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Serum Transforming Growth Factor-Beta 1 and Creatinine for Early Diagnosis of CKD of Unknown or Uncertain Etiology Phenotypes

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INTRODUCTION

The global burden of kidney diseases has disproportionally increased over the last 2 decades.[1] Although diabetes and hypertension are leading causes of chronic kidney disease (CKD), environmental risk factors contribute to a significant disease burden in hot spots of Mesoamerican countries, such as India, and Sri Lanka.[2,3] CKD associated with environmental risk factors, CKD of uncertain or unknown etiology (CKDu), is prevalent among farming communities living in these at-risk areas. Recently, a subcategory of patients has been reported from these at-risk areas for CKDu in Nicaragua and Sri Lanka.[4,5] There was tubulitis and significant interstitial cell infiltrate in the background of glomerular sclerosis, tubulointerstitial fibrosis and tubular atrophy in their biopsies, compatible to acute interstitial nephritis. CKDu patients with acute lesions (CKDu-A) subsequently transform into commonly encountered CKDu patients with chronic features (CKDu-NA),[6] or to a distinct subclinical phenotype (CKDu-S) with normal renal functions, besides irreversible histologic changes.[7]

Transforming growth factor-beta 1 (TGF-β1) activated in acute kidney injury, while positively or negatively regulating the process that is associated with cellular responses to the nature of toxin. Any persistent kidney injury causes a rise in TGF-β1, which promote kidney fibrosis and suppress the ongoing inflammation.[8] Therefore, TGF-β1 is an antiinflammatory as well as profibrotic biomarker that positively regulates the glomerular and tubulointerstitial fibrosis in CKD/CKDu.[9]

In this milieu, current study sought to evaluate the performance of serum TGF-β1 and serum creatinine (SCr) as potential candidate biomarkers to predict subphenotypes of CKDu. This is a cross-sectional case-control study where one-time serum TGF-β1 and corresponding SCr measurements were used. We recruited 38 CKDu-A cases (acute interstitial nephritis) and 45 CKDu-NA cases (chronic interstitial nephritis). Among the total cases (N = 83), there were 23 subclinical cases (CKDu-S). Cases were further divided into high-activity (18 cases with activity index ≥ 3/6) and low-activity (65 cases with activity index < 3) groups based on activity index grades of histologic classification. Healthy individuals from a CKDu nonendemic region were recruited as controls (N = 85). The case-control study design is depicted in Figure 1a. The predictive value of TGF-β1 and TGF-β1/SCr index (adjusted) for the differentiation of CKDu phenotypes were explored. More details about the "methods" are given in the Supplementary Methods.

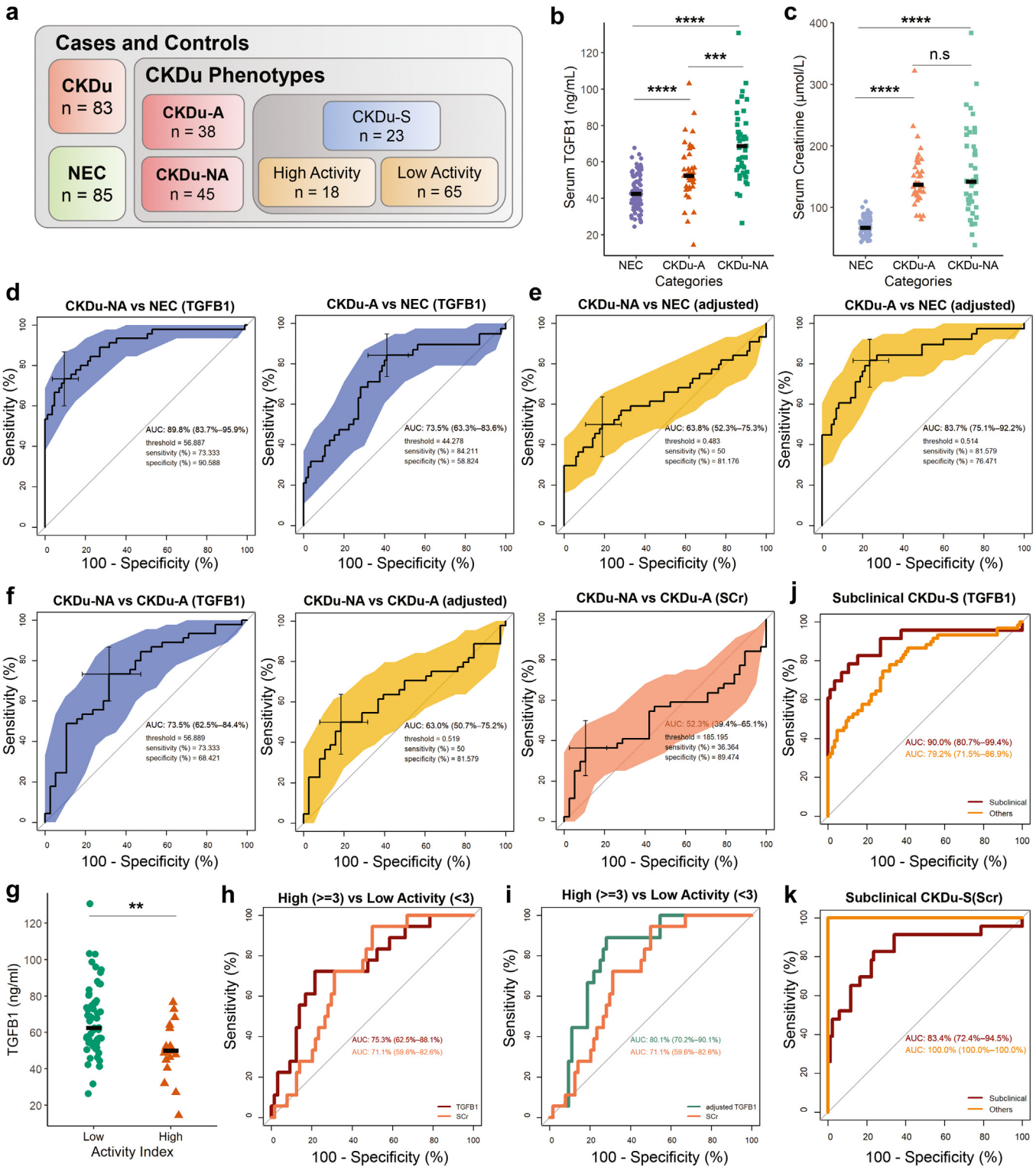

Figure 1. Performance evaluation of serum TGF-β1 for differentiating CKDu phenotypes. (a) Case-control study design is shown. (b) Concentration profiles serum TGF-β1 and (c) Serum creatinine, among the different disease and control categories, are shown as jitter plots. Statistical significance (*P*) comparing each biomarker category were determined by the Kruskal-Wallis *H*-test and Mann-Whitney *U*-test. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. (d) ROC analyses of serum TGF-β1 in CKDu-NA and CKDu-A groups are shown. (e) ROC analyses performed using adjusted TGF-β1:SCr index in CKDu-NA and CKDu-A groups. Analyses were performed against NEC representing healthy nonendemic controls. 95% CI is computed with 2000 stratified bootstrap replicates, and the colored region indicates the variation of sensitivity over specificity. The plot indicates the optimal threshold, sensitivity, and specificity based on Youden index. (f) Performance evaluation of serum TGF-β1, adjusted TGF-β1:SCr index, and SCr in segregating the CKDu-NA and CKDu-A subgroups are shown using ROC analysis. 95% CI is computed with 2000 stratified bootstrap replicates, and the colored region indicates the variation of sensitivity over specificity. The plot displays the optimal threshold, (continued)

RESULTS

We investigated the effectiveness of TGF-β1 as a biomarker for the transformation from initial injury to the chronic state of CKDu. The baseline characteristics and clinical profiles of the recruited cases and controls are summarized in Supplementary Table S1. Serum TGF-β1 profiles showed significant distribution differences among the different groups and was elevated in the disease groups, with the highest levels in CKDu-NA category (Figure 1b). Even though SCr efficiently differentiated healthy from disease groups, there were no significant differences between different CKDu phenotypic groups (Figure 1c).

TGF-β1 differentiated CKDu-NA and CKDu-A groups from healthy (CKDu nonendemic) with area under the receiver operating characteristic curves (AUC) of 89.8% and 73.5%, respectively (Figure 1d). Serum creatinine also differentiated both groups, with higher predictive performance for CKDu-A group at AUC 98.5% (Supplementary Figure S1). Therefore, we evaluated the performance capability of TGF-β1 in combination with serum creatinine to assess acute tubular interstitial damage. The adjusted TGF-β1: SCr index augmented the predictive ability for CKDu-A that improved to AUC 83.7%, mainly attributed to the recovery of sensitivity (Figure 1e). However, the adjusted TGF-β1/SCr index decreased the AUC of CKDu-NA to 63.3%. In addition, analysis of multinominal models using CKDu-NA, CKDu-A, and CKDu nonendemic showed an AUC of 73.8% for TGF- β1, 62.9% for adjusted TGF-β1, 83.7% for serum creatinine and the highest AUC of 92.1% for the full model of TGF-β1 adjusted with creatinine and confounders, age, and sex (Supplementary Figures S2 and S3).

Currently, there are no clinically validated biomarkers to assess the ongoing acute inflammatory process in CKDu. Although serum creatinine is a promising candidate in CKD, its predictive performance of segregating subphenotypes CKDu-NA versus CKDu-A was poor, with AUC 52.3% (Figure 1f). Serum TGF-β1 showed higher predictive ability in stratifying the above phenotypes with a AUC of 73.5% and sensitivity almost twice that of SCr (73.3% compared with 36.3%) (Figure 1f). The predictive performance incorporating adjusted TGF-β1:SCr index showed no improvement in differentiating these phenotypes (AUC = 63%) with sensitivity remaining low at only 50%. To assess the diagnostic accuracy of TGF-β1, we fit multivariable logistic regression models with adjustments for known CKD risk factors such as age and sex. This revealed significant association for TGF-β1 in distinguishing the subgroups even after adjusting for confounders (P = 0.00067) and the adjusted model showed a high AUC of 80.4% (Supplementary Figure S4A). Adjusted TGF-β1:SCr index or inclusion of serum creatinine did not improve the model performance (Supplementary Figure S4B and C). A bivariate analysis using linear regression revealed no significant association between age and TGF-β1 across all samples (P value = 0.754) or within only disease samples (P = 0.4133).

Among the different histologic activity groups, we observed highest levels of TGF-β1 in the low-activity groups (Figure 1g), and the difference in distribution was statistically significant. Adjusted TGF-β1:SCr index with an AUC of 80.1% performed better than either TGF-β1 (AUC = 75.3%) or serum creatinine (AUC = 71.1%) to distinguish these different inflammatory groups (Figure 1h and i). For identifying CKDu-S category, TGF-β1 displayed excellent performance with AUC of 90% (Figure 1j). This was superior to that of serum creatine which only achieved AUC of 83.4% (Figure 1k). Also, the serum creatinine cutoff of 78 μmol/l for optimal diagnostic ability is lower than the cutoff values (115 and 98 μmol/l for males and females, respectively) used in clinical practice for the diagnosis of CKDu, rendering it unfit for diagnosis. Adjusted TGF-β1:SCr index also did not perform well in distinguishing this group (AUC = 67.4%) (Supplementary Figure S5). Performance of age-adjusted and sex-adjusted model of TGF-β1 showed an AUC of 91.3%. A linear combination of TGF-β1 and SCr boosted the performance to AUC of 95.7%, offering improved predictive ability for distinguishing the CKDu-S group (Supplementary Figure S6). Further details of receiver operating characteristic analyses are described in Table 1.

Figure 1. (continued) sensitivity, and specificity based on Youden index. (g) Distribution of serum TGF-β1 concentrations in patient groups with "High" and "Low" histologic activity scorings are shown as a jitter plot (** $P < 0.01$). Statistical significance assessment was performed using Mann-Whitney U-test ($P = 0.001094$). (h) Comparison of serum TGF-β1 and serum creatinine, and (i) adjusted TGF-β1 index and serum creatinine in stratifying the "High" and "Low" activity groups of inflammation is shown. 95% CI is computed with 2000 stratified bootstrap replicates is indicated. (j) The performance of serum TGF-β1, and (k) Serum creatinine in distinguishing the subclinical CKDu-S category is shown. "Others" indicate all other patients who did not meet the criteria for subclinical categorization (based on eGFR and creatinine). ROC analyses were performed against NEC representing healthy nonendemic controls. 95% CI is computed with 2000 stratified bootstrap replicates. AUC, area under the receiver operating characteristic curve; CI, confidence interval; CKDu, chronic kidney disease of uncertain or unknown etiology; CKDu-A, chronic kidney disease of uncertain or unknown etiology and acute lesions; CKDu-NA, chronic kidney disease of uncertain or unknown etiology and no acute disease; CKDu-S, chronic kidney disease of uncertain or unknown etiology subclinical phenotype; eGFR, estimated glomerular filtration rate; NEC, nonendemic; n.s, not significant; ROC, receiver operating characteristic; SCr, serum creatinine.

Table 1. Details of receiver operating analyses in the diagnosis of different phenotypes of CKDu

Biomarkers and groups	AUC (95% CI) %	Sensitivity (%)	Specificity (%)	Threshold value
TGF-β1 in Figure 1d				
CKDu-NA vs. NEC	89.8 (83.7–95.9)	73.3	90.6	56.89 ng/ml
CKDu-A vs. NEC	73.5 (63.3–83.6)	84.2	58.8	44.3 ng/ml
TGF-β1/SCr (adjusted[a]) in Figure 1e				
CKDu-NA vs. NEC	63.8 (52.3–75.3)	50	81.2	0.48 ng/μmol/l
CKDu-A vs. NEC	83.7 (75.1–92.2)	81.6	76.5	0.514 ng/μmol/l
TGF-β1 in Figure 1f (left)				
CKDu-NA vs. A	73.5 (62.5–84.4)	73.3	68.4	56.89 ng/ml
TGF-β1/SCr (adjusted[a]) in Figure 1f (middle)				
CKDu-NA vs. A	63.0 (50.7–75.2)	50	81.6	0.519 ng/μmol/l
SCr in Fig. 1f (right)				
CKDu-NA vs. A	52.3 (39.4–65.1)	36.4	89.5	185.2 μmol/l
TGF-β1 in Figure 1h				
High[b] vs. Low[c] activity	75.3 (62.5–88.1)	72.2	78.5	52.50 ng/ml
TGF-β1/SCr (adjusted[a]) in Figure 1i				
High[b] vs. Low activity[c]	80.1 (70.2–90.1)	88.9	71.9	0.39 ng/μmol/l
TGF-β1 in Figure 1j				
CKDu-S vs. Others[d]	90.0 (80.7–99.4)	82.6	84.7	54.42 ng/ml
TGF-β1/SCr (adjusted[a]) in Figure S5				
CKDu-S vs. Others[d]	67.4 (54.4–80.4)	69.6	60.0	0.70 ng/μmol/l
SCr in Figure 1k				
CKDu-S vs. Others[d]	83.4 (72.4–94.5)	82.6	76.5	78.46 μmol/l

AUC, area under the receiver operating curve; CI, confidence interval; CKDu, chronic kidney disease of uncertain or unknown etiology; CKDu-A- patients with CKD of uncertain/unknown etiology and indicating features of acute disease (n = 38); CKDu-NA-patients with CKD of uncertain/unknown etiology and no acute disease (n = 45), CKDu-S, subclinical-biopsy proven CKDu patients ($n = 23$) after recovering of GFR to >60 ml/min/1.73 m^2 without proteinuria; NEC, healthy controls from CKDu nonendemic regions; n.s, not significant; ROC, receiver operating characteristic; SCr, serum creatinine; TGF-β1-transforming growth factor β1.
[a]TGF-β1/SCr index.
[b]High activity (18 cases with activity index ≥ 3/6).
[c]Low activity (65 cases with activity index < 3) groups based on grades of renal inflammation or biopsy activity index.
[d]Others- indicate all other cases ($n = 60$) who did not meet the criteria for subclinical categorization (based on eGFR and proteinuria).

DISCUSSION

The clinical spectrum of CKDu is broader than previously described. CKDu-A, an early manifestation of CKDu, represents a transient phase that often goes undetected because the symptoms are vague and nonspecific. Though SCr has been widely used in CKDu diagnostics, it does not exceed the normal ranges before having at least 40% to 50% of nephron loss. Our study revealed that TGF-β1 alone or in combination with serum creatinine displays excellent diagnostic potential for distinguishing CKDu phenotypes, while differentiating from healthy individuals. Diagnosing subclinical patients (CKDu-S) that have normal serum creatinine and no albuminuria in a routine evaluation is challenging. More importantly, there have been limited attempts to validate renal tubular markers for identifying subclinical CKDu. We report that TGF-β1 displays excellent diagnostic prospects for this subclinical group.

The acuteness of kidney disease is inferred from improvement or deterioration of kidney function in hours or days. Often, SCr measurements before the episode of acute kidney injury are unavailable or not accessible, especially in low resource setups such as CKDu endemic populations. Additionaly, kidney biopsy is an invasive procedure with limitations and there is a possibility to underdiagnose CKDu because of the patchy nature of their lesions. We acknowledge that sampling bias could have occurred when controls were drawn from a nonendemic region to prevent subclinical cases being selected as controls.

In summary, TGF-β1, which is implicated in fibrotic process of chronic kidney inflammation, is a credible indicator of overall tubular interstitial fibrosis, and therefore an attractive and less invasive alternative to kidney biopsy. This profibrotic marker is closely associated with CKDu progression and a promising biomarker for early diagnosis of CKDu.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

NN, ZB, and RH conceived the study. BF, RH, TH, and ZB performed sample collection and sample processing. NR, SW, and ZB reported biopsies with relevant scoring. BF and RH carried out biochemical analysis. AAS, JG, ZB, and NN designed the statistical data analyses and investigation methodologies. AAS carried out data and statistical analyses, regression analyses and visualizations of all the figures presented. ZB contributed to data analyses. AAS, JG, ZB, and NN interpreted and evaluated the data. ZB, AAS, and NN wrote the original manuscript draft. NN, NR, TA, JG, and ZB critically reviewed the manuscript. NN supervised the overall study. All authors read and approved the final version.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Performance of serum creatinine in identifying CKDu-NA and CKDu-A phenotypes of CKDu.

Figure S2. Performance of single-marker multinomial models for segregating different disease and healthy categories.

Figure S3. Performance of full multinomial models for segregating different disease and healthy categories.

Figure S4. Diagnostic potential of serum TGF-β1 in segregating the disease groups CKDu-NA and CKDu-A.

Figure S5. Assessment of adjusted TGF-β1:SCr index in stratifying subclinical CKDu-S phenotype.

Figure S6. Diagnostic assessment of serum TGF-β1 and creatinine in identifying subclinical CKDu-S disease group.

Table S1. The clinical profiles of cases and the control groups at the recruitment.

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