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Diabetic Nephropathy and Albuminuria in Type 2 Diabetic Patients in Enugu State Nigeria

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Running Title: Prevalence and Risk Factors of Diabetic Nephropathy in Type 2 Diabetes in Enugu State

Abstract: ***Diabetic nephropathy DN is a public health concern and reason for a significant reduction in the life expectancy of diabetic patient. There is dearth of information on the common risk factors of DN in type 2 diabetes in Enugu. Hence this study was carried out to emphasize on the need of regular screening of albuminuria and other biochemical parameters in type 2 diabetic patient. The study was conducted to know the prevalence of albuminuria, the most prevalent component of DN and the relationship of FBS with other biochemical markers. The work was carried out from February to August 2016 among sixty seven diabetic patient aged 40-75years. DN was defined by macro albuminuria. The most frequent component of diabetic nephropathy among the subject were albuminuria (61.2%), high cholesterol (59.7%), creatinine (59.7%) Urea (59.7%) and high blood pressure (56.7%). The prevalence of DN in type 2 diabetic patient was 61.2% while the mean level of biochemical parametes of the DN showed significant (P<0.05) difference except total protein when compared with subject without nephropathy. The most frequent cluster of the risk factors in the population studied was high abminuria, high cholesterol, high creatinine, high urea and high blood pressure.***

Keywords: Diabetic Nephropathy, Albuminuria and Biochemical profile.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by impairment in the metabolism of carbohydrates lipids and proteins. The chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys nerves, heart and blood vessels. Type 2 diabetes which comprises of 80-90% of all cases of diabetes mellitus has quickly become a global health problem due to rapidly increasing population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity (Chen et al, 2014).

The global prevalence of diabetes is expected to increase from 4% in 1995 to 5.4% by the year 2025 (King et al, 1998). Diabetes is a major cause of end-stage renal disease around the world accounting for nearly 44% of new case (USRDS, 2007). Diabetic nephropathy is the kidney diseases that occur as a result of diabetes and has been suggested that it is responsible for renal failure in one third of patient who undergo dialysis. Diabetic nephropathy is a public health concern and reason for a significant reduction in life expectancy of diabetic patients (Rita, 1999).

It is an important cause of morbidity and mortality worldwide. It is suggested that patients with common risk factors of diabetic nephropathy which include greater duration of diabetes, excretion of albumin, poor metabolic control, hypertension, hyperlipidemia, obesity, prothrombotic and proinflammatory states are more prone to diabetic complications (Daneman, 2005). Microalbuminuria is considered to be an early marker of diabetic nephropathy and predictor for cardiovascular disease (Go et al, 2004). There is dearth of information on these risk factors in our locality and diabetes mellitus has enormous medical, social and economic consequences. There is an urgent need to prevent diabetes and its complications. This study is to emphasize on the need of regular screening for albuminuria, and other biochemical markers in patient with type 2 diabetes.

2. Materials and Methods

Study Population

This study was conducted using diabetic patients clinically managed at the University of Nigeria Teaching Hospital Enugu, and Nsukka, a sub-urban town in Enugu State South-Eastern Nigeria. The study was conducted from February to August 2016. Sixty seven (67) diabetic patients aged 40-75years (32 males and 35 females). Ethical Consideration and protocols were approved by the Head of Department of medicine, University of Nigeria Enugu. Patients provided written informed consent according to Helsinki declaration (NCEP, 2002). The study was carried out in the Department of Chemical Pathology College of Medicine, University of Nigeria Enugu s. Subjects on lipid lowering antihypertensive and antihyperglycemic drugs were excluded from the study.

Anthropometric Measurement

The blood pressure was measured and the reading taken in nearest one millimeter of mercury (1mmHg) using sphygmomanometer.

Biochemical Measurements

Blood sample collection and Analysis

Six (6) milliliters of venous blood sample was collected from each patient after an overnight fast of 8-12hours using a standard method (Dacie and Lewis, 1975). Two (2) milliliters of the blood sample were placed in a fluoride oxalate bottle for the estimation of fasting blood sugar by glucose oxidaxe method (Trinder, 1969), while four (4) millitres were placed into centrifuge tubes, spun at 3000 rpm for 5 minutes then separated for biochemical analysis. Serum protein was analyzed using biuret method (Cornall et al., 1949). Estimation of creatinine was done using Jaffe

reaction method (Delanghe et al., 2012). Total cholesterol concentration was determined using the method (Allian, 1974) and urea concentration was analyzed or estimated using the method of Diacetyl Monoxime (Marsh et al., 1957).

24hr Urine Protein Measurement
Ten milliter of HCL was added in four (4) litres container as preservative, the bladder was emptied completely and exact time was noted, all the urine specimen passed thereafter were collected in the container for 24hrs for measurement of urine protein using biuret (Cornall et al., 1949).

Diagnosis of Diabetic nephropathy
Diabetic nephropathy (DN) is defined by macroalbuminuria – urinary albumin excretion of more than 300mg in a 24hour collection – or macroalbuminuria and abnormal renal function as represented by an abnormality in serum creatinine, cholesterol, high blood pressure and urea.

Subjects with macroalbuminuria and any other abnormal renal function parameters especially creatinine, urea, cholesterol were identified as having DN in this study

Statistical Analysis
Statistical package for social sciences (SPSS) version 16 and graph pad prism software were used to analyze the obtained. The results were presented as mean ± standard deviation for continuous variables while absolute numbers and percentages were used to present the categorical variables. Student's t-test and Pearson correlation were used to compare the data and inferences made at 95% confidence limit ($p<0.05$).

3. Results

Out of 67 subjects, 41 of them were identified as having of DN giving a prevalence of 61.2%. According to the result in table 1, the most frequent risk factor was albuminuria 61.2% of the population. The most frequent pattern of cluster of the risk factors seen in subjects with DN were, FBS, Chol, Creatinine, Urea and HBP. recorded the least frequency 56.7%. Therefore the most predominant cluster of the risk factors of DN in the population was high albuminuria, High Cholesterol, High Fasting Blood Sugar, High creatinine, Urea and High Blood Pressure.

Table 1: Frequency of the risk factors of diabetic nephropathy (DN) among all the subjects n = 67

Variables n (%)	DM with nephropathy (DM + N)	DM without nephropathy (DM – N)
Albuminuria	41 (61.2)	26 (38.8)
Fasting Blood Sugar	40 (59.7)	27 (40.3)
Cholesterol	40 (59.7)	27 (40.3)
Albumin	41 (61.2)	26 (38.8)
Globin	41 (61.2)	26 (38.8)
Creatinine	40 (59.7)	27 (40.3)
Urea	40 (59.7)	27 (40.3)
High Blood Pressure	38 (56.7)	29 (43.3)

Abnormality was identified using reference values [total protein 6.2 – 8.0g/100ml, albumin 3.6 – 5.2g/100ml, globin 1.8 – 3.2g/100ml, creatinine 0.5 – 2.2mg/100ml, urea 15 – 50mg/100ml, cholesterol 120-240 mg/100ml, blood pressure 120/80mmHg, albuminuria <150mg/24hrs] frequency is expressed in absolute number and percentage FBS = fasting blood sugar, chol = cholesterol, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, DM = Diabetic Mellitus Diabetic Mellitus with Nephropathy = DM + N Diabetic mellitus without Nephropathy = DM – N,

Table 2: comparison of the means of all the parameters studied between DM + N AND DM – N

Variables	DM + N S (n = 41)	DM – N S (n = 26)	P value
Age (years)	58.11 ± 1.10	57.6 ± 0.63	P>0.05
SBP (mmHg)	147.8 ± 2.5	134.2 ± 1.9	P<0.05
DBP (mmHg)	90.2 ± 1.47	82 ± 1.05	P<0.05
Total protein (g/100ml)	7.87 ± 1.48	7.56 ± 1.24	P>0.05
Albumin (g/100ml)	2.39 ± 0.69	4.57 ± 1.24	P<0.05
Globin (g/100ml)	5.59 ± 1.43	2.9 ± 0.93	P<0.05
Creatinine (mg100ml)	9.19 ± 4.2	1.16 ± 0.63	P<0.05
Urea (mg/100ml	161.9 ± 52	31.1 ± 9.83	P<0.05
Cholesterol (mg/100ml)	404.450 ± 105	198.65 ± 45.7	P<0.05
Fasting Blood Pressure (mg/100ml)	159 ± 20.7	93.8 ± 16.3	P<0.05
Urine Protein (albuminuria) g/24hrs	12.81 ± 8.44	0.659 ± 0.423	P<0.05

Variables are as mean ± standard deviation
Statistical significant at $p<0.05$

The mean value of Albumin, Globin, albuminuria, Fasting Blood Sugar, Cholesterol, Urea, High blood pressure and Creatinine were significantly ($P<0.05$) higher in DM compared to DM – N while total Prot was higher in DM + N compared to DM + N but not significantly ($P>0.05$).

Table 3: Correlation of FBS and biochemical risk factors of DN in population studied (n = 67).

Variables	Mean	r value	p value
Total protein (g/100ml)	7.75 ± 1.43	-0.024	P<0.05
Albumin (g/100ml)	3.24 ± 1.42	-0.697	P<0.05
Globin (g/100ml)	4.56 ± 1.81	0.578	P<0.05
Urine Protein (g/24hrs)	8.79 ± 8.78	0.539	P<0.05
Creatinine (mg/100ml)	6.03 ± 5.13	0.632	P<0.05
Urea (mg/100ml)	110.41 ± 76.35	0.724	P<0.05
Cholesterol (mg/100ml)	323.99 ± 132.99	0.651	P<0.05

(r) Pearson correlation

FBS was correlated with the risk factors of DM + N and the result in table 3 showed that all had positive significant correlation except total Prot (r = -0.24) and Alb (r = -0.629) that had weak correlation.

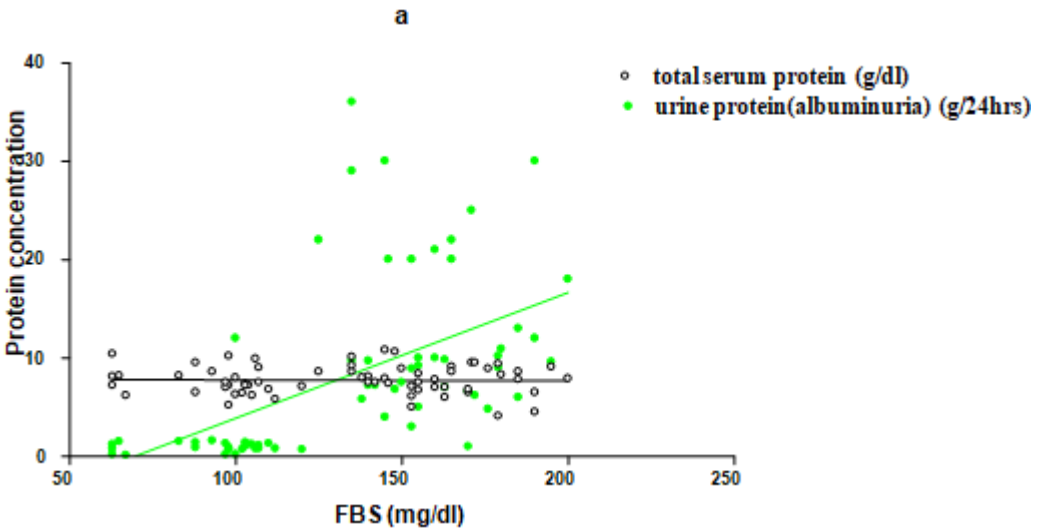


Figure 1 (a): Fasting blood sugar correlated with total protein and urine protein

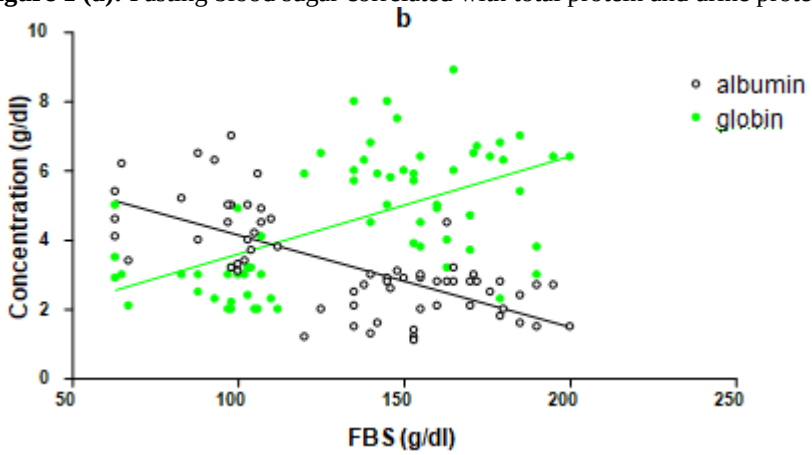
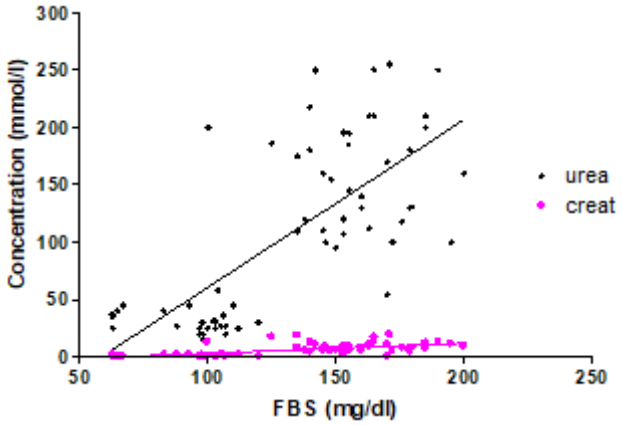


Figure 1 (b): Fasting blood sugar correlation with albumin and globin



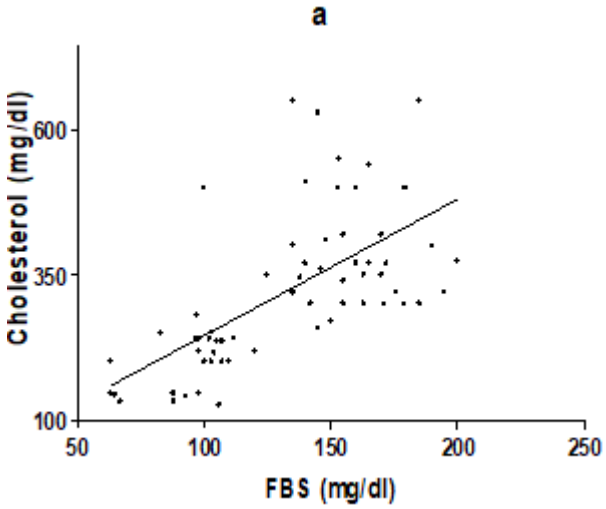


Figure 2 (a): Fasting blood sugar correlated with cholesterol

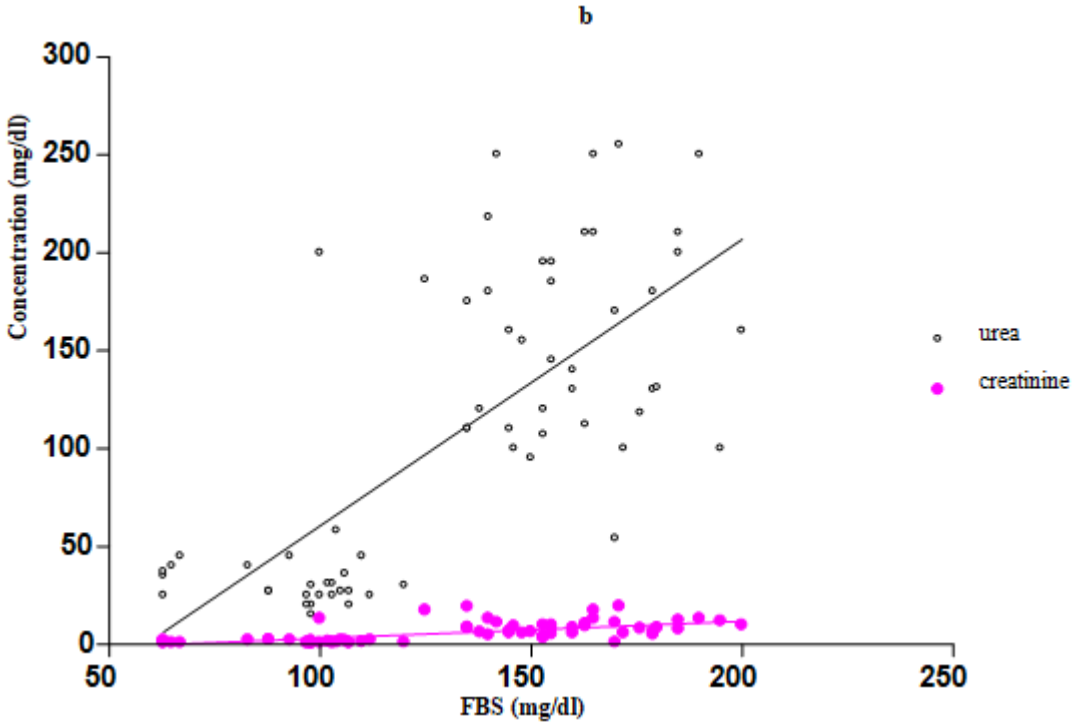


Figure 2 (b): Fasting blood sugar correlated with creatinine and urea

4. Discussion

In this study, it was observed that forty-one subjects (61.2%) had albuminuria the most frequent risk factors of DN in this population. The prevalence of DN (61.2%) recorded in this study is high compared to 30% in United Kingdom, while in Mexican Americans was 31% (Adler et al, 2003). Moreover studies conducted in Asian countries reported variability in the prevalence of microalbuminuria ranging from 14.2% in Iran, to 36.3% in India (Wu et al, 2005). The prevalence of microalbuminuria in European countries was 26.9% in Hungary, while microalbuminuria was 16% in Italy as well as Sweden, 9% in Germany (Bruno et al, 1996; Svensson et al, 2003).

These variations in the prevalence rate of albuminuria can be attributed to difference in several factors such as race, age, sex, diabetic duration and medication although in the locality studied, many people escape undiagnosed.

This shows an alarming high prevalence of albuminuria; it is a well known predictor of DN in a patient with type 2 diabetes, hypertension and cardiovascular disease. (Battisti et al, 2003).

This could be attributed with the increase in glomerular permeability which allowed plasma protein to escape in urine. Some of these proteins taken by the proximal tubular cell can initiate an inflammatory response in the kidney. It has been shown that various inflammatory cytokines e.g. tumor necrosis factor (TNF) $^{\alpha}$ produced plays a role in tissue damage (Blazka, *et al*., 1996). Leucocytes (Neutrophil and monocytes) as part of their defensive role might have contributed in renal injury in response to chemotactic factor during inflammation leading to kidney injury.

Hypoalbuninemia seen in the study is agrees with earlier study in Australia (Reuten et al 2011). It could be attributed to escape of albumin in the urine also globin shows significant increase (Fig 1b). It has been stated that in some inflammatory disease condition, release of (TNF) $^{\alpha}$ inhibit albumin synthesis but induces the synthesis of protein of acute phase response (Casteslino and Salern, 1997). FBS and Cholesterol had prevalence of 59.7% each. Hyperglycemia as a risk factor agrees with earlier work that demonstrated that DN is higher in patients with poor metabolic control (Parving et al, 2004). It could be attributed to the fact that hyperglycemia is a precondition of DN lesion and renal functional disturbances that resulted in the mesangial expansion and injury. Glucose can also bind to protein in the kidney and circulation to form advanced glucosylation end products which can form complex cross-links over year of hyperglycemia and can contribute to renal damage. Elevated levels of creatinine and urea were significant in this study. The shows that elevated levels in serum are specific for kidney disease. Creatinine is removed from the blood directly by the kidney, primarily glomerular filtration but also via proximal tubular secretion since the kidney has been damaged, accumulation of waste product will occur. Urea is not excreted normally and this increase can alter renal haemodynamics resulting in azotemia which can cause nephrotoxicity in the glomerulus leading to disruption of normal cellular function of mitochondria and membrane integrity.

The significantly high level of cholesterol in DN subjects agrees with earlier studies (Lurbe et al., 2002). The role of hyperlipidemia in the development of DN has been described in several studies (Lurbe et al, 2002). In the majority of the studies, Cholesterol showed a positive correlation with the degree of albuminuria. Cholesterol showed positive correlation with DN in this study (Fig. 2a). It could be attributed to uncontrolled DM, when fat is used as a source of energy in the absence of insulin.

The blood pressure was equally elevated in this study. Kalag et al., 1996, found that elevation of blood pressure was a strong independent risk factor for renal disease. The association between increased pressure and DN was recognized by most of the study (Schrier et al, 2002). This can be attributed to disordered autoregulation and systemic hypertension.

5. Conclusion

The prevalence of DN in type 2 diabetic patients was found to be very high, alarming the population, decision makers to develop strategies for prevention, detection and treatment. There is need for screening of microalbuminuria in type 2 DM. The presence of microalbuminuria is an indication to take steps to further prevent renal damage by correction of such factors as hyperglyceamia, hyperlipidemia and hypertension.

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