Gamma Glutamyl Transferase in Impaired Glucose tolerance and type 2 Diabetes Mellitus

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Abstract

Aim: The current study was undertaken, 1. to compare serum levels gamma glutamy transferase in among three groups namely type II diabetes mellitus, impaired glucose tolerance and apparently healthy controls; 2. to measure the association of gamma glutamy transferase levels and the risk of type II diabetes mellitus by logistic regression and to detect the best cut-off value for gamma glutamy transferase in type II diabetes mellitus and impaired glucose tolerance by ROC curve.

Materials and Method: The study was conducted at tertiary care hospital, Bagalkot, Karnataka, India. Under aseptic precautions 5 ml of fasting venous sample was collected and biochemical parameters FBS, PPBS, HbA1c, lipid profile, liver enzymes, serum gamma glutamy transferase were estimated. SPSS for window version; SPSS, 11.5 Inc, Chicago IL was used for statistical analysis.

Results: Only gamma glutamy transferase and BMI could fit in the model, whereas raised gamma glutamy transferase was associated with type II diabetes mellitus with odds ratio of 1.37 (P<0.001), whereas in the presence of raised BMI, odds ratio was 1.44(P<0.008). Even in impaired glucose tolerance, raised gamma glutamy transferase was associated with OR of 1.55 (p<0.001). The best cut-off value for gamma glutamy transferase was 24.3 mg/dL, in both type II diabetes mellitus and impaired glucose tolerance.

Conclusion: An increase in gamma glutamy transferase concentration within its physiological range is a sensitive and early biomarker for the development of impaired glucose tolerance and type II diabetes mellitus, with best cut-off value of 24.3 mg/dL.

Keywords: Diabetes mellitus, Impaired glucose tolerance, Gamma glutamyl transferase

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Introduction

Gamma-Glutamyl Transferase (GGT) is a cell-surface enzyme helps in extracellular metabolism of glutathione (GSH). Most serum GGT is contributed by the liver, even it is synthesized by most of the tissues in the body.[1] Serum GGT has been commonly used as a marker of alcoholic liver injury.[2] GGT is an intracellular antioxidant acts extracellular through glutathione.[3,4] GGT activity could be a marker of chronic inflammation.[5] Low-grade inflammation is found in a number of pathological conditions such as aging, obesity, physical inactivity, atherosclerosis, hypertension, dyslipidemia, and hyperinsulinemia by mediating or inducing oxidative stress,[6] it implies that increased GGT concentration is associated with metabolic syndrome.[6-8] Perry IJ et al in their cohort study on non-diabetic men found that serum GGT concentration was an independent predictor of incident type II diabetes mellitus (DM).[8] Many cross sectional studies have showed an association between diabetes mellitus and serum GGT levels independently of obesity.[9-11] The studies also showed that obesity is associated with high prevalence of diabetes among individuals with high-normal serum GGT levels, and not among low-normal serum GGT levels, it suggests that obesity itself may not be a sufficient risk factor for development of diabetes mellitus and estimation serum GGT levels can be helpful for detecting high risk individuals for type II diabetes mellitus.[12] Studies showed that increased serum GGT concentration can predict type 2 diabetes mellitus independently.[8,13-17]

Both ALT and GGT, even within the normal limit, have been reported to predict incident diabetes mellitus.[18] However, while some studies have demonstrated an association between GGT and diabetes than between alanine transaminase (ALT) and diabetes mellitus,[19,20] many prospective studies showed that raised levels of serum GGT levels within normal range, showed a dose–response relationship with type II diabetes (DM) incidence[14-16,21,22] irrespective of alcohol intake. Many studies have showed serum GGT concentration within higher normal range is a sensitive and early marker for the detection of diabetes mellitus. Only few studies have compared serum GGT concentration between DM and impaired glucose tolerance (IGT). The separate association of liver enzymes with IGT rather than type 2 DM has received less attention.[6]

Hence the current study was undertaken, 1. to compare levels of serum GGT among three groups namely type II DM, IGT and apparently healthy controls; 2. to measure the association of GGT levels

and the risk of type II DM by logistic regression and to find the best cut-off value for GGT in type II DM and IGT by ROC curve.

Materials and Method

The study was conducted at Hanagak Shri Kumareshwara hospital, Bagalkot from Feb 2015 to July 2015. The study was approved by institutional ethics committee. Informed consent was obtained from all the subjects.

The diagnosis of type 2 DM and IGT were based on WHO criteria. 60 subjects participated in each group (DM, IGT, Controls). Alcoholics, smokers, patients with diabetic complications, chronic liver diseases, chronic renal failure and patients with other systemic conditions were excluded from the study. Detailed history, genera physical examination and systemic examination was performed for all the participants. Under aseptic precautions 5 ml of fasting venous sample was collected and biochemical parameters FBS, PPBS, HbA1c, lipid profile, liver enzymes, serum GGT were estimated. Serum GGT was estimated by IFCC method kit supplied by Transasia (Normal 7-50 IU/L).

Statistical analysis: Study power was calculated (100%) retrospectively based on the mean serum GGT values in cases and controls. SPSS for window version; SPSS, 11.5 Inc, Chicago IL was used for statistical analysis. ANOVA followed by Posthoc Dunnet's test was applied to compare between three groups. ROC curve was constructed to know the diagnostic accuracy and best cut-off values for serum GGT in type II DM and IGT groups. Logistic regression analysis was done to know association between raised GGT and combination of raised GGT and BMI among type II DM and IGT groups.

Results

Base line characteristics and biochemical parameters in DM, Controls and IGT are shown in table 1. There was a statistical difference in all the parameters except age, low density lipoprotein (LDL), aspartate transaminase (AST) and alanine transaminase (ALT) (Table 1, highlighted by bold and italic).

BMI was significantly higher in cases when compared to healthy controls and there was no significant difference when compared between the IGT and control and between the type 2 DM and IGT groups.

The serum concentration of GGT was more in IGT group when compared with type II DM, but statistically not significant, whereas when compared with controls, GGT is raised significantly in both type II DM and IGT, similar results were with serum urea and uric acid. Serum HDL concentration was decreased in type II DM and IGT groups as compared to healthy controls.

Concentration of creatinine, TGL and VLDL were increased in type II DM as compared to controls and IGT group, but was not statistically high in IGT group compared to controls

All the variables were included in multiple logistic regression, and two models were developed, one for diabetes mellitus and another for impaired glucose tolerance. The criteria for entering and removing the independent variables from backward stepwise model was $p<0.05$. The risk factors which predict the DM are as follows, in model 1- All the risk factors were entered in regression model, only GGT and BMI could fit in the model, whereas raised GGT was associated with occurrence of DM with odds ratio (95% CI) of 1.37 (1.15-1.64)($P<0.001$), whereas in the presence of raised BMI, odds ratio(95%CI) was 1.44(1.10-1.89)($P<0.008$). There was association between GGT and DM, when all the factors were controlled (Table 2).

Even in IGT, raised GGT was associated with the clinical condition with OR (95%CI) of 1.55(1.11-2.16)($p<0.001$) (Table 3).

The area under the ROC curve for GGT at various cut-off was 0.95(95% confidence interval, 0.86-0.99; $P < 0.0001$) in DM, as shown in figure 1. Sensitivity and specificity of serum GGT concentration in type II DM at various cut-off values is shown in table 4. A maximum sensitivity of 94.74% and specificity of 93.75% were achieved in diabetes at the best cut-off of GGT greater than 24.3 mg/dL.

A receiver operator characteristics (ROC) curve was obtained. The area under the ROC curve for GGT in IGT patients at various cut-off was 0.984(95% confidence interval, 0.89-1.000; $p < 0.0001$) in (Fig. 2). Sensitivity and specificity of GGT for IGT at various cut-off values is shown in Table 5. A sensitivity of 100% and specificity of 93.75% were achieved to detect IGT greater than 24.3 mg/dL. At cut-off value of greater than 0.12, sensitivity was 100% but specificity reduced to 25%.

Table 1: Base line characteristics and biochemical parameters in DM, Controls and IGT

Parameter	Control	IGT	Type 2 DM	F	P ANOVA	Type 2 DM and & IGT
Age in years	***47.6±10.2***	***42.2±15.1 NS***	***45.0±13.2NS***	***1.2***	***0.302***	***NS***
BMI	21.1±2.6	23.7±5.6 NS	25.6±3.7***	9.3	0.000	NS
FBS *mg/dl*	87.6±12.2	117.2±5.2***	176.9±37.6***	93.5	0.000	***
PPBS *mg/dl*	112.4±14.4	176.2±18.6***	274.0±76.4***	80.8	0.000	***
GGT *mg/dl*	18.8±5.2	46.4±22.3***	44.5±15.4***	27.3	0.000	NS

HbA1c *%*	5.3±0.6	6.3±0.9**	7.8±1.4***	39.1	0.000	***
Uricacid *mg/dl*	3.6±0.9	6.7±0.7***	6.4±0.9***	76.5	0.000	NS
Urea *mg/dl*	20.9±6.5	31.7±7.4***	32.4±9.5***	16.7	0.000	NS
Creatinine *mg/dl*	0.9±0.1	0.9±0.1 NS	1.2±0.2***	12.4	0.000	***
TGL *mg/dl*	116.8±38.4	122.6±17.2 NS	151.2±32.8***	6.6	0.003	*
TC *mg/dl*	188.7±31.6	164.9±5.8*	191.2±36.5 *NS*	3.2	0.046	*
HDL *mg/dl*	50.5±5.1	36.6±4.1***	33.6±3.2***	98.7	0.000	NS
VLDL *mg/dl*	23.2±7.6	24.5±3.4 NS	30.2±6.5***	6.5	0.003	*
LDL *mg/dl*	**115.0±26.4**	**103.7±6.7 NS**	**122.4±33.6 NS**	**1.8**	**0.170**	**NS**
AST *mg/dl*	**17.7±6.6**	**20.8±4.6 NS**	**19.3±4.9 NS**	**1.35**	**0.26**	**NS**
ALT *mg/dl*	**15.1±4.2**	**17.1±3.6 NS**	**17.9±3.5 NS**	**2.7**	**0.070**	**NS**
hsCRP *mg/L*	1.8±1.03	5.3±6.2 *NS*	12.7±12.8***	12.2	0.001	**

***: p <0.05, **: p <0.01, ***: p <0.001, NS: Not significant**

Note: *,** and *** in cases column represent the p value of comparison between the type 2 DM and control, similarly in impaired column represent p value of comparison between the IGT and control. Column title type 2 DM and IGT represent the p value of comparison between the type 2 DM and IGT.

Table 2: Logistic regression for association of GGT and type II DM.

	Cases	Odd's ratio	95% CIP	P
Model 1	GGT	1.37	1.15-1.64	0.001
Model 2	GGT	1.44	1.10-1.89	0.008
	BMI	1.9	1.09-3.34	0.002

Table 3: Logistic regression for studying the association of GGT and IGT

	Odd's ratio	95% CIP	P
GGT	1.55	1.11-2.16	0.01

Table 4: Criterion values and coordinates of the ROC curve for DM cases

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=10.8	100	82.4 - 100.0	0	0.0 - 10.9	1	
>16.7	100	82.4 - 100.0	40.63	23.7 - 59.4	1.68	0
>18	94.74	74.0 - 99.9	40.63	23.7 - 59.4	1.6	0.13
>24.3 *	94.74	74.0 - 99.9	93.75	79.2 - 99.2	15.16	0.056
>29.7	78.95	54.4 - 93.9	93.75	79.2 - 99.2	12.63	0.22
>31	78.95	54.4 - 93.9	100	89.1 - 100.0		0.21
>84.2	0	0.0 - 17.6	100	89.1 - 100.0		1
* Criterion corresponding with highest Youden index						

Table 5: Criterion values and coordinates of the ROC curve for IGT

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>=10.8	100	73.5 - 100.0	0	0.0 - 10.9	1	
>24.3 *	100	73.5 - 100.0	93.75	79.2 - 99.2	16	0
>29.4	75	42.8 - 94.5	93.75	79.2 - 99.2	12	0.27
>31	75	42.8 - 94.5	100	89.1 - 100.0		0.25
>107.1	0	0.0 - 26.5	100	89.1 - 100.0		1
* Criterion corresponding with highest Youden index						

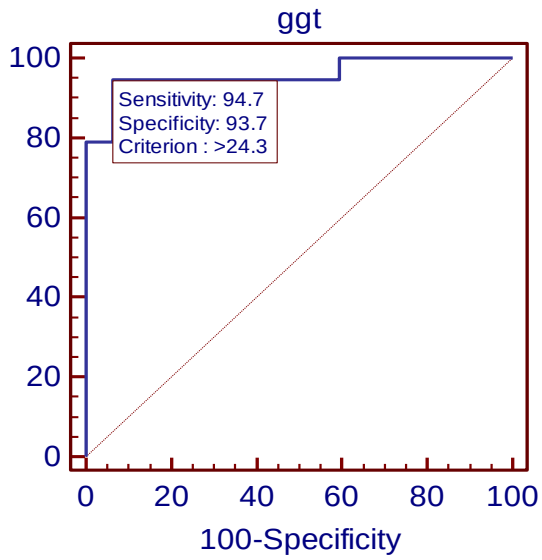

Fig. 1: ROC curve at various cut-off points for GGT in DM cases

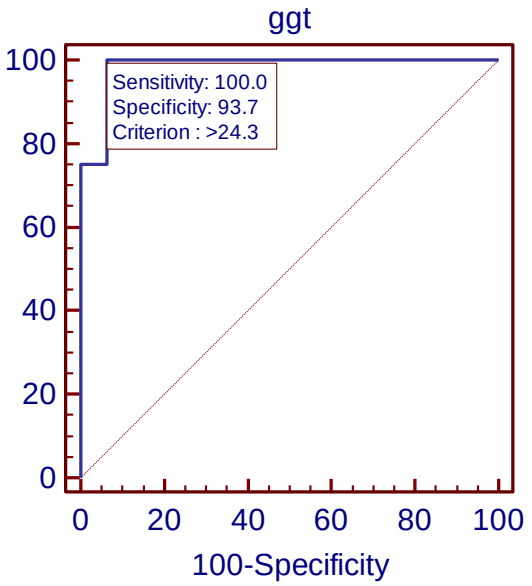

Fig. 2: ROC curve at various cut-off points of GGT for IGT

Discussion

In the current study, GGT was increased significantly in type II DM and IGT as compared to healthy control group, but there was no significant difference between type II DM and IGT. Our findings are similar to Zoppini et al,[12] Sabanayagam et al[23] and Nannipieri M et al,[6] where serum GGT concentrations were increased in type II DM patients compared to healthy controls. Nannipieri M et al there study found that serum GGT concentration was significantly increased in IGT compared to control group (p=0.0003), current study also observed similar results (p<0.001).

Modest increase in GGT within normal range has been found to predict hypertension, as well as incident cases of type II diabetes. Monica Nannipieri, et al[6] in their study found that GGT was the only parameter associated with DM and IGT, after controlling the other main predictors, with odds ratio of 2.05. Abigail Fraser et al and C. Meisinger et al[15] found that GGT may be better predictor of DM (Hazard ratio 1.24-1.55 and 1.85-2.27 respectively). D.H. Lee et al,[14,21] suggest that an increase in GGT concentration within its physiological range is a sensitive and early biomarker for the development of diabetes. Third National Health and Nutrition Examination Survey in the USA[24] and another Korean prospective cohort study[14] claimed that BMI was associated with diabetes only when GGT was in its high normal limit.[24] However, other prospective studies have failed to show a significant interaction between GGT and BMI on the incidence of DM[15,21] or have found a significant interaction only when the five year risk was analysed, and not for the total 15-year risk.[16] The increased serum GGT concentration elevated the incidence of DM in both genders after adjustment for several confounding factors including alcohol, BMI and ALT. In the current study also increased serum GGT concentration was associated with type II DM with odd's ratio of 1.37 (95% CI) (1.15-1.64) p<0.0001, where as in the presence of BMI odd's ratio was 1.44 (1.10-1.89).The interaction between serum GGT and BMI was associated with occurrence of DM, when all the risk factors were controlled (age, gender, hypertension, and dyslipidemia).

US adults study showed that serum GGT concentration was positively associated in a dose-dependent manner with diabetes mellitus, independent of age, sex, education, smoking, alcohol intake, waist circumference, hypertension and serum cholesterol concentration.[23] Further analysis employing nonparametric models, there was continuous positive association between serum GGT level and diabetes mellitus across the full range of serum GGT concentration.[25] In Hisayama Study, a population based prospective cohort study in Japan and a four-year follow-up prospective study have evaluated the effects of GGT on the risk of DM among groups stratified by their alcohol intake. They showed significant positive associations between GGT and DM in all of the stratified groups, while the association weaker in alcoholics than in non-alcoholics.[22] Subanayagam C et al showed the association between serum GGT concentration and diabetes mellitus was present even after adjusting for grams of daily alcohol intake, suggesting an effect independent of alcohol intake.[23] In the present study alcoholics were excluded.

In Pima Indian study, serum AST, ALT, and GGT concentrations were within normal limit and still hepatic enzyme levels were correlated with HOMA-IR, independent of age and gender. Only serum GGT

concentration persisted as a significant determinant of HOMA-IR after additional adjustment for anthropometric variables (Weight, BMI, or % Fat).[26]

GGT was a predictor of incident IGT/type 2 diabetes at follow-up, independently of anthropometric characteristics, and plasma glucose and proinsulin concentrations.[6]

Meisinger C et al in their study, from the general population, observed a significant positive association between serum GGT levels and incident type II diabetes mellitus in both genders[15] and association was independent of other predictors of type II diabetes, like alcohol use and BMI.[15] Similarly in the current study, increased GGT levels was associated with IGT with odd's ratio (95% CI) of 1.55 (1.11-2.16) with $p<0.01$ which was statistically significant.

The area under the ROC curve for GGT at various cut-off was 0.95 in DM. A maximum sensitivity of 94.74% and specificity of 93.75% were achieved in diabetes at the best cut-off of GGT greater than 24.3 *mg/dl*. In IGT patients the area under the ROC curve for GGT at various cut-off was 0.984. A sensitivity of 100% and specificity of 93.75% were achieved to detect IGT greater than 24.3 mg/dL. This means at the best cut-off value of 24.3 mg/dL, GGT will not difference between the DM and IGT. We could not find any references in literature explaining such findings.

Limitations of the present study were small sample size and diet history was not considered. Further longitudinal studies are required to find the relationship incident IGT and DM with GGT.

In conclusion an increase in GGT concentration within its physiological range is a sensitive and early biomarker for the development of IGT and DM, with best cut-off value of 24.3 mg/dL.

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