

**GAMMA GLUTAMYL TRANSFERASE AS PREDICTIVE MARKER OF CAROTID
INTIMA MEDIA THICKNESS IN TYPE 2 DIABETIC SUBJECTS****¹Dr. Farukhuddin*, Dr. Pooja Devi², Dr. Rabia Waseem Shaikh³, Dr. Bilal Razaque Memon⁴, Dr. Raheela Hassan⁵, Dr. Shahzore Gul⁶**¹MBBS, Dow Medical College, DUHS Karachi.

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ABSTRACT

Objective: To analyze the Gamma Glutamyl Transferase (γ-GT) as predictive marker of carotid intima media thickness (CIMT) in type 2 Diabetic subjects. **Study Design:** Case control study. **Place & Duration:** Department of Medicine - Liaquat University of Medical and Health Sciences Hospital from November 2015- August 2016. **Subjects & Methods:** A sample of 40 healthy controls and 45 type 2 diabetics was selected through non-probability (purposive) sampling by prior inclusion and exclusion criteria. Serum γ-GT was estimated by enzymatic colorimetric test. Carotid artery was examined with a 7.5-MHz linear-array transducer (Siemens Acuson x300) sonography. Data was analyzed using the SPSS 22.0 (USA) and Graph Pad Prism at 95% CI ($P \leq 0.05$). **Results:** Carotid intima media thickness (CIMT) in controls and cases was noted as 0.52 ± 0.07 and 0.75 ± 0.071 mm ($P=0.0001$). Significant γ-GT difference was noted between controls and cases i.e; 34.98 ± 5.47 and 42.71 ± 6.49 U/L ($P=0.001$). The γ-GT showed positive correlation with CIMT ($r=0.615$, $P=0.0001$). The γ-GT significantly predicted the CIMT by simple linear regression model ($F=50.37$, $P=0.0001$, $R^2=0.378$). **Conclusion:** The present study reports the serum γ-GT was increased in proportionate to CIMT. Serum γ-GT may be used as a non-invasive, inexpensive and easily available marker for atherosclerosis.

KEYWORDS: Gamma glutamyl transferase Carotid intima media thickness Atherosclerosis Diabetes mellitus.**INTRODUCTION**

Gamma-glutamyl transferase (γ-GT) plays pivotal role in regenerating the intracellular glutathione (GSSH) pool. GSSH is a cellular antioxidant which provides redox pairs. The γ-GT is a membrane bound enzyme. (Dominici et al. 2005; Emdin, Pompella, and Paolicchi 2005) Serum γ-GT is a clinical marker of liver disease, drug and alcohol consumption. Growing body of evidence suggest the serum γ-GT levels correlate with the cardiovascular disease (CVD). A predictive role of serum γ-GT has been suggested as an independent risk factor of mortality (Lee, Blomhoff, and Jacobs 2004; Lee et al. 2006; Ruttman et al. 2005). A previous meta-analysis reported that the γ-GT was associated with increased CVD related mortality (Du, Song, and Zhang 2013).^[6]

Type 2 diabetes mellitus (T2DM) is a risk factor for the CVD. It is reported that the T2DM subjects carry 2–4 times greater risk of CVD compared to non diabetic counterparts. (Du, Song, and Zhang 2013)⁷ T2DM subjects are recommended for a multi factorial

intervention of targeting hyperglycemia, hyperlipidemia, hypertension and CVD. (Eckel et al. 2006).

The conventional risk factors are not sufficiently helping in explaining the excess risk of CVD mortality observed in T2DM, hence there is increasing interest in making additional markers which should be non-invasive, cost effective and easy to perform for better CVD risk assessment in type 2 diabetics. (Lorber 2014).

Therefore, it is necessary to determine whether γ-GT level may be used as an independent marker atherosclerosis in T2DM patients which is a risk factor of CVD. Carotid intima-media thickness (CIMT) is a surrogate marker of changes in carotid vascular morphology and function. (H. Yoon et al. 2015) Carotid Ultrasonography is a simple, non-invasive technique used to measure CIMT with excellent predictive power for cardiovascular outcome. (Den Ruijter et al. 2012; Hung et al. 2009).

The γ-GT has been reported to correlate with carotid

intima media thickness.(Jung et al. 2011; Park et al. 2012; Saijo et al. 2008) Previous studies have reported conflicting results on the predictive value of γ -GT level and carotid IMT in diabetics.(Nuti et al. 2012) However, to our knowledge, no study from Pakistan has assessed serum γ -GT as surrogate marker of CIMT

Timely assessment of CIMT and serum γ -GT may be used to assess the consequences of atherosclerosis and cerebrovascular and cardiovascular risk. The present study, investigated the predictive significance of serum γ -GT levels for the carotid intima media thickness in type 2 diabetic subjects at our tertiary care hospital.

SUBJECTS AND METHODS

The study subjects of present case control study were selected from the Department of Medicine, Liaquat University Hospital Hyderabad/Jamshoro from November 2015- August 2016. 40 healthy controls and 45 type 2 diabetics. Study subjects were selected through non-probability purposive sampling according to inclusion and exclusion criteria.

Inclusion Criteria

45 patients between the age group of 40-70 years were randomly selected irrespective of sex, duration, glycemic control with or without hypertension and with or without diabetic macrovascular complication. Normal liver function test was also an inclusion criterion.

Exclusion Criteria

Patients with alcoholism, acute illness, congestive cardiac failure, chronic lung disease, chronic renal disease, liver disease and patients taking drugs – lipid lowering, vitamins, minerals, steroids, or hepatotoxic drugs were excluded.

Volunteers were facilitated to comply with the study protocol. 8-12 hour fasting was ensured for blood samples. Blood lipids, serum creatinine, and blood glucose and were analyzed (Cobas e 411 analyzer-Roche Diagnosis GmbH, Mannheim, Germany). Blood glucose was estimated by glucose oxidase method and Jaffe's method for serum creatinine. 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).

Measurement of γ -GT activity: Serum γ -GT was estimated by enzymatic colorimetric test at a temperature of $37^{\circ}C$.^[20] Roche/Hitachi analyzer (Mannheim, Germany) was used. γ -GT reference range is 3-55 U/L in our clinical laboratory.

Carotid Intima Media Thickness (CIMT): Patients were positioned in supine with extended neck. Pillow was put under the shoulder blades. Carotid artery was examined with a 7.5-MHz linear-array transducer (Siemens Acuson x300) sonography. Anterior and posterior walls of the carotid artery were displayed as 2 bright white lines separated by a hypoechogenic space on a longitudinal image. The CIMT was measured as the distance between first (lumen-intima interface) and second (media-adventitia interface) leading edge of bright lines. 3 sites were examined first the carotid artery bulb (1 cm proximal to the carotid bulb), second within the carotid bulb (maximum diameter) and third reading 1 cm distal to the carotid bulb in the direction of the internal carotid artery. (Expert Panel on Detection 2001; Mahashabde and Kothe 2015).

Statistical Analysis: Continuous and categorical data was analyzed by student's t test and Chi square test respectively on the SPSS 22.0 (USA) and Graph Pad Prism at 95% CI ($P \leq 0.05$). Simple linear regression model was used for predicting carotid intima media thickness from the serum gamma glutamyl transferase.

RESULTS

Study subjects were age, gender and body weight matched. Table 1 show the demographic and laboratory findings of study subjects. Carotid intima media thickness (CIMT) in controls and cases was noted as 0.52 ± 0.07 and 0.75 ± 0.071 mm ($P=0.0001$). Significant γ -GT difference was noted between controls and cases i.e; 34.98 ± 5.47 and 42.71 ± 6.49 U/L ($P=0.001$). Pearson's correlation of γ -GT showed positive correlation with CIMT ($r=0.615$, $P=0.0001$).

Linear regression analysis was performed by Enter method. A simple linear regression model was run to predict CIMT. The γ -GT significantly predicted the CIMT, $F=50.37$, $P=0.0001$, $R^2=0.378$. The results are shown in table 2.

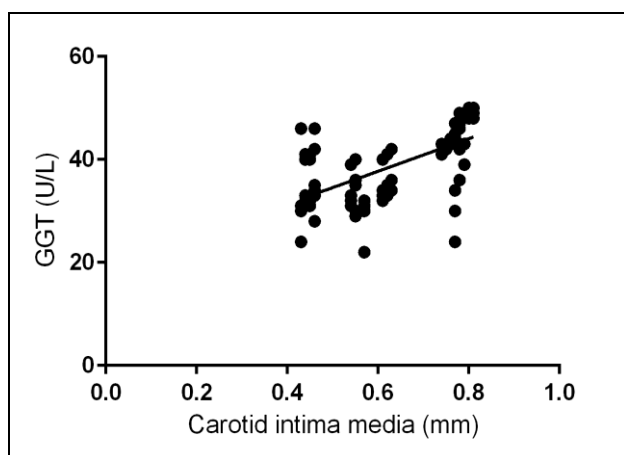
Table 1. Laboratory findings and Demography of study subjects				
	Study groups	Mean	SD	P-value
Age (years)	Controls	55.15	6.57	0.051
	Cases	56.00	3.81	
Body weight (kg)	Controls	78.63	12.14	0.56
	Cases	80.33	13.07	
Systolic BP (mmHg)	Controls	132.23	9.00	0.0001
	Cases	153.80	23.64	
Diastolic BP(mmHg)	Controls	69.25	6.75	0.0001
	Cases	94.11	13.02	

Blood Glucose (R) (mg/dl)	Controls	134.30	8.59	0.0001
	Cases	285.49	28.81	
A1C (%)	Controls	5.38	0.73	0.0001
	Cases	11.00	2.43	
S. Cr (mg/dl)	Controls	0.87	0.15	0.0002
	Cases	1.05	0.27	
S. Cholesterol (mg/dl)	Controls	169.00	28.28	0.0001
	Cases	215.87	40.87	
S. triglycerides (mg/dl)	Controls	197.85	22.08	0.0001
	Cases	366.84	129.34	
LDL-cholesterol (mg/dl)	Controls	97.93	26.61	0.031
	Cases	98.93	38.13	
HDL- cholesterol (mg/dl)	Controls	47.02	2.85	0.0001
	Cases	38.79	11.84	
CIM Thickness (mm)	Controls	0.52	0.07	0.0001
	Cases	0.75	0.07	
Y-GT (U/L)	Controls	34.98	5.47	0.0001
	Cases	42.71	6.49	

Table 2. Linear Regression Analysis Model

	B	SE B	β	t-value	P-value
(Constant)	0.177	0.067	0.615	2.65	0.001
Y-GT (U/L)	0.012	0.002		7.09	

a. Dependent Variable: Carotid intima media thickness (mm)



Graph. Correlation between GGT and Carotid intima media thickness.

DISCUSSION

The present prospective study is being reported for the first time from our tertiary care hospital on the predictive significance of Y-GT association with the CIMT. The present study reports a significant predictive value as observed by simple linear regression model ($F=50.37$, $P=0.0001$, $R^2=0.378$). Carotid intima media thickness (CIMT) was more in cases 0.75 ± 0.071 mm compared to 0.52 ± 0.07 mm in controls ($P=0.0001$). The Y-GT in cases was 42.71 ± 6.49 U/L compared to 34.98 ± 5.47 U/L in controls ($P=0.0001$). The Y-GT showed positive correlation with CIMT ($r=0.615$, $P=0.0001$). The findings of present study are in agreement with a recent study reported by Mahashabde et al 2015. They reported the serum Y-GT was raised in proportionate to CIMT thickness compared to controls. They reported

positive correlation of Y-GT and CIMT ($r=0.783$, $p=0.001$). (Mahashabde and Kothe 2015)

Other previous studies (Morling et al. 2014; Paolicchi et al. 2004; Davis et al. 2001; H. E. Yoon et al. 2016) reported the serum Y-GT increases with increase in CIMT. As the CIMT is a validated marker of atherosclerosis, hence Y-GT instead of it may be used as an easy and inexpensive method. The findings of above study are in agreement with present study. However, the present study analysed the linear regression analysis model for prediction of CIMT from Y-GT which is a new findings and not comparable to previous studies. Hence, Y-GT may be used as a simple marker of atherosclerosis vascular disease in clinical practice.

Recent studies (Emdin et al. 2002; Lee et al. 2003) had reported Y-GT as an independent risk factor in the coronary artery disease. These previous studies detected the Y-GT present within the atherosclerotic plaques in the carotid, cerebral, and coronary artery in histochemical examination of lesions. (H. E. Yoon et al. 2016).

Y-GT was extracted from the CD68⁺ foam cells (macrophages) of atheroma plaques. (Emdin et al. 2002) The Y-GT positive foam cells have been detected from arterial walls along with oxidized LDL in the coronary artery disease. (Mahashabde and Kothe 2015; Emdin et al. 2002; Lee et al. 2003).

It is stated that the glutathione hydrolysis by Y-GT triggers LDL oxidation, and induces reactive oxygen species (ROS) generation, both events likely promote the

atheroma plaque formation and related complications. A previous study concluded that the serum γ -GT is consumed up within atherosclerotic plaques.(Breitling et al. 2011).

Nuti et al reported positive association of γ -GT and CIMT. They further added that the age-adjusted CIMT did not affect the positive association. A previous study reported conflicting observations on the association of γ -GT with CIMT; however, they accepted these conflicting results were due to confounding factors of gender and age bias, low statistical strength and low sample size.(Völzke et al. 2005) Such confounding effect has been reported by previous studies.(McKimmie et al. 2008). The present study reports that the CIMT is a surrogate marker of atherosclerosis, but it needs particular sonography instrument and expert hence γ -GT may be used as easy to perform non-invasive which may be used in rural and remote areas of the country as a marker of atherosclerosis in type 2 diabetic subjects. The major limitations of present study include; a small sample of some particular ethnic group was studied, hence findings cannot be generalized and must be interpreted cautiously for other geographical areas and ethnic groups.

CONCLUSION

The present study reports the serum gamma glutamyl transferase was increased in patients with carotid intima media thickness which is a marker of atherosclerosis. Serum gamma glutamyl transferase may be used as a non-invasive, inexpensive and easily available marker for atherosclerosis. However, studies with large sample size are warranted.

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