Original Article

Urinary N-Acetyl-β-D-Glucosaminidase Excretion in Type – II Diabetes Mellitus

Noor-ul-Ain Waheed[1], Sumair Anwar[2], Uzma Jabbar[3]

Abstract

Renal complications develop in about 40% of all type II diabetic patients, characterized by persistent albuminuria, leading towards end stage renal disease. N-acetyl-β-D-Glucosaminidase (NAG) a large protein is released in the tubular lumen after proximal tubular damage thus considered as a tubular injury marker. Its raised levels were observed even in normoalbuminurics as the onset of microalbuminuria was considered a late finding. With the ***objective*** of comparing urinary NAG between normoalbuminuric and microalbuminuric type II Diabetes Mellitus patients, this study was planned.

Methods: This comparative cross-sectional study was conducted from January to June 2014 at Shaikh Zayed Hospital Lahore on 86 type-II diabetic patients. Out of known type-II diabetic patients from both genders coming to diabetic clinic for routine checkup, those having duration of diabetes for less than 10 years since its diagnosis made and aged 45 – 65 years, were selected through convenient sampling except those with any co-morbidity involving kidneys, pregnancy or history of nephrotoxic drug intake. After taking consent, their spot urine samples were collected. Those having urinary ACR (albumin creatinine ratio in mg/g creatinine) > 300 on dipstick termed 'macroalbuminuria' were also excluded. The albumin, creatinine and NAG assays were performed in the selected spot urine samples. On the basis of urinary ACR, patients were divided into normoalbuminurics (ACR < 30) as group 1 (n = 42) and microalbuminuric (ACR 30 – 300) as group 2 (n = 44). Urinary NAG level was measured in U/L and also expressed as NAG to creatinine ratio (U/gram creatinine). Data were analyzed through SPSS (v: 21.0) and described as tables and graphs for age, gender, duration of diabetes and urinary NAG. Five point pictorial comparison of NAG to creatinine ratio in both groups was presented through box-plot.

Results: Out of 86 subjects, mean age was 50.79 years in group 1 while it was 51.89 years in group 2. Majority subjects presented with disease history of 5 – 8 years, with male predominance (64% n = 55). Mean urinary NAG level was 18.69 U/L in group 1 and 42.36 U/L in group 2 and difference was significant (p = 0.01). Similarly NAG to creatinine ratio values revealed small sized box-plot in group 1 with lower minimum, maximum, median and interquartile range values as compared to group 2.

Conclusion: Among uncomplicated type-II diabetic patients in the first decade of their illness, the excretion of the urinary enzyme, 'N-acetyl-β-D-Glucosaminidase' was observed. Its levels were lower in normoalbuminurics with less variation as compared to microalbuminurics.

Key Words: Renal Tubular Enzyme, N-acetyl-β-D-glucosaminidase**,** Type II Diabetes Mellitus.

[1]Assistant Professor, Department of Biochemistry, King Edward Medical University, Lahore
[2]Associate Professor, Department of Community Medicine, Shalamar Medical & Dental College, Lahore
[3]Assistant Professor, Department of Biochemistry, Shalamar Medical & Dental College, Lahore

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Corresponding Author: Dr. Noor-ul-Ain Waheed, MBBS, M. Phil
Assistant Professor, Department of Biochemistry
King Edward Medical University, Lahore

Introduction

Diabetes mellitus (DM) is one of the most challenging health issues of this century.[1] Renal complications in DM develops in about 40% of all type II diabetic patients, characterized by persistent albuminuria, increased blood pressure and gradual decline in kidney function that leads towards end stage renal disease (ESRD).[2] By the end of 2013, there were 661,648 prevalent cases of ESRD and the prevalence was 2,034 per million in the US population.[3] Renal function impairment occurs in both type I and II DM. Creatinine excretion shows renal filtering ability, as it has a lot of reserve so it is not sensitive to acute or chronic kidney injury until it is sufficient enough to compromise the filtering capacity.[4] Renal tubulointerstitium plays major role in the development and progression of kidney disease in diabetes mellitus.[5]The start of microalbuminuria may be considered as a late finding in type II diabetes patients.[6] N-acetyl-β-D-Glucosaminidase (NAG) a large protein, is the most active of lysosomal exoglycosidases[7]with large size (130-150 kDa)cannot be filtered by glomerulus and it is released in the tubular lumen after damage of proximal tubule.[8]Urinary excretion of NAG is constant with minimal diurnal changes and it also remains stable against changes in temperature and pH.[9] Due to this it is considered as a tubular injury marker. Urinary NAG activity is increased in DM, renal lithiasis, tubulointerstitial nephropathies, pregnancy, drug induced nephrotoxicity, and renal allograft rejection.[10] Even in patients with normoalbuminuria level of urinary NAG was reported higher than healthy control subjects.[11] Renal complications in diabetes are affected by duration of DM and these renal changes tend to start after 5 to 10 years duration of illness.[12] Current study was conducted to find out NAG levels in urine of microalbuminuric and normoalbuminuric patients of DM in 1st decade of their illness. It will be helpful in detecting renal injury at earlier stage in such diabetic patients and indirectly in reducing morbidity and mortality.

The objective was to compare the urinary levels of N-acetyl-β-D-Glucosaminidase between normoalbuminuric and microalbuminuric type II diabetic patients.

Patients and Methods

In this comparative cross-sectional study carried out in the Biochemistry & Chemical Pathology Department of Shaikh Zayed Hospital, Lahore from January to June 2014, after approval of institutional review board. Out of known type II diabetic patients coming to diabetic clinic of Shaikh Zayed Hospital, Lahore for routine check-up, 86 study subjects were selected through non-probability convenient sampling. This sample size was calculated at 5% level of significance, keeping power of test at 80% and estimated diabetes cases having increased NAG level, with 34% showing normoalbuminuria and 63.7% showing microalbuminuria on urinalysis.[13] Patients aged 45 to 65 years from both genders with less than 10 years duration of type II diabetes mellitus since its diagnosis made were included in the study except those with any co-morbidity (acute and chronic kidney injury, renal transplant, hypertension, infections of urinary tract, any systemic disease, malignancy) or Pregnancy or with drug intake history (Angiotensin Converting Enzyme inhibitors or angiotensin receptor blocker and other nephrotoxic drugs i.e. cyclosporine, aminoglycosides, antibiotics, cisplatin, amphotericin B, beta-lactam antibiotics and indomethacin). Those having urinary ACR (albumin creatinine ratio in mg/g creatinine) >300 on dipstick termed 'macroalbuminuria' were also excluded from the study. After taking consent, the spot urine samples were collected in aseptic plastic containers from selected subjects. The biochemical tests performed in urine samples were albumin and creatinine quantitative estimation. They were divided into two groups (based upon albumin to creatinine ratio[14]) i.e. normoalbuminurics (ACR < 30) as group 1 (n = 42) and microalbuminuric (ACR 30 – 300) as group 2 (n = 44). NAG assay was performed on all selected urine samples. The method for determination of microalbuminuria[15] was based on an immunoassay (particle enhanced turbidimetric inhibition) that allowed direct quantification of urinary albumin in samples. Creatinine was estimated by Jaffe's kinetic method.[16] Utilizing ELISA plate washer (PW-40 model of BIORAD, USA) and ELISA absorbance reader (Multiskan EX, Germany) NAG assay[17] was performed. ELISA kit was provided for Human NAG estimation by Glory Science Co. Ltd., USA. Urinary NAG level was determined in U/L and also expressed as NAG to creatinine ratio (U/gram creatinine).The values of the above variables and assays were then entered in pre-tested Performa. Statistical analysis was done by SPSS version 21.0 and MS Excel. Distribution of patients was described separately on sex and age basis for each group. The data for age, duration of diabetes and urinary NAG were compared after finding minimum and maximum values, their mean with ± standard deviation (sd). The frequ-

ency of data in the groups was pictorially displayed through bar graphs for selected variables. The statistical difference was also found out through applying t-test where required. Five point pictorial comparison of NAG to creatinine ratio in both groups was presented through box-plot.

Results

In group 1 mean (± sd) age of study subjects was 50.79 (± 4.12) years with minimum age of 45 to maximum age of 60 years while it was 51.89 (± 5.39) years in group 2, with similar minimum age of 45 to maximum age of 64 years. Additionally more cases in age of 50 and 55 years were recorded in both groups (Figure 1).

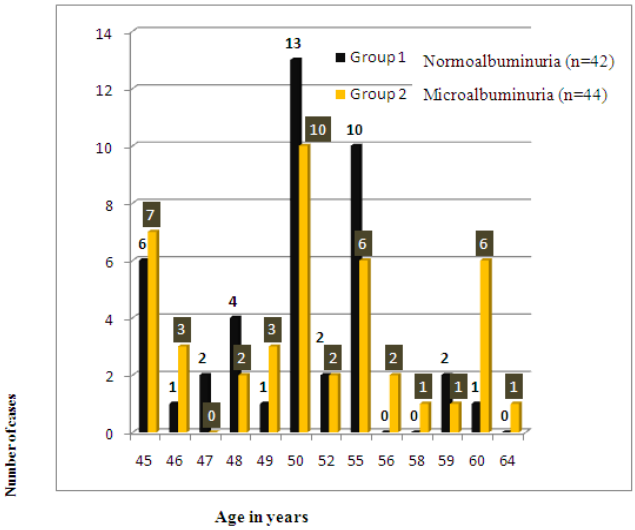

Figure 1: Group-wise comparison of frequency of cases according to their age

Figure 2: Group-wise comparison of frequency of cases according to Gender

Male cases (64% n = 55) were observed more in both groups. In the group 1, male subjects were 66.66% (n = 28) and in the group 2 they were 61.36% (n = 27) (Figure 2). This difference was not statistically significant (*p* value = 0.292).

Duration of diabetes mellitus considered in selection criteria was recorded as 3 to 9 years in both groups. Out of this recorded range, maximum cases had 5

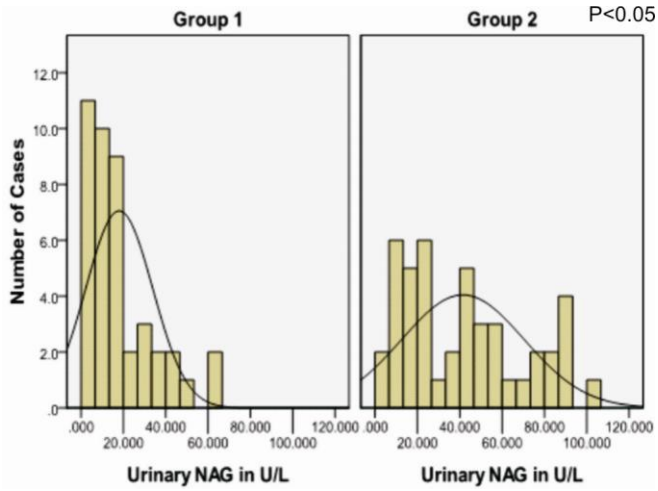

Figure 3: *Comparison of Distribution of Urinary NAG in Both Groups.*

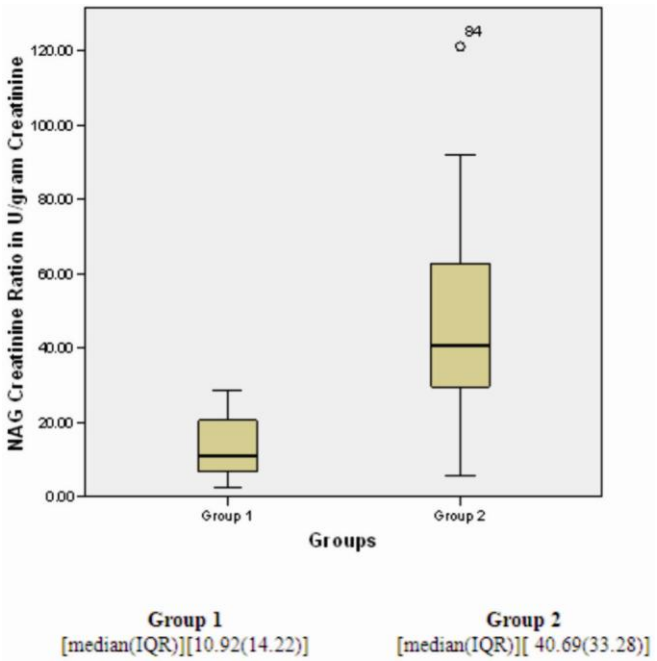

Figure 4: *Comparison of Box-Plots of Urinary NAG to Creatinine Ratio in Both Groups.*

to 8 years of duration in both groups. In group 1, mean (±SD) duration of diabetes mellitus was 6.81 (± 1.58) years while it was 6.52 (± 1.61) years observed in group 2. This difference was also not statistically significant in both groups (*p* value = 0.406).

Mean (± sd) of urinary NAG level was 18.69 (± 25.14) U/L in group 1 as compared to 42.36 (± 41.25) U/L in group 2. Cases with lesser NAG values were more in group 1, making distribution to be negatively skewed. Moreover, the increased NAG level in group 2 as compared to group 1 was statistically significant (p = 0.01) (Figure 3).

On graphic comparison of NAG to creatinine ratio values through box-plot, more variability or dispersion was observed in group 2 with higher median (40.69 U/gram creatinine) and interquartile range (33.28 U/gram creatinine) values as compared to group 1 (Figure 4).

Discussion

The type II diabetic patients of age 45 – 65 years were included in our study which was similar to a study done by Vlatkovic et al.[18]

Out of total cases in both groups, maximum number of study subjects (n = 13) was with 50 years age and (n = 10) with 55 years of age. Moreover, cases of these age groups were more in group 1 than in group 2. But no statistically significant difference was noted. This showed no confounding role of age between these groups. Such findings were reported in an Indian study[14] where mean age of study subjects in both groups was observed 54 years.

In our study male cases were more in number (64%, n = 55) than female cases in both groups (66.6% cases were male in group 1 and they were 61.3% in group 2). This finding was similar to another research work done in Japan[19] in which 56.1% type II diabetic study cases were male, and they were observed in a cohort study for 5 years to identify renal changes through determination of their urinary NAG levels.

Those type II diabetic patients were included in this study whose duration of disease was less than 10 years and in both groups majority cases were with 5 to 8 years duration of type II diabetes. However, there was no statistically significant difference. The cases of such duration were included in this study with the purpose to identify early renal changes before onset of microalbuminuria in type II Diabetes Mellitus. This was according to observation of the researchers[12] who reported that renal changes mostly occur after 5 to 10 years of diabetes mellitus history. In group 1 mean (± sd) duration of diabetes mellitus was 6.81 (± 1.58) years whereas it was6.52 (±1.61) years in group 2.This finding was almost same as that of work done by some researchers in 2013,[5] in which they assessed urinary NAG levels of type II diabetic patients grouped into normoalbuminurics, microalbuminurics and macroalbuminurics to evaluate the enzyme as screening marker for early identification of renal complications in diabetes. They found average duration of diabetes mellitus as 5.7 years, 8.7 years and 10.2 years respectively in the above mentioned groups. In our study urinary NAG level in group 1 was lower (p < 0.01) significantly than in group 2.Urinary NAG level was also recorded in the form of ratio with creatinine level as NAG to creatinine ratio. This ratio decreased the variation produced in excretion of enzyme related to time or volume. Similar findings related to both urinary NAG levels and urinary NAG to creatinine ratio, were observed by other researchers.[11,14,18]

Conclusion

Among uncomplicated type-II diabetic patients in the first decade of their illness, the excretion of the urinary enzyme, 'N-acetyl-β-D-Glucosaminidase' was observed. Its levels were lower in normoalbuminurics with less variation as compared to microalbuminurics. Excretion of this enzyme in normoalbuminuric study subjects may indicate the onset of renal complications before microalbuminuria in type-II diabetes mellitus.

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