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ORIGINAL ARTICLE

Correlation of Urinary Gamma Glutamyl Transferase to Creatinine Ratio with Albumin Creatinine Ratio in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetic Nephropathy is one of the most serious outcomes of Diabetes Mellitus worldwide. As tubular injury precedes albuminuria, tubular enzyme Gamma Glutamyl Transferase (GGT) may serve as a more sensitive diagnostic biomarker for diabetic nephropathy.

Objective: To determine and correlate urinary Gamma Glutamyl Transferase to creatinine ratio with urinary albumin creatinine ratio (uACR) in Type 2 diabetics based on gender.

Methods: A cross-sectional study was undertaken at Shaikh Zayed Hospital from March 2022 to April 2023. A total of 100 male and female participants were included in this study. The study participants included 75 type 2 diabetics and 25 controls. The diabetic group was subdivided into normoalbuminuric and microalbuminuric based on urinary albumin creatinine ratio. All subjects' fasting blood glucose, urinary albumin, urinary creatinine, and urinary GGT levels were measured on the automated chemistry analyzer. The data was analyzed by SPSS version 24. "t-test" was used to compare the variables between different groups. The Pearson correlation test was used to establish the correlation between Gamma Glutamyl Transferase to creatinine ratio and uACR.

Results: Urinary GGT (uGGT) levels were significantly higher in all type 2 diabetics as compared to controls ($p < 0.001$). Both urinary albumin and uACR were raised in male diabetics ($p<0.001$) and female diabetics ($p<0.005$) as compared to controls, in male diabetics more increase was observed ($p<0.001$). A highly significant positive correlation was observed between uGGT:Creatinine ratio and uACR of all normoalbuminuric patients ('r' males=0.837 & females=0.919) and microalbuminuric patients ('r' males=0.600 & females=0.636) at $p<0.001$.

Conclusion: Urinary Gamma Glutamyl Transferase to creatinine ratio is positively correlated with urinary albumin creatinine ratio and may serve as a more sensitive biomarker than urinary albumin creatinine ratio in diabetic patients with renal damage.

Key Words: Diabetes, Urinary Gamma Glutamyl Transferase to creatinine ratio, urinary albumin creatinine ratio

Doi: 10.53685/jshmdc.v5i1.139

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Received: 20.10.2023, I[st] Revision: 25.05.2024,
2[nd] Revision: 15.06.2024, Accepted: 27.06.2024

How to cite this article: Hameed S, Khan M , Ustrana S. Correlation of urinary gamma glutamyl transferase to creatinine ratio with albumin creatinine ratio in patients with type 2 diabetes mellitus. J Shalamar Med Dent Coll. 2024; 5(1): 42-48. doi: 10.53685/jshmdc.v5i1.139

INTRODUCTION

Diabetic Nephropathy (DN) is one of the major consequences of Diabetes Mellitus (DM). It is estimated that this microvascular complication develops in almost 40% of Type II diabetic patients.[1] It is manifested by persistent hypertension, albuminuria, and renal

dysfunction progressing to end-stage renal disease.[2]

Early detection is highly significant in the timely management of this disease and in avoiding its complications. Urinary albumin (microalbuminu ria) is by far the most common diagnostic test of nephropathy. It appears in urine after significant glomerular damage has already occurred.[3]

Moreover, it has been observed that it does not correlate well with renal function and lacks sensitivity and specificity in the early detection of diabetic nephropathy (DN).[4] Some of the recent studies have reported that tubular impairment precedes glomerular involvement in diabetic patients.[5] Hence, tubular injury markers have an important diagnostic role with subsequent therapeutic implications in DN. Moreover, tubular biomarkers are shown to be much more effective in detecting renal dysfunction than microalbuminuria, which is currently taken as the gold standard in the diagnosis of DN.[6]

Additionally, urinary biomarkers are a preferable choice because they are non-invasive and more sensitive. Gamma Glutamyl Transferase (GGT) is an enzyme found in the liver and pancreas but it is most abundant in the luminal brush border of the proximal tubular cells. This enzyme is not excreted by Glomerular filtration, so when it is detected in urine it is strongly indicative of tubular damage.[7]

Urinary Gamma Glutamyl Transferase (uGGT), owing to its presence mainly at the luminal border, gives early and marked response to renal tubular injury and may prove to be an even more sensitive and cheap biomarker for renal injury in patients of type II DM. In addition, GGT has been suggested as a sensitive marker of oxidative stress that can reflect any pathophysiological process.[8]

Keeping in view these observations, this research was planned to evaluate the role of uGGT in the early detection of Diabetic Nephropathy. For this purpose, uGGT was assessed, and uGGT: creatinine ratio was calculated and correlated with the urinary albumin ratio (uACR) of type II diabetic patients.

The main objective was to ascertain if DN could be detected by uGGT before microalbuminuria sets in.

METHODS

Ethical approval was obtained from the Institutional Review Board of Shaikh Zayed Hospital (IRB #5689) on February 21, 2022. This cross-sectional study was conducted at Shaikh Zayed Hospital from March 2022 to April 2023. It was a time bound sampling collected in a period of three months. A total of 100 subjects were enrolled in three months after taking informed consent. The diabetic group included 75 type-2 diabetics (34 females and 41 males) randomly selected from the diabetic clinic of Shaikh Zayed Hospital, whereas, the control group comprised 25 healthy individuals (10 females and 15 males) recruited from the general population.

The inclusion criterion for the diabetic group was diagnosed type II diabetics for 10-18 years including both genders between 30-65 years of age. Type II diabetics who had any pre-existing renal disorder due to reasons other than DM, any other systemic disease, or malignancy was excluded from the excluded.

Five ml venous blood was collected from all the study participants after 10-12 hours of fasting. After clotting these samples were centrifuged and serum was stored in aliquots at -20°C. Simultaneously, midstream urine samples were collected and stored in the given aseptic urine containers. fasting blood glucose,[9] Urinary albumin,[10] urinary creatinine[11,] and Urinary GGT[12] of all the subjects were measured on the Dimension clinical chemistry autoanalyzer after calibration using Siemens kits. ACR was calculated as mg/gm of creatinine and uGGT: creatinine ratio as U/gm of creatinine to compensate for the effect of diuresis.[13]

The diabetic group was subdivided into normoalbuminurics (<30mg albumin/gm of

creatinine) and microalbuminurics (30-300 mg albumin/gm of creatinine) based on the urinary albumin creatinine ratio.[14]

Statistical Analysis

Statistical analysis was performed on SPSS 24 Variable were expressed as mean±SD. Comparison of variables between all groups was done using ''t-test''.

Pearson correlation test was used to assess the relationship between uGGT: creatinine ratio and uACR. The correlation coefficient 'r' was calculated between uACR and uGGT: creatinine ratio of the controls and the two diabetic groups namely normoalbuminuric and macroalbuminuric. A 'p' value < 0.05 was considered statistically significant.

RESULTS

The basic characteristics of the diabetic and control groups are given in Table 1. The fasting blood glucose, uGGT, urinary albumin and uGGT:Creatinine ratio were significantly higher in the diabetic group compared with the control group at p<0.001(Table 1).

On subgroup comparison, urinary GGT and uGGT : Creatinine ratio were elevated in both male and female diabetics compared to controls (p <0.001) but significant increase was seen in females compared to male with normoalbuminurea and microalbuminurea as shown in Table 2.

Both urinary albumin and urinary albumin creatinine ratio were raised in male diabetics (p<0.001) and female diabetics (p<0.005) as compared to controls. There was marked increase in urinary albumin and urinary albumin creatinine ratio in males compared to female patients with normo and microalbuminurea (Table 3). Urinary GGT and uGGT: The Creatinine ratio was raised ssignificantly in females and urinary albumin creatinine ratio was significantly raised in males (p<0.05) Table 4.

A significantly positive correlation was observed between uACR and uGGT: creatinine ratio of both normoalbuminuric and microalbuminuric diabetics as given in Table 5.

Table 1: Basic characteristics of the diabetic and control group

Parameter	Control Group (mean±SD)	Diabetic Group (mean±SD)
Age	50.80±8.2	51.4 ± 6.19
BMI (kg/m^2)	25.5±3.7*	28.5 ± 2.5*
Systolic BP (mmHg)	118.52±8.4	122.25±10.2
Diastolic BP (mmHg)	81.94±7.5	82.98±9.5
FBG (mg/dl)	81.84±8.2	228.89±15.2***
uGGT (U/L)	3.32±2.54	34.85±5.5***
uGGT: Creatinine Ratio (u/gm)	3.19±1.12	49.17±5.12***
UrinaryAlbumin (mg/dl)	3.7±1.12	207.51±35.14
Urinary Albumin Creatinine Ratio (uACR) (mg/gm)	2.83±1.1	394.29±94.59***

*t-test was used for group comparison; *p <0.05 is statistically significant, ***p<0.001 highly significant.*

Table 2: Comparison of urinary uGGT and urinary uGGT:creatinine ratio in normoalbuminuric and microalbuminuric groups

Group	Control		Normoalbuminuric		Microalbuminuric	
	Male (mean±SD)	Female (mean± SD)	Male (mean±SD)	Female (mean±SD)	Male (mean±SD)	Female (mean±SD)
uGGT (U/L)	3.60±2.84	3.30±2.9	21.73±14.83	29.83±11.34*	31.30±19.09	42.43±17.43*
uGGT:Creatinine Ratio (U/gm)	3.63±1.01	2.75±2.6	14.90±7.62*	18.36±6.04*	34.00±16.01*	38.40±17.42*

*t-test was used for group comparison; ***p <0.05 is taken as statistically significant*

Table 3: Comparison of urinary albumin & urinary albumin creatinine ratio of normoalbuminuric and microalbuminuric diabetics with control group based on gender

Group	Control		Normoalbuminuric		Microalbuminuric	
	Male (mean±SD)	Female (mean±SD)	Male (mean±SD)	Female (mean±SD)	Male (mean±SD)	Female (mean±SD)
Urinary Albumin (mg/dl)	4.31±1.44*	3.09±1.65	6.74±1.01*	4.90±1.16	55.50±9.45*	44.73±12.68
Urinary Albumin: Creatinine ratio (mg/gm)	2.35±0.72	3.31±1.53	4.53±1.82*	4.29±1.16	76.70±30.29*	70.20±25.54

t-test was used for group comparison; *p <0.05 is statistically significant

Table 4: Comparison of study parameters between the diabetic and control group based on gender

Parameter	Control Group Male (15)	Diabetic Group Male (41)	Control Group Female (10)	Diabetic Group Female (34)
Age	50.80±6.6	52.10 ± 6.9	50.80± 9.5	50.44± 6.29
BMI (kg/m^2)	24.47±3.1	28.32 ± 2.3	26.50± 3.7	28.68± 2.62
Systolic BP (mmHg)	117.33±7.04	121.95±12.1	118.00±4.2	123.24±9.45
Diastolic BP (mmHg)	80.33±6.7	82.68±9.5	82.60±4.3	83.82± 9.85
FBG (mg/dl)	81.27±8.0	239.80±42.5*	82.40±8.9	226.94±40*
uGGT (U/L)	3.60±2.84	31.05±4.63*	3.00±2.9	36.44±6.39*
uGGT:Creatinine Ratio (u/gm)	3.63±1.01	42.22±4.74*	2.75±2.6*	56.12±5.63*
Urinary Albumin (mg/dl)	4.31±1.44	221.76±38.97*	3.09±1.65	193.27±57.68*
Albumin:Creatinine Ratio (mg/gm)	2.35±0.72	469.90±119.94*	3.31±1.53	318.69±91.59*

*t-test was used for group comparison *p <0.05 is taken as statistically significant*

Table 5: Correlation between Urinary Albumin Creatinine Ratio (uACR) and Urinary GGT Creatinine Ratio (uGGT: creatinine ratio) in Normoalbuminuric & Microalbuminuric Diabetics

Group Compared	Urinary Albumin:Creatinine Normoalbuminuric		Microalbuminuric	
	Male "r"	Female "r"	Male "r"	Female "r"
uGGT : Creatinine Ratio	0.837**	0.919**	0.600*	0.636**

*Pearson correlation test was used; *p <0.05 there is significant correlation **p <0.01, there is highly significant correlation.*

DISCUSSION

Diabetic Nephropathy is a major concern of health practitioners due to significant morbidity and mortality reported worldwide.[2] Numerous renal biomarkers including both glomerular and tubular markers, that predict the onset or the progression of diabetic nephropathy have been reported and have gained immense significance in clinical diagnostics. Currently used biomarkers for DN include serum creatinine, serum urea and urinary albumin.[15]

The time-lapse reported between the elevation of these markers and actual renal insult reflects that these markers are highly insensitive. A major degree of renal impairment has already occurred even before the appearance of albumin in the urine.[12] Therefore, the identification of more sensitive and effective biomarkers that are capable of detecting renal injury in its earliest phase, is the need of the hour. Many studies have reported that tubular injury markers precede the rise in urinary albumin.[16] Potential tubular markers of renal injury include luminal border enzyme GGT which indicates injury to the proximal renal tubules.[17] This research was devised to evaluate the role of uGGT as a potential renal function biomarker in type 2 DM by correlating it with ACR. Raised Albumin Creatinine Ratio is currently considered a gold standard for the indication of kidney dysfunction. The main aim of this study was therefore to investigate the correlation between the uGGT: creatinine ratio in subjects with type II DM and severity of proteinuria as shown by the uACR.

There was no significant difference observed between the age and BMI of the diabetics and control groups but the fasting serum glucose of the diabetic group was significantly raised (Table1). The previous studies conducted on type II diabetic patients with nephropathy have reported a correlation between renal dysfunction and hyperglycemia.[18,19] uGGT levels when measured, were significantly higher in both genders of the diabetic group as compared to the control group (Table 2). These results were in close agreement with the results reported in previous studies that reported that type II diabetic patients with similar duration of diabetes had also shown significantly higher values of uGGT in comparison to the controls.[12.20]

To correlate uGGT:Creatinine ratio with uACR the diabetic group was further divided into two groups based on their albumin creatinine ratio i.e. normoalbuminuric and microalbuminuric. When the correlation coefficient was calculated, the uACR of males and females of the normoalbuminuric group and females of the microalbuminuric group showed a highly significant correlation with uGGT: creatinine ratio , while the uACR of microalbuminuric males, showed a less significant correlation with uGGT: creatinine ratio . A similar study conducted previously by Dai et al had also shown a significant positive correlation between serum GGT and uACR of the normoalbuminuric group.[20] Moreover, our findings were also relatable with the work of Ambade et al who demonstrated increased uGGT: creatinine ratio both in normoalbuminuric and microalbuminuric diabetic patients.[12]

The most important aspect of the current study is that it has shown elevated uGGT levels in normoalbuminuric patients. This manifests that uGGT precedes the rise of albumin in the urine. This interesting and significant observation indicates the emerging hypothesis of a tubular phase in DN that precedes the occurrence of the classic glomerular lesions.

Moreover, it is demonstrated that tubular hypertrophy is already apparent before the onset of albuminuria.[21] In this perspective, the increase in uGGT: creatinine ratio values may express the degree of subclinical tubular impairment much earlier than uACR, thus representing a more sensitive index of the renal injury. If diabetic nephropathy is detected and diagnosed earlier, effective treatment can help to reduce the

mortality and morbidity associated with it. uGGT: creatinine ratio can help in the earlier diagnosis of diabetic nephropathy & therefore can prove to be a useful, non –invasive and cost-effective biochemical marker for the detection of renal dysfunction in the early phase of DM.

Among some limitations of the study were a smaller sample size and the cross-sectional study design. A similar study performed with a larger sample size and a prospective study of longer duration including other risk factors like insulin resistance, and physical activity can further enhance the quality of the results.

CONCLUSION

Raised levels of uGGT in diabetics and a significant positive correlation between uGGT to creatinine ratio and uACR in the diabetic patients suggests that urinary GGT:creatinine ratio may be considered a more sensitive biomarker of nephropathy as compared to urinary albumin creatinine ratio in type II diabetic patients.

Limitations of Study

Sample size in diabetics and control group was not equal and patients with grades of renal damage were not identified. Future studies with large sample size and with grades of nephropathy should be conducted.

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AUTHOR'S CONTRIBUTIONS

SH: Idea conception, drafted manuscript critically reviewed the manuscript
MK: Designed the study, analyzed data, interpretation,
SU: Designed study, drafted work, critically reviewed the manuscript
All authors approved the final version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

CONFLICT OF INTEREST:
All authors declared no conflict of interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE:
No specific grant was taken for this research from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT:
The data are available from the corresponding author upon reasonable request.