ORIGINAL ARTICLE

pISSN: 1907-3062 / eISSN: 2407-2230

The role of urine albumin creatinine ratio and serum β2 microglobulin as biomarkers of chronic kidney disease

Augustine Onovuakpo Eguvbe[*@], Marcellinus Uchechukwu Nwagu[**], Eshiotseme Sylvester Idogun[***], and Adeyinka Abdulrasaq Akande[†]

ABSTRACT

BACKGROUND

Chronic kidney disease (CKD) is an increasing burden on individuals and on the healthcare system. The need to identify more sensitive and specific markers of CKD cannot be overemphasized to facilitate detection and appropriate intervention. β2 microglobulin is one of such markers of CKD. The aim of this study was to investigate the sensitivities and specificities of serum β2 microglobulin and major biochemical markers of CKD, namely creatinine and urine albumin.

METHODS

This was a hospital-based cross-sectional study involving 124 subjects with CKD and 124 healthy controls. Participants were categorized in two groups : group 1 the CKD based on persistent reduction in GFR <60 mL/min/1.73 m2 and group 2 healthy subjects as controls. Blood (serum) samples of participants were analyzed for serum creatinine and serum β2 microglobulin while their urine samples were analyzed for creatinine and albumin. Urine albumin creatinine ratio (UACR) was calculated from the results of the analyses.

RESULTS

There was a very strong positive correlation of serum β2 microglobulin with serum creatinine (r=0.750; p=0.000) and UACR (r=0.775; p=0.000), respectively. Also, there was a very strong negative correlation between serum β2 microglobulin and eGFR (r=-0.866; p=0.000). UACR had the highest sensitivity and specificity as shown by receiver operating curve characteristics (ROC) analysis.

CONCLUSION

In CKD, UACR and serum β2 microglobulin had the best diagnostic value. Periodic renal assessment of renal patients is mandatory as they may be affected by hidden renal dysfunction.

Keywords: β2 microglobulin, serum creatinine, urine albumin creatinine ratio, chronic kidney disease

[*]Department of Chemical Pathology, Edo University, Iyamho, Edo State, Nigeria
[**]Department of Haematology and Blood Transfusion and Acting Dean, Faculty of Clinical Sciences, Edo University, Iyamho, Edo State, Nigeria
[***]Department of Chemical Pathology, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria
[†]Department of Chemical Pathology and Immunology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria

Correspondence
Augustine Onovuakpo Eguvbe
Department of Chemical Pathology, Edo University, Iyamho, Edo State, Nigeria.
E-mail: austinoforlife@yahoo.com
ORCID ID: https://orcid.org/ 0000-0002-2722-2186

Date of first submission, June 22, 2019
Date of final revised submission, November 16, 2019
Date of acceptance, November 19, 2019

Cite this article as: Eguvbe AO, Nwagu MU, Idogun ES, Akande AA, et al. The role of urine albumin creatinine ratio and serum â2 microglobulin as biomarkers of chronic kidney disease. UnivMed 2019;38:172-8. doi: 10.18051/UnivMed.2019.v38. 172-178

DOI: http://dx.doi.org/10.18051/UnivMed.2019.v38.172-178

INTRODUCTION

Chronic kidney disease (CKD) is an increasing burden on individuals and on the healthcare system, with an increasing prevalence and incidence. It is linked with a poor outcome and high cost of treatment.[1] Chronic kidney disease is defined as "an abnormality of kidney structure or function that has been present for three months or more and that has implications for health". The diagnostic criteria for CKD include a GFR of <60 ml/min/1.73 m^2, the presence of kidney damage (proteinuria, albuminuria, urinary sediment abnormalities, pathological imaging abnormalities, genetic disorders, or a history of renal transplantation).[2]

The prevalence of CKD in Nigeria varies between 7.8% and 18.8%. Hospital based prevalence is 8–10%. CKD stages 1, 2, 3, 4 and 5 accounted for 2.4%, 0.6%, 3.0%, 1.2% and 0.3% of participants respectively.[3-6] Mortality rate of CKD in Nigeria varies between 40–50%.[7] CKD was documented to be the 18th highest cause of death worldwide in 2010.[8]

β2 microglobulin , an 11.8-kDa protein, is the light chain of the major histocompatibility complex class I (MHC I) molecule that is present on the cell surface of all nucleated cells. It is freely filtered by the glomeruli and reabsorbed and broken down by proximal tubular cells.[9] It is not affected by age, gender, or muscle mass. However, it is influenced by infectious or inflammatory process, proliferative syndromes, and hepatic and autoimmune illnesses.[10,11] In a study assessing the biomarker value of serum β2 microglobulin, cystatin c, and lipocalin-2 in detecting renal impairment in Fabry disease, serum β2 microglobulin was found to be the best biomarker.[12] Lack of further studies in the last decade however has limited the utility of B2M in clinical practice.

Creatinine is a 113 Da amino acid derivative, is easily filtered by the glomerulus, and is the most commonly used for assessing GFR. Also, it is produced at an approximately constant rate from muscle creatine. However, it is secreted at variable rates by the proximal tubule. Consequently, creatinine clearance exceeds the GFR.[13,9]

Microalbuminuria is the main laboratory indicator for kidney structure damage.[14,15] It was initially defined as the presence of small quantities of albumin in urine, too little to be detected by standard "dip stick" methods.[16] It is now defined as "urinary albumin excretion between 30 and 300 mg/day, if measured in a 24 hour urine collection, 20–200 ug/min, if measured in a timed urine collection or 30–300 mg/g, if measured with the use of urinary albumin to creatinine ratio in a spot urine collection".[17] Furthermore, early diagnosis of CKD can provide an opportunity to modify some of the risk factors and reduce the rate of progression to end stage renal disease. This study was to investigate the diagnostic value of serum B2M and major biochemical markers of CKD such as serum creatinine and urine albumin.

METHODS

Research design

This was a hospital-based cross-sectional study carried out between April and June 2016 at the University of Benin Teaching Hospital.

Research subjects

Participants diagnosed with CKD based on persistent reduction in GFR <60 mL/min/1.73 m^2, albuminuria and abnormalities based on histology or imaging studies, at the renal unit of the University of Benin Teaching Hospital, were included in the study. Consecutive sampling was used to recruit participants. The number of subjects in each group required to complete the study with α= 0.05 and β=0.20, and standardized effect size of 0.40 and to account for dropouts was 124 subjects per group.[18] Therefore, the study comprised 124 participants with CKD and 124 healthy controls. The exclusion criteria for this study were chronic hemodialysis, lymphoproliferative and myeloproliferative

disorders, glucocorticoid therapy, inflammatory conditions and patients who have had kidney transplant. Written informed consent was obtained from each study participant. The study used a structured interviewer-administered questionnaire, which included identification number, age, gender, weight, height, medical history and laboratory results

SAMPLING

Blood sample

About five to ten milliliters (5–10 ml) of blood was collected from each participant by venipuncture into a plain bottle and allowed to clot. The specimen was centrifuged at 3000 rpm. The serum was separated from the cells using a Pasteur pipette and stored at -20^{0}C until analysis. AM and PM temperature monitoring was used to monitor the temperature of the freezer. Serum level of â2 microglobulin was analyzed by Enzyme Linked Immunosorbent Assay (ELISA), according to the manufacturer's protocol, while serum creatinine and urine creatinine were analyzed on a spectrophotometer using the kinetic modification of the Jaffe procedure.[19]

Urine sample

About ten milliliters of random urine was collected into plain bottles for one week for batching at 2 to 8℃ until analysis for urine albumin and creatinine. Urine albumin was analyzed on a spectrophotometer using the immunoturbidimetric assay.[20] Urine albumin creatinine ratio (UACR) was calculated by dividing urine albumin concentration in milligrams by urine creatinine concentration in grams.

Statistical analysis

Data was analyzed with SPSS version 16. Results were expressed as proportions, mean and standard deviation. Student's independent t-test was used to compare numerical variables while Chi-square and Fisher's tests were used to compare qualitative variables. Pearson correlation coefficient was used to correlate serum β2 microglobulin with other markers of renal disease. A receiver operating characteristic (ROC) curve with a 95% CI was used to determine the sensitivity and specificity of the renal markers. The p value was set at 0.05.

Ethical clearance

This study was approved by the ethics and research committee (protocol number: ADM/E 22/A/VOL. VII/11200) of the University of Benin Teaching Hospital, Benin City.

RESULTS

A total of 248 participants enlisted for this study consisting of 124 participants with CKD and 124 healthy controls. The mean and standard deviation of serum β2 microglobulin, serum creatinine and UACR are contained in Table I. Mean serum creatinine was significantly higher in CKD participants than in controls (147.67 ± 81.38 µmol/l versus 96.45 ± 12.78 µmol/l; p<0.001). Similarly, β2 microglobulin was higher in CKD participants than in controls (4.51 ± 2.74 mg/l versus 1.77 ± 0.86 mg/l; p<0.001) (Table 1). Urine albumin and UACR were also significantly higher among participants with CKD than in controls.

Serum β2 microglobulin had a very strong negative correlation with eGFR in CKD participants (r=-0.866; p<0.001). Conversely, serum creatinine had a very strong positive correlation with β2 microglobulin in participants with CKD (r=0.758; p<0.001). UACR had a very strong positive correlation with β2 microglobulin in CKD participants (r=0.775; p<0.001) (Table 2).

Figure 1 and Table 3 show the performance of various markers. The sensitivities of UACR, β2 microglobulin and serum creatinine in detecting CKD were 98.6%, 95.7% and 87.8% respectively while their specificities were 94.4%, 79.0% and 13.7% respectively. This is based on the following cut-offs: an eGFR of 0.87 ml/s/m^2; UACR of 3.4 mg/mmol; β2 microglobulin of 2 mg/L; serum creatinine of 115 µmol/L.

Table 1. Comparison of demographic and biochemical parameters between the CKD and control groups

Variable	CKD (n=124)	Controls (n=124)	p value
Age (years)			
<30	3 (2.7)	17 (13.7)	0.762
30 – 39	34 (27.4)	36 (29.0)	
40 – 49	34 (27.4)	33 (26.6)	
50 – 59	23 (18.9)	19 (15.3)	
≥60	30 (24.3)	19 (15.3)	
Gender			
Male	54 (43.2)	63 (50.8)	0.303
Female	70 (56.8)	61 (49.2)	
Serum Cr* (µmol/L)	147.67 ± 81.38	96.45 ±12.78	0.000
β_2 microglobulin (mg/L)	4.51 ± 2.74	1.77 ± 0.86	0.000
Urinary Cr (µmol/L)	5244.00±1126.56	9768.70±1286.56	0.018
Urine albumin(mg/l)	67.00 ± 40.01	0.75 ± 0.20	0.000
UACR* (mg/mmol)	13.31 ± 8.04	0.08 ± 0.03	0.000
eGFR (ml/s/m^2)	0.53 ± 0.19	0.77 ± 0.02	0.000

*Data presented as Mean ±SD, except for age and gender (n,%); Abbreviations: SD: Standard deviation, Cr: creatinine; UACR: urinary albumin creatinine ratio; eGFR: estimated glomerular filtration rate

DISCUSSION

In the present study, the serum concentrations of β2 microglobulin, creatinine and UACR were higher in CKD patients compared to controls, implying that they can all serve as markers of renal insufficiency. However, their sensitivities differ, which is the focus of this study. The reasons for the differences in their sensitivities may be attributed to the fact that increasing levels of microalbuminuria has been associated with an increased cardiovascular mortality in subjects with normal renal function.[16] β2 microglobulin maybe a better marker of CKD than serum creatinine because of its low molecular weight, constant production by all nucleated cells and its independence of age, gender or muscle mass.[21]

Table 2. Correlation of serum β2 microglobulin with other biochemical markers

Variable	β 2 microglobulin	
	r	p value
Serum Cr (µmol/L)	0.758*	0.000
UACR (mg/mmol	0.775*	0.000
eGFR (ml/s/m2)	-0.866†	0.000

*strong positive correlation, †strong negative correlation; Abbreviations: Cr: creatinine; UACR: urinary albumin creatinine ratio; eGFR: estimated glomerular filtration rate

β2 microglobulin, a low molecular weight protein, has been assessed as a potential marker of CKD. [9] In the present study, mean serum levels of β2 microglobulin were significantly higher in patients with CKD than in the control group and its level progressively increased with decreasing GFR. Many studies have confirmed the relationship between β2 microglobulin and eGFR. In a study involving forty biomarkers for the prediction of rapid decline in renal function in type 2 diabetes, β2 microglobulin and kidney injury molecule 1 showed the most consistent effects.[22]

The present study showed a strong association between serum β2 microglobulin and serum creatinine in both CKD participants and healthy controls. β2 microglobulin also had a strong positive association with UACR and a strong negative association with eGFR. From these results, it is obvious that serum creatinine is a less sensitive marker of renal injury. The results also show that β2 microglobulin is a more sensitive marker of CKD. These findings are supported by a cross-sectional study on the correlation of β2 microglobulin with serum

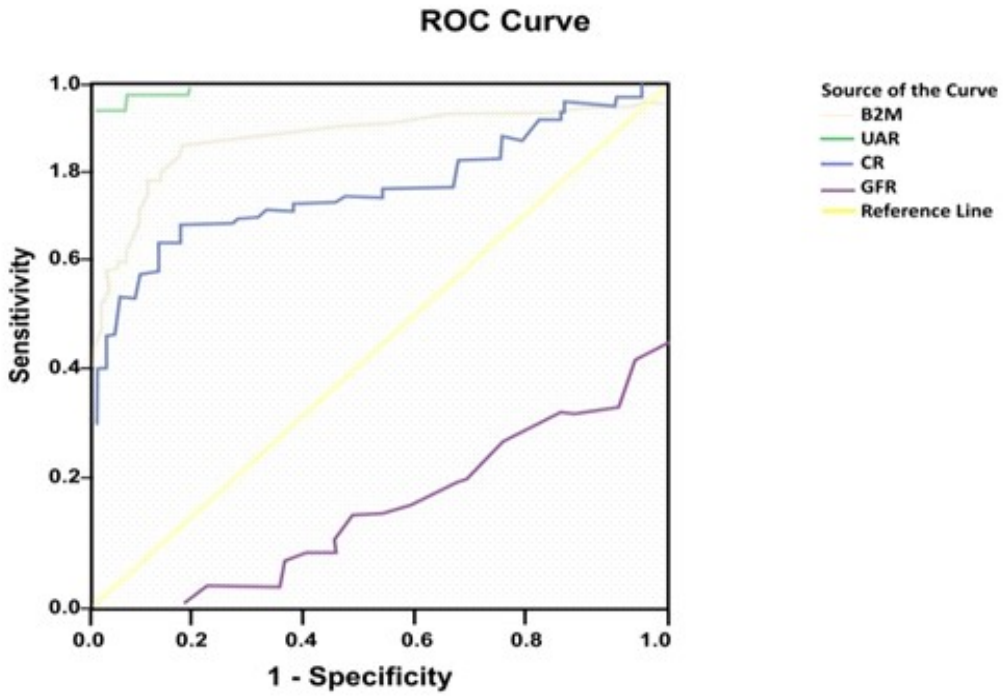

Figure 1. Receiver operator curve comparing UACR, β2 microglobulin and serum creatinine, based on eGFR as markers of CKD

creatinine and creatinine clearance in patients with different levels of renal function, which reported that β2 microglobulin was a more sensitive and accurate biomarker for the assessment of renal function as compared to creatinine.[23] Furthermore, a study conducted among sickle cell disease patients reported that β2 microglobulin had a higher sensitivity and specificity than serum creatinine.[24] Similarly, in the present study, β2 microglobulin had a higher sensitivity and specificity than serum creatinine. The results of the ROC analysis revealed that serum β2 microglobulin had a better diagnostic accuracy than serum creatinine. The AUC for serum β2 microglobulin was also higher than that of serum creatinine.

It has been well documented that albuminuria is a sensitive marker of CKD caused by diabetes mellitus, hypertension and glomerular diseases.[25] In such cases, the first sign of glomerular disease is increased urinary excretion of albumin.[26] The results of the present study confirm the reports that urinary albumin is a sensitive marker of CKD. The results show that

Table 3. Diagnostic accuracy of creatinine, β2 microglobulin, UACR, and eGFR as diagnostic tools for chronic kidney disease

	AUC*	SE†	p value	95% CI	Sensitivity	Specificity
UACR	0.996	0.003	0.000	0.991 – 1.001	98.6%	94.4%
β2M	0.884	0.027	0.000	0.830 – 0.937	95.9%	79.0%
Cr	0.809	0.036	0.000	0.739 – 0.879	87.8%	13.7%
eGFR	0.171	0.031	0.000	0.109 – 0.232	1.4%	83.1%

*area under the curve, †standard error of the mean; Abbreviations: CR: creatinine; UACR: urinary albumin creatinine ratio; β2M: β2-microglobulin; eGFR: estimated glomerular filtration rate

mean UACR was much higher in CKD than in controls. Also, the UACR was elevated in all the stages of CKD. In addition, the ROC plots confirmed that the UACR was the most sensitive marker of CKD.

The present study also reported that the UACR was significantly higher in patients with advanced CKD compared to those with early disease. In a cross-sectional study of North American children with CKD, it was shown that the level of proteinuria tended to be higher as the level of iohexol GFR decreased irrespective of the cause of CKD. [27] A significant relationship between UACR and end stage renal disease was reported in a cohort study. In the latter, higher UACR and lower eGFR were associated with increased risk of end stage renal disease.[28]

Furthermore, in a collaborative meta-analysis of eGFR and albuminuria on outcome of CKD, it was reported that higher albuminuria was associated with higher risk of end stage renal disease, independent of lower eGFR and of traditional cardiovascular disease risk factors.[29]

Although serum β2 microglobulin is not as good as UACR as a biomarker of CKD, its clinical value is justified because of its analytical reliability. Measurement of serum β2 microglobulin along with serum creatinine for renal evaluation should be considered. One limitation of this study was that elevation of serum β2 microglobulin can occur in certain conditions, which may limit its use as a biomarker of CKD.

CONCLUSION

In CKD, UACR had the best diagnostic value, followed by serum β2 microglobulin and serum creatinine. Periodic renal assessment of renal patients are mandatory as they may be affected by hidden renal dysfunction.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest concerning this article.

ACKNOWLEDGEMENT

The authors thank the staff of the Renal Unit of University of Benin Teaching Hospital.

CONTRIBUTORS

AOE and MUN made significant contributions to conception, design, experimentation, acquisition and interpretation of data and writing of manuscript. ESI and AAA made substantial contribution in interpretation of data and revising the manuscript for intellectual content. All authors read and approved the final manuscript.

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