ejpmr, 2020,7(1), 70-77

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ASSESSMENT OF URINARY ALBUMIN-TO-CREATININE RATIO IN PATIENTS WITH DIABETIC NEPHROPATHY IN SOUTH-SOUTH NIGERIA

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Article Received on 30/10/2019 **Article Revised on 20/11/2019** **Article Accepted on 10/12/2019**

ABSTRACT

Introduction: Screening for diabetic nephropathy (DN) facilitates early detection, evaluation, and treatment. A spot urine sample used to measure the albumin to creatinine ratio (ACR) accurately reflect the total 24 hour level of urine albumin excretion and the ACR is now recommended by the American Kidney Foundation as the screening tool for patients with diabetes. There are however many drawbacks to the cut off value of 30mg/g. Thus this study aimed to assess the diagnostic accuracy of spot Urinary albumin-to-creatinine ratio in diabetic nephropathy based on the cut off value of 30mg/g. **Method:** A hospital based cross sectional study involving 80 DN cases being managed in the University of Benin Teaching Hospital. The controls were 80 non diabetic persons from the University of Benin Teaching Hospital. The cases were selected using systematic sampling method and data collected using structured questionnaire after obtaining informed consent. They were sampled for Serum Creatinine, Serum Urea, Urinary Albumin and Creatinine. Urinary albumin-to- creatinine ratio and creatinine based glomerular filtration rate were calculated from the results. Data was analyzed using IBM SPSS version 25 and presented in form of tables, charts and graphs. **Results:** Of the total of 160 patients, 80 were DN cases and 80 were non diabetic controls. The study showed a significant difference between the mean levels of serum creatinine and urea, urinary creatinine and albumin, urinary albumin –to- creatinine ratio, and eGFR between the cases and controls. The diagnostic accuracy of UACR was higher with cut off value 57.8182mg/g. **Conclusion:** Serum UACR was shown to have a higher diagnostic accuracy in diagnosing diabetic nephropathy at 57.8182mg/g compared to 30mg/g.

KEYWORDS: Urinary Albumin-to-Creatinine Ratio(UACR), Diabetic Nephropathy(DN), Estimated Glomerular Filtration Rate(eGFR), Urinary Albumin, Urinary Creatinine.

BACKGROUND

Development of chronic kidney disease (CKD) in patients with diabetes adds significantly to the morbidity and mortality and significantly increases health care costs, even before the development of end stage renal disease (ESRD). Diabetic nephropathy may be accurately diagnosed by renal biopsy. But a biopsy is usually only done after suspicion of a kidney disease. Literature has shown a pathological classification of DN developed by the Renal Pathology Society based on glomerular lesions, with a separate evaluation for interstitial and vascular lesions as well.[1] In this classification, progression evolves from GBM thickening to mesangial expansion to so-called Kimmelstiel–Wilson (KSW) lesions, and finally to global glomerulosclerosis in different glomerular DN classes. However the diagnosis of diabetic nephropathy is more popularly primarily based on the development of increased albuminuria usually associated with preexisting retinopathy and decreasing GFR. Hyperglycemia, has been shown to cause increase in the level of urine albumin.[2] Thus the term microalbuminuria was coined to describe a small increase in the level of albumin of normal urine protein without an associated significant rise in the total urine protein level.[3] Initial definitions were based on an hourly excretion rate of urinary albumin and subsequently the 24 hour excretion of 30-300 mg of albumin was adopted as the microalbuminuric range that correlated to the hourly definitions. More recent studies showed that a spot urine sample used to measure the albumin to creatinine ratio (ACR) accurately reflected the total 24 hour level of urine albumin excretion and the ACR is now recommended by the American Kidney Foundation (AKF) as the screening tool for patients with diabetes. The spot ACR in a first-morning void has been shown to be superior to a 24-hour urine collection in predicting renal events in patients with type 2 diabetes and nephropathy.[4] Using the ACR, microalbuminuria is defined as being between 30-300 mg/g.[5] In the 1980's based on studies in type 1 diabetic patients with DN, Mogensen and colleagues developed a staging classification for the evolution of DN that became the widely accepted clinicopathologic classification for diabetic nephropathy (Table 1).[6]

Table 1: Staging System for Diabetic Nephropathy.

Stage 1.	Glomerular Hyperfiltration. The earliest observation in development of nephropathy is an increase of up to 50% in the glomerular filtration rate (GFR).
Stage 2.	Thickening of the glomerular capillary basement membrane (BM) is found histologically.
Stage 3.	Development of microalbuminuria (20-200 mcg/min or 30-300mg/24 h, not detectable by routine urine dipsticks).
Stage 4.	Overt diabetic nephropathy and macroalbuminuria (>200mcg/min or >300mg/24 h, that is detectable by routine dipsticks
Stage 5	End-stage renal disease (ESRD) (usually 25-30 years after diagnosis) with glomerular closure and resultant decrease in proteinuria.

Recent studies suggest that elevated urinary albumin excretion, even within the normal range, is also associated with a greater risk of diabetic complications.[7,8] Drawbacks of using an arbitrary cut off value of 30 mg/g to define normal urinary albumin excretion was shown in a Japanese study which stated that higher baseline ACR predicted a faster decline in eGFR among diabetics even within the normal range (<30 mg/g).[9,10] This study was done to assess the diagnostic accuracy of spot Urinary albumin-creatinine ratio in diabetic nephropathy based on the cut off value of 30mg/g.

MATERIALS AND METHODS

The study was conducted in the Department of Chemical Pathology of the University of Port Harcourt Teaching Hospital. River State, South south Nigeria. The state has a total of 23 Local Government Areas (LGAs) with a population density of 470 square kilometers and total population of 5, 198, 716 (2006 census). It is bounded in the south by the Atlantic Ocean, north by Imo, Abia and Anambra states, east by Akwa-Ibom state and west by Bayelsa and Delta states. Port Harcourt being the capital of Rivers State is cosmopolitan in nature and habours people of different backgrounds. Ethical approval obtained from the University of Port Harcourt Teaching Hospital. An Informed written consent was equally obtained from the study participants as well as the control subjects.

The study population was made up of adult diabetic nephropathy patients. The cases included both recently diagnosed patients and patients on admission. Controls were non diabetic persons from the University of Benin Teaching Hospital. Eighty (80) diabetic nephropathy patients (cases) and 80 non diabetic controls were used for this study. The sample size was calculated from the standard formula.[11]

About ten millilitres (10 mls) of venous blood was collected with syringe and needle from the ante-cubital vein of all patients and divided into lithium heparin and plain bottles as whole blood. The contents of the lithium heparin bottles were centrifuged at 2000-3000 revolutions per minutes (rpm) and plasma were then collected into plain 5 ml labeled bottles with Pasteur pipette. The contents of the plain bottles were allowed to clot for 2 hours. This was followed by a 10 minutes centrifugation at 2000-3000 rpm, and sera were then collected into 5ml labeled plain bottles. The samples were labeled with a unique identification number, and date of collection. All sample collection was done under standard operating procedure. Every batch of sample was processed alongside a control. Access to patient data/information was restricted to the researcher/supervisor and assessors.

Urea: Serum levels of urea was measured by Urease-Berthelot Method.[12] Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.[12]

The albumin concentration of urine samples were measured by Turbidimetric Immunoassay.[12] In this method, determination of urinary albumin is based on the principle of agglutination reaction. The test specimen is mixed with the activation buffer (R1) and anti-human antibody solution (R2) and allowed to react.[24] Presence of albumin in the test specimen forms an insoluble complex producing a turbidity, which is measured at wavelength 340 nm. The resulting turbidity corresponds to the concentration of albumin in the test specimen.

Levels of creatinine were measured by Modified Jaffe's kinetic Reaction.[13] Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed is directly proportional to the amount of creatinine present in the sample. Creatinine + Alkaline Picrate gives an orange coloured complex.[13]

UACR is a measure of Albumin Excretion Rate (AER). UACR is a ratio between two measured substances. Unlike a dipstick test for albumin, UACR is unaffected by variation in urine concentration. Albuminuria is used to diagnose and monitor kidney disease.

$$\textbf{UACR} = \frac{\textbf{Concentration of Urinary Albumin (mg)}}{\textbf{Concentration of Urinary Creatinine (g)}}$$

The UACR categorized subjects as follows: normoalbuminuria (ACR < 30 mg/g), microalbuminuria (ACR = 30–299 mg/g), and macroalbuminuria (ACR > 300 mg/g).[9]

The estimated glomerular filtration rate (eGFR) is used to screen for and detect early kidney damage, to help diagnose chronic kidney disease (CKD), and to monitor kidney status. It is a calculation based on the results of a

blood creatinine test along with other variables such as age, sex, and race, depending on the equation used. The following equation is applicable to Africans:[14]

$$\frac{(140\text{-Age in years}) \times \text{Body weight (Kg)}}{72 \times \text{Serum creatinine (mg/dl)}} \times 0.83 \text{ for females}$$

Control Sera level 2 was added to each run. Control sera was analyzed ten times and coefficient of variation calculated to determine the precision. The coefficient of variation for serum creatinine, serum urea, urinary creatinine, and urinary albumin were 2.94%, 4.3%, 4.8% and 2.1% respectively with precision of 97.1%, 95.7%, 95.2%, and 97.9% respectively.

Data obtained during the course of the study were analyzed using statistical package for social sciences (SPSS) version 22. Results were expressed as mean, standard deviation and proportions and presented in tables and charts as appropriate. Categorical variables were compared using chi squared test and fisher's exact. Continuous variables were compared using t test. Scatterplot was used to depict the relationship between eGFR with UACR. Receiver operating characteristics curve (ROC) analysis was used to determine sensitivity and specificity of each assay. UACR results were analyzed using receiver operating characteristic (ROC) analysis by SPSS 25; cut-off (CO) values for the test were set according to the diagnostic accuracy. Accordingly, the maximum CO value for UACR was set 57.8182 which is equal to the point where Y is maximum (Y = (sensitivity + specificity)/2). Level of probability was set at $P \leq 0.05$.

RESULTS

A total of 160 cases participated; 80 cases being DN patients and 80 being controls. The findings of the results were as follows.

Table 1: Socio-demographic characteristics of Age Sex distribution of study subjects.

	DN n = 80	Control n = 80	P value
Age group			
40 – 49	4 (5.0)	7 (8.8)	
50 – 59	33 (41.2)	44 (55.0)	***0.004****
60 – 69	39 (48.8)	18 (22.5)	
>=70	4 (5.0)	11 (13.8)	
Sex			
Male	42 (52.5)	37 (46.2)	0.429
Female	38 (47.5)	43 (53.8)	
Weight			
≤68.417	7 (8.8)	22 (27.5)	***0.006****
68.418 – 76.238	19 (23.8)	16 (20.0)	
76.239 – 84.058	49 (61.3)	33 (41.3)	
≥84.059	5 (6.3)	9 (11.3)	

Significant

Table 2: Mean values of the cases and controls study subjects.

	DN Mean±SD	Control Mean±SD	t value	P value
Age	59.73±6.43	58.13±8.41	-1.351	0.179
Weight	75.04±9.53	77.43±5.40	-1.958	0.052
Serum Creatinine	4.85±2.67	1.02±0.21	-12.805	< ***0.001****
Serum Urea	113.73±54.5	25.7±7.09	-14.326	***0.001****
Urinary Creatinine	58.86±19.43	129.12±29.01	17.995	< ***0.001****
Urinary Albumin	11.36±3.35	3.13±0.77	-21.424	< ***0.001****
UACR	222.03±103.51	26.05±11.06	-16.840	< ***0.001****
eGFR	25.47±15.19	97.30±14.10	30.998	< ***0.001****

Significant

Thirty nine (48.8%) of the cases were aged 60 – 69 years compared to 44 (55.0%) of the controls aged 50 – 59 years. There was a statistically significant association between the age of the patients and their diagnostic groups (p = 0.004).

Forty two (52.5%) of the cases were males compared to 43 (53.8%) of the controls that were females. This association was however not statistically significant (p = 0.429).

The mean age of the cases was 59.73 ± 6.43 years compared to 58.13 ± 8.41 years of the controls. There were no statistically significant differences between the mean age of the cases and controls (p = 0.179).

The mean weight of the cases was 75.04 ± 9.53 kg compared to 77.43 ± 5.40 kg of the controls. There was also no statistically significant difference between the means (p = 0.052).

The mean serum creatinine value of the cases was 4.85 ± 2.67 mg/dl compared to 1.02 ± 0.21 mg/dl of the controls. There was a statistically significant difference between the means (p < 0.001).

The mean value of serum urea among the cases was 113.73 ± 54.5 mg/dl compared to 25.7 ± 7.09 mg/dl among the controls. This difference between the means was statistically significant (p = 0.001).

The mean value for urinary creatinine among the cases was 58.86 ± 19.43 mg/dl compared to 129.12 ± 29.01 mg/dl of the controls. This difference was also statistically significant (p < 0.001).

The mean value for urinary albumin among the cases was 11.37 ± 3.35 mg/dl compared to 3.13 ± 0.77 mg/dl of the controls. This difference was statistically significant (p < 0.001).

The mean value for urinary albumin-to- creatinine ratio among the cases was 222.03 ± 103.51 mg/g compared to 26.05 ± 11.06 mg/g among the controls. This difference was statistically significant (p < 0.001).

The mean value for eGFR among the cases was 25.47 ± 15.19 ml/min compared to 97.30 ± 14.10 ml/min among the controls. There was a statistically significant difference between the means (p < 0.001).

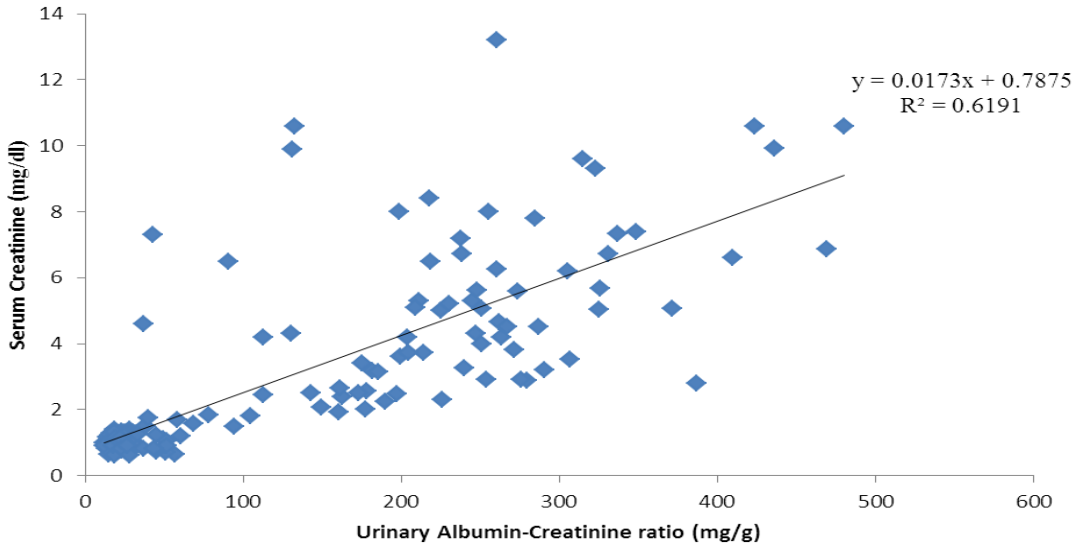

Figure 1: Scatterplot showing the relationship between Urinary albumin-to-creatinine ratio and serum creatinine concentration.

There was a direct relationship between the urinary albumin-to-creatinine ratio and serum creatinine concentration.

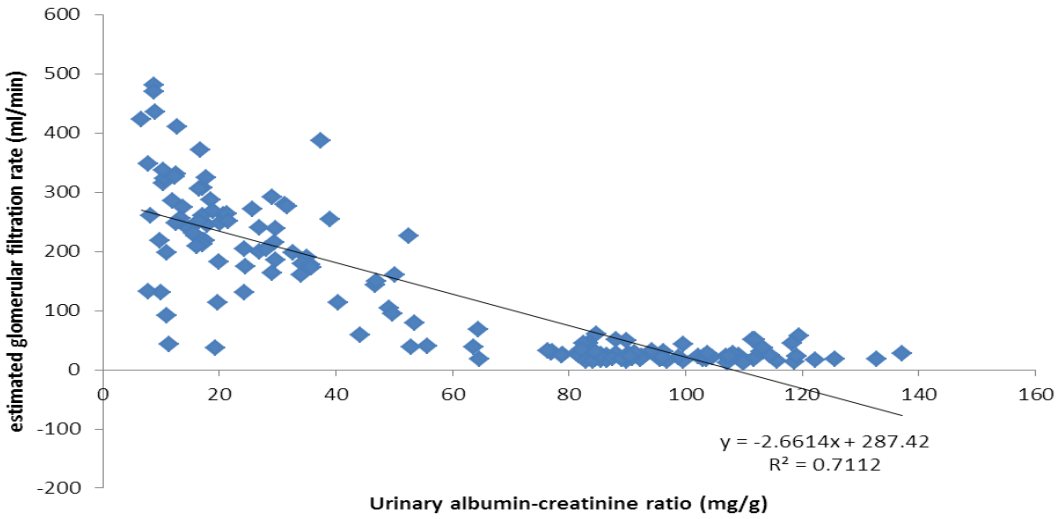

Figure 2: Scatterplot showing the relationship between eGFR and Urinary albumin-to-creatinine ratio.

There was an inverse relationship between eGFR and urinary albumin-to-creatinine ratio.

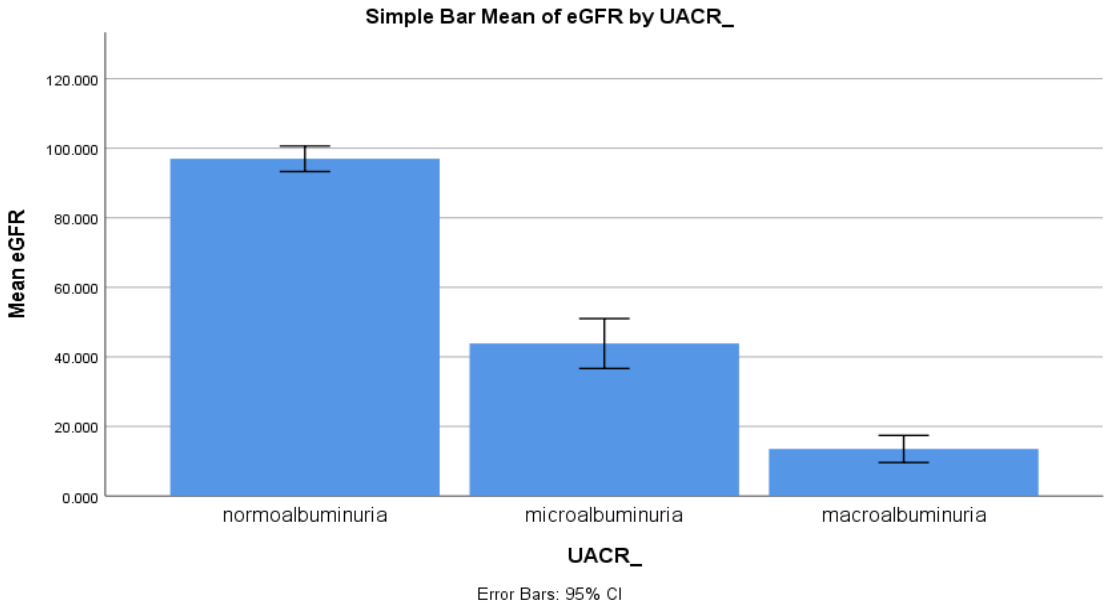

Figure 3: Bar chart comparing the mean eGFR in the different classes of Urinary albumin-to-creatinine ratio.

The mean eGFR tended to decrease with increase in urinary albumin-to-creatinine ratio. the mean eGFR was 96.95±14.45ml/min, 43.84±32.65ml/min, and 13.50±7.31ml/min among the normoalbuminuria, microalbuminuria and macroalbuminuria.

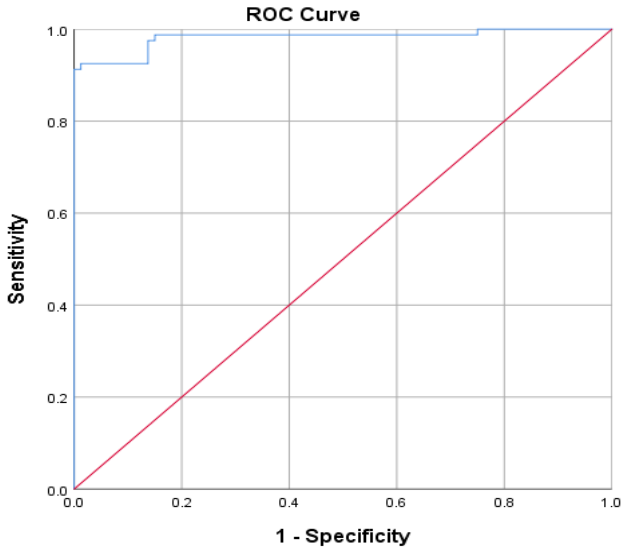

Figure 3: Receiver operating characteristics curve showing Urinary Albumin–to-Creatinine ratio prediction of diabetic nephropathy (diagnosed using eGFR).

Table 4: Receiver operating characteristics interpretation.

Assays	Area under the curve	Standard error	p value	95% confidence interval	
				Lower	Upper
UACR	0.982	0.010	< ***0.001****	0.961	1.000

Significant

Table 5: Cutoff point and diagnostic parameters of UACR assay in Diabetic Nephropathy.

Assay	Cutoff point	Sensitivity	Specificity
AKF cut off	30.000*	98.8 %	76.2%
Study cut off	57.8182	92.5%	98.7%

Using the American Kidney Foundation cutoff point of 30.000mg/g, the ROC curve yielded a sensitivity and specificity of 98.8% and 76.2% respectively in the study population. This cut off point had a diagnostic accuracy of 87.5%.

However a cutoff point of 57.8182 mg/g with sensitivity and specificity of 92.5% and 98.7% respectively and a diagnostic accuracy of 95.62.

Table 6: Cross tabulation between UACR with the eGFR diagnosed groups with the different cut off points.

Assay	DN (Positive) n = 80	Control (Negative) n = 80	Total n = 160	Stat test	P value
Cut off (30.000mg/g)					
Positive	79 (98.8)	19 (23.8)	98 (61.3)	χ^2=94.799	***< 0.001****
Negative	1 (1.2)	61 (76.2)	62 (38.8)		
Cut off (57.8182mg/g)					
Positive	74 (92.5)	1 (1.2)	75 (46.9)	χ^2=133.747	***< 0.001****
Negative	6 (7.5)	79 (98.8)	85 (53.1)		

****Significant***

Using the cutoff mark of 30.000mg/g, 79 (98.8%) of the cases were diagnosed positive for DN while 1 (1.2%) was diagnosed negative. This was statistically significant ($p < 0.001$).

Seventy four (92.5%) of the cases were diagnosed positive by the cut off value of 57.8182mg/g while 6 (7.5%) were negative. This was also significant ($p < 0.001$).

Table 7: Summary of diagnostic accuracy of UACR in Diabetic nephropathy.

Performance variable	Cut off = 57.8182mg/g	Cut off = 30.0000mg/g
Positive predictive value	98.67%	80.6%
Negative predictive value	92.94%	98.4%
Positive Likelihood ratio	71.15	4.15
Negative Likelihood ratio	0.08	0.02
Accuracy	95.62%	87.5%

DISCUSSION

In this comparative cross sectional study, urinary albumin-to-creatinine ratio was assessed in diabetic nephropathy patients. A total of 160 subjects participated, of which, 80 were persons diagnosed with DN using eGFR and 80 were non diabetic control persons.

In the present work, the variation in blood urea, serum creatinine, and urinary creatinine in patients of varying duration of DN were compared to the normal subjects. It was observed that there was a significant relation between blood urea, serum creatinine, urinary creatinine and albumin with diabetic nephropathy. This is in line with a previous study done in India.[15]

The mean serum creatinine and urea were significantly higher in those with DN while urinary creatinine was significantly lower. These are expected in nephropathy as the kidney has the primary function of excreting these metabolites. Increase in blood urea and creatinine levels are usually seen when there is damage to the kidney. This increase in blood urea and creatinine levels in the presence of high blood sugar level in diabetic patients indicate damage to the kidneys. Often, plasma creatinine and urea are markers routinely used in monitoring DN patients on therapy, especially in deciding on need for renal dialysis, with creatinine more than urea fulfilling most of the requirements of an established marker. Thus these metabolites are used in monitoring disease progression in DN and the need for dialysis.[16]

Estimated glomerular filtration rate is a measure of the rate at which the kidneys' two million glomeruli filter plasma in order to process it and remove waste products from it. If the kidneys are injured by chronic kidney disease, the GFR gradually declines, and the amount of remaining kidney function can be estimated by measuring or calculating the GFR. Thus this study showed significant decrease in eGFR among the cases compared to the controls.

Diabetic nephropathy is a clinical hall mark of microangiopathy and is the most important single disorder leading to renal failure in adults.[17] The earliest clinical evidence of nephropathy is the appearance of

low but abnormal levels of albumin in the urine and the patients with micro-albuminuria are referred to as having incipient nephropathy. Thus it is not surprising that there was higher levels of urinary albumin among the cases (11.37 ± 3.35 mg/dl) compared to the control (3.13 ± 0.77 mg/dl). In addition to its being the earliest manifestation of nephropathy, albuminuria is a marker of greatly increased cardiovascular morbidity and mortality for patients with DM. Thus, the finding of micro-albuminuria is an indication for screening for possible vascular disease and aggressive intervention to reduce all renal and cardiovascular risk factors. The study also found that the mean value for urinary albumin –to-creatinine ratio among the cases was significantly higher in those with DN. The cut off value of 30.0mg/g was set by the American kidney foundation and has gained universal acceptance. However, using this cutoff point, UACR had 98.8% sensitivity for the detection of DN and 76.2% specificity compared to 92.5% and 98.8% respectively for the cut off of 57.8182mg/g (ROC selected value with highest diagnostic accuracy. This implies a higher sensitivity but comparatively higher number of False Positive results would be obtained using the former against the latter cut off value. The performance of any test in a population is influenced by Positive and Negative Predictive values. Predictive values vary among populations such that PPV are higher and NPV are lower in disease prevalent areas.[18] In this study a PPV of 98.67% was recorded for the study's cut off value (57.8182mg/g) explaining the higher False Positives and the lower negative prediction of the test compared to the universal cut off value in diagnosing DN. This suggests that ~1.33% of people who test with UACR in such study settings with similar characteristics as Edo State will receive False Positive results. This is much lower than 19.4% false positives using the AKF cut off. The study cut off for UACR had a higher diagnostic accuracy (95.62%) compared to the AKF cut off (87.5%). This has remarkable implications in the diagnosis and management of diabetic nephropathy. It does not necessarily translate into rejection of the former cut off for UACR assay but is a pointer for need for more research to be done to adjust the cut off values for Africans and Nigerians specifically. Of note is the fact that some of the diagnostic indices such as the PPV of a screening test is determined not only by factors that determine the validity of the test, but also by the prevalence of disease in the population. Thus a case control study such as this one is not the most suited to assess the diagnostic strength of any test.

CONCLUSION

The study showed that UACR was significantly higher among patients with diabetic nephropathy. There was a significant increase in serum creatinine and urea levels in the diabetic patients compared to the non-diabetic control individuals. The study also recorded increased urinary albumin and reduced urinary creatinine among the diabetic nephropathy patients. Thus a significantly higher urinary albumin-to-creatinine ratio and lower eGFR were recorded among diabetic nephropathy patients. Using a higher cut off value for UACR of 57.8182mg/g was shown to have a higher diagnostic accuracy in diagnosing diabetic nephropathy compared to 30mg/g.

Recommendation

Long-term, prospective study which takes into recognition suspected risks or protection factors during disease pathogenetic period will be needed to substantiate the study findings and recommend new cut off values and ranges for UACR. Not neglecting the current study which could serve as a foundation for the use of an adjusted UACR value in the diagnosis of DN among Africans.

ACKNOWLEDGEMENT

The authors wish to appreciate the contribution of Dr.Moses Akpoghene of Federal Medical centre, Jalingo, for statistical analysis and typing of manuscript. Acknowledgement also goes to Prof. C.G. Orluwene of the department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State.

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