (Bogdan et al, 1982). Specifically, we sought to understand how potential causes of the CKD epidemic identified in the interviews compared with our pre-existing hypotheses, and how perception of these causes might potentially inform future research activities.

5.3 Results

All physicians and pharmacists approached by our team agreed to be interviewed, demonstrating a high level of awareness and concern regarding the disease, societal effects and implications for their practice. We interviewed ten physicians and nine pharmacists who were diverse with regard to geographic areas and locations (Table 5.1). Six of the nine pharmacists reported a degree in Pharmacy and/or Chemistry, with three reported no formal training. The results of our analysis are organized by headings that reflect key findings of physician and pharmacist opinions, experiences and practices.

Table 5.1 -Characteristics of interviewees

	Physicians n=10	**Pharmacists n=9**
Sex		
Women	6 (60)	8 (89)
Men	4 (40)	1 (11)
Region		
Leon	5 (50)	5 (56)
Chinandega	5 (50)	4 (44)
Location		
Rural	6 (60)	6 (67)
Urban	4 (40)	3 (33)
Health Institution		
Health Center	7 (70)	NA
Hospital/2nd level health center	3 (30)	NA
Physician Specialty		
General Practitioner	6 (60)	NA
Nephrologist/Internal Medicine	4 (40)	NA
Years of experience	19 (9-30)	10 (1-25)
Interview length (minutes)	41 (11)	18 (4)

Results are number (%) for all variables, except median (range) for years of experience and mean (sd) for interview length. NA: Not Applicable

5.3.1 CKD: Prevalence, Diagnosis and Prognosis of a serious and increasing problem in Nicaragua

All interviewees stated that CKD is a serious problem. With the exception of one pharmacist, all interviewees were of the opinion that the number of CKD cases has increased each year, although four physicians suggested that this may be partially explained by improvements in monitoring and diagnoses.

All physicians indicated that the observation of decreased kidney function over time would be the basis for the CKD diagnosis. Seven of the ten physicians observed that patients with CKD had no or very little

proteinuria. Six physicians relied on serum creatinine to classify a patient with CKD while four also calculated estimated glomerular filtration rate. Seven physicians indicated that, while ideal, kidney ultrasounds require referral to a hospital or private clinic and are usually not feasible. In terms of treatment, seven of the ten physicians said they do not have access to national or regional guidelines for the diagnosis and treatment of CKD and therefore relied on guidelines for other chronic diseases (hypertension or diabetes), their own familiarity with CKD, or, in one instance, an "ad hoc" protocol developed by local physicians. Three physicians who said they had access to CKD-specific diagnosis and treatment guidelines referred to protocol of Nicaragua's Nephrology Association or the Ministry of Health, or international standards published by the National (USA) Kidney Foundation's Kidney Disease Outcomes Quality Initiative. All physicians indicated that patient survival depends on stage of CKD at diagnosis, self-care, and nutrition as well as access to dialysis or transplant, neither of which are widely available in Nicaragua.

5.3.2 CKD Causes: Strong sun, hard work, and… water?

All ten physicians and six of nine pharmacists described men as the most affected population. More than half of the physicians volunteered that the causes of the CKD epidemic did not include the traditional risk factors of diabetes or hypertension. Four physicians suggested that, while women experience CKD, the majority of cases in women can be explained by underlying risk factors. The affected population was most often described as under age 45.

Seven of the ten physicians identified agricultural workers (fruit pickers and workers on banana, cotton, melon, peanut, rice, and sugar plantations) as most affected by CKD. Eight of nine pharmacists explicitly identified what they thought was an occupational association, with one stating: "Those who are not diabetic are almost all males who work in the fields." Other non-agricultural occupations mentioned included miners, construction workers and bricklayers.

Nine physicians referred to exposures at work, with seven identifying exposure to sun and heat as the major occupational factor associated with CKD. All ten physicians described many possible contributors to CKD, with six using the term "multi-factorial." Chemicals used in agriculture were identified as a possible cause by half the physicians, and three talked about contaminated drinking water, with two referring specifically to heavy metals.

Water was the common denominator in eight of nine pharmacist responses regarding the causes of CKD; however, there were two distinct perspectives on the role of water. Four pharmacists thought that insufficient water intake was the primary problem, while four other pharmacists believed that drinking contaminated water was the problem. Three of the four pharmacists who thought dehydration was the problem also thought it was not the only cause, or else, as one said, "everyone would be dead." Another pharmacist summarized beliefs about causes:

> "I think that [CKD] is from the dehydration they get in the fields, their work is hard, hard, and they ruin themselves taking diuretics and further draining their body water."

Other causes of CKD mentioned by more than one interviewee (in order of frequency) were the use of nephrotoxic medications, alcohol, and poor nutrition. Other possible contributors mentioned included leptospira infection and volcanic ash.

5.3.3 "Chistata" and UTI: Physician perspectives on their diagnosis and treatment, and associations with CKD

All physicians said that "chistata" is a colloquial term used to characterize a constellation of symptoms including "pain," "burning" and the "urgent" need to urinate, with chistata approximating the clinical term dysuria. Nine physicians opined that dehydration is a probable cause of chistata, while eight also cited UTIs. There was disagreement among physicians as to how closely infection was related to chistata, with some equating the two, and at least one insisting, "they cannot just be considered synonymous."

When queried further about UTI, four of the ten physicians claimed to diagnose UTI based on the results of a urine culture. However, two of these physicians acknowledged that they do not have the facilities to analyze a urine culture and, as one stated, it would be "at the cost of the patient." Instead, these two along with five others admit to diagnosing UTI based on a urine exam indicating the presence of leukocytes, bacteriuria and/or nitrites via dipstick when available, under a microscope, or a combination of both practices. One physician suggested UTIs are probably over diagnosed:

> "What happens is that we have been doing a bad management of the urinary tract infections… sometimes we consider that the appearance of white blood cells, leukocytes, in urine is enough. But it is not like that."

When asked about the relationship among chistata, UTI and CKD, seven physicians said that UTIs may be associated with CKD. Five thought the connection had to do with repeated, non-treated or inadequately treated UTIs and regular use of broad spectrum antibiotics.

There was no agreement regarding the relationship of nephrolithiasis with CKD. Four physicians said that stones are not frequent, while six said they are very frequent. Three physicians named kidney calculi as a possible cause of chistata, with two associating them with "too concentrated urine" or "sandy urine" due to dehydration.

When asked how chistata is treated, seven physicians said they recommend oral rehydration solutions, and seven recommended urinary analgesics such as phenazopyridine or the antibiotic nitrofurantoin. Five physicians specified that they would not treat chistata without further tests. Antibiotics that they

might then prescribe included fluoroquinolones, amoxicillin, and penicillin, with one physician naming aminoglycosides.

Treatment of UTIs included the same urinary analgesics and antibiotics enumerated for chistata plus other beta-lactam antibiotics and cephalosporins. One physician stated that gentamicin should be used only in very select cases, cautioning of its nephrotoxicity. When answering a question regarding the uses of gentamicin later in the interview, most physicians responded that it was reserved for severe UTI or sepsis patients. Two noted its nephrotoxicity, with one noting he sometimes had to use gentamicin due to the unavailability of other medications.

Eight physicians acknowledged the possible nephrotoxic effects of long-term intake of NSAIDs, with six saying they would not prescribe them (or would restrict their use) in CKD patients. However, two physicians said NSAIDS are prescribed for back pain.

5.3.4 Pharmacist perceptions of UTI, Chistata, CKD and their treatment

Pharmacists did not make the distinctions that physicians did among the symptoms or conditions of low back pain, chistata, UTI, and CKD. Three pharmacists referred to CKD as an infection. Three said that UTI and chistata are the first step, or "warning," of CKD. Three other pharmacists suggested that CKD results from UTIs that were either not treated or treated ineffectively.

When asked specifically about prescriptions filled for CKD, eight of nine pharmacists named phenazopyridine (not known to be nephrotoxic) in addition to antibiotics. One pharmacist offered the following: "For kidney insufficiency, doctors from health centers here commonly prescribe ampoules called gentamicin, trimethoprim-sulfa..." This contradicts what the majority of physicians indicated as per gentamicin prescription. For the most part, however, pharmacists described giving the same medications as the physicians for treatment of chistata and UTIs plus some antispasmodic drugs and NSAIDs. Pharmacists did not mention oral rehydration solutions.

Pharmacists' responses revealed a range of opinions and practices regarding their role in the diagnosis and treatment process. On one end of the spectrum are those who would not prescribe anything until a patient is diagnosed by a physician, and on the other end are those willing to provide treatment directly. However, most said they would first recommend the patient obtain a urinalysis and have it interpreted by a physician. In some cases pharmacists offered to interpret lab results. Four pharmacists described the practice of selling antibiotics without a doctor's prescription or a formal diagnosis. One pharmacist described the pressure to sell antibiotics over the counter with no diagnosis: "They'll say, 'Why can't you give me one directly? Why do I have to go and spend money to get an exam?' So I recommend them cefadroxil."

Three pharmacists said that they would first recommend diuretics: "Furosemide is the most common, I sell it every day." Recognizing that diuretics could further tax the kidney, another pharmacist explained:

> "When I sell it, I tell people to drink plenty of fluids. But at the end, that is the decision of people, right? But I let them know that [the medication] can damage the kidney."

Several physicians opined that pharmacists are enabling patients to self-medicate and selling potentially harmful medication: "The problem is that NSAIDs and gentamicin are sold in the markets of cities like candies" and "agricultural workers with back pain get shots of gentamicin by vendors in stores." Taking diuretics for chistata was also described by two physicians:

> "… these patients that normally have chistata are dehydrated most of the time, so what they do is take a furosemide to get rid of [chistata]. And later, they arrive with cramps, more dehydrated, and it becomes chaos."

Table 5.2 . Further illustrative interview quotes

CKD: A serious and growing problem in Nicaragua
"In the last five years they've been dropping like flies, almost one person dead or very, very sick with this illness each day." (Pharmacist) *"More men are affected than women… This doesn't mean that women are not… but these are women who have a chronic pathology to begin with." (Physician)* *"What caught my attention was that there are young people, very young, and that they are rapidly getting worse. We have them age 20, 23, 24, and I am telling you we have detected it in people that maybe have two years, and they have deteriorated, significant health deterioration…" (Physician)* *"If we had family support… they are very cooperative, but they're people of few resources, they become like a burden for their family, and many of them are also patients who are alone, they're not retired, they're in poverty, so really, that kind of speeds up the suffering." (Physician)* *"Well, I see dehydration, with faces like [scrunched up], uncomfortable. When they have had the illness for a while and are deteriorating, they look yellow, thin. There are others that swell. When cases like this come in, I send them to the health center to get a doctor." (Pharmacist)*
Causes of CKD(in Nicaragua)
"Normally, they don't present symptoms [of CKD], but the symptoms that they do present with when they arrive for the first time, are generally heat strokes, people are working and have cramps, nausea, vomiting, with fever. …After re-hydrating them, people improve and the creatinine levels after three or four months can lower from 2.8 or 3, to normal ranges. And they get reintegrated to work, but they still suffer from heat strokes. So there are possible consequences from dehydration." (Physician) *"Patients have worked in agriculture - more than anything they have been exposed to the sun a lot, they have been exposed to dehydration, they work with sugar cane, they work in the cotton fields." (Physician)* *"The majority are young men who have worked under the sun, under the heat, and maybe they haven't been drinking enough liquid when they have sunstroke or dehydration." (Physician)*

"Men have other factors associated with deterioration of kidney function… the use and abuse of substances, alcohol or drugs, strenuous exercise or work, not necessarily exposure to pesticides, but work in the fields, or work in areas with many hours exposed to the sun…" (Physician) *"Those who are not diabetic are almost all males who work in the fields…. The most affected ones are, well, from the rural counties who work with pesticides and insecticides…. And then there are those from the city who are diabetic." (Pharmacist)* *"I insist that I believe there is an interstitial nephritis, caused by something in the environment that we have been unable to determine." (Physician)* *"Well, now we have leptospira, which means renal damage and it is also a public health problem. We had patients admitted here with abnormal creatinine, with confirmed diagnosis of leptospira. That is frequent." (Physician)*
Perceptions of UTI, Chistata, CKD and their treatment
"[With the general urine exam] we can have data that give us signals of a chronic kidney infection, just starting or advanced. CKD signs will be changes in the casts, a change in urine density; we can find a change in the pH, within the important phases, and the leukocyturia, that don't have an established etiologic cause. That should get our attention and start us to investigate." (Physician) *"Here people say, 'sell me something for chistata', the majority want you to sell them furosemide pills. These pills dehydrate them. It's my duty to explain to them that this pill is not for that. 'Take the phenazopyridine for that.' 'No because that stains me (the urine), give me the half white ones please… look, give me the white ones, if not I'll go shop somewhere else'." (Pharmacist)*

5.4 Discussion

Interviews of physicians and pharmacists in Western Nicaragua were consistent with the existence of an epidemic of CKD, describing characteristics similar to those noted in the prevalence studies conducted in the region (Torres, 2010; Laux, 2011; O'Donnell, 2011). These include CKD being more frequent among men, starting in young adulthood, and associated with mainly agricultural work but also occurring in miners and construction workers. The sentiment that diabetes and hypertension cannot explain the CKD epidemic in Nicaragua was also expressed in the interviews. If this sentiment is in based on empirical observations of physicians and pharmacists, these findings provide additional verification of the findings of local studies.

The primary contribution of this study, however, is highlighting medication usage patterns described by physicians and pharmacists that have not previously been well characterized in cohort studies and government data, and which may lead to increased risk of CKD among younger men in this region.

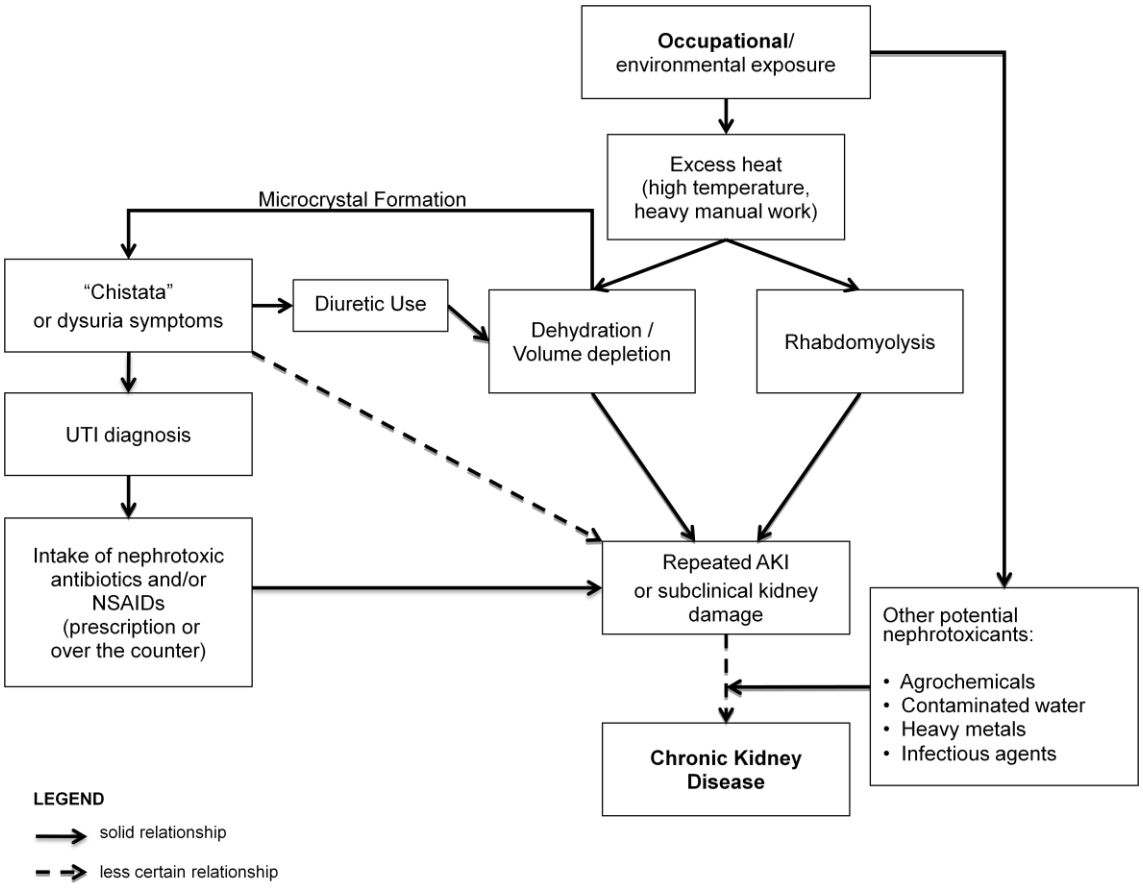
Interviews indicate that patients with CKD, or at high risk for CKD, may be receiving nephrotoxic drugs for the syndromes of chistata and UTI. In particular, aminoglycoside antibiotics and chronic use of NSAIDs are associated with acute kidney injury (AKI) in a dose- and duration-dependent manner

(Harirforoosh et al, 2009; Sweileh, 2009; Lopez-Novoa et al, 2011). This association is particularly notable in the setting of volume depletion, which may be further aggravated due to the use of diuretics. Reports that attempt to quantify regional NSAIDs use vary widely, with self-reported use ranging from 10 to 75% of study populations (Torres et al, 2010; O'Donnell et al, 2011; Orantes et al, 2011).

Despite the media attention given to the potential role of agrichemicals in causing CKD, physicians and pharmacists were much more likely to cite exposure to heat, physical work and dehydration as key factors responsible for CKD; a common combination of exposures among men in this region and in Central America (Delgado-Cortez, 2009; Crowe et al, 2009; Kjellstrom et al, 2011; McClean et al, 2011). This opinion is consistent with local and regional studies, which find that CKD appears more common among occupations where strenuous work undertaken at high ambient temperatures is typical (Torres et al, 2010; Sanoff et al, 2010, O'Donnell et al, 2011; Laux, 2011; Peraza, 2012; Weiner et al, 2012; Brooks et al, 2012). Although not a recognized cause of CKD, heat stress is associated with volume depletion and may also be associated with muscle damage (rhabdomyolysis), both of which may predispose individuals to AKI, particularly in the presence of nephrotoxic medications (Schrier et al, 1967; Demos et al, 1974). Critically, there is a growing body of literature that suggests that AKI, even if mild or if the serum creatinine recovers to baseline, may result in residual structural damage and ultimately progress to clinically recognized CKD (Coca et al, 2007; Levin et al, 2008; Venkatachalam et al, 2010; Coca et al, 2012; Bedford et al, 2012).

Figure 5.1 demonstrates the relationships, with different degrees of certainty, identified by interviewees regarding several key causal hypotheses, beginning with the relationship between occupational exposure to heat and manual labor, followed by the occurrence of Chistata and diagnosis of UTI, use of medications, and eventual CKD, while including other potentially nephrotoxic exposures in what is largely considered to be a multi-factorial disease.

Figure 5.1– Conceptual Model of Excess Heat Hypothesis



Our interviews provide inconsistent yet important information regarding the perceived role of drinking water in CKD, with half the pharmacists believing that not drinking enough water leads to CKD, and the other half believing that CKD may be caused by drinking contaminated water. These beliefs, taken at surface value, would result in contradictory recommendations to area residents and workers. However, it is too early to say which view is correct and the truth may lie somewhere in between. Even if drinking water contaminants are not known to directly cause CKD, it cannot be concluded that drinking water quality is acceptable in various areas. Additionally, the emphasis of interviewees was that dehydration is due in part to perceptions of water and water consumption practices. The question remains, is hydration in general recommended or water consumption specifically? If lacking clean water, should workers and the general public be advised to hydrate with soda or fruit juices? What are the risks and benefits of hydrating with these high-sugar fluids compared with unclean water? To the extent that the contradictory views of pharmacists may represent those in the broader community, further examination of these questions would be beneficial before public health professionals design interventions with messages simply exhorting people to drink more water.

According to both physicians and pharmacists, UTI is one of the main causes of chistata. However, in medical practice elsewhere UTI is very uncommon among younger men with the rare cases typically

associated with congenital urinary tract abnormalities or obstruction such as from nephrolithiasis. Urine cultures analyzed by our group from 47 male workers whose urine dipstick results were positive for leukocyte esterase or who had complained of dysuria within the past 24 hours were all negative for bacterial growth (McClean et al, 2012), supporting the impression of some physicians that UTI is likely over-diagnosed.

Exposures to agrichemicals, heavy metals, alcohol consumption and infectious agents were also mentioned in physician and pharmacist interviews and are the focus of additional causal hypotheses in the region (Torres et al, 2010; O'Donnell et al, 2011; Sanoff et al, 2011; Orantes et al, 2011). In particular, infection by leptospirosis, although mentioned by only one interviewee, is considered an occupational hazard for people who work outdoors, including sugar-cane field workers, and a high seroprevalence of leptospirosis has been described in northwestern Nicaragua (Ashford et al, 2000; WHO, 2003). Manifestations of leptospirosis range from subclinical infection to severe disease accompanied by acute kidney failure. To date, there has been little research regarding the potential for leptospirosis infection to cause chronic tubular dysfunction or CKD in the absence of severe acute illness (Daher et al, 2004; Cerqueira et al, 2008; Ganoza et al, 2010).

Limitations of our study include the inability to determine the truthfulness and accuracy of interviewee responses, and the small number of physicians and pharmacists interviewed. Qualitative studies are rarely designed to have statistically representative samples of the population, but for several of our questions we could have learned more about the general practice among physicians in particular if we had been able to conduct more interviews and achieved a higher degree of redundancy in the responses. However, these interviews have highlighted several potential contributors to CKD in Nicaragua including heat stress and use of potential nephrotoxic medications, supporting the plausibility of a multi-factorial cause of CKD. These findings also provide impetus for future cohort studies designed to examine such exposures and diagnoses.

Recommendations

Many features of CKD in Central America require further elucidation. These include identifying the true cause of "chistata" and UTI-like symptoms, evaluating potential associations among heat stress, UTI diagnosis and nephrotoxic medication use, evaluating the role of systemic infectious diseases, and assessing the potential contribution of all of these factors to the development of CKD. However, until such time, the possible association between the occupational exposure to heat and the use of potentially nephrotoxic medications may be a beneficial focus of regional preventive health programs (Ramirez-Rubio, 2013a), including at the workplace, at the community level, and in health care settings. Surveillance systems of worker symptoms of heat exposure and workload, together with hydration practices, could be consolidated by governmental agencies, periodically assessed for lessons learned/best practices, and shared with employers. Similarly, there have been several assessments of water quality in specific areas. These assessments could be compiled with a similar analysis, identifying and addressing data gaps with further testing, so (a) public health professionals can make scientifically-

based recommendations regarding consumption of water, and (b) local health agencies could come up with rapid and inexpensive processes to check for local variability (e.g., arsenic may be an issue in some areas, not in others). Overall, research into current hydration practices and promoters, and obstacles to greater hydration, could help shape public health recommendations.

Common self-medication reported by pharmacists and physicians of widespread (across the community and particularly workers) dysuria related symptoms with diuretics, NSAIDs and antibiotics could be the target of greater oversight and health protection measures from government agencies. The contradiction between pharmacists reporting that physicians prescribe gentamicin, and physicians reporting otherwise, could also be explored via observations at pharmacies where prescriptions are filled. Finally, we recommend increased and targeted education for pharmacists and physicians on the diagnosis and treatment of heat exposure symptoms, UTIs and CKD, along with strategies that may assist pharmacists when confronted with patients who demand medications that may be nephrotoxic.

Conclusions

High mortality has been documented due to CKD among young adult men in Northwest Nicaragua, and specific areas of Central America including regions of El Salvador and Costa Rica, the cause of which remains unknown. This study identifies perceived causes of the CKD epidemic in Nicaragua by physicians and pharmacists working on the front line. Our analysis articulates perceptions of physicians and pharmacists that heat stress and subsequent volume depletion experienced by manual laborers plays a role in the frequent occurrence of dysuria-like symptoms, which are often treated with non-steroidal anti-inflammatory drugs, diuretics and antibiotics that may be further nephrotoxic.

CHAPTER VI: KIDNEY DAMAGE AMONG NICARAGUAN ADOLESCENTS

"All grown-ups were once children... but only few of them remember it."

— Antoine de Saint-Exupéry, The Little Prince

6.1 Introduction

An epidemic of chronic kidney disease of unknown cause/s (CKDu), also called Mesoamerican Nephropathy (MeN), is occurring across that region (Weiner et al, 2013; Ramirez-Rubio et al, 2013a, Wesseling et al, 2013). CKD constitutes the second leading cause of death among adult males in El Salvador (El Salvador MoH, 2010), and the prevalence of the disease is 20% or greater in some communities, following a cluster pattern (Torres et al, 2010; Peraza et al, 2010). In addition to the toll on the health of the population, the epidemic has had a substantial negative impact on the social and economic well-being of the affected communities.

Community prevalence studies indicate that relatively young men who engage in strenuous outdoor work (e.g. agriculture, mining, etc), and live in the lowlands of the Pacific coast are among the most affected (Garcia-Trabanino et al, 2005; Torres et al, 2010; O'Donnell et al, 2010; Sanoff et al, 2010; Orantes et al, 2011; Laux et al, 2011; Peraza et al, 2012). These studies have also helped to characterize CKDu/MeN, mainly, as a tubulo-interstitial non-proteinuric nephropathy, where diabetes and hypertension do not explain the vast majority of the cases.

The etiology is presumably multifactorial (Wesseling et al, 2013; Ramirez-Rubio et al, 2013a), and there are a variety of hypothesized causes: heat and heavy physical work load (chronic volume depletion), heavy metals such as arsenic or cadmium, agrichemicals, systemic infectious diseases such as leptospirosis, and chronic intake of potential nephrotoxic medications such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics or aminoglycoside antibiotics (Soderland 2010) that are used for a wide range of symptoms (Ramirez-Rubio et al, 2013b). It has been hypothesized (Brooks et al, 2012, Wesseling et al, 2013) that chronic volume depletion coupled with muscular damage, although not a recognized cause of CKD, might result in repeated bouts of clinical or subclinical acute kidney damage (AKI), that eventually could lead to CKD (Bedford et al, 2012).

Studies to date have focused on the general adult population or on workers in occupational settings. However, because many of the affected adults are diagnosed with CKD at very young ages, and because most of the hypothesized etiologic factors could already be present (perhaps with less intensity) earlier in life, we hypothesized that initial damage to the kidney may begin in childhood. Markers of tubular damage may appear before there is a sufficient reduction in glomerular filtration to result in an elevation of serum creatinine, the most conventional biomarker of CKD. The aim of this exploratory study was to investigate novel biomarkers of early kidney damage among Nicaraguan adolescents who have not yet entered the workforce, in order to further explore factors associated with the initiation and progression of CKD in this context.

6.2 Methods

6.2.1 Study design

We conducted a cross-sectional study among adolescents aged 12-18 in four different schools in Nicaragua. Schools were selected to represent a range of demographic, geographic, and environmental factors that have been associated with CKD mortality among adults. Among others, altitude of the community (i.e. lower altitude has been associated with a higher prevalence of CKD in adults), and occupation (i.e. workers at the sugar cane industry have showed higher CKD prevalence). Based on these factors, schools were ranked in an increasing order of a priori risk of CKD. School 1 was located in the department of Jinotega (adult CKD mortality rate of 5 per 100,000 inhabitants in 2010) at an altitude of 800 meters above sea level and surrounded by coffee plantations. School 2 was located in the department of Masaya (2010 CKD mortality rate = 12 per 100,000) at 220 meters above sea level near the Pacific in an area dedicated mainly to small farms and predominantly artisan-related occupations. School 3 and 4 were both based in the same city in the department of Chinandega (2010 CKD mortality rate = 95 per 100,000), an area in the Pacific region located almost at sea level, and characterized by production of crops such as sugar cane, rice, and peanuts. School 4 participants were restricted to students whose parents (one or both) had worked in the sugar cane industry. At the remaining schools, we excluded children if either parent had ever worked in the sugar cane industry.

The study protocol was approved by the Institutional Review Boards at the Boston University Medical Center and the Nicaraguan Ministry of Health. As the study population was mainly younger than 18 years old, the parents of all study subjects provided written informed consent and adolescents were asked to provide assent prior to participation in the research activities.

6.2.2 Study population

To be eligible, students must have never worked in a manual labor job (defined as at least one month of manual labor, whether paid or not). Students who had previously participated in sports or vigorous exercise on the same day as samples were being collected, and female students who were currently menstruating, were also excluded.

The recruitment process was planned in coordination with the Ministry of Education's (MOE) officials, including the heads of the MOE at the Department and municipal levels, and the schools' teachers. Our goal was to recruit at least 50 students at each school, with an equal distribution of male and female, and older (16-18 years) and younger (12-15 years) students. A flow chart of the study population at different stages is shown in Figure 2. Information regarding the purpose, procedures, and inclusion and exclusion criteria was briefly described to students in their classrooms. A packet of material containing a letter of invitation to the study, a parental consent and permission form, and a brief questionnaire was sent home with interested students.

After review of the invitation letter, interested parents returned the signed consent form the following day, together with a parental questionnaire, which solicited information on eligibility criteria and general family characteristics, including parents' vital status, education, length of residence in the area, current and longest occupation, whether either parent had ever held a job in the sugar cane industry, parents' medical history (CKD, kidney stones, hypertension and diabetes), and brief questions about the child's kidney health and medical history.

We included all students who met eligibility criteria based on the parental questionnaire. Study staff then asked the children to assent to participate. Those children who signed the assent form were then asked to complete a questionnaire (student's demographics, past work history, prior participation in sports or vigorous exercise on that day, length of time walked to school in the morning, current health problems, and frequency of urinary symptoms), which was reviewed to assess final eligibility (current menstruation status was obtained verbally by a female study staff member).

6.2.3 Sample collection

Urine samples were collected by the field study personnel in the morning before classes started to increase the likelihood that all samples would be taken before students became physically active. Prior to providing urine samples, participants were asked to wash their hands with soap and clean the perineal/genital area with an alcohol pad. Each adolescent was asked to void into a sterile, 100-mL red-topped container (Nipro). Urine samples collected at Schools 1 and 2 were immediately placed in a cooler and transported on the day of the collection to the National Laboratory Center for Diagnostic and References (CNDR) in Managua for storage at -80 °C. Samples in schools 3 and 4 were transported to the local Health Center, where they were aliquoted and stored at -20 °C (for a period of days to a week maximum) until transported to the CNDR.

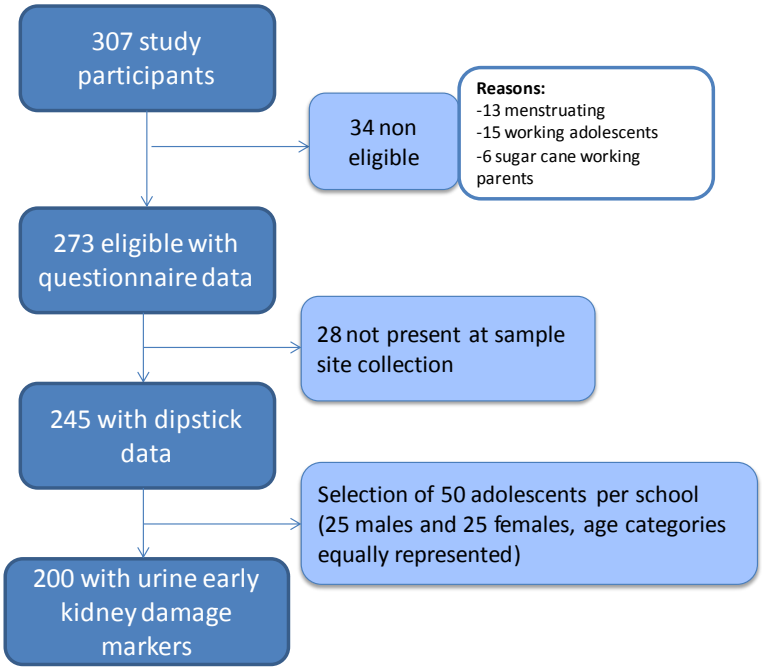
6.2.4 Sample analysis

Urine dipstick analyses were conducted on all 245 students to semi-quantitatively assess specific gravity, pH, leukocyte esterase, nitrite, protein, glucose, ketones, urobilinogen, bilirubin, and blood. A urine dipstick (Combur 10UX®, Roche Diagnostics) and a urine strip reader (Urisys 1100, Roche Diagnostics) were used.

Biomarkers of kidney damage were measured in urine samples collected from 200 participants (i.e. 50 participants randomly selected at each of the four schools equally distributed by sex and age category) (see Figure 2). Urine samples were shipped to the Division of Nephrology and Hypertension at the Cincinnati Children's Hospital Medical Center (Cincinnati, OH, USA), and were analyzed for albumin (µg/mg creatinine), a standard marker of increased glomerular permeability and damage, (NKF, 2002); neutrophil gelatinase-associated lipocalin (NGAL) (mU/mg creatinine), a marker increased when kidney tubules are acutely damaged (Hasse, 2009; Devarajan, 2008); N-acetyl--D-glucosaminidase (NAG)

(ng/mg creatinine), a marker of proximal tubular epithelial cells damage (Skalova, 2005); and interleukin-18 (IL18) (pg/mg creatinine), a cytokine indicative of tubule inflammation and necrosis (Parikh, 2005). Urine creatinine (mg/ml) was also determined in order to normalize the urinary biomarkers according to urine concentration. Albumin and creatinine were measured by immunoturbidimetry and a colorimetric modification of the Jaffe reaction, respectively, on a Siemens Dimension Xpand plus HM chemistry analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). NAG activity was measured using a colorimetric assay (Roche Diagnostics, USA). NGAL (Bioporto, Gentofte, Denmark) and IL18 (MBL, Intl., Woburn, MA) were measured by enzyme-linked immunosorbent assay (ELISA). The time between urine collection and analysis of biomarkers ranged from 30-45 days.

Figure 6.1- Adolescent study population flow chart



6.2.5 Data analysis

Information from questionnaires, urine dipsticks, and kidney biomarkers was merged and analyzed using SPSS statistical software (version 18). The distribution of each biomarker was characterized using graphical displays and summary statistics. When the reported value was less than the limit of detection (LOD), the LOD divided by the square root of 2 was substituted. The exception was for NAG since no LOD was available; we substituted them using a value equal to the lowest reported value divided by the square root of two. All biomarkers exhibited a lognormal distribution and were natural log-transformed prior to analysis to satisfy normality assumptions.

Linear regression models were used to assess potential predictors of biomarkers of kidney damage. Because the biomarker concentrations were natural log-transformed for the purpose of analysis, the exponentiated β estimates (eβ) can be interpreted as multiplicative differences or "mean ratios" for each index variable category compared to the mean of the reference variable category. We used directed acyclic graphs (DAG) to identify potential confounders (Hernan, 2006). One of the main objectives was to evaluate differences in urinary biomarkers by school (four categories), which served as a proxy for the region of residence and for differential environmental exposures and/or parents' occupations. Fathers' education (as a proxy for socio-economic status), and length of residence (as a proxy for accumulation of community environmental or parents' occupational exposures) were entered in the models to adjust for confounding based on their effect on the estimate of association (>10% change) between school and the kidney damage biomarkers. Although sex and age were not confounders because they were balanced across schools as part of the study design, which we confirmed empirically, we included both as covariates in all multivariate adjusted models because it increased the precision of the estimates for some biomarkers. We also conducted linear regression analyses of the association between kidney injury biomarkers and selected parameters measured by urine dipstick.

6.3 Results

6.3.1 Population characteristics

A total of 245 students (51% females) were enrolled and provided questionnaire information. Table 6.1 summarizes the demographics of the study population and relevant information on the adolescents and their parents' health.

The sex distribution among students was quite similar across schools, while the proportion of younger students was slightly higher at School 1 and 4. We also assessed the type of transport and time walked to get to the school. Among those who walked, the median time to get to school was 10 minutes, with a minimum of 1 and a maximum of 60 minutes. Only 5% of children at Schools 1 and 4 walked ≥30 minutes compared to 13%-18% at Schools 2 and 3. Regarding self-reported urinary symptoms and "kidney" disease, a total of 23 parents reported their child (12 boys and 11 girls) to suffer from kidney

problems, with no significant differences by school (p=0.967). ¨Kidney" related reasons for hospitalization reported by parents included: "kidney infections" (17), "urinary problems" (2), urinary tract infections (1), "kidney problems" (1), "kidney stones" (1), and "kidney insufficiency" (1). A total of 15 children (10 girls and 5 boys) reported kidney problems, with no significant differences by school (p=0.269). In particular they used terms such as: "kidney infection" (5), "kidney problems" (4), "Pain or burning while urinating" (4), and "kidney pain" (2). The proportion of students who reported "suffering from pain while urinating" frequently (1-2 times per month or weekly), were 10% in School 1, 23% in School 2, 24% in School 3, and 15% in School 4. Urine biomarkers of kidney injury were not associated with self-reported frequent dysuria, nor with kidney or urinary problems reported by either parents or children (results not shown).

Parents had resided in the same region within Nicaragua for a median of 33 years (range 2-57), with no significant differences among schools. Almost no parents of students at School 4 had completed a university or professional education compared to a range of 17%-30% at the other schools. Schools 2 and 4 showed the highest percentage of fathers (51 and 54% respectively) who did not have education beyond primary school, compared to about a third of those at the other schools. Among mothers, the percentage with no more than a primary school education was much higher at School 4 (62%) than among the other schools (28%-42%). At School 4 (where almost all parents were sugar cane workers as per the study design), 13% of students' fathers had died compared to 5%-6% at the other schools. The same pattern was observed for self-reported CKD, with relatively low rates (2-6%) among mothers across all schools, while 36% of fathers at School 4 reported CKD compared to 2%-6% at the other schools.

Table 6.1 Characteristics of the study population

Schools	1. Jinotega	2. Masaya	3. Chinandega	4. Chinandega	TOTAL
	n=63	n=52	n=63	n=67	N=245
	n (%)	n (%)	n (%)	n (%)	n (%)
ADOLESCENTS' CHARACTERISTICS					
Sex					
Male	33 (52%)	25 (48%)	32 (51%)	31 (46%)	121 (49%)
Female	30 (48%)	27 (52%)	31 (49%)	36 (54%)	124 (51%)
Age group					
12-15y	37 (59%)	25 (48%)	33 (52%)	38 (57%)	133 (54%)
16-18y	26 (41%)	27 (52%)	30 (48%)	29 (43%)	112 (46%)
Minutes walked to school					
<10	15 (26%)	7 (16%)	8 (15%)	37 (62%)	67 (31%)
10-29	39 (69%)	30 (67%)	38 (72%)	20 (33%)	127 (59%)
≥30	3 (5%)	8 (18%)	7 (13%)	3 (5%)	21 (10%)
Symptoms					
Pain while urinating					
Never	38 (60%)	21 (40%)	30 (48%)	44 (66%)	133 (54%)
1-2/year	19 (30%)	19 (37%)	17 (27%)	13 (19%)	68 (28%)
1-2/month	5 (8%)	8 (15%)	11 (18%)	5 (7.5%)	29 (12%)
1/week	1 (2%)	4 (8%)	4 (7%)	5 (7.5%)	14 (6%)
"Kidney" problems	7 (11%)	8 (15%)	9 (14%)	8 (12%)	32 13%)
Reported by child	4 (6%)	6 (12%)	2 (3%)	3 (4%)	15 (6%)
Reported by parent	6 (10%)	5 (10%)	7 (11%)	5 (7%)	23 (9%)
PARENTS' CHARACTERISTICS					
Years parents lived in the same region (department)					
<5	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (0.4%)
5-9	0 (0%)	0 (0%)	2 (3%)	0 (0%)	2 (0.8%)
10-19	10 (16%)	13 (27%)	19 (31%)	17 (26%)	59 (24.7%)
20+	53 (84%)	35 (71%)	40 (66%)	49 (74%)	177 (74.1%)
Father education					
No school	1 (1.5%)	2 (5%)	4 (7%)	6 (10%)	13 (6%)
Primary	15 (24.5%)	19 (46%)	15 (27%)	28 (44%)	77 (34.5%)
Secondary	31 (51%)	14 (33%)	23 (41%)	27 (43%)	95 (43%)
University/professional	14 (23%)	7 (17%)	14 (25%)	2 (3%)	37 (16.5%)
Mother education					
No school	0 (0%)	1 (2%)	0 (0%)	3 (5%)	4 (2%)
Primary	17(28%)	17 (40%)	20 (34%)	34 (57%)	88 (39%)
Secondary	26 (43%)	16 (37%)	28 (47%)	23 (38%)	93 (42%)
University/professional	18 (29%)	9 (21%)	11 (19%)	0 (0%)	38 (17%)
Sugar cane parent job					
Father or mother worked in sugar cane	0 (0%)	0 (0%)	1 (2%)	63 (94%)	64 (26%)

Parents' health reported background					
Either parent reported CKD	7 (11%)	3 (6%)	4 (6%)	26 (39%)	40 (16%)
Father					
CKD	4 (6%)	1 (2%)	4 (6%)	24 (36%)	33 (16%)
Kidney stones	7 (11%)	1 (2%)	1 (2%)	6 (9%)	15 (7%)
Hypertension	8 (13%)	6 (12%)	6 (10%)	12 (18%)	32 (13%)
Diabetes	7 (11%)	3 (6%)	5 (8%)	3 (5%)	18 (9%)
Deceased	3 (5%)	3 (6%)	3 (5%)	9 (13%)	18 (8%)
Mother					
CKD	4 (6%)	2 (4%)	1 (2%)	3 (5%)	10 (5%)
Kidney stones	6 (10%)	4 (8%)	1 (2%)	3 (5%)	14 (7%)
Hypertension	11 (18%)	11 (21%)	8 (13%)	12 (18%)	42 (20%)
Diabetes	2 (3%)	2 (4%)	4 (6%)	4 (6%)	12 (6%)
Deceased	0 (0%)	0 (0%)	3 (5%)	2 (3%)	5 (2%)

6.3.2 Dipstick

Dipstick data were available on all 245 study participants (Table 6.2). A higher proportion of girls than boys had values of urine specific gravity in the three highest hexiles of urine specific gravity (34.7% vs. 22.3%, respectively; p=0.0005), indicating that girls had more concentrated urine. Both leukocyte esterase and nitrites were only very rarely detected in the study population. Nitrites were detected only in 5 adolescents (2%). 23 (9.4%) adolescents had a positive leukocyte esterase, of whom 22 were females evenly distributed among schools. Protein (7 cases or 2.9%) or glucose (2 cases or 0.8%) was only rarely present in the urine of study participants.

Table 6.2- Urine dipstick measures by school and sex (n=245)

	SCHOOL (Department)				SEX		TOTAL
	1. Jinotega	2. Masaya	3. Chinandega	4. Chinandega	Females	Males	Total
	n=63	n=52	n=63	n=67	n=124	n=121	N=245
Urine dipstick measures	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
SPECIFIC GRAVITY							
1,005	4 (6%)	2 (4%)	7 (11%)	16 (24%)	13 (10%)	16 (13%)	29 (12%)
1,010	14 (22%)	19 (37%)	18 (29%)	25 (37%)	30 (24%)	46 (38%)	76 (31%)
1,015	18 (29%)	12 (23%)	20 (32%)	20 (30%)	38 (31%)	32 (27%)	70 (29%)
1,020	19 (30%)	12 (23%)	14 (22%)	6 (9%)	29 (23%)	22 (18%)	51 (21%)
1,025	4 (6%)	3 (6%)	3 (5%)	0 (0%)	6 (5%)	4 (3%)	10 (4%)
1,030	4 (6%)	4 (8%)	1 (2%)	0 (0%)	8 (7%)	1 (1%)	9 (3%)
pH							
5	41 (65%)	25 (48%)	26 (41%)	19 (28%)	66 (53%)	45 (37%)	111 (45%)
6	10 (16%)	11 (21%)	19 (30%)	24 (36%)	27 (22%)	37 (31%)	64 (26%)
6.5	1 (2%)	3 (6%)	3 (5%)	7 (10%)	4 (3%)	10 (8%)	14 (6%)
7	8 (13%)	9 (17%)	10 (16%)	12 (18%)	18 (15%)	21 (17%)	39 (16%)
8	3 (5%)	4 (8%)	5 (8%)	5 (8%)	9 (7%)	8 (7%)	17 (7%)
LEUKOCYTE ESTERASE							
Neg	57 (90%)	46 (88%)	58 (92%)	61 (91%)	102 (82%)	120 (99%)	222 (91%)
+1	3 (5%)	2 (4%)	0 (0%)	1 (1.5%)	6 (5%)	0 (0%)	6 (2%)
+2	1 (2%)	4 (8%)	2 (3%)	1 (1.5%)	7 (6%)	1 (1%)	8 (3%)
+3	2 (3%)	0 (0%)	3 (5%)	4 (6%)	9 (7%)	0 (0%)	9 (4%)
NITRITE							
Neg	63 (100%)	51 (98%)	61 (97%)	65 (97%)	122 (98%)	118 (97%)	240 (98%)
Pos	0 (0%)	1 (2%)	2 (3%)	2 (3%)	2 (2%)	3 (3%)	5 (2%)
BLOOD							
Neg	55 (87%)	45 (86%)	57 (90%)	60 (90%)	102 (82%)	115 (95%)	217 (89%)
+1	2 (3%)	4 (8%)	1 (2%)	3 (4%)	9 (8%)	1 (1%)	10 (4%)
+2	1 (2%)	3 (6%)	2 (3%)	2 (3%)	5 (4%)	3 (2%)	8 (3%)
+3	2 (3%)	0 (0%)	2 (3%)	0 (0%)	4 (3%)	0 (0%)	4 (2%)
+4	3 (5%)	0 (0%)	1 (2%)	2 (3%)	4 (3%)	2 (2%)	6 (2%)
PROTEIN							
Neg	63 (100%)	48 (92%)	61 (97%)	66 (99%)	122 (98%)	116 (96%)	238 (97%)
+1	0 (0%)	3 (6%)	2 (3%)	0 (0%)	1 (1%)	4 (3%)	5 (2%)
+2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
+3	0 (0%)	1 (2%)	0 (0%)	1 (1%)	1 (1%)	1 (1%)	2 (1%)
GLUCOSE							
Neg	63 (100%)	50 (96%)	63 (100%)	67 (100%)	123 (99%)	120 (99%)	243 (99%)
+1	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	1 (0.5%)
+2	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	1 (0.5%)
KETONES							

	Neg	63 (100%)	52 (100%)	62 (98%)	67 (100%)	124 (100%)	120 (99%)	244 (99.5%)
	+1	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	1 (1%)	1 (0.5%)
UROBILINOGEN								
	Neg	63 (100%)	52 (100%)	60 (95%)	67 (100%)	124 (100%)	118 (98%)	242 (99%)
	+1	0 (0%)	0 (0%)	2 (5%)	0 (0%)	0 (0%)	3 (2%)	3 (1%)
BILIRUBIN								
	Neg	63 (100%)	52 (100%)	63 (100%)	67 (100%)	124 (100%)	121 (100%)	245 (100%)

6.3.3 Urinary biomarkers

200 adolescents (50 per school) had their albumin, IL-18, NGAL, and NAG in urine evaluated. All analyses were adjusted for urinary creatinine to adjust for urine concentration. The median urinary creatinine concentration value in male adolescents was lower as compared to female adolescents, 72 mg/dl (IQR 45-122) vs. 105 mg/dl (IQR 47-155) respectively (P<0.01), which was consistent with the differences between males and females in urine specific gravity by dipstick.

Sixteen students (8%) had an albumin in the range of microalbuminuria (≥30 ug/mg of creatinine), including only one higher than 300 ug/mg (a boy from School 4).

Linear regression models evaluated the association between the biomarkers concentration and sex, age, and school, adjusted for confounding (table 6.3). Boys had similar albumin creatinine ratio (ACR) mean concentrations compared to girls (0.86, 95%CI: 0.64-1.15). Among girls, the relative mean ACR concentration was lowest at School 2 and School 3.

Mean concentrations of NGAL in boys were one quarter of those in girls (0.25, 95%CI: 0.20-0.32). As age increased the mean concentration of NGAL increases by 9% per year (1.09, 95%CI: 1.02-1.16). The mean values of NGAL were at least 40% higher in School 4 than in School 1, both in males (1.56, 95%CI: 1.01-2.40) and females (1.42, 95%CI: 0.84-2.41).

Mean concentrations of NAG for boys were 21% lower than for girls (0.79, 95%CI: 0.59-1.04), and decreased 8% for each year of age (0.92, 95%CI: 0.85-0.99). The mean ratio concentrations of NAG were elevated among students at all schools (compared to School 1), particularly at the two schools in the department of Chinandega. School 3 had a mean concentration 1.97 times higher (95%CI: 1.34-2.88) and School 4 2.77 times higher (95%CI: 1.85-4.16) than School 1. Similar results were found when females and males were analyzed separately.

Mean concentrations of IL-18 were 72% lower in boys than in girls (0.28, 95%CI: 0.22-0.36), and there was no association with age. Mean concentrations of IL-18 were lower in all schools compared to School 1. However, when analyzed independently, females showed a decreased mean ratio values in all three

schools, while males in School 4 showed an increased mean ratio values when compared to School 1 (1.52, 95%CI: 0.93-2.50).

We also examined whether individuals with higher values of one biomarker were more likely to also have higher values of the other biomarkers as well. Taking the 75th percentile as a cutoff point for each of the biomarkers, 33% of the population had one biomarker above P75, 20% had two, 7% three, and 1% all four biomarkers. The percentage of students with at least at two biomarkers above P75 differed by school: 32% at School 1, 8% at School 2, 28% at School 3, and 44% at School 4 (p=0.001). Non-parametric correlations between the four biomarkers yielded mostly significant but weak correlation coefficients from 0.1 to 0.54.

We further analyzed whether leukocyte esterase positivity in females was associated with biomarkers of kidney injury. The mean of NGAL values was 2.13 (95% CI 1.46-3.12) times higher in leukocyte esterase positive females compared to leukocyte esterase negative females, but there were no differences for any of the other biomarkers. 28 (11.4%) adolescents tested positive for hemoglobin, with 22 being females evenly distributed among schools. IL-18 showed a higher relative mean (1.64, 95% CI 1.10-2.44) when we analyzed whether hematuria was associated with higher values of biomarkers of kidney injury in females. Lastly, urine biomarkers of kidney injury were not associated with any kidney or urinary problems reported by either parents or children.

Table 6.3- Urine biomarker's median and relative mean concentrations, by sex, age, and school (n=200)

		IL-18			NGAL			NAG			ACR		
	N	Median (IQR) pg/mg Creatinine	Relative mean* (95% CI)	Relative mean (95% CI)	Median (IQR) ng/mg Creatinine	Relative mean* (95% CI)	Relative mean (95% CI)	Median (IQR) mU/mg Creatinine	Relative mean* (95% CI)	Relative mean (95% CI)	Median (IQR) ug/mg Creatinine	Relative mean* (95% CI)	Relative mean (95% CI)
Overall	200	56.7 (28.9-122.8)	N/A	N/A	13.3 (6.3-28.1)	N/A	N/A	1.5 (1.0-2.2)	N/A	N/A	6.2 (4.6-10.5)	N/A	N/A
Sex													
F	100	117.7 (66.5-178.8)	Ref	Ref	25.7 (15.6-49.6)	Ref	Ref	1.6 (1.2-2.2)	Ref	Ref	7.5 (5.4-11.5)	Ref	Ref
M	100	32.3 (21.3-53.1)	0.28 (0.22-0.35)	0.28 (0.22-0.36)a	7.0 (4.2-10.9)	0.26 (0.21-0.32)	0.25 (0.20-0.32)a	1.4 (1.0-2.1)	0.79 (0.59-1.06)	0.79 (0.59-1.04)a	5.2 (4.1-7.7)	0.80 (0.61-1.04)	0.86 (0.64-1.15)a
Age													
1 y incre.	200	N/A	0.97 (0.89-1.06)	0.97 (0.90-1.04)b	N/A	1.10 (1.01-1.19)	1.09 (1.02-1.16)b	N/A	0.90 (0.83-0.98)	0.92 (0.85-0.99)b	N/A	0.96 (0.89-1.04)	0.96 (0.88-1.04)b
School													
1 Jinotega	50	54.1 (34.5-165.4)	Ref	Ref	12.0 (6.8-26.1)	Ref	Ref	1.3 (0.5-2-2)	Ref	Ref	5.3 (4.0-17.4)	Ref	Ref
2 Masaya	50	41.9 (20.4-119.3)	0.63 (0.42-0.96)	0.66 (0.46-0.95)c	13.1 (6.2-21.9)	0.88 (0.58-1.32)	0.95 (0.68-1.33)c	1.3 (0.9-1.6)	1.20 (0.82-1.76)	1.26 (0.84-1.88)c	7.1 (5.1-8.8)	0.95 (0.65-1.39)	0.96 (0.63-1.47)c
3 Chinandega	50	48.2 (25.4-115.3)	0.76 (0.51-1.15)	0.76 (0.54-1.08)c	11.4 (5.2-38.7)	0.98 (0.65-1.47)	0.96 (0.70-1.32)c	1.6 (1.3-2.1)	2.01 (1.37-2.94)	1.97 (1.34-2.88)c	6.1 (4.6-10.3)	0.89 (0.61-1.30)	0.89 (0.59-1.33)c
4 Chinandega	50	78.8 (38.3-121.1)	1.01 (0.67-1.53)	0.93 (0.65-1.34)c	15.1 (8.2-34.2)	1.23 (0.82-1.85)	1.42 (1.02-1.99)c	2.2 (1.4-3.1)	2.57 (1.75-3.77)	2.77 (1.85-4.16)c	6.0 (4.7-10.8)	0.92 (0.63-1.35)	0.91 (0.59-1.40)c
Sex:FEMALE													
School													
1 Jinotega	25	161.2 (103.1-22.2)	Ref	Ref	26.1 (17.3-47.0)	Ref	Ref	1.2 (0.5-2.0)	Ref	Ref	8.6 (5.4-26.1)	Ref	Ref
2 Masaya	25	118.6 (47.7-237.2)	0.68 (0.43-1.05)	0.67 (0.41-1.10)a	18.0 (11.7-27.5)	0.67 (0.43-1.04)	0.76 (0.46-1,26)a	1.4 (0.9-1.7)	1.34 (0.87-2.05)	1.25 (0.84-1.86)a	7.2 (5.0-9.0)	0.63 (0.40-1.01)	0.67 (0.40-1.12)a
3 Chinandega	25	112.6 (57.9-142.5)	0.63 (0.40-0.98)	0.64 (0.39-1.04)a	37.6 (18.7-67.0)	1.27 (0.81-1.98)	1.28 (0.78-2.12)a	1.8 (1.4-2.5)	2.04 (1.33-3.12)	2.01 (1.36-2.96)a	7.2 (5.6-11.1)	0.68 (0.43-1.08)	0.73 (0.44-1.21)a
4 Chinandega	25	93.4 (52.2-147.3)	0.61 (0.39-0.95)	0.48 (0.29-0.80)a	26.1 (16.3-77.3)	1.24 (0.79-1.93)	1.42 (0.84-2.41)a	2.2 (1.7-3.9)	2.75 (1.79-4.21)	2.52 (1.67-3.79)a	7.4 (5.5-10.9)	0.74 (0.47-1.17)	0.87 (0.51-1.47)a
Sex: MALE													
School													
1 Jinotega	25	36.3 (24.3-42.8)	Ref	Ref	6.9 (5.0-9.7)	Ref	Ref	1.5 (0.4-2.7)	Ref	Ref	4.7 (3.5-5.3)	Ref	Ref
2 Masaya	25	22.2 (9.1-38.24)	0.60 (0.38-0.93)	0.60 (0.36-1.00)a	7.4 (3.7-13.9)	1.15 (0.77-1.72)	1.35 (0.86-2.11)a	1.2 (0.7-1.5)	1.07 (0.56-2.05)	1.44 (0.71-2.94)a	7.1 (5.3-8.9)	1.43 (0.79-2.62)	1.49 (0.74-3.01)a
3 Chinandega	25	25.5 (21.6-42.2)	0.93 (0.59-1.45)	0.87 (0.55-1.37)a	5.5 (2.9-6.8)	0.75 (0.50-1.12)	0.77 (0.52-1.15)a	1.5 (1.2-1.9)	1.98 (1.04-3.77)	2.17 (1.15-4.09)a	4.9 (4.1-7.5)	1.16 (0.64-2.11)	1.08 (0.58-2.02)a
4 Chinandega	25	68.2 (27.4-97.3)	1.69 (1.08-2.65)	1.52 (0.93-2.50)a	8.3 (6.4-13.1)	1.23 (0.82-1.83)	1.56 (1.01-2.40)a	1.9 (1.3-2.9)	2.40 (1.26-4.58)	3.17 (1.59-6.33)a	5.5 (3.9-9.7)	1.15 (0.63-2.10)	1.10 (0.55-2.17)a

Abbreviations: IQR, interquartile range; CI, confidence interval

* Crude relative means

Adjusted relative means:

a. Adjusted for age, parents' length of residence in the same address, father's education

b. Adjusted for sex, parents' length of residence in the same address, father's education

c. Adjusted for sex, age, parents' length of residence in the same address, father's education

6.4 Discussion

This study is the first, to our knowledge, to focus on adolescents in the context of the CKD epidemic of unknown causes in the Central American region.

In epidemiological studies, CKD is usually measured by use of the estimated glomerular filtration rate (eGFR), which is primarily determined by the concentration of serum creatinine. However, creatinine is not sufficiently sensitive to detect early kidney damage, and a new series of biomarkers have arisen in the last years to measure AKI, and predict progression to adverse clinical outcomes (Devajaran, 2011), and even a long term kidney function decline (Shilipak et al, 2013), We used some of these novel urine biomarkers of kidney damage, which had been employed in a previous study of the kidney function of sugar cane workers through a harvest season in Nicaragua. In that study, those workers engaged in tasks with the most exposure to heat and strenuous work showed a more pronounced decreased in their kidney function, associated with an elevation of some of these urine biomarkers (McClean et al, 2012).

For this study, we recruited adolescents in schools from three different regions of Nicaragua. Children from Schools 3 and 4 (in school 4 a child's inclusion was restricted to parents with experience working on the sugar cane industry) resided in an area of high mortality and prevalence of CKDu. Results confirmed this fact; a third of fathers at school 4 self-reported CKD, and 13% had died. Our main *a priori* hypothesis was that students who attended school in areas of higher adult CKDu mortality would have higher levels of biomarkers than students in areas of lower mortality.

Results from the study were generally in accordance with our a priori hypotheses, with result to risk of kidney injury by school. The results for NAG were most consistent in this regard, with the relative concentrations rank ordered by school according to risk profile in both boys and girls. Boys at the school 4, which we had hypothesized would have the highest prevalence of kidney damage, had elevated levels of NGAL, NAG, and IL-18 compared to boys at the school 1. Both boys and girls at both of the two schools in the Chinandega region (schools 3 and 4) had elevated levels of NAG compared to children at school 1. Thus, both schools in Chinandega demonstrated some evidence of kidney damage among both boys and girls, but only boys at school 4 exhibited the most consistent evidence across all three tubular biomarkers. It is notable that the biomarker ACR, which is primarily associated with glomerular damage, did not demonstrate these patterns, and macroalbuminuria measured with dipstick, was only present in one of the students.

However, contrary to our expectations (since adult males generally have a higher prevalence of CKDu in Nicaragua), girls had higher mean levels than boys for all biomarkers, with particularly large differences for ACR and IL-18. These findings remained whether or not we adjusted the biomarkers concentrations for urine creatinine. Girls had higher urine creatinine concentrations and urine specific gravity than boys. If urine volumes were similar in boys and girls, we would expect urine creatinine concentration to be higher in boys, since boys usually excrete significantly more creatinine compared with girls before and during puberty (reflecting greater muscle mass) (Skinner et al., 1996; Trachtenberg et al, 2007). We hypothesize that girls

may suffer from a relative dehydration compared to boys, which may simply be from fluid restriction due to the greater propensity for girls (for hygiene or cultural reasons) to avoid using the toilet in a public setting. This phenomenon has been reported anecdotally in Nicaragua and other countries (e.g. cartoon vignettes). NGAL, for example, has been proposed in preliminary research as a very sensitive biomarker even in minimal and silent prerenal kidney dysfunction due to dehydration (Damman et al, 2011; Antonopoulos et al, 2011). In our study, girls had a median (IQR) of 26.1 ng/mg Creatinine (17.3-47), compared to 6.9 ng/mg Creatinine (5-9.7) in males. If adolescent girls turn out to be, in fact, at a greater risk of early kidney damage, this would imply that there is a later exposure to a progression factor for CKD that disproportionately affects males. Although adult women in the most affected communities have a higher prevalence of CKD compared to women in communities not affected (i.e., the same pattern as for males), the prevalence is still significantly lower than for males in the same communities (Torres et al, 2010; O'Donnell, 2010; Peraza et al, 2012). While it is perhaps likely that males have greater exposure than females to many of the hypothesized risk factors for CKDu (e.g., heat with accompanying volume depletion, leptospirosis, arsenic, agrichemicals) during adulthood as a result of gender differences in types of occupational, we have no evidence that exposure to such factors would be greater among girls than among boys among adolescents who have not yet entered the workforce (we excluded adolescents who had ever worked manually, whether paid or unpaid). The possible causes of the higher biomarkers among girls remain unknown and need to be taken into consideration in future studies.

NSAIDs and antibiotics are widely used in the region to relieve a common dysuria-like syndrome in the community, and particularly among workers, locally called "chistata", which is probably associated with dehydration and exposure to high temperatures (Ramirez-Rubio et al, 2013b). Although there were not significant differences by school, remarkably, a high percentage of children reported pain while urinating (46%), and "kidney" problems (13%), which included several symptoms and diseases, sometimes equating the terms urinary tract infections and kidney "insufficiency". Dipstick results, though, did not show evidence of lower tract infections, since leukocyte esterase positivity appeared in around 10% of the population (mostly girls, with no differences between schools), and nitrites were mostly absent.

One of the main limitations of this study is the absence of well-validated normal values for these biomarkers (except for albumin). In order for these biomarkers to serve as a screening tool of kidney damage, data available for comparison from a healthy reference population is needed. In this case, there are few studies in healthy schoolchildren or adolescents (Waring et al, 2011; Mazaheri, 2011; Misurac et al, 2013). Furthermore, lack of standardized laboratory methods, non-Gaussian distribution, and the absence of consensus reporting guidelines (e.g. units, excretion vs. concentration, adjusted or not for urine creatinine, etc) make comparisons with the published literature difficult (Jung, 1992; Csathy, 1995). The lack of well-defined standard values of these biomarkers in a comparable population, drove us, like it has been done in other settings (Kaneko et al, 2003; Stefanovic et al, 2009), to design the study such as the selected schools would represent different a priori CKD risks, with the one hypothesized with lowest risk as the reference school. Our sample size has enabled us to observe differences among schools, but may not be enough to explain the risk factors involved.

Also, another limitation of our study is that we were not able to access overnight or first morning urine samples, those are the preferred samples as exercise or changes in posture may affect urine biomarkers concentration outputs. Instead, we asked for early morning samples at the school, prior to any strenuous physical activity. We were also concern that some children may have walked long distances from home, but it turned out that only 21 (10%) walked for more than 30 minutes, and certainly, School 4 (the one with most a priori risk for kidney damage) presented the least percentage (5%) of children walking longer time.

Overall, our results suggest some evidence of early-stage kidney damage in adolescents in Nicaragua with higher frequency in those schools and regions within Nicaragua that were defined with a priori more CKD risk. This finding is preliminary and needs confirmation with further investigation, but, if corroborated, it will be an important piece of information, not only for the children's health, but also to investigate the susceptibility factors and causes of the CKD epidemic in Nicaragua and throughout Central America in the general population.

CONCLUSIONS/CONCLUSIONES

"It is more than 10 years now that someone dies of CKD every week around me and no one does anything".

"Hace 10 años que cada semana alguien muere de IRC a mi alrededor y nadie hace nada".

-Donald Cortez

This thesis report have yielded the following conclusions:

Regarding Objective 1:

1.1-There is an epidemic of CKD of unknown causes, recently named Mesoamerican Nephropathy (MeN), which probably extends from South Mexico to Panama. Mortality rates and prevalence studies have confirmed that communities where agriculture, mining and port-related occupations are prevalent, and particularly those in lowlands of the Pacific coast are the ones most affected. Also, contrary to the typical distribution of a chronic [renal] disease, it affects primarily men in their 20-40s (relatively young). Women, although less affected, follow also the same distribution as men (e.g. higher prevalence's in low altitude).

1.2-Risk factors (susceptibility, initiation and/or progression) associated with CKDu in the different prevalence and case control studies to date have brought researchers to think that etiology is presumably multifactorial. The variety of hypothesized causes include: heat and heavy physical work load (chronic volume depletion), exposure to heavy metals such as arsenic or cadmium, agrichemicals, systemic infectious diseases such as leptospirosis, and chronic intake of potential nephrotoxic medications such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics or aminoglycoside antibiotics.

1.3-The epidemiological information on MeN/CKDu is still very limited, but has helped to describe the distribution of the disease and propose some risks factors. Main limitation of the mortality data is the variation in the extent of ascertainment of mortality data in the different countries and even within country regions, with plausible under-estimation in those areas where no active screening programs are taking place. Community- prevalence studies also present a number of limitations including its cross-sectional design (which generally does not allow any causal inference, and estimates prevalence with only one point in time measure), and the strong questionnaire-based assessments of risk factors with potential for exposure misclassification, such as recall bias).

1.4-Further public health and research priorities to address gaps in knowledge include: 1) incident cases based on surveillance systems and a CKDu case definition, 2) basic information on the natural history and the clinical progression of the disease, 3) prevalence studies in additional areas selected on a systematic basis, 4) longitudinal studies capable of assessing incidence, progression, 5) burden of disease studies, quality of life, and social determinants of the disease.

Regarding Objective 2:

2.1-Semi-estructured interviews with Nicaraguan physicians and pharmacists working in the front line of the epidemic in Western Nicaragua were consistent with the existence of an epidemic of CKD, describing characteristics similar to those noted in the prevalence studies conducted in the region, including CKD being more frequent among men, starting in young adulthood, and associated with mainly agricultural work but also occurring in other manual occupations, and usually not with diabetes and hypertension.

2.2-Our analysis articulated perceptions of physicians and pharmacists in a causal framework where heat stress and subsequent volume depletion experienced by manual laborers plays a role in the frequent occurrence of dysuria-like symptoms (locally called “chistata”), which are often treated with non-steroidal anti-inflammatory drugs, diuretics and antibiotics that may be further nephrotoxic.

Regarding Objective 3:

3.1- Adolescents recruited at four schools from different regions of Nicaragua, showed biomarkers of kidney tubulo-interstitial damage in accordance with our a priori hypothesis (ranked by mortality rates in the general population). For example, the results for NAG were most consistent in this regard, with the relative concentrations rank ordered by school according to risk profile in both boys and girls.

3.2-Albumin-Creatinine Ratio, which is primarily associated with glomerular damage, did not demonstrate these patterns, and macroalbuminuria measured with dipstick, was virtually absent.

3.3-An unusual finding is that girls had higher levels than boys for all biomarkers, with particularly large differences for ACR and IL-18. We hypothesize that girls may suffer from a relative dehydration compared to boys, which may simply be from fluid restriction due to the greater propensity for girls (for hygiene or cultural reasons) to avoid using the toilet in a public setting, but this fact may need further confirmation.

3.4-Results from this pilot study are preliminary and need to be the focus of further investigation, but if confirmed, it may indicate that early risk factors for CKD in the region may be present during teenage, prior to other additionally exposures.

Las conclusiones que derivan de la investigación presentada en esta tesis son las siguientes:

Respecto al Objetivo 1:

1.1 La información basada en las tasas de mortalidad y en estudios de prevalencias confirman que ciertas comunidades dedicadas a la agricultura, minería, y trabajadores portuarios presentan prevalencias de ERC elevadas, particularmente aquellas comunidades localizadas a nivel del mar en la Costa del Pacífico. Al contrario de la típica distribución de enfermedad renal crónica, NeM afecta principalmente a hombres jóvenes de 20-40 años (relativamente jóvenes). Las mujeres, aunque se encuentran menos afectadas, también presentan una distribución similar a los hombres (Ej. mayor prevalencias en comunidades localizadas a menor altitud).

1.2 Los factores de riesgo (susceptibilidad, iniciación y/o progresión) asociados a NeM en los diferentes estudios de prevalencia y de casos y controles hasta la fecha, así como aquellas causas ambientales conocidas de ERC que pudieran tener un papel en esta epidemia, han llevado a pensar que la etiología podría ser multifactorial. La variedad de las hipótesis causales incluye: exposición al calor y trabajo manual intenso (depleción de volumen crónica), exposición a metales pesados como cadmio, o a arsénico, agroquímicos, enfermedades infecciones sistémicas como la leptospirosis, el uso crónico de medicamentos potencialmente nefrotóxicas, tales como antiinflamatorias no esteroideas (AINEs), analgésicos o antibióticos aminoglucósidos.

1.3 La información sobre la epidemiología de NeM es aún escasa y presenta múltiples limitaciones, pero ha sido esencial para describir la distribución de la enfermedad y proponer algunos factores de riesgo. La limitación principal que presentan los datos de mortalidad es la variabilidad existente entre los diferentes países o incluso entre regiones dentro de cada país en cuanto a la vigilancia y registro de muertes, con probables subestimaciones en las zonas donde no hay programas de detección de casos activos. Los estudios comunitarios de prevalencia también presentan una serie de limitaciones, tales como su diseño transversal (que, por lo general, no permite ninguna inferencia causal, y cuyas estimaciones de prevalencia se basan en una única medida), y las evaluaciones de factores de riesgo basadas en cuestionarios con potencial para la clasificación errónea de exposición como, por ejemplo, el sesgo de memoria).

1.4-Algunas de las prioridades de salud pública e investigación para hacer frente a las lagunas de conocimiento incluirían: 1) definición de caso de NeM y registro de casos incidentes por sistemas de vigilancia, 2) información básica sobre la historia natural y progresión clínica de la enfermedad, 3) estudios de prevalencia en otras regiones seleccionadas de forma sistemática, 4) estudios longitudinales que permitan evaluar la incidencia y progresión de la enfermedad, 5) estudios de carga de enfermedad, calidad de vida, y determinantes sociales de la enfermedad.

Respecto al Objetivo 2:

-Los médicos y farmacéuticos entrevistados que trabajan en la primera línea de la epidemia describen la epidemia de manera consistente con los resultados llevados a cabo en la región.

-Nuestro análisis articuló las percepciones de médicos y farmacéuticos en un esquema de red causal donde el estrés por calor y la depleción de volumen subsecuente que experimentan los trabajadores manuales podrían jugar un papel en la aparición frecuente de síntomas similares a la disuria (llamados localmente “chistata”), que son normalmente tratados con antiinflamatorios no esteroideos, diuréticos y antibióticos que pueden ser también nefrotóxicas.

Respecto al Objetivo 3:

3.1- Los adolescentes de cuatro colegios en tres regiones diferentes de Nicaragua mostraron biomarcadores de daño renal túbulo-intersticial en un orden creciente igual a aquel que muestran las tasas de mortalidad por ERC en la población general de las regiones mencionadas. Por ejemplo, los resultados para NAG fueron las más consistentes en este sentido, con concentraciones medias relativas por colegio ordenadas según el perfil de riesgo basado en las tasas de mortalidad por ERC en su región, tanto para chicos como para chicas.

3.2- La razón albúmina-creatinina urinaria (RAC), asociada principalmente con daño glomerular, no demostró estos patrones, y no hubo evidencia de macroalbuminuria medida con tira reactiva de orina.

3.3- Un hallazgo inusual fue que las chicas presentaron niveles superiores respecto a los chicos para todos los marcadores, con diferencias particularmente notorias para IL-18 y RAC. Nuestra hipótesis es que las chicas podrían sufrir de una deshidratación relativa comparada con los chicos, debido quizás a una restricción de fluidos debido a la mayor propensión de las chicas (por razones de higiene y culturales) para evitar usar el baño en lugares públicos, pero este hecho debe ser confirmado en el futuro.

3.4- Los resultados de este estudio piloto son preliminares y requieren ser el foco de investigación en el futuro, pero si se confirman, podrían indicar que algunos factores de riesgo temprano para NeM podrían estar presentes durante la adolescencia, previamente a otras exposiciones adicionales.

RECOMMENDATIONS: OUTLINING PUBLIC HEALTH INTERVENTIONS FOR MeN/CKDu

"Knowing is not enough; we must apply. Willing is not enough; we must do."

—Goethe (1749-1832)

Public health responses to the epidemic cannot wait until efforts to identify the causes are completed, especially because treatment is not widely available, and poverty, stigma, and premature death are key elements that have turned this disease into a real public health crisis of regional dimensions.

Responses should address the various levels of actions proposed in the eco-health theoretical model (See Chapter II). The following recommendations are based on the general knowledge of the situation in Central America (including discussion with other researchers and public health officers) that I have acquired during the last five years, and the evidence presented in this document. These recommendations are also compiled in the annexed document "Brief Policy Paper: Epidemic of Chronic Renal Disease in Central America", that was commissioned in May 2012 by Instituto de Salud Carlos III, as a request by the Ministry of Health of Costa Rica in the context of bilateral cooperation projects that both institutions share (see annexes).

Multisectoral and multilevel public policies in Central America: CKD as a regional public health alert

- From a macro perspective, mainstreaming health and environmental issues should be included and prioritize into development policies, with the aim to promote sustainable and equitable patterns of production (including working conditions) and consumption.
- In particular, there is a need to build capacity to monitor and manage ecosystems and contaminants or hazardous exposures to human health (such as agrichemicals, heavy metals or extreme temperatures).
- Institutions involved in these tasks should include an ecosystem perspective instead of operate just in human constructs and boundaries such as municipalities, and provinces or departments.
- Strengthening field disease surveillance, joint animal-human epidemiological studies (essential to study, for example, leptospirosis) and health services development, seems essential to monitoring CKD incidence and its determinants at the population level. Ad-hoc surveillance of workers' symptoms of heat exposure and workload, together with hydration practices, could be consolidated by governmental agencies and main employers.

Health promotion and primary prevention linked with applied research

- Target of community preventive health programs (including in settings such as the workplace or health centers) should be the possible association between the occupational exposure to heat and the use of potentially nephrotoxic medications.
- Research into current hydration practices and promoters, and obstacles to greater hydration, could help shape public health recommendations.

- Common self-medication of widespread (across the community and particularly workers) dysuria related symptoms with diuretics, NSAIDs and antibiotics should be the target of greater oversight and health protection measures from government agencies.
- Particularly important is the training of health workers and pharmacists, and the development of context-specific guidelines and protocols addressing a correct differential diagnosis and treatment of heat exposure symptoms, UTIs and CKD, and structured around patient safety and rational use of medicaments issues.

Health Care Services: early detection and comprehensive treatment

- Strengthen health care systems to provide both primary health care and renal replacement therapies (dialysis and/or transplantation), which constitute a huge burden in these Central American countries.
- Develop evidence-based and context-oriented CKD clinical practice guidelines, and education of health professionals on their implementation.
- Clinical practice should focus on early detection, regular monitoring and control of progression factors, nutritional aspects, social protection, and counseling for patients and families.
- Working with communities on CKD related education and dissemination, improving understanding of the disease and existing treatments, and thus, tackling stigma.

Regional Action Plan

- CKDu/MeN has a wingspan of a regional public health problem, thus actions should have the same scope.
- Fortunately, there have been very recently initiatives with this spirit:
    - Workshop on MeN nephropathy 2012, followed by a "declaration statement" and the creation of a "Research Consortium" with a Board of Directors, November 2012.
    - "High-level meeting on Chronic Kidney Disease of non-traditional causes in Central America", and its "San Salvador Declaration", organized by El Salvador Ministry of Health, and the participation of the Central American Ministries of Health (COMISCA), April 2013.
    - PAHO 152nd Executive Committee Meeting (June, 2013) discussed "chronic kidney disease in agriculture communities in Central America" and set up the conditions for a Regional Cooperation Program on this topic.
- Actions should involve at different levels important organizations and stakeholders: government bodies, universities and researchers, health workers and patients, NGOs, employers and worker unions and associations, etc.).

Taken together, the research and public health agendas described here constitute a significant task, but one that is achievable if these epidemics receive sufficient attention from scientific, public health, and development agencies. As expressed by one of the CKD patients in

Nicaragua: “We are a poor country, but it is very amazing to see how they mobilize money and human resources when it comes to malaria, which barely exists in my community, or influenza. But it is more than 10 years now that someone dies of CKD every week around me and no one does anything”. Simple words that describe a complex problem. The interrelated need for science and public health action are hardly unique to this epidemic, and the global health community has repeatedly demonstrated its capability to tackle this type of problem. It is our hope that the community will bring its considerable intellectual resources to identify the causes of this puzzling epidemic (paragraph taken from Ramirez-Rubio, 2013a).

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ABBREVIATIONS INDEX

ACR-Albumin Creatinine Ratio

AKI- Acute kidney injury

ASOCHIVIDA- Asociación Chichigalpa por la vida

BEN- Balkan endemic nephropathy

BQSC- Brookline-Quezalguaque sister city

BUSPH- Boston University School of Public Health

CAO- Compliance, advisory, ombudsmen

CKD- Chronic Kidney Disease

CKDu- Chronic Kidney Disease of unknown causes

CISTA- Centro de Investigación en Salud, Trabajo y Ambiente (UNAN-Leon)

COMISCA- Consejo de Ministros de Salud de Centroamérica

CVD- Cardiovascular Disease

DALY- Disability Adjusted Life Years

GFR/eGFR- Glomerular filtration rate/ estimated glomerular filtration rate

IL-18- Interleukin 18

IRB- Institutional Review Board

ISA- Ingenio San Antonio

KDIGO- Kidney Disease: improving global outcomes

K/DOQI- Kidney Disease outcome quality initiative

MOE- Ministry of Education

MoH- Ministry of Health

MDRD- Modification of diet in renal disease study equation

MeN- Mesoamerican Nephropathy

NAG- N-acetyl-D-glucosaminidase

NGAL- Neutrophil gelatinase-associated lipocain

NHANES- National health and nutrition examination survey (USA)

NKF- National Kidney Foundation

NSAIDs- Non-steroidal anti-inflammatory drugs

NSEL- Nicaragua Sugar States Limited

OR- Odds Ratio

PAHO- Pan American Health Organization

RRT- Renal replacement therapy

SALTRA- Programa de Salud Trabajo y Ambiente en Centroamérica

SCr- Serum Creatinine

UNAN-Leon- Universidad Nacional de León (Nicaragua)

UTI- Urinary tract infection

WHO- World Health Organization

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ANNEXES: publications, prizes, and communications to conferences

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