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SERUM LEVELS OF B2 MICROGLOBULIN, CYSTATIN C AND CREATININE IN PATIENT WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Diabetic nephropathy has been incriminated to be the most important cause of end stage renal diseases and death in patients with diabetes mellitus. This study was aimed at evaluating β2 microglobulin, cystatin c and creatinine in patient with type 2 diabetes mellitus. A total of 40 subjects comprising of 20 diabetic patient and 20 healthy subjects were recruited randomly for the study, and their blood sugar level was determined. Five milliliters (5 ml) of blood was then collected into the vaccutainer tube with minimal stasis. The blood was allowed to clot at room temperature, and serum separated and harvested into a clean dry well labeled sample bottles following centrifugation at 3000 rpm for 5 minutes. The sample was stored in a freezer at -20^{oc}, prior to use. β2 microglobulin and cystatin c was determined using ELISA techniques while and creatinine was determined using Jaffe-slot Alkaline picratecolometric method. Results were presented in mean ± standard deviation (SD). All data obtained in the study were analyzed using student t-test (spss.20) and Pearson correlation coefficent. The level of significance was set at $p < 0.05$. There was a significant increase (p=0.000) in the mean value of β2microglobulin in Diabetic patients (2.52±0.22 mg/L) when compared to controls (1.54±0.23 mg/L). The mean value of Cystatin C was significantly increased (p=0.009) in Diabetic patients (1.10±0.87 mg/ml) when compared to Controls (0.79±0.18 mg/ml). The mean value of Creatinine was significantly increased (p=0.023) in Diabetic patients (1.10±0.87 mg/ml) when compared to Controls (0.79±0.18 mg/ml). There was a non-significant negative correlation of serum $\beta_2$microglobulin with serum cystatin C and Creatinine (r= -0.638, p=0.247 and r=-0.115, p=0.853). Diabetes mellitus is associated with increased level of β_2Microglobulin, Cystatin C and Creatinine. The changes observed in these parameters are critical as this may suggest their use as early risk predictors of diabetic nephropathy in patients diagnosed of diabetes mellitus.

Keywords: B2 microglobulin, cystatin c and creatinine, patient, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels, which result from defects in insulin secretion, action, or both [1]. Over time, Diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as micro vascular disease. Diabetes is also an important factor in accelerating hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel disease [2]. Diabetes affects approximately 17 million

(about 8% of the population) in Nigeria. One third or more of the diabetes mellitus patients develop Diabetic Nephropathy (DN) with progressive deterioration of renal function and structure in their life time [3]. Diabetic Nephropathy is the leading cause of endstage renal disease (ESRD) worldwide [18]. The earliest clinical evidence of DN is the appearance of low but abnormal levels (30 to 300 mg/day) of albumin in the urine, referred to as Microalbuminuria [4]. In addition, an estimated additional 12 million people in Nigeria have diabetes and don't even know it. Diabetes is the third leading cause of death in Nigeria after heart disease and cancer [5]. Beta2-microglobulin is plentiful on the surface of many cells. Increased production or destruction of these cells causes Beta2-microglobulin levels in the blood to increase. This increase is seen in people with cancers involving white blood cells, but it is particularly meaningful in people newly diagnosed with multiple myeloma [6].

Change in the plasma levels of B2-microglobulin can be also be detected in kidney disease. When kidney disease is suspected, comparing blood and urine levels of Beta2-microglobulin helps identify where the kidney is damaged. Beta2-microglobulin normally is filtered out of the blood by the kidney's glomeruli, only to be partially reabsorbed back into the blood when it reaches the kidney's tubules [5] [18]. Cystatin C, a novel serum biomarker that rises in the setting of kidney injury, may be a better surrogate marker for assessing kidney function in patients with liver impairment. In comparison to serum creatinine, Cystatin C was less affected by age, gender, and muscle mass. It potentially offers better accuracy in assessing GFR and could be utilized in future studies involving patients with alcohol use disorder [6].

Studies by Mittal *et al.*, [7] reported that over time, high blood sugar levels damage millions of nephrons - tiny filtering units within each kidney. A raised serum uric acid and creatinine levels in diabetics clearly indicate that prolonged hyperglycaemia causes irreversible damage to nephrons of kidney, but the mechanism at which this is achieved is not clear. Studies by Wu *et al.*, [8] revealed that Serum B2 microglobulin levels are also elevated in patients with diabetes due to several factors, such as declining renal function, repeated bouts of ischemia and reperfusion in the legs, and vascular inflammation, but it is still unclear whether B2 microglobulin, cystatin C and creatinine could play in the pathogenesis of diabetic nephropathy. Due to the paucity of information on this area of research, the present study evaluated the level of B2 microglobulin, cystatin C and creatinine in patient with diabetes mellitus.

MATERIALS AND METHODS

Study Area

The study was conducted at the federal medical centre, Owerri, Imo state.

Study Population

Sample size determination

Sample size was determined in accordance to Araoye, (2004).

Approximately 20 individuals were selected from the subject population.

Selection Criteria

A. Inclusion criteria

1. Subjects that signed the informed consent.
2. Type 2 diabetic patients within the age 18 years to 60 years.
3. Confirmed type 2 diabetic patients without nephropathy, retinopathy, neuropathy or other terminal complications.
4. Subjects confirmed suffering from Diabetes mellitus.

B. Exclusion criteria

1. Subjects who did not sign the informed consent
2. Type 2 diabetics below the age of 18 years
3. In all cases, subjects with associated diseases that could alter the results of the analyzed parameters (infections, liver disease, uncontrolled hypothyroidism, nephrotic syndrome, gastrointestinal surgery, and protein-losing enteropathies) were excluded.

Study Design

This cross-sectional study was conducted in the month of August 2018 and all eligible subjects who filled the questionnaire and gave a written informed consent for the study period were sampled. A total of 40 subjects comprising of 20 diabetic patient and 20 healthy subjects were recruited randomly for the study, and their blood sugar level was determined.

Sample Collection

Five milliliters (5 ml) of blood was then collected into the vaccutainer tube with minimal stasis. The tube was properly labeled with the subject's name, sample number and date of collection. The blood was allowed to clot at

room temperature, and serum separated and harvested into a clean dry well labeled sample bottles following centrifugation at 3000 rpm for 5 minutes. The sample was stored in a freezer at -20^{oc}, prior to use.

Biochemical Assay

Blood glucose level was determined using the glucose oxidase method, β2 microglobulin and cystatin c was determined using ELISA techniques while creatinine was determined using Jaffe-slot Alkaline picrate method.

Statistical Analysis

Results were presented in mean ± standard deviation (SD). All data obtained in the study were analyzed using student t-test (spss.20) and Pearson correlation coefficient. The level of significance was set at $p < 0.05$.

RESULTS

Table 1 shows that there was a significant increase (p=0.000) in the mean value of microglobulin in Diabetic patients (2.52±0.22 mg/L) when compared to controls (1.54±0.23 mg/L). The mean value of Cystatin C was significantly increased (p=0.009) in Diabetic patients (1.10±0.87 mg/ml) when compared to Controls (0.79±0.18 mg/ml). The mean value of Creatinine was significantly increased (p=0.023) in Diabetic patients (1.10±0.87 mg/ml) when compared to Controls (0.79±0.18 mg/ml). There was a non significant negative correlation of serum $\beta_2$microglobulinwith serum cystatin C and Creatinine (r= -0.638, p=0.247 and r=-0.115, p=0.853) (Table 2).

Table 1: Microbulin, Cystatin C and Creatinine in Patients with Diabetes Mellitus (Test)

Parameter	Test	Control	P-value
B_2 M (mg/L)	2.52±0.22	1.54±0.23	0.000
Cystatin C (mg/ml)	1.10±0.87	0.79±0.18	0.009
Creatinine (mg/dl)	1.84±0.49	1.16±0.21	0.023

Results are means and standard deviations, $p<0.05$ is statistically significant; $p>0.05$ is not statistically significant

Table 2: Correlation of Serum $\beta_2$microglobulin with Cystatin C and Creatinine in Patients with Diabetes Mellitus

Dependent Variable	N	R	P-value
Cystatin C	20	-0.638	0.247
Creatinine	20	-0.115	0.853

$p<0.05$ is statistically significant; $p>0.05$ is not statistically significant

DISCUSSION

Hospital based studies in various parts of Nigeria have shown diabetes mellitus as the third commonest cause of end stage renal disease following chronic glomerulonephritis and hypertensive nephrosclerosis [11].

The present study revealed that there was a significant increase (p=0.000) in the mean value of β_2 microglobulin in Diabetic patients when compared to controls. The mechanism behind the increase is not clear, but previous studies have stated that microglobulin is elevated in renal dysfunctions and can be used as a marker of renal function. B2M is also part of the major histocompatibility complex (MHC) class 1 complex and an initiator of inflammatory response that triggers the inflammatory process [10]. For example, B2M can induce the expression of cytokines, adhesion molecules, and metalloproteinase. Increased β2m levels in diabetics have been reported by other studies just as other studies have found proportions of diabetic tubulopathy in type 2 diabetes in the range of 55 to 57% using urinary β2m as a measure of tubulopathy [12]. In this study**,** the mean value of Cystatin C was significantly increased in Diabetic patients when compared to Controls. One of the complications of diabetes is nephropathy, and Cystatin C is produced at a constant rate by all nucleated cells. Because of its small size, it is freely filtered by the glomerulus and is not secreted but is fully reabsorbed and broken down by the renal tubules. This means that the primary determinate of blood cystatin C levels is the rate at which it is filtered in the glomerulus, making it an excellent GFR marker and hence can be use to predict the outcome of renal diseases. The result of this study is in agreement with the study carried out by Alebiosu and Ayodele, [13] who reported that Cystatin C is considered to have a useful monitoring role in diabetes with respect to detecting kidney disease progression and evaluating treatment effects.

And lastly, the current study showed that the mean value of Creatinine was significantly increased ($p<0.05$) in Diabetic patients when compared to Controls. Increase in the level of serum creatinine indicates the progression towards diabetic nephropathy, increased serum creatinine levels in diabetics clearly indicate prolonged hyperglycaemia which causes irretrievable damage to nephrons of the kidney [10]. Serum creatinine is filtered by the glomerulus, therefore, serum creatinine level is used as an indirect measure of glomerular filtration. As glomerular filtration rate (GFR) diminishes, there is a rise in plasma concentrations of serum creatinine. Raised serum creatinine and reduced GFR has become fairly reliable indicators of kidney dysfunction. The result of this

study is in agreement with the report by Kamal, [15] but a study carried out by Harita *et al*., [16] hypothesized that, lower serum creatinine is associated with an increased risk of type 2 diabetes, which might reflect a lower volume of skeletal muscle. Various factors such as skeletal muscle size, diet, exercise and treatment regimen may be the reason behind the disparity in result. Serum levels of creatinine can be used as prognostic markers and predictors of renal damage in diabetic patients [17-29]. Effective control of blood sugar levels can stop progression to diabetic nephropathy and thus remarkably reduce the morbidity and mortality associated with this metabolic disease. There was a non-significant negative correlation of serum $\beta_2$microglobulinwith serum cystatin C and Creatinine, which clearly indicates that there is no relationship between $\beta_2$microglobulin, cystatin C and creatinine.

CONCLUSION

Diabetic nephropathy, especially related to type 2 diabetes has become the single most important cause of end stage renal disease worldwide. The increase in the serum levels of β_2Microglobulin, Cystatin C and Creatinine, observed in this study may be useful in early prediction of risk and prognosis in patients with diabetes mellitus. β_2Microglobulin, Cystatin C and Creatinine should be included early in the panel of test for effective monitoring, treatment and management of diabetes mellitus.

CONSENT AND ETHICAL APPROVAL

With a letter of introduction and ethical committee approval of the study, the consents of the volunteer subjects were sort. Each subject signed an informed consent form after which the procedure and implication were explained using a language the subjects would understand.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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