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ASSOCIATION OF URINARY N-ACETYL-BETA-GLUCOSAMINIDASE WITH MICROALBUMIN FOR EARLY DETECTION OF NEPHROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Background: The urinary N-acetyl-beta-Glucosaminidase can served as a early detectible marker for nephropathy in patients with type 2 diabetes mellitus. **Materials and Methods:** This cross-sectional study included total ninety (90) subjects, whereas sixty were type 2 diabetes mellitus cases and remaining thirty were healthy controls. For all the subjects biochemical, clinical and experimental parameters are studied. **Results:** The type 2 diabetes mellitus patients showed a significant increased levels of urinary N-acetyl-beta-Glucosaminidase when compared to healthy controls. Among, the cases the type 2 diabetes mellitus patients with microalbuminuria showed a significantly higher levels of urinary N-acetyl-beta-Glucosaminidase than the type diabetes mellitus with normoalbuminuria. The urinary N-acetyl-beta-Glucosaminidase levels are significant positive correlation with microalbumin and negative correlation with eGFR. **Conclusion:** The urinary N-acetyl-beta-Glucosaminidase determination might be used as early predictable marker for type 2 diabetic nephropathy.

Keywords: HbA1c, Type 2 Diabetes Mellitus, Microalbumin, and N-acetyl-beta-Glucosaminidase.

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Introduction

Type 2 diabetes mellitus (T2DM) is a complex disorder characterized by hyperglycemia due to impaired insulin secretion by pancreatic beta cells as well as insulin resistance in the target tissues such as liver and skeletal muscle. This chronic metabolic disorder affects the metabolism of carbohydrates, protein, fat, water, and electrolytes (1). With time hyperglycemia damages the basement cell membrane of the blood vessels causing dysfunction and failure of various organ systems in the body, especially the eyes, kidneys, nerves, heart and blood vessels leading to multi system failure (2).

Diabetic Nephropathy (DN) occurs as a result of functional and structural changes in the glomerulus, such as glomerular hyper filtration, thickening of glomerular basement membrane and an expansion of extra cellular matrix in mesangial areas (3). It has recently been recognized that proximal tubular cell atrophy and tubulointerstitial fibrosis are also important than glomerulosclerosis in terms of renal prognosis (4). Need biomarkers require for to predict early onset of nephropathy than the microalbumin.

N-acetyl-beta-Glucosaminidase (NAG) is an enzyme eliminated into the urine in higher quantities preceding the appearance of microalbuminuria and is considered as a sensitive tubular biomarker. NAG is a high-molecular weight enzyme found in the lysosomes of the proximal tubule epithelial cells and cannot pass through the glomerular filtration normally and hence high NAG activity in urine might indicate an early sign of renal disorder (5-6). NAG is reported as predominant biomarker of proximal tubular damage, but may also be considered as biomarker of injury to other parts of the nephron (7). A recent study found that urinary NAG levels were significantly higher in type 2 diabetic patients with normo, micro- and macro albuminuria than in non-diabetic controls, and its levels were increased in parallel with the severity of renal involvement (8). Another showed that urinary NAG levels were significantly increased in microalbuminuria group compared to normoalbuminuria group and correlated positively with ACR, leading to the conclusion that urinary NAG can be considered complementary marker for early detection of DN in type 2 diabetes (9). Hence, the present study aimed to evaluate the association of urinary podocalyxin with microalbumin in patients with type 2 diabetes mellitus.

Materials and Methods

A case-control prospective observational study was undertaken to study urinary markers in early detection of diabetic nephropathy in patients with type 2 diabetes mellitus. A total of ninety (90) subjects, thirty (30) healthy controls and sixty (60) patients who were diagnosed with T2DM as per American Diabetes Association (ADA) criteria (10) during the period of March 2022 to December 2023 and attending Endocrinology& metabolism and Nephrology OPD at Akash Institute of Medical Sciences, Bangalore were included into the study. Thirty healthy controls were included in Group 1. The patients were divided into two groups of thirty each based on the albuminuria levels as type 2 diabetes mellitus patients with normoalbuminuria (Group2) and type 2 diabetes mellitus patients with microalbuminuria (Group3). A questionnaire was administered to all the study subjects to collect details of their age, physical data, ethnic origin, dietary habits, duration of diabetes and treatment history. Informed consent of the subjects was taken after explaining the study in their vernacular

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language. The study was conducted after obtaining approvals by the Institutional thesis protocol approval committee and Institutional ethics committee.

Criteria of the study

Inclusion Criteria: Type 2 diabetes mellitus patients with normoalbuminuria (UACR < 30 mg/g creatinine) for Group 2 and type 2 diabetes mellitus patients with microalbuminuria (UACR 30-300 mg/g creatinine) for Group 3 according to kidney disease improvement global outcomes (KDIGO) (11) criteria.

Exclusion Criteria: Subjects with history of other forms of diabetes mellitus, hypertension, liver disease, thyroid disorders, smoking, alcoholism, chronic kidney disease, pregnancy and lactation.

Sample collection: Five (5) milliliters of fasting blood sample were collected from each study subject and also 3 milliliters of post prandial blood sample were collected. The blood samples were transferred into properly labelled vacutainers and plasma and serum were separated from the blood samples by centrifugation at 3000 rpm for 10 minutes and stored until biochemical analysis was done. Along with the blood sample, a spot urine sample was also collected. Urinary albumin and creatinine were immediately analyzed after the urine sample was centrifuged at 3000 rpm for 10 minutes. Later, 1 mL of urine transferred appropriate label aliquots.

The fasting blood sugar (FBS), post prandial blood sugar (PPBS), serum urea, serum creatinine, and microalbumin was measured by using laboratory standard methods. The eGFR was calculated by modified diet in renal diseases (MDRD) formula. The urinary N-acetyl-beta-Glucosaminidase was measured by using enzyme linked immunosorbent assay.

Statistical analysis

The data was expressed as mean and standard deviation (SD). Analysis of Variance (ANOVA) was used to assess differences between the three groups under investigation, and it was followed by post hoc multiple testing using Tamhane's or Bonferroni's tests, if necessary. The correlations between the markers were examined using Pearson's correlation analysis. The Microsoft Excel and SPSS were used for the statistical analysis and P value is <0.05 was considered statistically significant.

Results

Table 1: Comparison of study variables in between cases and controls

Parameter	**Controls**			**Cases**			**P-Value**
	Mean ± SD			**Mean ± SD**			
Age	47.86	±	6.34	51.64	±	4.66	0.21
FBS	87.21	±	5.21	167.23	±	27.25	0.001**
PPBS	114.68	±	8.37	197.86	±	13.10	0.001**
HbA1c	4.7	±	0.27	9.5	±	2.39	0.001**
Microalbumin	10.3	±	1.78	69.7	±	5.96	0.001**
eGFR	96.21	±	7.49	65.14	±	7.51	0.001**
N-acetyl-beta-Glucosaminidase	6.25	±	1.23	23.58	±	3.16	0.001**

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The mean and standard deviation of FBS, PPBS, HbA1c, microalbumin, and eGFR, significantly elevated in T2DM cases when compared to healthy controls (P=0.001**). The mean levels of age not showed significant between T2DM patients when compared to controls (P=0.21). The urinary N-acetyl-beta-Glucosaminidase levels significantly elevated in patients with T2DM when compared to controls (P=0.001**).

Table 2: Comparison of study variables between the groups

Parameter	**Controls**			**T2DM Normoalbuminuria**			**T2DM Microalbuminuria**			**P-Value**
	Mean ± SD			**Mean ± SD**			**Mean ± SD**			
Age	47.86	±	6.3	50.12	±	5.5	55.67	±	4.1	0.03*
FBS	87.21	±	5.2	148.13	±	19.8	165.77	±	13.6	0.001**
PPBS	114.68	±	8.3	171.23	±	10.3	196.54	±	12.6	0.001**
HbA1c	4.7	±	0.9	7.2	±	1.9	10.3	±	2.2	0.001**
Microalbumin	10.3	±	1.7	15.4	±	4.9	72.36	±	6.7	0.001**
eGFR	96.21	±	7.4	93.55	±	5.4	59.34	±	13.5	0.001**
N-acetyl-beta-Glucosaminidase	6.25	±	1.2	13.41	±	3.4	19.66	±	2.7	0.001**

Table 2 illustrates the comparison of biochemical, clinical and experimental parameters between the groups. The mean levels of age showed significant between T2DM with micro and normoalbuminuria when compared to controls (P=0.03*). The mean and standard deviation of FBS, PPBS, HbA1c, microalbumin, and eGFR, significantly elevated in T2DM with micro and normoalbuminuria when compared to healthy controls (P=0.001**). The mean and standard deviation of microalbumin, and eGFR, significantly elevated in T2DM with microalbuminuria when compared to T2DM with normoalbuminuria and healthy controls (P=0.001**). The urinary N-acetyl-beta-Glucosaminidase levels significant progressively elevated in T2DM with micro and normoalbuminuria when compared to controls (P=0.001**).

Table 3: Correlation of urinary podocalyxin with clinical markers

Parameters	**Urinary N-acetyl-beta-Glucosaminidase**	
	R	**P**
FBS	0.369	0.001**
HbA1c	0.572	0.001**
Microalbumin	0.366	0.001**
eGFR	-0.657	0.001**

Table 3 illustrates that the pearson's correlation analysis of urinary N-acetyl-beta-Glucosaminidase with clinical markers. There was a significant positive correlation between urinary N-acetyl-beta-Glucosaminidase and FBS, PPBS, and microalbumin, respectively the P value is 0.001**. The urinary N-acetyl-beta-Glucosaminidase was negatively correlated with eGFR, respectively the P value is less than 0.001**.

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Discussion

Diabetic nephropathy is one of the leading causes of ESRD and is becoming more prevalent globally. The current gold standard test for early detection of diabetic nephropathy is persistent microalbuminuria (30-300 mg/day) that is confirmed on at least 2 occasions 3-6 months apart (12). But it has limitations such as low sensitivity, its plasma concentration is affected by several factors and it also has high inter individual variation which is 47% (13). The identification of novel biomarkers for the early detection of DN and progression towards ESRD is thus necessary to reduce the burden of chronic kidney disease in T2DM.

In the present study, microalbumin in the controls, T2DM patients with normoalbuminuria and T2DM patients with microalbuminuria were showing a statistically significant increase across the groups ($p < 0.001$). The eGFR levels showed a decrease across the three groups which was found to be statistically significant ($p < 0.001$) and this levels also differed between the various groups studied ($p < 0.001$) except between Type 2 diabetes mellitus patients with normoalbuminuria and control group. eGFR estimation is still largely creatinine based and thus cannot be considered as

an early marker of renal dysfunction. Hence there is a need for identification of novel biomarker for early diagnosis of DN. Accordingly the present study estimated N-acetyl-beta-Glucosaminidase in in urine of healthy controls as well as T2DM patients with normo- and microalbuminuria.

In the present study, urinary NAG levels were found be higher in patients with T2DM with normoalbuminuria as well as microalbuminuria ($p < 0.001$) when compared to controls. Also, the increase in microalbuminuria diabetic patients was more when compared to the normoalbuminuric counterparts. As NAG is basically a tubular injury marker, increase in urinary NAG along with a decrease in eGFR implies glomerulo-tubular damage in diabetic nephropathy as evidenced in the present study (14). U. NAG was found to be increased progressively with the degree of albuminuria. Among those with diabetes higher levels of U.NAG/Cre were observed in patients with microalbuminuria, which was significantly different from those in the normoalbuminuria group. It was reported that in T2 DM, urinary NAG excretion increases proportionally to the duration of diabetes and earlier to albumin thus becoming early tubular biomarker (15). This finding is supported by Assal *et al.* who reported that urinary NAG is the most sensitive biomarker for detecting early damage in diabetic patients (16). Another cross-sectional study also observed that urinary NAG has clinical significance as an early biomarker of DN (17). Thus, majority of reports point to the likelihood of urinary NAG getting into clinical use as an early marker for identifying the presence of DN.

Conclusion

Based on the study findings, the present study concludes significant elevation of N-acetyl-beta-Glucosaminidase in type 2 diabetes mellitus might be used as a early predictable marker for nephropathy than microalbumin.

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