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GAMMA GLUTAMYL TRANSFERASE AS A PREDICTIVE MARKER OF MICROALBUMINURIA IN DIABETIC NEPHROPATHY

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ABSTRACT

Objective: To determine predictive value of gamma glutamyl transferase (ϒ-GT) for microalbuminuria in diabetic nephropathy. **Study Design:** Case control study. **Place & Duration:** Department of Medicine –Jinnah Post Graduate Medical Center, Karachi from December 2015- August 2016. **Subjects & Methods:** 45 healthy controls and 55 type 2 diabetics were selected through non-probability purposive sampling. Microalbuminuria can be diagnosed from a 24-hour urine collection. BUN, serum creatinine, albumin, bilirubin, uric acid, blood glucose, HbA1c, serum creatinine and blood lipids were analyzed. Urinary albumin was estimated by immunoturbidometry method and ϒ-GT by IFCC method. Data was analyzed by statistical package *SPSS 22.0 and Graph Pad Prism*. **Results:** Albuminuria and ϒ-GT in controls and cases were found as 19.73±5.21 and 150.47± 102.43 mg/dL and 21.62±5.36 and 36.27± 6.33 U/L (P=0.001) respectively. ϒ-GT showed positive correlation with Microalbuminura (r= 0.871, P=0.0001). ϒ-GT, on linear regression model, predicted the microalbuminuria, F= 221.82, P= 00001, R^2 = 0.694. In binary logistic regression analysis, ϒ-GT was strongly predicted the microalbuminuria (AUC=0.914, Odd ratio= 1.366, P = 0.0001). **Conclusion:** The ϒ-GT may be used as alternative screening test for predicting the microalbuminuria in type 2 diabetics. ϒ-GT levels along with urinary albumin may help in early detection of diabetic nephropathy.

KEYWORDS: ϒ-Glutamyl transferase Microalbuminuria Diabetic nephropathy.

INTRODUCTION

Diabetes mellitus is a serious public health problem because of its growing Prevalence and chronic macro and microvascular complications ending up in an increase in mortality and morbidity. (Sun et al. 2014; Nunes et al. 2012) In diabetic patients with proteinuria, the relative mortality is 40 times higher than in diabetic without proteinuria. (Viswanathan, Tilak and Kumpatla 2012) Microalbuminuria is an indicator of vascular endothelial dysfunction and an independent risk factor for progressive renal damage. This holds true for diabetes mellitus patients and indicates who are likely to develop macrovascular disease and progressive renal impairment.

Microalbuminuria arises from increased passage of albumin through filtration barrier of vascular endothelium. Serum gamma glutamyl-transferase (ϒ-GT) is a nonprotein antioxidant present in serum, plasma membranes of all cells except erythrocytes, catalyses degradation of extracellular glutathione. (Yilmaz et al. 2013) ϒ-GT has commonly been used as a marker for excessive alcohol consumption and liver disease. (Koenig and Seneff 2015; Wickham et al. 2011) Emerging evidence suggest, ϒ-GT as a predictor of incident diabetes and systemic hypertension.

Few studies have also shown that ϒ-GT predicts microalbuminuria and may also act as a predictor of microvascular complications in diabetes patients. Increased serum ϒ-GT activity observed in diabetic patients may be a response to oxidative stress that occurs during the course of the disease. (Cho 2010; Lim et al. 2004) Accumulating data state that oxidative stress alters many functions of the endothelium leading to microalbuminuria.

As the incidence and prevalence of DM is increasing in Pakistan and similarly the diabetic nephropathy load is going to incline hence there is need to establish cost effective and easy markers for screening, diagnosis and prognosis of glomerular injury in Diabetic at the earlier

stages. There is paucity of data relating ϒ-GT and albuminuria type 2 diabetes mellitus patients. The present case control study was performed to determine the predictive value of serum gamma glutamyl-transferase for the microalbuminuria in type 2 Diabetic patients.

SUBJECTS AND METHODS

The study subjects of present case control study were selected from the Department of Medicine – Jinnah Postgraduate and Medical Center, Karachi from December 2015- August 2016. A sample of 45 healthy controls (Group A) and 55 type 2 diabetics (Group B) was selected as cases. Study subjects were selected through non-probability purposive sampling according to inclusion and exclusion criteria.

INCLUSION CRITERIA

All the patients attending the Diabetic clinic diagnosed as per the American diabetic association criteria were considered for the study. Only those patients who were positive for albumin (microalbuminuria) were included. Microalbuminuria was diagnosed from a 24-hour urine collection (between 30–300 mg/24 hours) or, more commonly, from elevated concentrations in a spot sample (30 to 300 mg/L). Both were measured 2-3 times over 2-3 months period. (Bakker 1999).

EXCLUSION CRITERIA

Patients with Urinary tract infection, acute illness, congestive cardiac failure, patient on ACE inhibitor for hypertension and pregnant women were excluded. Samples were not collected after exercise. Cardiac failure, chronic kidney disease (CKD), acute & chronic liver disease and smokers were exclusion criteria. Intake of diuretic therapy, vitamin and mineral supplements and hypolipidemic therapy was also exclusion criteria.

BLOOD SAMPLING & BIOCHEMICAL TESTING

Subjects were facilitated to comply with protocol. 8-12 hour fasting was ensured for blood samples. BUN, serum creatinine, albumin, bilirubin, uric acid, blood glucose, HbA1c and blood lipids were analyzed (Cobas e 411 analyzer- Roche Diagnosis GmbH, Mannheim, Germany). Serum creatinine was estimated by Jaffe`s method. Triglycerides and cholesterol were determined by enzymatic colorimetric (CHOD-PAP & GPO-PAP) methods. Precipitant method was used for HDL-Cholesterol. Friedewald's formula (LDL-C = TC - HDL-C – (TG/5) was used for LDL-Cholesterol. (Expert Panel on Detection 2001) "*Glucose oxidase*" method was employed for glucose determination. Urinary albumin was estimated by immunoturbidometry method. This method is based on anti-human albumin antibodies reaction with the antigen human albumin in the sample to form antigen- antibody complexes which is then measured by turbidometry. Gamma glutamyl-transferase was estimated by IFCC method. GGT catalyze the transfer of glutamic acid to acceptor glycyl-glycine, the reaction releases 5-amino 2-nitrobenzoate, which is measured at 405nm. Data was analysed on Graph Pad Prism and SPSS 22.0 (IBM, Incorporation, USA).

Continuous and categorical data was analyzed by student's t test and Chi square test respectively. Simple linear regression and logistic regression were used for detecting the predictive value of GGT for microalbuminuria. All data was analyzed at 95% confidence interval ($P \leq 0.05$).

RESULTS

Demographic characteristics and biochemical findings are shown in table 1. Of 45 controls and 55 cases, male were 28 and 34 and female were 17 and 21 respectively. Compared to controls, 38 cases urine was positive for microalbuminuria (X^2= 50.1, P=0.0001) (table 2). Albuminuria in controls and cases was noted as 19.73±5.21 and 150.47± 102.43 mg/dL (P=0.0001). ϒ-GT showed statistically significant different between controls and cases. ϒ-GT in controls was 21.62±5.36 compared to cases as 36.27± 6.33 U/L (P=0.001). Spearman`s correlation of ϒ-GT showed strong positive correlation with Microalbuminura (r= 0.871, P=0.0001).

Linear regression analysis was performed by Enter method. A simple linear regression model was run to predict microalbuminuria. The ϒ-GT statistically significantly predicted the microalbuminuria, F= 221.82, P= 00001, R^2 = 0.694. The results are shown in table 3.

In binary logistic regression analysis, ϒ-GT was strongly predicted the microalbuminuria strongly as shown in table 4. Area under curve (ROC) = 0.914, Sensitivity = 79% and specificity 93%, (Odd ratio= 1.366, P = 0.0001).

Table 1. Demographic characteristics and biochemical findings

	Study groups	Mean	SD	P-value
Age (years)	**Group A. Controls**	52.11	6.47	0.73
	Group B. Cases	52.49	4.83	
Body weight (kg)	**Group A. Controls**	75.89	11.40	0.011
	Group B. Cases	82.27	12.90	
Systolic BP (mmHg)	**Group A. Controls**	132.27	9.07	0.001
	Group B. Cases	152.71	23.65	
Diastolic BP(mmHg)	**Group A. Controls**	69.22	6.39	0.001
	Group B. Cases	90.18	15.12	

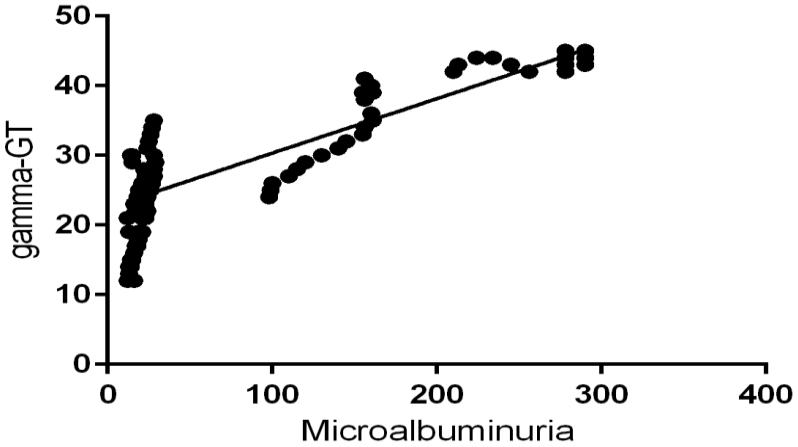
RBG (mg/dl)	**Group A. Controls**	134.36	8.56	0.001
	Group B. Cases	269.58	54.68	
A1C (%)	**Group A. Controls**	5.46	0.73	0.001
	Group B. Cases	11.09	2.26	
Serum Creatinine (mg/dl)	**Group A. Controls**	0.88	0.16	0.001
	Group B. Cases	1.09	0.26	
Serum cholesterol (mg/dl)	**Group A. Controls**	175.16	32.99	0.001
	Group B. Cases	225.80	34.05	
Serum triglycerides (mg/dl)	**Group A. Controls**	198.29	20.99	0.001
	Group B. Cases	387.51	115.00	
LDL-c (mg/dl)	**Group A. Controls**	94.93	26.61	0.001
	Group B. Cases	185.38	40.25	
HDL-c (mg/dl)	**Group A. Controls**	46.83	2.97	0.001
	Group B. Cases	33.04	11.73	
Microalbuminura (mg/dl)	**Group A. Controls**	19.73	5.21	0.001
	Group B. Cases	150.47	102.43	
ϒ-GT (U/L)	**Group A. Controls**	21.62	5.36	0.001
	Group B. Cases	36.27	6.33	

Table 2. Gender and microalbuminuria distribution

	Group A. Controls	**Group B. Cases**	**X^2 –value**	**P value**
Male	28	34	0.02	0.96
Female	17	21		
Microalbuminuria-ve	45	17	50.1	0.0001
Microalbuminuria +ve	0	38		

Table 3. Linear Regression Analysis Model

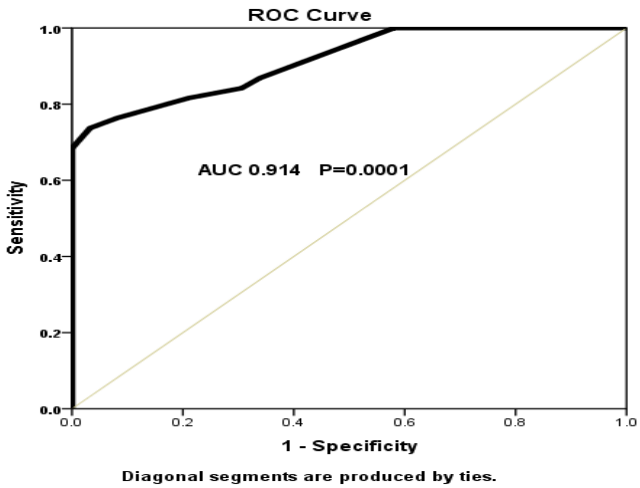
	B	**SE B**	**β**	**t-value**	**P-value**
(Constant)	-171.54	18.52	0.833	-9.259	0.0001
ϒ-GT (U/L)	8.86	0.59		14.894	
a. Dependent Variable: microalbuminuria (ng/dl)					



Graph 1. ϒ-GT (U/L) and microalbuminuria (mg/dL) correlation

Table 4. Logistic regression model for prediction of microalbuminuria

	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**
ϒ-GT	0.312	0.063	24.491	1	0.0001	1.366
Constant	-10.266	2.013	26.018	1	0.0001	0.0001



Graph 2. Receiver operating characteristic curve

DISCUSSION

It is for the first time, the present study reports a case control study on the predictive value of ϒ-GT for microalbuminuria in type 2 diabetics. World prevalence of DM was 2.8% in year 2000 and estimations for 2030 are reported as 4.4%. Renal disease (Diabetic nephropathy) is a common complication of type 2 diabetics. Prevalence of DN is very high and 5 - 15% enters the stage of ESRD. (Safdar and Mahmud 2015) DN is increasing in type 2 diabetics due to more prevalence of type 2 DM. Timely intervention and early screening and diagnosis is a clinical tool to prevent ESRD. Earlier most stage of DN is called incipient DN which may be subclinical when it can be detected by minor urinary albumin amounts of 30 – 300mg/day). (Viswanathan, Tilak and Kumpatla 2012)**.**

This range of urinary albumin is known as the microalbuminuria. Microalbuminuria is a strong predictor of DN and an independent risk for the coronary artery disease. (Chadban et al. 2010; Chowta, Pant and Chowta 2009) Microalbuminuria has association with the duration of type 2 DM. Chronic hyperglycemia contributes to the advanced glycosylation end product (AGE) which cause endothelial dysfunction and albuminuria ensues. (Manjrekar Poornima, Shenoy and Hegde 2010).

If microalbuminuria is detected earlier, then timely intervention may prove helpful in dampening the generalized endothelial dysfunction. This will reduce the burden of chronic complications of type 2 DM and body organ damage. (Satchell and Tooke 2008) Chronic hyperglycemia increases oxidative stress to the vascular endothelium. (Şarlı et al. 2013; Onal 2014; André et al. 2007) Vascular endothelium dysfunction of kidneys is the primary lesion of DN which is aggravated by oxidative stress too. The ϒ-GT plays role in the anti oxidative stress mechanisms through glutathione homeostasis. ϒ-GT is found nearly all of body cells. Previous studies reported positive association of ϒ-GT with insulin resistance in type 2 diabetics. (Zoppini et al. 2009; Grundy 2007; Sabanayagam et al. 2009).

The present study was conducted to analyze the predictive value of ϒ-GT microalbuminuria which is a marker of endothelial dysfunction. In the present study, the ϒ-GT was within high normal range in cases (P=0.001) and showed positive correlation with microalbuminura (r= 0.871, P=0.0001). The ϒ-GT predicted the microalbuminuria significantly (F= 221.82, P= 00001, R^2 = 0.694). The ROC curve showed AUC= 0.914, sensitivity = 79% and specificity 93%, Odd ratio= 1.366 and P = 0.0001. The ϒ-GT levels in DN were in the upper normal physiological range.

The finding of ϒ-GT is consistent with previous studies (Vijayasamundeeswari and Sudha, n.d.; Sun et al. 2014; Nunes et al. 2012) which reported similar observations. A previous study reported the ϒ-GT was positively correlated with the microalbuminuria in DN. (Vijayasamundeeswari and Sudha, n.d.). Lee et aI reported the rise in serum ϒ-GT levels is a compensatory response to oxidative load and endothelial injury which is the initial lesion causing microalbuminuria. They reported that the large increase in serum ϒ-GT may help in predicting the oxidative load. However, the Lee et al was not able to analyze the predictive significance of ϒ-GT. (Lee et al. 2005).

Evidence based finding of ϒ-GT of present study is worth finding, indicating high normal range of serum ϒ-GT may be used for predicting the incipient DN in type 2 diabetics. Raised serum ϒ-GT in type 2 diabetics may be exploited as a screening test for early detection of microalbuminuria and DN and then preventive measures may be taken at the earliest to prevent ESRD. The observations of present study are in agreement with previous studies. (Zoppini et al. 2009; Sabanayagam et al. 2009; Grundy 2007; Lee et al. 2005) The ϒ-GT, even within high normal range, may be used for predicting the microalbuminuria. It is concluded from the evidence based findings of present study supported by statistical significance that the serum ϒ-GT may be used not only

as a marker of oxidative stress, but also an indicator of vascular endothelial dysfunction and microalbuminuria in type 2 diabetics. The ϒ-GT may prove a reliable, easy, and inexpensive parameter in clinical practice, but it needs large sample size prospective studies. The major limitation of present study is a small sample size, different ethnicity and race; hence findings should be cautiously interpreted for the other geographical areas.

CONCLUSION

The gamma glutamyl transferase (ϒ-GT) may be used as alternative screening test for predicting the onset of microalbuminuria in type 2 diabetics. ϒ-GT levels along with urinary albumin should be routinely performed at regular intervals for earlier detection of diabetic nephropathy.

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