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Point Prevalence of Chronic Kidney Disease of Uncertain Etiology in a Suspected Endemic Region Determined Using Predefined Cutoffs for Markers of Kidney Disease: A Pilot Study

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Abstract

Background: Chronic Kidney Disease of uncertain etiology (CKDu) affecting many tropical countries in the world has reached epidemic proportions. Studies on the diagnostic accuracy including data on the performance of existing renal tests in targeted population screenings of CKDu are limited.

Objectives: This study aimed to determine the point prevalence of CKDu using selected screening markers of kidney disease with predefined cutoffs established by the authors (Ratnayake et al., 2017).

Method: One hundred individuals from a population of 5718 who lived in a CKDu-endemic region were selected. After the exclusion of diabetes, hypertension, and known renal diseases, dipstick proteinuria, serum cystatin C, serum creatinine, and urine albumin creatinine ratio (ACR) were estimated on fifty individuals recruited. Individuals with test results above the predefined cutoff values were considered as having or suspected to be having CKDu.

Results: The respective determinations of diagnostic accuracy of each test at the optimal cutoff for a CKDu population, and detection of positive cases as a percentage of total tests were: serum cystatin-C accuracy - 0.8, detects 14% at ≥ 1.22 mg/L, serum creatinine accuracy - 0.73, detects 6% at ≥ 115 and ≥ 96 $\mu\text{mol/L}$ for males and females respectively, and ACR accuracy - 0.68, detects 8% and 26% at ≥ 30 mg/g and ≥ 12.6 mg/g cutoffs respectively.

Conclusion: In the targeted population screening of CKDu, detection of suspected cases, or point prevalence by serum creatinine was lower than that of cystatin C and ACR.

Keywords: Chronic Kidney Disease of uncertain aetiology, screening markers, Creatinine, Albumin creatinine ratio, cystatin C.

INTRODUCTION

Chronic kidney disease of uncertain origin, particularly affecting farmers has been reported from specific geographical regions in Central America, Egypt, India, and Sri Lanka [1]. Despite numerous studies the aetiology is still obscure [2]. Chronic interstitial nephritis of the agricultural community (CINAC) and chronic kidney disease of multifactorial origin (CKDmfo) are synonyms for the identical condition.

Chronic kidney disease (CKD) is defined as abnormal kidney structure or function lasting more than three months with associated health implications. Diagnosis of CKDu is made in long term residents in specific geographical locations such as the North Central Province of Sri Lanka after the exclusion of known renal causes of CKD. The last comprehensive case definitions for CKDu in Sri Lanka was published in 2019 [3]. Although albuminuria and hypertension are common manifestations of CKD it is rare in CKDu. Renal biopsy studies done on late stage disease have shown interstitial fibrosis and tubular atrophy to be predominant lesions, along with glomerular sclerosis, with no evidence of immune mediated or other glomerular pathology [4, 5]. Subsequently, an acute phase of CKDu or acute interstitial nephritis (AIN) has been reported in CKDu-endemic regions from Sri Lanka and Nicaragua, which is believed to be the earliest stages of the disease [6, 7]. In these acute scenarios, the serum creatinine, which is initially elevated, gradually return to baseline or near normal till such time fibrosis becomes established [8]. Thus, early stages of CKDu may not be detectable by testing serum creatinine alone, and novel renal markers could improve the detection.

Screening to detect early disease provides the advantage of being able to treat or slow down the progress of the disease and also plan future management. Proteinuria is the first test generally used in the population targeted screening of CKD. If positive, serum creatinine (GFR), renal ultrasonography, and renal biopsy if indicated are done. CKD screening is done in patients with diabetes, hypertension, and cardiovascular diseases. Also, age >60 years, hyperlipidaemia, metabolic syndrome, etc. are considered in certain guidelines [9]. Further, chronic exposure to certain environmental risk factors such as manual labour under hot and humid climatic conditions leading to dehydration and salt loss, drinking water containing

high ionicity, irrational use of agrochemicals, and frequent or prolonged use of non-steroidal anti-inflammatory drugs are considered in the screening of CKDu. Athuraliya et al. described CKDu as a proteinuric kidney disease after using dipstick proteinuria (DPU) that detects urine proteins in the range of macroalbuminuria (>300mg/g) as the indicator of renal impairment [10]. Subsequently, in the WHO CKDu study Jayatilake et al. have used persisting albuminuria for screening (spot urine ACR >30mg/g in two visits) [11]. However, the manifestation of albuminuria in early CKDu is either low (10-30mg/g), within the microalbuminuria range (30-300mg/g) or negligible [12,13]. Thus, a single proteinuria test of the targeted population may not detect non-proteinuric patients with renal impairment. Markers of tubular damage are an attractive alternative with some supportive evidence of superiority over traditional markers. But their utility it is yet to be confirmed [14].

The diagnostic accuracy of existing renal markers for CKDu was estimated by the authors [15]. The estimated diagnostic accuracy of serum cystatin C, serum creatinine, and ACR were 0.93, 0.92, and 0.74 respectively for cases vs “non-endemic” (Raththota) healthy controls [15]. The diagnostic accuracy of the same markers was reduced to 0.8, 0.73, and 0.68 respectively when cases were compared with “endemic” (Girandurukotte) healthy controls [15]. This study aims to estimate the point prevalence of CKDu using renal tests with predefined cut-offs established by Ratnayake et al. [15].

MATERIALS AND METHODS

Individuals from the village “Nagadeepa” located in the dry zone of Sri Lanka were selected for this study as many patients with CKDu had been diagnosed from this region. The case definition including the cut-off for diagnostic tests cystatin C, ACR, and HbA1C were as described by Ratnayake et al. [15]. We invited 100 randomly selected subjects out of a population of 5718, (50 males and 50 females >18 years of age) for renal disease screening. Individuals with diabetes mellitus, prediabetes, hypertension, and kidney diseases and with two blood pressure readings >140/90 mmHg were excluded. HbA1C was used to detect prediabetes and diabetes which were considered as independent risk factors for incident CKD [16]. A total of 50 persons formed the cohort of this study. A single measurement of DPU, serum

creatinine, serum cystatin C and ACR was done. Subjects who had serum creatinine $\geq 115 \mu\text{mol/L}$ for males and $\geq 96 \mu\text{mol/L}$ for females, and/or cystatin C (serum) $\geq 1.22 \text{ mg/L}$, and/or ACR (spot urine) $\geq 12.6 \text{ mg/g Cr}$, and/or DPU $\geq 1+$ were diagnosed as or suspected as having CKDu. The point prevalence (number of persons testing positive on the date of examination, as a percentage of the study population) was calculated. The diagnostic accuracy of the renal tests was estimated so that each renal test measurement of "endemic" (Girandurukotte) healthy controls were compared against CKDu cases using the receiver operating characteristic (ROC) curves [15]. The estimated optimal area under the ROC curve (accuracy of the test) for serum cystatin C, serum creatinine, and ACR were 0.8, 0.73, and 0.68, respectively [15].

Morning first void spot urine was collected into polypropylene tubes for DPU and ACR. Venous blood was collected for HbA1C, serum creatinine, and cystatin C. All samples were stored at 4°C until analysis. Cystatin C was measured by particle enhanced immuno-turbidimetry using the Dako cytometry assay kit (DAKO Ltd, Code No. LX002, Denmark). HbA1C and ACR were analyzed by a Bio-Rad D-10 HPLC and Hitachi 911 and 912 auto analyzers, respectively. Creatinine was measured using Jaffe's method and isotope dilution mass spectrometry, (IDMS) not traceable. The modified diet in renal disease (MDRD) formula [$\text{GFR (ml/min/1.73m}^2\text{)} = 30849 \times \text{Standardized SCr (}\mu\text{mol/L)}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \text{ (if black)} \times 0.742 \text{ (if female)}$] was used to estimate glomerular filtration rate (eGFR). CKDu categories based on estimated GFR (mg/min/1.73M^2) are G1 ≥ 90 , G2 60-89, G3a 45-59, G3b 30-44, G4 15-29 and G5 <15 . Descriptive statistics were used. The study was carried out from December 2013 to February 2014.

RESULTS

15 out of 50 individuals were positive for at least one of the three screening markers. The DPU was negative in all. The male to female ratio of screening positives was 8:7. The point prevalence of suspected CKDu for each test was 6% (3/50 positive cases) for serum creatinine at ≥ 115 and $\geq 96 \mu\text{mol/L}$ cut-off for males and females respectively with 0.73 accuracy, 14% (7/50 cases positives) for serum cystatin C at $\geq 1.22 \text{ mg/L}$ cut-off with 0.8 accuracy, and 26% (13/50 positive cases) for ACR at 12.6 mg/g cut-off with 0.68 accuracy. When cut-off for ACR was $>30 \text{ mg/g}$, the

point prevalence was reduced to 8% (4/50 positives). 18% (9/50 individuals) had low-grade albuminuria ($10\text{--}30 \text{ mg/g}$). 6% (3/50 individuals) had microalbuminuria ($>30\text{--}300 \text{ mg/g}$) and normal eGFR ($\text{GFR} < 60 \text{ ml/min per } 1.73 \text{ M}^2$). When the CKD indicators, ACR $>30 \text{ mg/g Cr}$ and/or $\text{GFR} < 60 \text{ ml/min per } 1.73 \text{ M}^2$ cutoff in the current clinical practice guidelines was used, the point prevalence was 12% (6/50 positives).

DISCUSSION

In this pilot study, a one-time measurement of specified renal tests was done on fifty individuals from a CKDu endemic region after the exclusion of known causes for renal disease. The detection of suspected CKDu was 14% for cystatin C, 6% for creatinine and 26% and 8% for ACR at 12.6 mg/g (low-grade) and $>30 \text{ mg/g}$ cut-off, respectively.

There is no empirical evidence to support that general population screening for CKD is beneficial. The screening of high-risk populations for CKD and CKDu is recommended [17]. Proteinuria has been used as a single test in many at-risk population screening programs [18]. Similarly, DPU or ACR as a single test has been used in population screening of CKDu, despite proteinuria being low grade and intermittent in these patients [10, 11]. In our study DPU was negative in all screening positive individuals. The diagnostic accuracy of ACR was 0.68 for the CKDu endemic population and it detected 8% of cases at $>30 \text{ mg/g}$ and 26% of cases at 12.6 mg/g cut-offs. The optimal cut-off for ACR in discriminating normal from suspected-CKDu was 12.6 mg/g , which is lower than the current reference standard ($>30 \text{ mg/g}$) for CKD. A few studies have reported that cardiovascular mortality risk is increased with low-grade albuminuria [19]. Furthermore, another study using the artificial intelligence approach reports that the detection of low-grade albuminuria in prediabetes followed by early treatment may improve the outcome of diabetic kidney disease [20]. The accuracy of the lowered cut-off value of ACR is dependent on the respective assay, the linearity of the standard curve at a lower level of detection, and the laboratory standards [21, 22].

18% (9/50 individuals) of the study population had low-grade albuminuria ($10\text{--}30 \text{ mg/g}$), indicating a potential future CVD risk which needs further evaluation in the CKDu endemic population. Also, there were 3/50 (6%) patients with

microalbuminuria (>30 -300mg/g) and normal renal function (eGFR <60 ml/min per 1.73 M²), suggesting that there is an advantage of using albuminuria over GFR in detecting patients with renal damage before the deterioration of renal function. Measurement of ACR, on two consecutive days in spot urine, may reduce the number of false positives. In this study ACR was measured once which could have included false positives. Tubular proteins, micro globulins (alfa and beta), neutrophil gelatinase-associated lipocalin (NGAL), or kidney injury molecule-1 (KIM-1) have been proposed by different investigators in the early detection of CKDu[23].

Serum creatinine is the most widely used renal function test in clinical practice, although the level of creatinine in the blood is affected by the gender, ethnicity, muscle mass and the protein content of the diet and certain medications taken. Many GFR formulas have been developed to overcome these effects on serum creatinine [24]. Cystatin C is an alternative biomarker of renal function. It displays less variation than creatinine due to muscle mass, and offers greater accuracy of GFR estimation, and improves prediction of adverse outcomes in CKD stage 3 including end-stage renal failure [25]. The overall result of this study indicates that the accuracy of serum creatinine and cystatin-C was 0.72 and 0.8 respectively for a CKDu endemic population, and each test detected 6% and 14% of cases, respectively. Even though cystatin-C is superior to serum creatinine and ACR, there is limited experience in clinical practice. Further studies are needed before cystatin C in CKDu screening is recommended. International CKD guidelines recommend the use of cystatin-C in the confirmation of the diagnosis in people with GFR 45–59 ml/min/1.73 m² and no albuminuria (CKD G3aA1) [26].

Some limitations of the present study is the small sample size, estimation of GFR from MDRD formula using Jaffe serum creatinine assay and application of screening markers only once without review by 3 months of follow up.

CONCLUSION

In conclusion with these limitations, the detection of suspected CKDu cases by serum creatinine is lower than cystatin C and ACR. The recommended clinical practice cut-off value for ACR is >30mg/g. Low-grade albuminuria (10-30mg/g) observed in this study needs further clinical evaluation.

Author declarations

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Author contributions

NN and TA designed the study. SR, ZB, and LG collected the data. All authors contributed to the interpretation of data. ZB prepared the manuscript. NN, SR, NR and ZB revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The title of the dataset is Nagadeepa screening data. The datasets are available at the Open Science Framework repository [https://osf.io/sg78b/?view_only=0199276e8ab5463990c52fe9b102fbbf]

Ethics approval and consent to participate

The Institutional Ethical Review Committee (IERC) of the Faculty of Medicine, University of Peradeniya approved this study. All subjects who participated in this study have given informed written consent.

Competing interests

No conflicts of interest.

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