Research Article

Correlation of Urinary N-Acetyl-beta-D-Glucosaminidase with Albuminuria in Type-II Diabetic Patients

[*]Noor-ul-Ain Waheed[1], Riffat Yasmin[2], Sumair Anwar[3]

[1]Assistant Professor, Department of Biochemistry, King Edward Medical University, Lahore; [2]Ex-Professor of Biochemistry, Head of Biochemistry & Chemical Pathology Department SZPGMI Lahore, Currently Professor of Biochemistry, Rehbar Medical and Dental College Lahore Pakistan; [3] Professor and Head of Community Medicine Department, Gujranwala Medical College, Gujranwala, Pakistan*

Abstract | Background: Urinary N-acetyl-β-D-Glucosaminidase (NAG) level, if raised shows tubular damage in type-II diabetics. Its positive correlation with albumin creatinine ratio (ACR) at microalbuminuria stage of nephropathy, suggests role as early screening marker. With these objectives, we determined correlation of urinary NAG activity with albuminuria in both normoalbuminuric & microalbuminuric type-II diabetics and sensitivity of this biomarker as a screening test.

Methods: In this cross-sectional analytical study of 6 months on 86 type-II diabetics, subjects with less than 10 years duration of diabetes were included and those with macroalbuminuria, other kidney disease, and co-morbidity were excluded. Study subjects were grouped into normoalbuminuria (group 1) & microalbuminuria (group 2). Correlation of urinary NAG to creatinine ratio (NCR) with ACR and their sensitivity were determined.

Results: Out of all, group 1 and group 2 included 42 and 44 subjects with male to female ratio 2:1 & 1.6:1 respectively. We found lower mean levels of both urinary NCR and ACR in group 1 than in group 2 (P <0.00). In both groups they were positively correlated (r=0.65 in group 1 & r=0.53 in group 2). 95% of subjects with nephropathy were identified by NCR while only 51% by ACR.

Conclusion: Levels of urinary NAG were more positively correlated with urinary albumin in normoalbuminuric than microalbuminuric type-II diabetics. It was more sensitive to detect nephropathy than microalbuminuria level when used as a screening test.

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[*]Corresponding Author | Dr. Noor-ul-Ain Waheed, Assistant Professor, Department of Biochemistry, King Edward Medical University, Lahore **Email:** noorwaheed80@gmail.com

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Keywords | N-acetyl-β-D-Glucosaminidase,Type-II diabetics, Nephropathy, ACR

Introduction

Diabetic nephropathy is one of the most common complications of Diabetes Mellitus, that affects the quality of life as well as survival of these patients.[1] 20% of the type 2 diabetic nephropathy patients developed end stage renal disease when observed for five years in a study.[(2)] Pathological albuminuria constitutes the consequence of diabetes induced glomerular damage.[(3)] As microalbuminuria is thought to be a biomarker of glomerular injuryso currently it is the gold standard in the diagnosis of diabetic kidney disease and the evaluation of its progression.[(4)] The occurrence of microalbuminuria is almost one fourth in type 2 diabetes mellitus patients that includes recently as well as already diagnosed cases.[(5)] If detection of renal injury is possible early then it results in early management which has better

consequences.[6] With the development of newer technologies, to study protein excretion in urine in more detail is now possible, that facilitates detection of disease early.[7] Early renal proximal tubular damage is followed by glomerular permeability in Diabetes that become possible due to assay of site-specific biochemical markers in urine.[8]The increase activity of urinary N-acetyl-beta-D-glucosaminidase (NAG) at the microalbuminuria stage of diabetic nephro-pathy (DN) suggests that tubular dysfunction is already present in this period.[9] Urinary NAG is strongly positive correlated with ACR ($r=0.74$, $p<0.001$), and therefore it can be used as early screening marker of renal disease in diabetes mellitus.[10] NAG has both diagnostic value in the early detection of diabetic nephropathy and role in monitoring the advancement of nephropathy.[11] In a study urinary NAG activity more than 4.0 U/g creatinine was considered abnormal.[12] Using this cut off value, another researcher classified the study subjects with normoalbuminuria into 2 groups i.e. normal (up to 4.0 U/g creatinine) vs. increased (>4.0 U/g creatinine).[13] Therefore, instead of using urinary albumin alone, if combined with biomarker for tubular damage, may prove to be a useful method for early identification of diabetic nephropathy.[14]

Our study was aimed to find out association of N-acetyl-β-D-Glucosaminidase activity in urine with albumin in urine through determining correlation coefficient in both normoalbuminuric & micro-albuminuric type-II diabetics and also to determine the sensitivity of this biomarker if used as a screening test.

Materials and Methods

In this cross-sectional analytical study which was carried out for six months in 2014, 86 already diagnosed type-II diabetic patients reporting at Shaikh Zayed Hospital Lahore were included, estimated at 5% level of significance, keeping power of test to 80% with expected cases having raised NCR of 34% & 63.7% among normoalbuminurics and microalbuminurics subjects respectively.[15] The subjects included in our study were of both genders, 45-65 years of age, having less than 10 years duration of diabetes selected through non-probability sampling. They were thoroughly examined by diabetologist to exclude the subjects with any other kidney disease & co-morbidity on the basis of already available medical records. Spot urine samples of study subjects were collected in aseptic disposable containers during their short stay at OPD. All those type 2 diabetic patients, whose spot urine samples gave positive result with dipstick, were excluded. Rest of them who fulfilled the inclusion criteria was included in the study. Informed consent was taken from every subject included in the study. It was developed for them and translated in Urdu to understand the detail of this research study. Urinary measurements of biochemical parameters including albumin, creatinine & NAG were done by immuno-turbidimetric method, Jaffe's method employing spectrophotometer and Enzyme-linked Immuno-sorbent assay (ELISA) test respectively. In both groups, albumin creatinine ratio (ACR) in mg / g creatinine and NAG to creatinine ratio (NCR) in U/g creatinine were calculated.[16] In order to nullify the disparity of concentration of albumin in spot urine samples, we calculated the creatinine in these samples beside albumin concentration. NCR cuts the effect of variation in enzyme excretion regarding volume or time.[17] Study subjects were divided into 2 groups based on albuminuria (42 normoalbuminurics as group 1 (ACR < 30mg /g creatinine) and 44 microalbuminurics as group 2 (ACR 30-299 mg /g creatinine).The values of the above mentioned parameters were then recorded in pre-designed proforma. This was done by SPSS version 22.0 and MS Excel. Distribution of subjects was described on the basis of duration of diabetes in each group through bar chart. The levels of urinary NCR & ACR were compared in both groups with their means & standard deviation of both groups for both genders. Difference was also analyzed statistically after applying t-test of significance. The median (interquartile range) was also given as data was not normally distributed. Finally correlation of these 2 ratios with each other was given in both groups separately, using spearman rank-order correlation coefficient and displayed graphically through scatter plots. Age and duration of diabetes being possible confounders were also controlled through application of partial correlation. We compared sensitivity of NCR (using 4.0 U/g creatinine as cut-off) with ACR (using 30.0 mg/g creatinine as cut-off) for diagnosis of diabetic nephropathy. This research project was approved by institutional review board of Sheikh Zayed Medical

Complex (Letter No. F-39/NHRC/Admn/IRB/36)for ethical aspects and concerns.

Results

The age range of 86 subjects (42 in group 1 and 44 in group 2) was 15 years in group 1 and 19 years in group 2. Male to female ratio of subjects was 2:1 in group 1 and 1.6:1 in group 2 (Table I).In figure I, bar chart shows comparison of distribution of study subjects in both groups on the basis of duration (in years) of type-II DM. Maximum no. of cases had duration of 8 years in group 1 (n=13) whereas in group 2 majority (n=11) subjects have 7 years duration of diabetes. The biochemical parameters were compared in group 1 and group 2 in this study on 86 type-II diabetics. On comparing the mean (± sd) values of urinary ACR with NCR in both groups and in both genders (Table I) we found lower levels of both urinary ACR and NCR in group 1 as compared to group 2 (P <0.00) (Table I).The levels of urinary NCR in both groups and in both genders were showing positive skewness(Table I).In group 1, mean (± sd) level 13.42 (± 8.05) U/L was more than median (IQR) level 10.92 (14.22) U/L. Similarly in group 2, mean (±sd) level 44.92(±23.75) U/L was more than median (IQR) which was 40.69(33.28) U/L (Table I). Correlation between urinary NCR and urinary ACR in both groups separately using spearman rank-order correlation coefficient was found positive (Table II) and this was also pictorially displayed through scatter plots (Figure II). In group 1 correlation was moderately strong positive (r=0.65) while in group 2 cases, moderately positive correlation (r=0.53) was observed (Table II). After controlling the two confounding variables i.e. age & duration of DM (on partial correlation), again correlation was observed to be moderately positive both in group 1 (r=0.573) and in group 2(r=0.408).On comparing the sensitivity of ACR with NCR, we determined that ACR was able to ascertain 51% of type-II diabetics with nephropathy while NCR was able to find 95% of type-II diabetics with nephropathy (Figure III& Table III).

Table 1: *Distribution of Urinary ACR & NCR in Both Groups & Both Genders Separately*

Measured Variables	Group 1		Group 2	
	Male N=28	Female N=14	Male N=27	Female N=17
	Mean (± sd)	Mean (± sd)	Mean (± sd)	Mean (± sd)
Urinary Albumin to Creatinine Ratio mg/g creatinine	13.72 ± 9.58*	11.02 ± 9.4*	138.72 ± 86.9*	96.62 ± 67.1*
Urinary NAG to Creatinine Ratio U/g Creatinine	12.87 ± 8.2*	14.51± 7.9*	46.47 ± 26.97*	42.45 ± 17.9*

*P<0.000

Table 2: *Correlations (Spearman's RHO) Between Urinary ACR & NCR in Both Groups*

		Group 1
		NAG to Creatinine Ratio U/g creatinine
Urinary Albumin to Creatinine Ratio mg/g creatinine	Correlation Coefficient	**0.656****
	Sig. (2-tailed)	.000
	N	42
	Group 2	
		NAG to Creatinine Ratio U/g creatinine
Urinary Albumin to Creatinine Ratio mg/g creatinine	Correlation Coefficient	**0.531****
	Sig. (2-tailed)	.000
	N	44

**Correlation is significant at the 0.01 level (2-tailed)

Table 3: *Comparison of Sensitivity of Urinary ACR (A Well-Established) with NCR (As New) Biomarker of Diabetic Nephropathy in Type-II Diabetes Mellitus (n=86)*

Biomarkers		Albumin to Creatinine Ratio (ACR)		Urinary NAG to Creatinine Ratio	
Result		mg/g creatinine	n	U/g creatinine	n
	Positive	≥ 30 to < 300	44	> 4	82
	Negative	<30	42	<4	4
		Total	86	Total	86
Sensitivity		**51.16%**		**95.34%**	

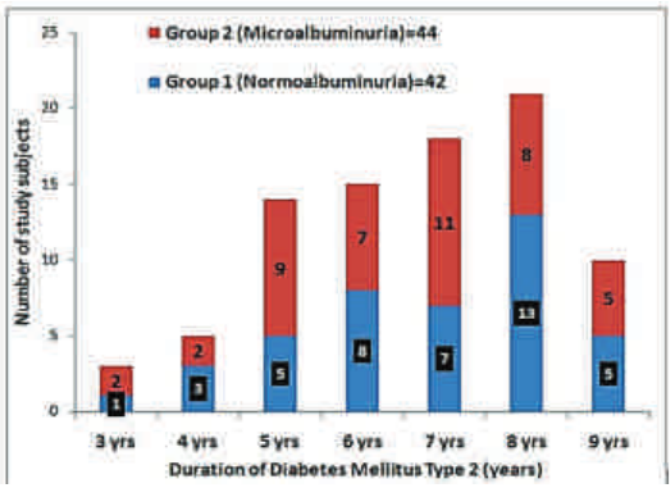

Figure 1: *Comparison of Distribution of Study Subjects in Both Groups on the Basis of Duration (Years) of Type-II Diabetes Mellitus*

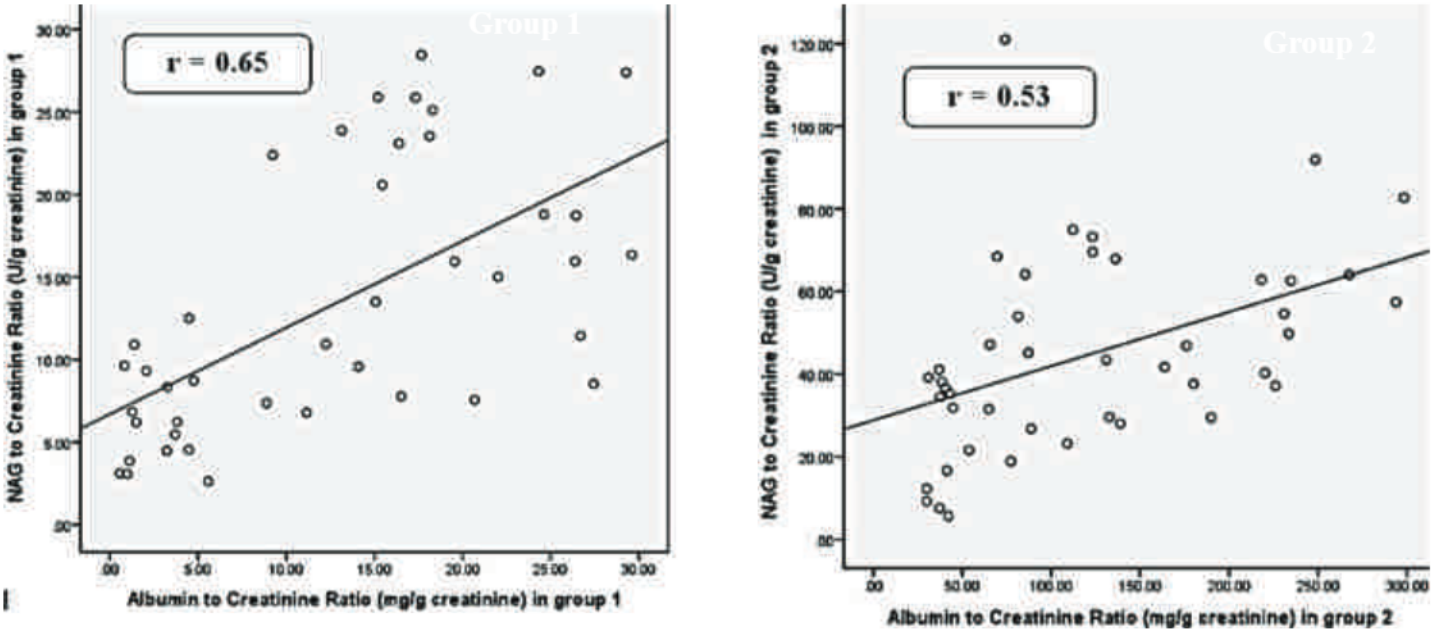

Figure 2: *Comparison of Association Between Urinary ACR & NCR in Both Groups*

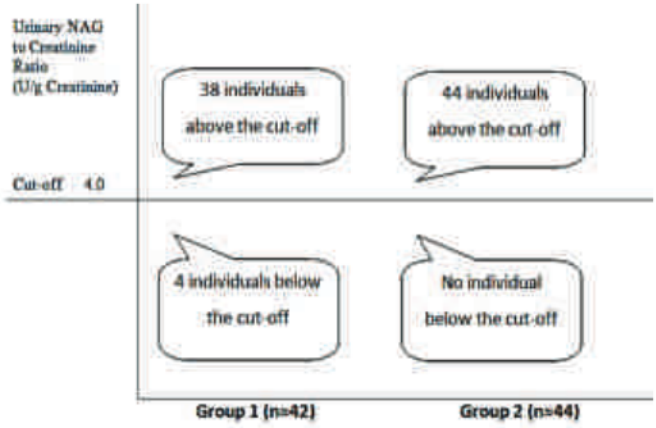

Figure 3: *Schematic Diagram of Type-II Diabetic Individuals With or Without Microalbuminuria and Urinary NCR using 4.0 U/g Creatinine as a Cut-Off for Diagnosis of Diabetic Nephropathy*

Discussion

Our study demonstrated the role of a lysosomal enzyme NAG as proximal tubular injury marker in detection of DN earlier than microalbuminuria. The key feature of our study is that in addition to find out the correlation of urinary NAG & albuminuria we also determined the validity of new biomarker in terms of sensitivity. Male to female ratio and duration of diabetes of 7-8 years in majority subjects in our study are similar to that of another study done in Korea.[13] Urinary ACR and NCR in our study was higher in group 2 (mircoalbuminuric) than group 1 (P <0.01). This is similar to other studies on NAG level in type II diabetic patients.[18,19] In both groups, values of urinary NCR showed skewed distribution on positive side as we selected study subjects only from hospital. In our study, using spearman correlation moderately strong positive (r=0.65) was found in group 1 while in group 2 moderately positive correlation (r=0.53) was determined. Our results are in agreement with both of the studies carried out in Argentina & Ghana on type-II diabetic patients which showed moderately positive correlation of urinary NAG with ACR (r = 0.628; p < 0.0001) & r = 0.49, p<0.001) respectively.[9,20] The work done by Udomah FP et al also recorded correlation (r=0.74) that was strongly positive between NCR and urinary ACR in both diabetic patients as well as healthy controls in 1:2 ratio.10Similarly in a recent study the levels of urinary NAG showed moderate positive correlation with the levels of urinary ACR in type-II DM (r=0.46).[13] However, our results were different to an Indian study by Ambade V et al, They reported weak positive correlation between urinary NCR and ACR in normoalbuminurics (r=0.312) and microalbuminurics (r=0.278).[15] The difference in findings of this study with our study might be because of two reasons. First difference is about sample size. It was larger (n=196) than ours (n=86) and second difference is about duration of diabetes mellitus. In Indian study, researcher included diabetic subjects with longer duration (from 1 month to 40 years), while in our study it was less than10 years.

On controlling the effect of two confounding variables of age & duration of DM, slight decrease in positive correlation was observed in group 1 ('r' value decreased from 0.65 to 0.573 on partial correlation) and in group 2 ('r' value decreased from 0.53 to 0.408 on partial correlation). This showed little confounding effects of confounders on variables

of interest in our study. In our study sensitivity of this new biomarker urinary NAG was 95.34% whereas that of microalbuminuria was 51.16%. This finding is very close to the result of other researchers who reported sensitivity of urinary NAG as 96.1% on type 2 diabetic patients.[21] Another researcher determined sensitivity of urinary NAG as 83.3% on subjects with type-II diabetes mellitus but sample size of this study was small as it included only 20 & 25 subjects with normoalbuminuria & microalbuminuria respectively.[3]

Conclusion & Recommendation

In patients of type-II diabetes mellitus, levels of urinary excretion of N-acetyl-β-D-Glucosaminidase were more positively correlated with urinary albumin in normoalbuminuric as compared to microalbuminuric. Moreover, it was able to pick 95.34% of type-II diabetics with nephropathy than 'microalbuminuria' (51.16%) when used as a screening test. This may predict early tubular damage due to diabetic nephropathy and can be used as diagnostic biomarker.

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