International Journal of Clinical Biochemistry and Research 2019;6(4):458–463

Content available at: iponlinejournal.com

International Journal of Clinical Biochemistry and Research

Journal homepage: www.innovativepublication.com

Original Research Article

Association of gamma glutamyl transferase activity with fasting glucose among first degree relatives of type 2 diabetes patients

C M Maithri[1,*], P Manoj[1], S K Mahadev[1], Tejaswani[1]

[1] *Dept. of Biochemstry, Mandya Institute of Medical Sciences, Mandya, Karnataka, India*

ARTICLE INFO

Article history:
Received 05-09-2019
Accepted 11-10-2019
Available online 14-12-2019

Keywords:
Gamma glutamyl transferase
First degree relatives
Euglycemia
Predia betes
Diabetes

ABSTRACT

Introduction and Objectives: Serum gamma glutamyl transferase (GGT) activity has been considered to play an important role in the pathogenesis of diabetes. Therefore, the objectives of our study was to determine the association of GGT with fasting glucose among first degree relatives (FDR's) of type 2 diabetes patients and to correlate GGT with body mass index (BMI), Blood pressure (BP), waist circumference (WC) and lipid profile parameters among FDR's of type 2 diabetes patients.
Materials and Methods: In this study, 150 non-diabetic FDR's of type 2 diabetic patients were enrolled. The analysis included anthropometric measur ements, BP and estimation of fasting glucose value, lipid profile and GGT. Based on fasting glucose value and according to American Diabetic Association criteria, the study subjects were classified into euglycemic, prediabetic and diabetic groups.
Results: Among 150 FDR's of type 2 diabetic patients, 36%, 31.3% and 32.7%subjects were euglycemic, pre diabetic and diabetic respectively. There was a positive correlation between GGT and systolic BP ($r=0.205$, $p=< 0.05$), GGT and total cholesterol ($r=0.198$, $p< 0.05$) and GGT Vs LDL ($r=0.198$, $p< 0.05$). There was a significant negative correlation between GGT and HDL($r=-0186$, $p<0.05$). There was a significant association between fasting glucose and area under the curve (AUC) of GGT (AUC= 0.722, $P<0.001$). Receiver operating characteristic curve analysis shows that the cutoff of GGT as a predictive value for type 2 diabetes was 29U/L.
Conclusion: Elevated levels of GGT, although in normal ranges, especially at higher quartiles play an important role as a predictor of diabetes among FDR's of type 2 diabetic patients.

1. Introduction

Serum Gamma glutamyl transferase (GGT) is a well known marker commonly used to diagnose liver disease and alcoholism. It is present on the cell membranes of many tissues like liver, kidney and pancreas. GGT catalyzes the transfer of gamma glutamyl group from glutathione to other acceptors and is thus responsible for the extracellular catabolism of glutathione. Thus GGT is involved in glutathione homeostasis. Glutathione acts as an antioxidant by inactivating free radicals formed inside red blood cells. Hence increased level of GGT may be linked to greater oxidative stress. Increased oxidative stress has been implicated in β cell dysfunction leading to reduced insulin action. Therefore, serum GGT activity could reflect several different processes relevant to diabetes pathogenesis. [1]

In the World Health Organization (2014) report, it has been mentioned that globally about 347 million people are diagnosed with type 2 diabetes and the number is increasing steadily. [2] Serum GGT levels and type 2 diabetes may be biologically linked through circulating insulin level, oxidative stress, and non-alcoholic fatty liver disease. [3]

Several studies have proposed that high level of GGT is an independent risk factor of metabolic syndrome, diabetes, cardiovascular disease, stroke and hypertension. [4–6] In addition to alcohol, obesity has been found to have a major effect on serum GGT. Non alcoholic fatty liver disease is a condition where there will be excess deposition

* *Corresponding author*.
E-mail address: drmaithricm@gmail.com (C. M. Maithri).

https://doi.org/10.18231/j.ijcbr.2019.096

of fat in the liver. This disease is closely associated with increased levels of serum GGT, hyperinsulinemia and insulin resistance. These interrelations between serum GGT and other metabolic disorders raise the possibility that elevated serum GGT can help in predicting the development of metabolic syndrome and type 2 diabetes.[7]

Many prospective studies have demonstrated that GGT is a strong and independent predictor of type 2 diabetes.[7,8] But in a study by Tohidi et al, they demonstrated that GGT was not independently associated with diabetes, but after adjustments for family history, anthropometric factors and blood pressure, it had relationship with type 2 diabetes.[9]

In a study by Nannipieri M et al, it was presented that GGT enzyme was clustering with most of the features of metabolic syndrome and at follow up it was proved that GGT is a predictor of incident type 2 diabetes.[8]

Nakanishi N et al, in their cohort study investigated the association of serum liver enzymes like a lanine transaminase, aspartate transaminase, alkaline phosphatase and GGT. They concluded that among the liver enzymes GGT was the most powerful indicator of risk of incidence of the metabolic syndrome and type2 diabetes.[7]

Genetic factors have been known to influence variation in liver enzyme levels and in adults, heritability estimates for GGT range between 32-69%. Lee YS et al, in their Mendalian randomization study on general Korean population arrived at a conclusion that there is some genetic evidence for causal relationships between elevated GGT levels and increased risk of type 2 diabetes.[3]

In another study by Onat A et al, it was shown that GGT activity modestly predicted hypertension, metabolic syndrome and diabetes in each sex, independent of multiple confounders including BMI, but the strongest hazard ratio existed for diabetes.[10]

Considering the increasing rate of type 2 diabetes worldwide and its genetic association, this study was planned to assess the association between serum GGT levels and fasting glucose among the first degree relatives (FDR's) of type 2 diabetes patients.

2. Materials and Methods

This cross-sectional study was conducted after taking written informed consent from the study subjects. The relevant ethical clearance was obtained from Institutional Scientific Committee and Institutional Ethics Committee of MIMS, Mandya. The study was conducted at the Clinical Biochemistry section of Central diagnostic laboratory, MIMS, Mandya. In this study, 150 participants who were the first degree relatives of type 2 diabetic patients aged between 25-65 years were enrolled after taking detailed family history, past history and treatment history. All participants with co-morbid conditions like alcoholism, hepatitis, liver disorders, myocardial infarction, thyroid disorders, renal disease, inflammatory disease or taking any other medications were excluded from the study.

Anthropometric measurements included height (cm) which was measured by stadiometer and weight by using analog weight scale. Body mass index (BMI) was calculated by the formula (weight in kg / height in m^2) - BMI was classified according to WHO as normal (18.0 to 22.9), overweight (23.0 to 24.9), obese ($\geq$ 25) respectively. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest. Blood pressure was measured by using mercury sphygmomanometer.

Patients were advised to fast at least 8-10 hrs before lab tests and not to take any medications that affect carbohydrate metabolism. Under aseptic precautions 3ml of fasting venous blood w as drawn and after appropriate processing it was used for the estimation of glucose, lipid profile parameters like total cholesterol (TC), triglycerides (TG), Low density lipoprotein (LDL), High density lipoprotein (HDL) and GGT in fully automated Abbott Architect analyzer (ci4100) at Clinical Biochemistry Lab, MIMS Mandya. Fasting plasma glucose was measured by hexokinase method. Total cholesterol was analyzed by cholesterol oxidase peroxidase CHOD-POD method. Triglyceride levels were estimated by glycerol peroxidase (GPO-PAP) method. Direct enzymatic assay was used for the estimation of HDL. GGT was measured by enzymatic method. The reference range of GGT by this method is 12-64 U/L. LDL cholesterol was calculated by using Friedwald's formula.

After analyzing the fasting glucose (FPG) value and applying ADA criteria, the study subjects were classified into euglycemic, prediabetic and diabetic groups. Subjects with FPG<110mg/dl were considered as euglycemic, FPG 110mg/dl $\leq$ 125mg/dl were considered as prediabetic and with FPG >126mg/dl were considered as diabetic.

2.1. Statistical methods

The data was analyzed using descriptive and inferential statistics by SPSS 22.0, ver.3.2.2. To generate graphs and tables, Microsoft word and Excel have been used. Results are presented as Mean $\pm$ SD for continuous variables and on categorical measurements as Number (%).

Analysis of variance (ANOVA), Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. P value <0.05 were considered statistically significant. The study subjects were divided into quartiles based on GGT levels as follows: <16.5U/L, 16.5-21.9U/L, 22 -30.5U/L and >30.5U/L and the proportion of normal, prediabetic and diabetic patients were assessed according to the quartiles of serum GGT level. Receivers operating characteristic curve (ROC) analysis and Area under the ROC curve of the logistic regression model were used to predict the outcome.

3. Results

In the present study, out of 150 non diabetic FDR's of type 2 diabetes patients 51% were females and 49% of them were males. Considering their fasting glucose value and according to ADA criteria, 36%, 31.3% and 32.7% subjects were euglycemic, pre diabetic and diabetic respectively. This is shown graphically as pie chart (Figure 1).

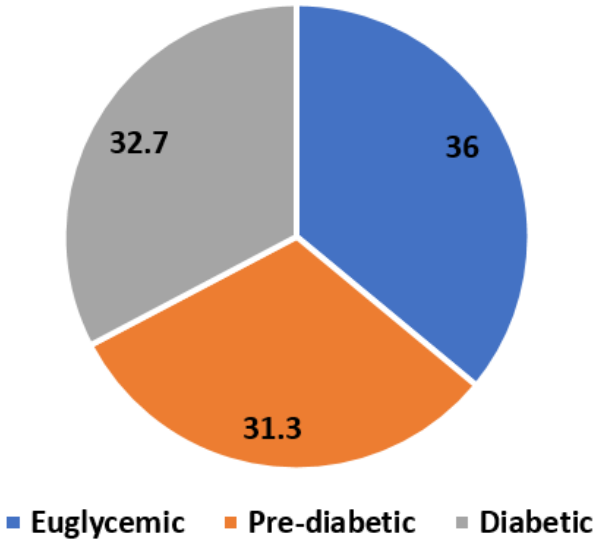

Fig. 1: Distribution of subjects by fasting plasma glucose ($p > 0.05$ statistically not significant)

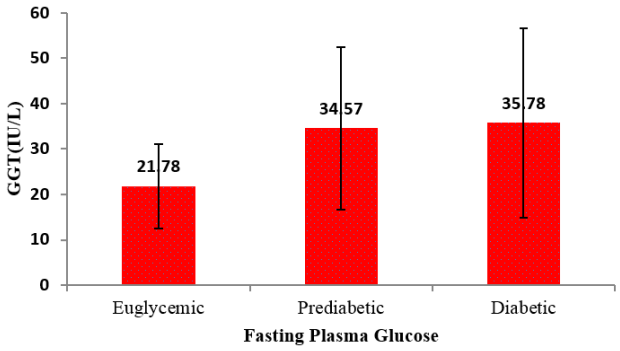

Fig. 2: Comparison of GGT according to FBS of patientsstudied

In our study it was found that the mean and SD values of WC, systolic blood pressure (SBP) and diastolic blood pressure (DBP) showed statistically significant higher levels among prediabetic and diabetic when compared to euglycemic FDR's. When lipid profile parameters were compared, the mean±SD values of TG was high among prediabetic and diabetic FDR's compared to euglycemic FDR's of type 2 diabetic patients and the difference was statistically significant with p<0.001 (Table 1).

The mean values of GGT was 21.78±9.35, 34.57±20.82 and 35.78±17.67 among euglycemic, prediabetic and diabetic respectively and the difference was statistically significant with P<0.001 as shown in Figure 2.

Study subjects were categorized into quartiles based on GGT values as <16.5U/L, 16.5-21.9U/L, 22-30.5U/L and >30.5U/L. The proportion of euglycemic, prediabetic and diabetic patients according to the quartiles of GGT is presented in Figure 3. It was found that there was more proportion of prediabetic and diabetic study subjects in the higher quartiles of GGT.

In our study it was found that the SBP, TC, TG and LDL steadily increased across GGT quartiles (Table 2). The difference in the mean values of the mentioned study variables across GGT quartiles was statistically significant.

In Pearson correlation analysis (Table 3) serum GGT levels showed significant positive correlation with SBP (r= 0.205, P<0.05) TC (r=0.198, P<0.05) and LDL (r=0.198, P<0.05). There was a statistically significant negative correlation between GGT and HDL (r=-0.186, P<0.05).

In the present study it was observed that on applying receiver operating characteristic curve (ROC) analysis of the logistic regression models, cutoff of GGT as a predictive value for type 2 diabetes was 29U/L (Table 4).

From the ROC curve (Figure 4) it was found that, the area under the curve (AUC) for GGT was 0.722. Hence in our study population GGT emerged as a biomarker to assess the risk of development of diabetes among FDR's of type 2 diabetes patients.

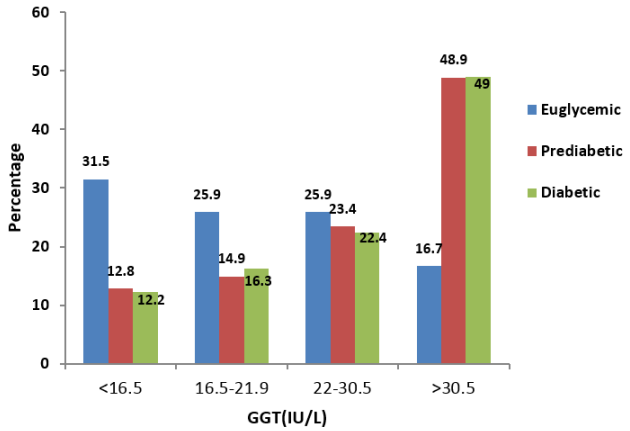

Fig. 3: Proportion of normal, prediabetic and diabetic according to quartiles of GGT

4. Discussion

Diabetes mellitus has emerged as one of the most challenging health problems of the 21st century. Type 2 DM has a strong genetic component. Individuals with a parent with type 2 DM have an increased risk of diabetes. The disease is polygenic and multifactorial since in addition to

Table 1: Comparison of clinical variables according to FBS of patients studied

Variables	Fasting Plasma Glucose			p value
	Euglycemic	Prediabetic	Diabetic	
BMI (kg/m2)	26.40±4.03	26.24±4.50	26.06±4.09	0.920
Waist Circumference	88.32±12.17	94.00±7.82	94.18±6.66	0.002**
SBP (mm Hg)	122.22±12.57	132.89±12.59	133.04±15.75	<0.001**
DBP (mm Hg)	83.26±9.01	86.34±8.32	88.51±8.57	0.010**
Total Cholesterol (mg/dl)	185.89±36.19	194.11±33.63	199.18±33.61	0.146
TGL (mg/dl)	147.26±58.12	177.45±67.34	196.27±67.89	0.001**
HDL (mg/dl)	37.31±9.00	36.08±8.45	37.25±8.76	0.739
LDL (mg/dl)	117.56±30.52	121.15±36.42	120.67±31.09	0.832
GGT(IU/L)	21.78±9.35	34.57±17.95	35.78±20.82	<0.001**

** $p < 0.01$ statistically significant

Table 2: Comparison of clinical variable according to GGT of patients studied

Variables	GGT(IU/L)				Total	p value
	<16.5	16.5-21.9	22-30.5	>30.5		
BMI (kg/m2)	26.05±4.45	27.98±4.88	25.70±4.09	25.79±3.52	26.24±4.17	0.093
Waist Circumference	90.40±12.51	93.03±10.52	91.64±9.49	92.57±7.62	92.02±9.68	0.717
SBP (mm Hg)	122.17±15.48	127.07±13.53	130.94±12.32	132.55±14.84	129.10±14.55	0.011*
DBP (mm Hg)	83.03±10.98	86.38±7.88	85.19±8.17	87.70±8.35	85.94±8.87	0.130
FBS (mg/dl)	122.83±70.16	137.34±80.54	131.11±64.32	140.71±44.65	134.30±62.42	0.634
Total Cholesterol (mg/dl)	184.72±40.09	191.66±32.56	184.28±30.74	203.07±33.60	192.81±34.79	0.033*
TGL (mg/dl)	135.69±56.23	184.62±79.56	179.81±60.69	181.20±64.34	172.73±67.16	0.010**
HDL (mg/dl)	38.55±9.91	39.07±10.86	34.65±5.68	36.39±8.23	36.91±8.71	0.142
LDL (mg/dl)	119.16±32.98	111.09±31.17	110.99±27.72	130.03±33.51	119.70±32.48	0.015*

*$p < 0.05$, **$p < 0.01$ statistically significant

Table 3: Pearson correlation of GGT with other study variables

Pair	r value	p value
GGT(IU/L)vs Waist Circumference	0.015	0.859
GGT(IU/L)vs SBP (mm Hg)	0.205	0.012*
GGT(IU/L)vs DBP (mm Hg)	0.106	0.196
GGT(IU/L)vs Total Cholesterol (mg/dl)	0.198	0.015*
GGT(IU/L)vs TGL (mg/dl)	0.155	0.058
GGT(IU/L)vs HDL (mg/dl)	-0.186	0.023*
GGT(IU/L)vs LDL (mg/dl)	0.198	0.015*

*$p < 0.05$ statistically significant

Table 4: ROC curve analysis

Variables	ROC results to predict diabetic				Cut-off	AUROC	SE	p value
	Sensitivity	Specificity	LR+	LR-				
GGT (IU/L)	53.06	83.3	3.18	0.56	>29	0.722	0.049	<0.001**

**$p < 0.01$ statistically significant

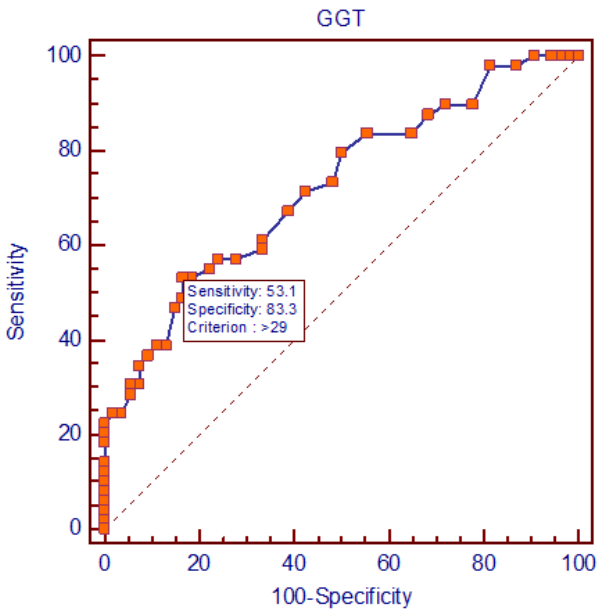

Fig. 4: Receiver operating characteristic (ROC) curve of GGT with fasting plasma glucose

genetic susceptibility, environmental factors such as obesity, nutrition, and physical activity modulate the phenotype.[11]

Oxidative stress has been implicated in the pathogenesis of diabetes. The cause of oxidative stress is both increased production of reactive oxygen species and sharp reduction in antioxidant defenses system of our body. Both these phenomenon leads to altered cellular redox status. Hyperglycemia through multiple mechanisms may lead to an increased generation of free radicals. Serum GGT is an ectoplasmic enzyme and plays an important role in glutathione homeostas is thus protecting cells against oxidative stress. Many studies have demonstrated that serum concentration of GGT could be used as a marker for increased oxidative stress in humans.[12] Many studies have reported an association of GGT concentration with incidence of type 2 diabetes.[13,14]

The present study demonstrates that the mean values (Table 1) of WC, systolic blood pressure and diastolic blood pressure was significant higher in prediabetic and diabetic FDR's compared to euglycemic FDR's. Increased WC is one of the risk factor for metabolic syndrome and Diabetes. This was in accordance with the study by Music M et al where they observed increased systolic and diastolic BP in patients with type 2 diabetes compared to healthy controls. The cause of hypertension in diabetes is multifactorial. Hyperglycemia, insulin resistance and inflammation may have contributory role in the development of hypertension in diabetic patients.[14]

In our study we observed that the mean values of lipid profile parameters like TC, TG, LDL were higher and HDL values were lower in prediabetic and diabetic FDR's compared to euglycemic FDR's. But only TG values showed statistically significant difference in the mean values (Table 1). Chahil et al demonstrated that prevalence of high plasma TG levels was seen in individuals with DM.[15] This occurs when insulin deficient or insulin resistance is present as lipolysis is accelerated and plasma non esterified fatty acids concentration rise.

Our study showed that the mean± SD for GGT among euglycemic, prediabetic and diabetic was 21.78±9.34, 34.57±17.95 and 35.78±20.82 respectively, indicating increased mean values among prediabetic and diabetic (Figure 2) which was statistically significant with p value of <0.001. (Table 1) This was in total agreement with the study by Haghighi S et al where they showed that the mean GGT values were higher in prediabetic and diabetic FDR's compared to euglycemic FDR's.[1]

When the study subjects were categorized based on the quartiles of GGT it was observed that the proportion of prediabetic and diabetic subjects was more in higher quartiles of GGT (Figure 3). The study parameters of participants, graded by GGT quartiles are shown in Table 2 and systolic BP, TC, TG and LDL steadily increased across GGT quartiles indicating that the risk factors associated diabetes increased with higher quartiles of GGT. This was in contrast with the study done by Zoppini G et al and they showed that BMI and serum Triglycerides steadily increased across the quartiles of GGT but BP, TC, LDL and HDL concentrations were not different across GGT quartiles.[16]

Our study showed a significant correlation between GGT and other variables. The correlation analysis showed that there was statistically significant positive correlation between GGT and SBP, GGT Vs TC and GGT Vs LDL (Table 3). There was a statistically significant negative correlation between GGT and HDL indicating that there is a relation between serum GGT levels and risk of development of type 2 diabetes. Meena SK et al showed similar findings in their study.[17]

According to the results (Table 4) ROC curve analysis, cutoff of GGT as a predictive value for type 2 diabetes was 29U/L. In contrast to our finding, Highighi S in their study found that the cutoff of GGT was 14U/L[1]. The area under the curve for GGT was 0.722 (Figure 4). This indicates that there was an association between GGT and risk of development of type 2 diabetes.

Lee YS et al in their Mendelian randomization study showed that there is some genetic evidence for causal relationship between elevated GGT levels and increased risk of type 2 DM in general Korean population.[3] Many theories have been put forward to explain the possible mechanisms on how serum GGT increases the risk of type 2 diabetes. Nonalcoholic fatty liver disease, a condition with excess deposition of fat in the liver may lead to elevation of serum GGT through hepatic insulin resistance

and hyperinsulinemia. GGT serves as an oxidative stress marker through its mediation on glutathione homeostasis and hence plays an important role in antioxidant system. Hence, Raised GGT concentrations could be a marker of oxidative stress, which might be implicated in the cause and development of diabetes.[18]

Nakanishi et al conducted a study on middle aged Japenese men and found that the risk of development of IFG or type 2 diabetes increased as serum GGT increased and this increase was in dose dependent manner middle.[7] Serum GGT showed a strong and graded relation with DM according to a study by Mesinger et al.[18] Our results are consistent with most of these studies.

The limitation of the current study is that, sample size was small. Our results would have been more conclusive if the sample size was larger.

5. Conclusion

In conclusion, our study showed that elevated serum GGT levels were associated with risk of development of diabetes among FDR's of type 2 diabetes patients. This indicates that serum GGT can be used as an additional biomarker in subjects with high risk for development of diabetes. Although more population based and research on the underlying pathophysiology is needed, measurement of serum GGT is easy and inexpensive and could have practical and clinical implications in the management of FDR's of type 2 diabetes patients.

6. Conflict of interest

None

7. Source of support

Nil

References

1. Haghighi S, Amini M, Pournaghshband Z, Amini P, Hovesepian S. Relationship between gamma-glutamyltransferse and glucose intolerance in first degree relatives of type 2 diabetic patients. *J Res Med Sci*. 2011;16(2):123–129.
2. 2.World Health organization. (2014) Global status report on noncommunicable diseases 2014. WHO publication. Geneva, World Health Organization ;. .
3. Lee YS, Cho Y, Burgess S, Smith GD, Relton CL, Shin SY. Serum gamma glutamyltransferase and risk of type 2 diabetes in the general Korean population: a Mendelian randomization study. *Human Molecular Genetics*. 2016;25(17):3877–3886.
4. Lee DH, Jacobs DR, Gross M, Kiefe CI, Roseman J, Lewis CE. Gamma- glutamyl transferase is a predictor of incident diabetes and hypertension: the coronary Artery Risk Development in young adults (CARDIA) Study. *Clin Chem*. 2003;49:1358–1366.
5. Jousilahti P, Tuomilehto RD. Serum g-Glutamyl transferase, self - reported alcohol drinking, and the risk of stroke. 2000;31:1851–1855.
6. Iqbal A, Iftikhar U, Ali FA, Memon S, Zuberi N. Comparison of gamma glutamyltransferase in normal and type 2 diabetics. *J Pak Med Assoc*. 2010;60(11):945–948.
7. Nakanishi N, Suzuki K, Tatara K. Serum γ- Glutamyltransferase and risk of metabolic syndrome and type 2 Diabetes in middle-aged Japanese men. Diabetes Care. *Diabetes Care*. 2004;27(6):1427–1432.
8. Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K. Liver enzymes,the metabolic syndrome and incident diabetes. *Diabetes care*. 2005;28(7):1757–1762.
9. Tohidi M, Harati C, Hadaegh F, Mehrabi Y, Azizi F. Association of liver enzymes with incident type 2 diabetes: a nested case control study in an Iranian population. *BMC Endocr Disord*. 2008;8(5). PubMed 18533046.
10. Onat A, Can G, Ornek E, Cicek G, Ayhan E, Dogan Y. Serum γ glutamyl transferase: Independent predictor of risk of Diabetets, Hypertension, Metabolic syndrome, and Coronary disease. *Nature Pub Obes*. 2012;20(4):842–848.
11. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Lango DL, et al. Harrison's principles of internal medicine. 16th ed. McGraw-Hill: Medical publishing division ; 2008,.
12. Bozbas H, Yildirir A, Karacaglar E, Demir O, Uls T, Eroglu S. Increased gamma- glutamyl transferase activity in patients with metabolic syndrome. *Arch Turk Soc Cardiol*. 2011;39(2):122–128.
13. Ahn HR, Shin MH, Nam HS, Park KS, Lee YH, et al. The association between liver enzymes and risk of type 2 diabetes: the Namwon study. *Diabetol Metab Syndr*. 2014;6(14).
14. Music M, A D, Pepic E, Lepara O, Fajkic A, Buturovic BA. Metabolic syndrome and serum liver enzymes levels at patients with type 2 diabetes mellitus. *Med Arh*. 2015;69(4):251–255.
15. Chahil TJ, Ginsberg HN. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am*. 2006;35:491–510.
16. Zoppini G, Targher G, Trombetta M, Lippi G, Muggeo M. Relationship of serum - glutamyltransferase to atherogenicdyslipidemia and glycemic control in type 2 diabetes. *Nature Publishing Group*. 2009;17(2):370–374.
17. Meena SK, Meena A, Ahuja J, Bohra VD. A study of gamma glutamyl transferase (GGT) in type 2 diabetes mellitus patients and its risk factors. *IJCR*. 2012;04(11):89–95.
18. Meisinger C, Lowel H, Heier M, Schneider A, Thorand B. Serum - glutamyltransferase and risk of type 2 diabetes mellitus in men and women from general population. *J Internal Med*. 2005;258:527–535.

Author biography

C M Maithri Assistant Professor

P Manoj Final Year MBBS Student

S K Mahadev Associate Professor

Tejaswani Tutor

Cite this article: Maithri CM, Manoj P, Mahadev SK, Tejaswani . Association of gamma glutamyl transferase activity with fasting glucose among first degree relatives of type 2 diabetes patients. *Int J Clin Biochem Res* 2019;6(4):458-463.