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Urinary N-acetyl-beta-d-glucosaminidase levels in diabetic adults

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Abstract:

Diabetes mellitus (DM) is known to be one of the most common causes of end-stage renal disease. The disease is usually not detected on time, because of the large functioning reserve of the kidney. Currently used markers (serum creatinine, creatinine clearance, urea, and electrolytes) remain relatively normal even when more than 50% of the renal nephron is not functioning. The aim of this study was to determine the level of urinary N-acetyl-beta-d-glucosaminidase (NAG) in diabetic adults in comparison with some currently used markers. A total of 56 diabetic patients between the ages of 23 and 63 were used for this study and 30 nondiabetic between the ages of 18 and 62 were used as control. The diabetic patients were classified into three groups based on how long they have been diagnosed: <2 years (25), 2–5 years (30), and >5 years (25). Spot midstream urine samples were collected into sterile containers, and blood samples were collected into plain tubes. All the analyses were done spectrophotometrically. Creatinine clearance was calculated using the Cockcroft–Gault Equation. There was a significant increase ($P < 0.01$) in NAG values of 2–5 years and above 5 years and control. The urinary microalbumin concentration of controls was significantly different ($P < 0.05$) only with those who have had DM for <2 years. Urinary creatinine concentration of control was significantly higher ($P < 0.05$) than values of all the diabetic groups. There was a significant increase ($P < 0.01$) in creatinine clearance of control group and those who have had DM for <2 years. It is thus concluded that urinary NAG can be used as an early marker in the diagnosis of diabetic nephropathy since urinary NAG increases first before the other markers analyzed in this current study begins to increase.

Key words:

Creatinine clearance, diabetic nephropathy, microalbumin, N-acetyl-beta-d-glucosaminidase

Introduction

Diabetes mellitus (DM) prevalence increases continually around the world; it is one of the major global health problems in the developed and developing countries. Six to seven percent of world's population had been affected by diabetes. It is the most common cause of kidney failure, accounting for nearly 44% of new cases.[1] Diabetes can lead to chronic kidney failure even when it is controlled.[2] Glomerular filtration rate (GFR) is the standard indicator of renal function; normal values for GFR are 130 ± 15 ml/min in males and 120 ± 15 ml/min in females. In both sexes, a GFR of 56 ml/min–100 ml/min constitute mild renal failure.

Serum creatinine concentration is useful in the determination of renal disease. The normal serum creatinine concentration in males is <1.3 mg/dl and <1 mg/dl in females. When the serum creatinine values are within normal range, but different from the person's first value, it is an indication of underlying renal failure. Thus, an increase in serum creatinine from 0.6 to 1.2 mg/dl

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Table 1: Anthropometric data Age, height, weight and BMI of control and diabetics patients. Values are means±SEM

Parameters	Control mean±SEM	< 2yrs Diabetic mean±SEM	2-5 yrs Diabetic mean±SEM	> 5yrs Diabetic mean±SEM	*P*
Age (years)	48±2.2	49.5±2.5	53±2.4	58.5±2.5	*P*>0.05
Height (m)	1.64±0.08	1.58±0.06	1.57±32.61	1.58±0.07	*P*>0.05
Weight (kg)	74.27±13.20	73.44±15.77	78.10±15.15	75.00±10.55	*P*>0.05
Body mass index (BMI)	28.41±5.35	29.75±6.38	31.12±5.78	29.89±4.15	*P*>0.05

P>0.05 – Not Significant

Table 2: Serum; Urinary Creatinine Concentrations and Creatinine Clearance of Control and Diabetics Patients. Means±SEM

Parameters	Control mean±SEM	< 2yrs Diabetic mean±SEM	2-5 yrs Diabetic mean±SEM	> 5yrs Diabetic mean±SEM	*P*
Serum Creatinine (mg/dl)	0.8±0.0.03	0.9±0.0.06	0.8±0.56	0.92±0.73	*P*>0.05
Urine Creatinine (mg/dl)	78.30^{a}±7.04	27.81^{b}±1.36	30.66^{b}±1.39	26.24^{b}±1.6	***P*<0.001
Creatinine Clearance (ml/min)	134.51^{a}±9.3	97.57^{b}±8.18	100.98^{b}±8.61	95.87^{b}±9.42	***P*<0.01

***P*<0.01-Highly Significant, *P*>0.05 – Not Significant

Table 3: Urinary N-acetyl-beta-d-Glucosaminidase (NAG), and urinary micro albumin concentrations of control subjects and classes of diabetics' patients, Means±SEM

Parameters	Control mean±SEM	<2 yrs Diabetic mean±SEM	2-5 yrs Diabetic mean±SEM	>5 yrs Diabetic mean±SEM	*P*
NAG (IU/L)	21.80±1.5	24.84±4.42	30.53^{a}±3.65	38.54^{a}±5.69	***P*<0.01
Urine microalbumin (mg/l)	2.840^{b}±0.24	1.2391^{a}±5.44	3.939^{b}±1.09	5.615^{b}±1.55	**P*<0.05

***P*<0.01-Highly Significant, **P*<0.05- Significant

Table 4: Test of relationship of the DM variables using Pearson's Correlation

Variable (s)	*r* (Correlation Coefficient)	*P*
Serum Creatinine VS Creatinine Clearance	-0.624**	*P*<0.01
Serum Creatinine VS NAG	0.084	*P*>0.05
Creatinine Clearance VS NAG	-0.035	*P*>0.05
Albumin/Cr VS Serum Creatinine	0.328**	*P*<0.01
Albumin/Cr VS NAG	0.080	*P*>0.05
Note: *r* (0.05)(df=84)=0.220, *r* (0.01) (df=84)=0.286		

***P*<0.01-Highly Significant, **P*<0.05- Significant

represents a 50% decline in renal function, which might be missed out if only the recent value is noted since it lies within the normal value.[3]

Routinely used measures of renal function such as levels of blood urea and serum creatinine increase significantly only after substantial kidney injury occurs.[4] Creatinine reflects renal filtering capacity which has a lot of reserves and is therefore not sensitive to acute or chronic kidney injury unless the injury is substantial enough to compromise the filtering ability.

N-acetyl-beta-d-glucosaminidase (NAG) is a lysosomal enzyme originating in renal tubules and its excretion is elevated in various renal disorders. Tubular abnormalities are present early in the course of type 1 diabetes and early increase in urinary excretion of albumin may be at least tubular in origin and may be influenced by glycemic control.[5] An increase in NAG activity in urine is a sensitive test for renal tubular damage since its relative molecular mass (130,000) precludes its filtration by the glomerulus, and it is the most active glycosidase found in the lysosome in the proximal tubule.[6] NAG can thus be used as a marker in the assessment of renal tubular toxicity.

The need for an early marker to aid in the early diagnosis of diabetic nephropathy is of urgent need, as currently used parameters (serum creatinine, proteinuria, and creatinine clearance) in the diagnosis of renal nephropathy do not begin to increase until 50% of renal cells have gone bad, hence the need for a study like this.

The aim of this study is therefore to determine the level of urinary NAG in diabetic adults attending the clinic in University of Benin Teaching Hospital (UBTH) in comparison with urinary microalbumin, serum creatinine, and GFR as an early marker of diabetic nephropathy. Measurement of urinary NAG, microalbumin, serum and urinary creatinine was done. The GFR using clearance of creatinine was also determined.

Experimental Procedure

Patients attending Endocrinology Clinic at the UBTH were used for this study. The controls were staffs of University of Benin. Patients' consent and approval was sort and questionnaire was used to get information on their sex, age, duration of diagnosis, and the type of medication they were taking. Ethical approval was obtained from the UBTH Human Research and Ethics Committee. A total

of 86 known patients with diabetes were used for this study. The diabetic patients were classified into three groups based on how long they have been diagnosed: <2 years (28), 2–5 years (33), and >5 years (25). The age range of the patients was between 20 and 63 years: >60 years (23), 20–40 years (14), and 41–60 years (49). The controls were 30 in number (15 males and 15 females) between the age range of 25 and 65.

Sample Collection

Spot midstream urine samples were collected into clean and sterile universal container, while the blood sample was collected through vena puncture into plain tubes and allowed to clot. Serum was thereafter collected. Serum samples were analyzed for creatinine, while the urine samples were analyzed for microalbumin, creatinine, and NAG. All analyses were performed spectrophotometrically. Creatinine was assayed by two point's kinetic and modified Jafee increasing reaction.[7]

While determination of urine microalbumin was done spectrophotometrically.[8]

NAG was analyzed spectrophotometrically.[9]

Creatinine clearance was then calculated using Cockcroft–Gault equation.

$$\text{Ccr} = \frac{\text{Gender} \times 140 - \text{Age}}{\text{Serum Cr} \times \text{Weight}/72}$$

Statistical analysis of results was performed using ANOVA and regression analysis. The $P < 0.05$ was taken to be statistically significant.

Results

Results are presented as means and standard error of mean in the tables below.

When age, height, weight, and body mass index of control and diabetic patients were compared, there was no significant difference between these parameters.

When the serum creatinine concentration of control was compared with that of diabetic patients, there was no significant difference between them. However, there was a significant difference ($P < 0.001$) between urinary creatinine concentrations of control and diabetic group. There was no significant difference between values of the various classifications of diabetic groups.

For the creatinine clearance, there was a significant difference ($P < 0.01$) between the values of control group and those who have had DM for <2 years, but no significant difference between control and those who have had DM for 2–5 years and >5 years.

For the urine microalbumin concentration, there was a significant difference ($P < 0.05$) between control and those who have had DM for <2 years. No significant difference between control and 2–5 years as well as those above 5 years was observed. When urinary NAG values of control were compared with that of DM, there was a significant difference ($P < 0.01$) between control and DM groups of 2–5 years and >5 years. However, there was no significant difference between NAG values of control and those who have had DM for <2 years.

There was a correlation between creatinine clearance and serum creatinine. This correlation implies that as serum creatinine was increasing, creatinine clearance was reducing. However, there was no correlation between NAG and serum creatinine as well as creatinine clearance. Furthermore, there was no correlation between urinary NAG and urinary albumin [Tables 1-4].

Discussion

For several years, many studies have demonstrated that excreted urinary enzymes are useful biomarkers for evaluation and diagnosis of tubular dysfunction or injury, especially NAG.[6,10,11] In the current findings, NAG values increased in patients who have had DM for 2–5 years and >5 years when compared with the controls. An increase in urinary NAG in early stages of DM even before there was any clinical evidence of renal involvement have been established.[12,13] They concluded that excretion of NAG is helpful in the diagnosis of diabetic nephropathy. In their study, they found that urinary NAG values increased in the early stages of diabetic nephropathy. In our study, urinary NAG concentrations increased significantly even when there was no significant difference in serum creatinine concentrations of control and DM patient. Routinely used measures of renal function such as levels of blood urea and creatinine increase significantly only after substantial kidney injury occurred.[4] Creatinine reflects renal filtering capacity which has a lot of reserves and is therefore not sensitive to acute or chronic kidney injury unless the injury is substantial enough to compromise the filtering ability.

In this study, we also observed a significant difference between urinary microalbumin concentrations of control and DM patients of <2 years but not between those who have had DM for 2–5 years and >5 years. This can be attributed to the fact that patients used in this study are on medications already. The use of some drugs, for example, angiotensin-converting enzyme inhibitors have

been shown to reduce urinary albumin excretion.[14,15] The presence of elevated NAG in all the diabetic patients and elevated albumin in some of the diabetic patients suggests that they have a high risk of developing diabetic nephropathy. Early diabetic glomerulopathy has been detected by electron microscopy in normoalbuminuric patients and found to be more advanced in those with microalbuminuria and proteinuria.[16] Our findings did not show any correlation between urinary microalbumin concentrations and urinary NAG concentrations, this did not agree with findings of Abdel Shakour *et al*.[17] who reported that there was a positive correlation between urinary NAG levels and microalbuminuria.

There was also no correlation between creatinine clearance and urinary NAG in our study, urinary NAG excretion preceded the increase in albumin excretion,[12] what this implies is that even before microalbumin concentrations begin to appear in urine, urinary NAG concentration is already increased. This was reflected in our study as there was no relationship between urinary NAG concentrations and urinary microalbumin concentrations in the diabetic patients. There was also no relationship between urinary NAG concentrations and creatinine clearances as well as serum creatinine concentration, this call for concern as in clinical trials, decrease in creatinine clearance, increase in serum creatinine, and appearance of microalbumin in urine are keys for diagnosis and treatment of diabetic nephropathy. What this simply implies is the need for early marker in the diagnosis of renal impairment. Of all the parameters treated in this study, urinary NAG was seen to have increased across all the classes of DM and was highly significant when compared with control.

Conclusion

Urinary NAG can be used along with other early markers, for example, cystatin C in the early diagnosis of diabetic nephropathy. With the increasing rate of renal problems, especially among the patients with diabetes, there is a serious need for an early marker to aid in early diagnosis of renal impairment, and from our findings, urinary NAG can be used as an early marker in the diagnosis of diabetic nephropathy.

Based on our findings, we thus recommend that emphasis should be placed on researches targeted toward getting early markers to aid in the diagnosis of renal problems in this part of the world, as early diagnosis will go a long way in reducing the increasing incidence of renal problems in developing countries.

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Conflicts of interest
There are no conflicts of interest.

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