

Original article:

Evaluation of relationship between gamma glutamyl transpeptidase (GGT) and diagnosed cases of type2 diabetes mellitus: A cross sectional study in a tertiary health care centre

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Abstract:

Introduction -Gamma glutamyl transpeptidase (GGT) is responsible for transferring glutamyl groups linked through the gamma carboxylic acid from peptides such as glutathione to acceptors. Increased GGT activity, may be a response to oxidative stress, which can increase the transport of glutathione precursors into cells is an independent predictor of many diseases, including cardiovascular diseases, type 2 diabetes and inflammation.

Aim was to estimate levels of GGT, fasting plasma glucose (FPG) and glycated hemoglobin (HbA_{1c}) in type2 DM subjects and to investigate possible correlation of GGT with glycemic control (FPG and HbA_{1c}) in diabetic subjects.

Methods – Forty(40) diagnosed type2 Diabetes Mellitus patients between age group of 40-60 yrs, and forty(40) age and sex matched healthy individuals were included in this cross sectional study. FPG, HbA_{1c} and GGT were estimated using fully autoanalyser VITROS 5600 considering p value<0.05 as significant.

Observations- We found highly significant increase in mean BMI, waist: hip ratio, serum GGT, FPG and HbA_{1c} levels in type2 DM patients compared to controls (p<0.05). We also found significant positive correlation of GGT levels with BMI (p<0.05), waist to hip ratio (p<0.05), Disease duration (p<0.001), FPG (p<0.0001) and HbA_{1c} (p<0.0001) diabetic cases.

Results with Conclusion- oxidative stress due to poor glycemic control and it's positive correlation with GGT probably indicates antioxidant role of GGT in response to oxidative stress. Therefore, it may be useful to determine serum GGT as a marker of oxidative stress in type2 DM patients with poor glycemic control.

Key Words- Gamma glutamyl transpeptidase, glycated hemoglobin, fasting plasma glucose, oxidative stress.

Introduction:

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. Hyperglycemia in diabetes results from defects in insulin secretion, insulin action or most commonly both⁽¹⁾ The prevalence of type2 diabetes mellitus is increasing regularly with International Diabetes Federation(IDF) reported in 2013 that there were 387 million diabetic patients all over the world which is projected to increase up to 592 million in 2035; a 53% increase⁽²⁾. For India, **ICMR-INDIAB** national study in 2011 reported that there were 62.4

million people with type 2 diabetes (T2DM) and 77 million people with pre-diabetes in India⁽³⁾& these numbers are projected to increase to 101 million by the year 2030⁽⁴⁾.

Previous studies like **Chait A. et al.** showed that increased level of oxidative damage to lipids in diabetes and their presence was correlated with development of complications⁽⁵⁾. Some recent experimental findings have also suggested the involvement of overproduction of reactive oxygen species (ROS) in the initiation and development of vascular complications in diabetes⁽⁶⁾⁽⁷⁾.

Gamma glutamyl transpeptidase (GGT) is a membrane bound enzyme responsible for transferring glutamyl groups linked through the gamma carboxylic acid from peptides such as glutathione to acceptors. It's main physiological function is to make cysteine available for regeneration of intracellular glutathione and protect against ROS causing oxidative stress⁽⁸⁾. **Whitfield JB. et al.**⁽⁹⁾ also reported that increased GGT activity may be a response to oxidative stress, which can increase the transport of glutathione precursors into cells. Type 2 diabetic patients are highly prone to liver function tests abnormalities than non-diabetic individuals. Previous studies have indicated that circulating concentration of liver function tests like gamma glutamyl transpeptidase (GGT) , alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are increased in individuals with insulin resistance and the metabolic syndrome^{(10),(11)(12)}. Numerous studies have found that GGT is not just a marker of alcohol consumption, but is an independent predictor of many diseases, including cardiovascular diseases, type 2 diabetes and inflammation and possibly underlies oxidative stress^{(10),(13),(14),(15)(16)}

Also a recent meta-analysis have showed that both elevated ALT and GGT were associated with increased risk of diabetes, while GGT might be a stronger risk factor than ALT⁽¹⁷⁾.

Meisinger et al. (2005) postulated that GGT is possibly a marker for increased risk of type 2 diabetes as- (a) elevated serum GGT could indicate excess fat deposits in the liver, which may cause hepatic insulin resistance and increase the risk of type 2 diabetes by contributing to systemic insulin resistance; (b) increased GGT is a marker for oxidative stress; and (c) increased GGT may be the expression of inflammation⁽¹⁸⁾. **Lee H. et al.**⁽¹⁹⁾ (2003) studied a group of 4,088 healthy, male Korean workers and found a strong dose response

relationship between serum GGT levels at baseline and incident type 2 diabetes after 4 years of follow up.

Therefore considering the unique geographical location, ethnicity, distinct culture and dietary habits of the people of North Eastern part of India, the aim was to estimate and compare serum levels of GGT, FPG (Fasting plasma glucose) and HbA_{1c} in type2 diabetic patients with that in age and sex matched healthy controls to see any significant changes of these measured parameters in type2 DM patients in this part of the country. The aim was also to find out any possible correlation of GGT with other measured biochemical parameters in type2 diabetic patients.

Materials and methods:

The study was conducted after the approval from institutional ethics committee. 40 diagnosed patients of type2 Diabetes Mellitus between age group of 40-60 yrs, of either sex, attending OPD of Gauhati Medical College & Hospital and 40 age and sex matched healthy individuals were included in this cross sectional study. Informed consent duly signed by each participant was taken. Persons with history of infectious and hepatocellular diseases, renal diseases, Cardio-vascular diseases, alcoholism and smoking and female taking oral contraceptives were excluded from the study. Taking aseptic and antiseptic precautions, about 6 ml of fasting blood was collected by venous puncture and transferred to appropriate sterile vials. For estimation of glycated hemoglobin and fasting plasma glucose, blood was collected in EDTA and fluoride vials respectively while for estimation of GGT, blood was collected without any anticoagulant. The serum/plasma was separated by centrifugation at 3000 r.p.m. for 5 minutes in a centrifuge machine and immediately used for analysis on fully auto analyzer VITROS 5600. GGT was estimated by method recommended by

the International Federation of Clinical Chemistry (IFCC)⁽²⁰⁾ and plasma glucose was estimated by hexokinase method⁽²¹⁾ while HbA_{1c} was estimated by turbidometric immunoassay method⁽²²⁾.

Statistical analysis:

Statistical analysis was done using GraphPad InStat version 3.0. Data were expressed as mean \pm SD. Comparisons between case group and control group were done by unpaired T-test while Correlations were observed by using Pearson's correlation coefficient and probability (p value) < 0.05 was considered significant.

Observations and results:

On comparing the baseline characteristics of our study groups, we found highly significant increase in mean BMI ($p < 0.0001$) and Waist: Hip ratio ($p < 0.05$) in type2 Diabetes Mellitus patients (case group) compared to age and sex matched controls (Table1, Fig 2,3) . Also there was a significant positive correlation of GGT levels with BMI ($r = 0.46, p < 0.05$), waist to hip ratio ($r = 0.43, p < 0.05$) and with Disease duration ($r = 0.59, p < 0.001$) in diabetic patients of our study (Table8, Fig 9-11).

On comparing the biochemical parameters, we found highly significant increase in mean serum gamma glutamyl transpeptidase (GGT) level in type2 Diabetes Mellitus patients (case group) compared to age and sex matched controls ($p < 0.0001$) (Table 4, Fig 5). Also, both mean fasting plasma glucose (FPG) level and glycated hemoglobin (HbA_{1c}) concentration were found significantly increased in type2 Diabetes Mellitus patients ($p < 0.0001$) compared to controls (Table4, Fig 6,7) and there was a highly significant positive correlation of GGT with FPG ($r = 0.72, p < 0.0001$) and HbA_{1c} ($r = 0.69, p < 0.0001$) in diabetic patients of our study (Table 12, Fig 13,14).

Discussion:

The study was conducted with a purpose to estimate and compare serum GGT level in type2

DM with that in age and sex matched healthy controls so as to evaluate the effect of hyperglycemia on GGT, a liver enzyme. In our study, BMI and Waist to hip ratio, the indicators of obesity were found to be increased significantly in diabetic patients compared to healthy controls ($p < 0.0001$ and $p < 0.05$ respectively) and there was a positive correlation between serum GGT level and BMI as well as with waist to hip ratio in diabetic patients (case group).

The finding of our study was in corroboration with studies by **Lawlor DA. et al.**⁽²³⁾, **Kang YH. et al.**⁽²⁴⁾ and **Nakanishi N. et al.**⁽¹²⁾ who found similar increase in GGT values when compared with increase in waist hip ratio and BMI in diabetic patients. It can be explained by observation of **Iqbal A et al.**⁽²⁵⁾ that this enzyme acts as an intervening factor in the association between obesity and diabetes and that generation of free radicals, which can occur in fatty liver and central obesity, may deplete intracellular glutathione and thus induce the activity of GGT in the circulation as GGT has a known protective effect in maintaining the hepatic glutathione levels, which are crucial in antioxidant defences⁽²⁶⁾ .

In our study, we also found significantly increased mean level of serum GGT, FPG and HbA_{1c} in type2 DM patients when compared with control group ($p < 0.0001$) and GGT levels showed a highly significant positive correlation with FPG and HbA_{1c} in diabetic patients ($p < 0.0001$). The findings of our study were in corroboration with the study by **Gohel M.G. et al.**⁽²⁷⁾ who found increased level of serum GGT and it's significant positive correlations with markers of glycemic control (Fasting Blood Sugar and HbA_{1c}) in diabetic patients compared to healthy controls. Similar study by **Khubchandani A et al** also found increased level of GGT and it's significant

correlation with increased blood sugar level in diabetic patients compared to normal controls.

The mean levels \pm SD of FPG and HbA_{1c} were **196 \pm 54.28 mg/dl** and **8.50 \pm 1.85 %** respectively indicating poor glycemic control which might cause long term complications due to formation of advanced glycation products and oxidants in diabetic patients of our study. Our observation can be supported by **Dyck P.J. et al**⁽²⁸⁾ who explained that the toxic effects of hyperglycemia and its pathophysiologic derivatives such as oxidants, hyperosmolarity or glycation products can be exerted indirectly on tissues or can cause sustained alteration in cell signalling pathways (such as changes in phospholipids or kinases) induced by the products of glucose metabolism. Elevated serum GGT could also indicate excess fat deposits in the liver, which may cause hepatic insulin resistance and increase the risk of type 2 diabetes by contributing to systemic insulin resistance⁽¹⁸⁾. Primary function of GGT is to maintain the intracellular concentrations of glutathione (GSH), a critical antioxidant defence for the cell. So increased activity of GGT can be a response to oxidative stress, facilitating increased transport of GSH precursors into cells. In addition, GGT is leaked into the serum possibly because of various mechanisms such as increase in oxidative stress, proteolysis, glycosylation, and endothelial cell damage⁽²⁹⁾.

Numerous studies have found that GGT is not just a marker of alcohol consumption, but is an independent predictor of many diseases, including cardiovascular diseases, type 2 diabetes, inflammation possibly underlying oxidative stress.^{(10),(13),(14),(15)(16)} Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to

nonenzymatic protein glycosylation, autooxidation of glucose, impaired glutathione metabolism alteration in antioxidant enzymes, lipid peroxides formation and decreased ascorbic acid levels. Oxidative stress and inflammatory markers should be used in addition to HbA_{1c} for assessment of increased cardiac risk in uncontrolled diabetic patients because of accelerated atherosclerosis due to free radical injury⁽³⁰⁾. Significantly increased level of FPG and HbA_{1c} in diabetic patients of our study probably signifies poor glycemic control which could lead to oxidative stress and it's positive correlation with GGT probably indicates it's antioxidant role in response to oxidative stress. It can be explained by observations of **Karp DR. et al**⁽³¹⁾ and **Lee DH. et al**⁽¹⁹⁾ that raised GGT concentrations could be a marker of oxidative stress, which might also play a role in the cause and development of diabetes and its complications.

Limitation:

Limitation of our study was small sample size.

Conclusion:

In summary, this study indicates that increased levels of serum GGT is significantly correlated with increased levels of FPG and HbA_{1c}, markers of glycemic control of patients with type2 DM. Also significant correlation between GGT and increased levels of both BMI and waist to hip ratio probably indicates an association between obesity, insulin resistance and increased activity of GGT in response to depletion of hepatic glutathione levels in diabetic patients of our study. Therefore, it may be useful to determine serum GGT as a marker of oxidative stress in type2 DM patients with poor glycemic control to prevent it's long term complications though further studies are necessary in a large scale of populations to confirm the findings of our study

Table 1: Showing comparison of baseline characteristics between control group and case group.

BASELINE CHARACTERISTICS	CONTROLS(n=40) Mean± SD	CASES(n=40) Mean± SD	P Value
BMI	24.22 ± 0.70	26.22 ± 1.95	<0.0001
W:H Ratio	0.81 ± 0.029	0.83 ± 0.041	<0.05
DISEASE DURATION (YEARS)		7.06 ± 2.76	

*BMI- Body Mass Index, † W:H - waist To hip Ratio

Fig 2: Showing means of BMI in the control and case group.

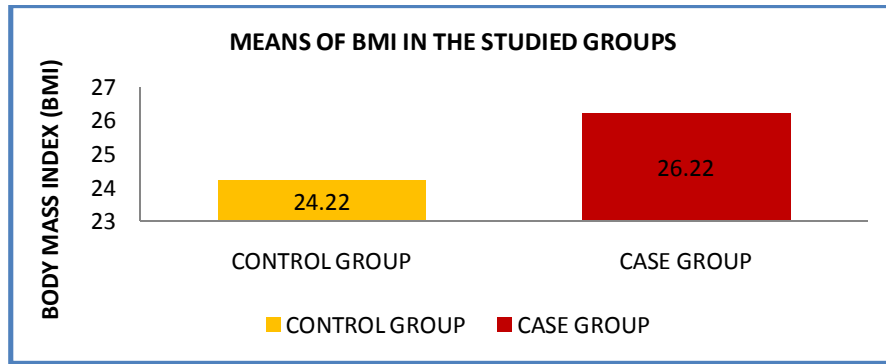


Fig3: Showing means of waist to hip ratio in the control and case group.

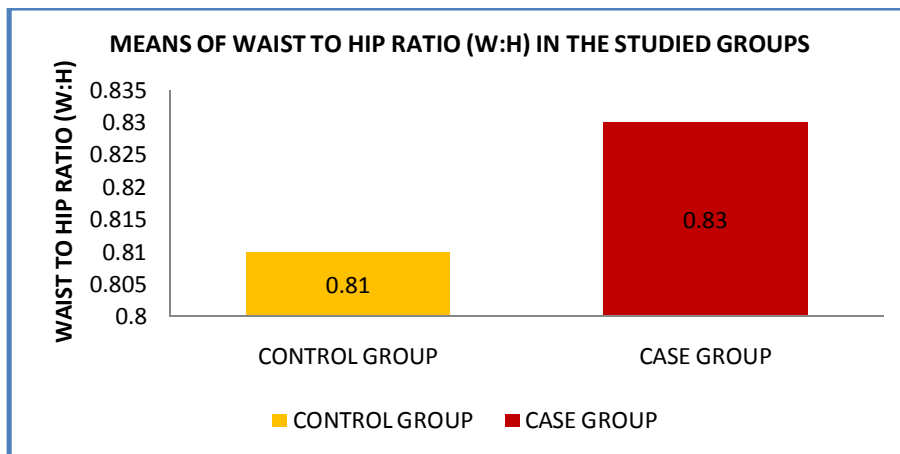


Table 4: Showing comparison of biochemical parameters between control group and case group.

BIOCHEMICAL PARAMETERS	NORMAL VALUES	CONTROLS(n=40) Mean± SD	CASES(n=40) Mean± SD	P Value
FPG (mg/dl)	70-110	90.43 ± 9.86	196.76 ± 54.28	<0.0001
HbA _{1C} (%)	<6.0	5.11 ± 0.286	8.50 ± 1.85	<0.0001
GGT (U/L)	12-58	25.73 ± 6.84	44.36 ± 12.05	<0.0001

*FPG- Fasting plasma glucose, † HbA_{1c}- Glycated hemoglobin, ‡ GGT-Gamma glutamyl transpeptidase.

Fig 5: Showing means of GGT in the control and case group.

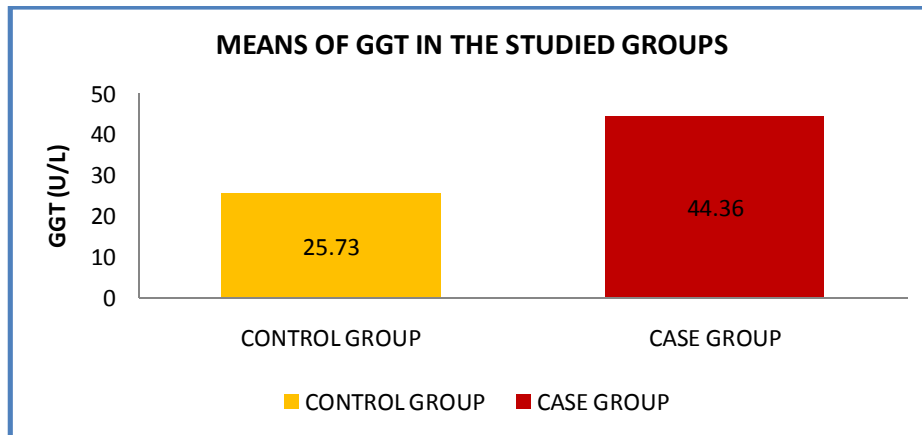


Fig 6: Showing means of fasting plasma glucose in the control and case group.

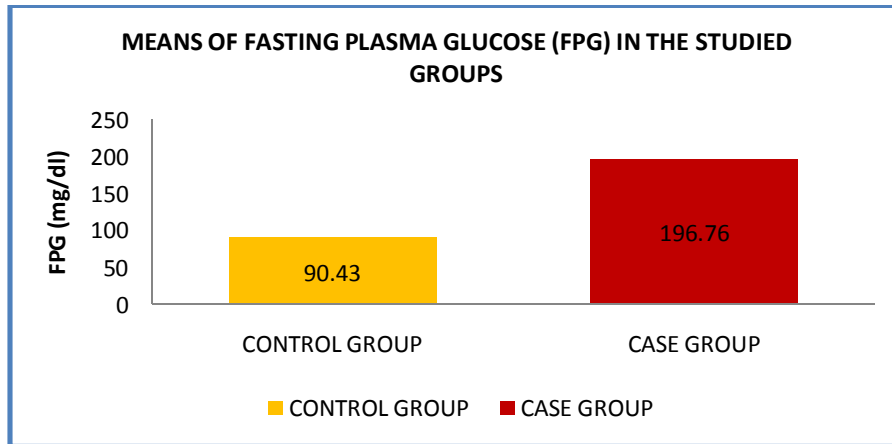


Fig 7: Showing means of HbA_{1c} in the control and case group

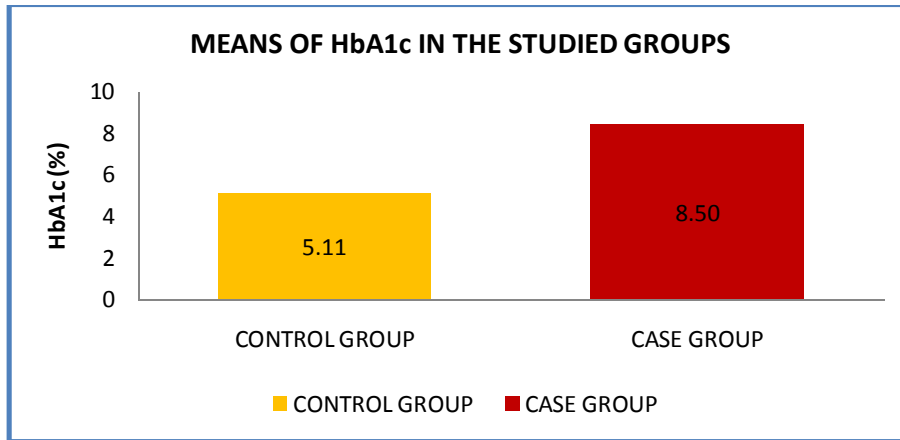


Table 8: Showing Pearson's correlation between GGT and baseline characteristics in case group.

PARAMETER	PARAMETERS	CORRELATION COEFFICIENT (r value)	TWO TAILED p VALUE
GGT	BMI	0.46	<0.05
	W:H Ratio	0.43	<0.05
	Disease Duration	0.59	<0.001

*BMI- Body Mass Index, † W: H - waist To hip Ratio.

Fig 9– Showing Pearson’s correlation between GGT and BMI in case group.

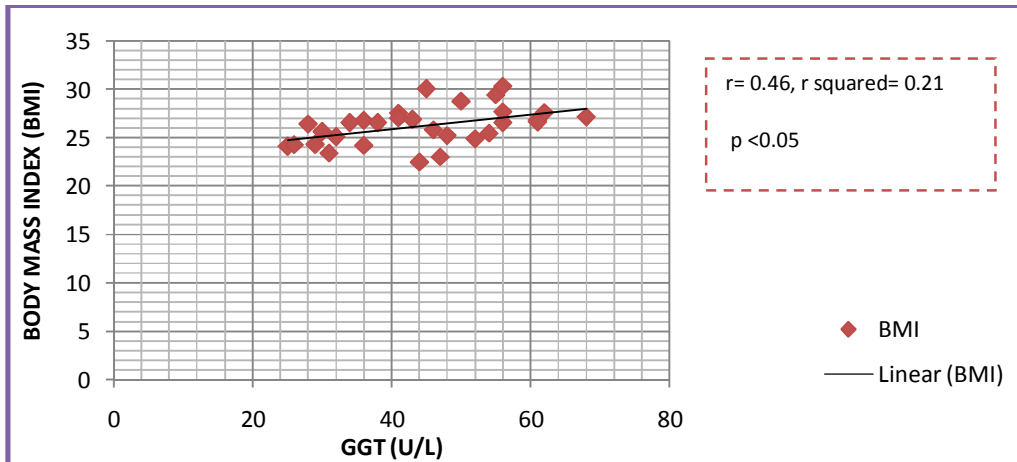


Fig 10– Showing Pearson’s correlation between GGT and W:H ratio in case group

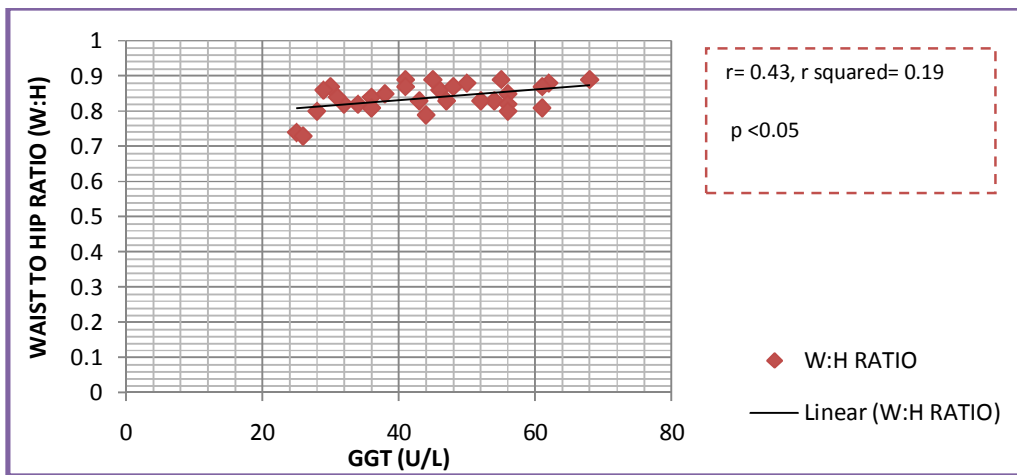


Fig 11– Showing Pearson’s correlation between GGT and disease duration in case group

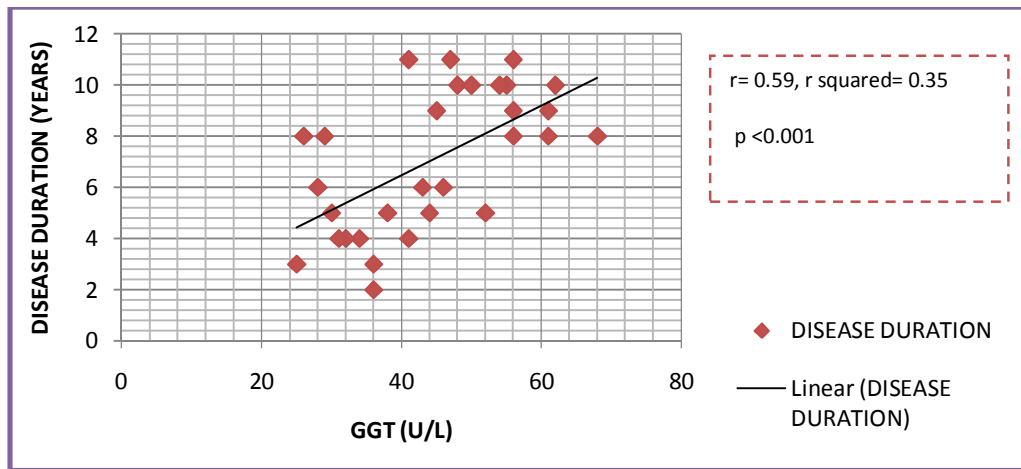


Table 12: Showing Pearson’s correlation between GGT and other biochemical parameters in case group.

PARAMETER	PARAMETERS	CORRELATION COEFFICIENT (r value)	TWO TAILED p VALUE
GGT	FPG	0.72	<0.0001
	HbA _{1c}	0.69	<0.0001

*FPG- Fasting plasma glucose, †HbA_{1c}- Glycated hemoglobin, ‡GGT-Gamma glutamyl transpeptidase.

Fig 13 – Showing Pearson’s correlation between GGT and FPG in case group

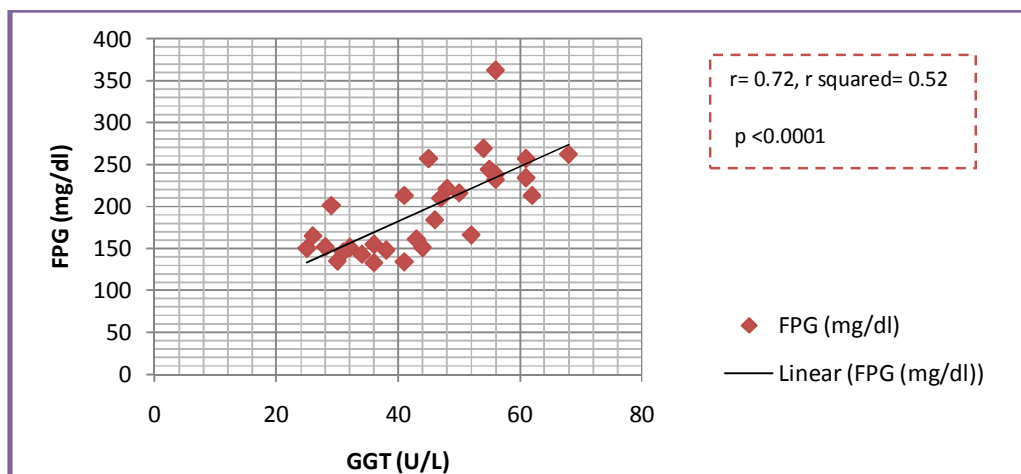
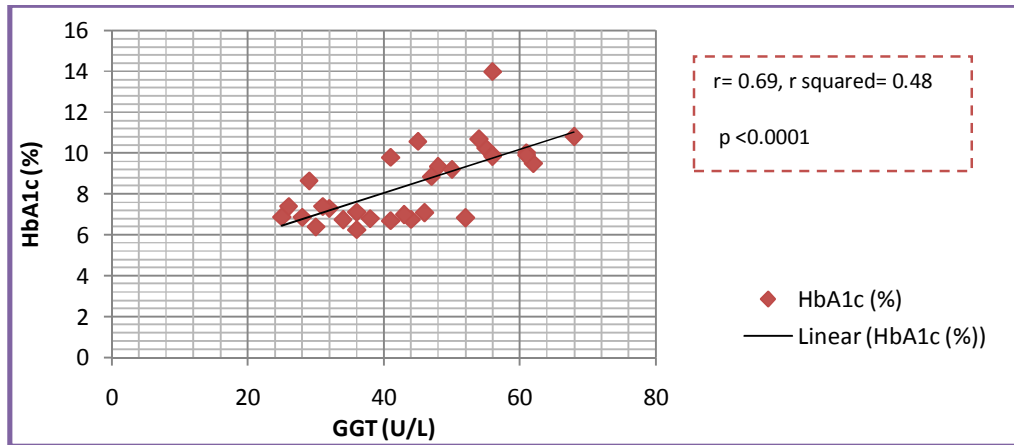


Fig 14– Showing Pearson’s correlation between GGT and HbA_{1c} in case group



References

1. Kumar V, Abbas AK, Fausto N, Mitchell. chapter-Diabetes mellitus. In Robbin’s Basic Pathology. Philadelphia, New-York: Elsevier Saunders p. p-775-776.
2. Cho N.H.,WhitingD.,GuariguataL.,Aschner P., Forouhi N.,Hemblemton H. *et al*, IDF Diabetes Atlas. 6TH ed.ISBN(2013): www.idf.org/diabetesatlas.
3. Anjana RM, Pradeepa R, Deepa M, Datta M, Sudha V, Unnikrishnan R. On behalf of the ICMR–INDIAB Collabo.Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India:Phase I results of the Indian Council of Medical Research-India DIABetes (ICMR-INDIAB) study. Diabetologia. 2011; 54: p. 3022-7.
4. Whiting DR, Guariguata L, Weil C, Shaw J. IDF Diabetes Atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract. 2011;(94): p. 311-21.
5. Chait A, Brazg R, Tribble D, Krauss R. Susseptibility of small,dense, low-density lipoproteins to oxidative modification in subject with the atherogenic lipoprotein phenotype, pattern B. Am J Med. 1993; 94: p. 350-356.
6. Gupta M, Chari S. Prooxidant and antioxidant status in patients of type2 diabetes mellitus with IHD. Indian journal of clinical biochemistry. 2006; 21(2): p. 118-122.
7. Jakus V. The role of free radical, Oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl. Lek.Listy. 2000; 101(10): p. 541-551.
8. Rita P, Berg M, Benjamin B. Loci affecting gamma-glutamyl transferase in adults and adolescents show age interaction and cardio metabolic associations. Human Molecular Genetics. 2012; 21(2): p. 446–455.
9. Whitfield J. Gamma glutamyl transferase. Crit Rev Cli Lab Sci. 2001; 38: p. 263-355.
10. Wannamethee S, Shaper A, Lennon L, Whincup P. Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Care. 2005; 28: p. 2913–2918.
11. Hanley A, Williams K, Festa A, Wagenknecht L, D’Agostino RJ. Elevations in markers of liver injury and

- risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*. 2004; 53: p. 2623–2632.
12. Nakanishi N, Suzuki K, Tatara K. Serum gammaglutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. *Diabetes Care*. 2004; 27: p. 1427–1432.
 13. Bo S, Gambino R, Durazzo M, Guidi S, Tiozzo E, Ghione F, et al. Associations between gamma-glutamyl transferase, metabolic abnormalities and inflammation in healthy subjects from a populationpopulation basedcohort: A possible implication for oxidative stress. *World Journal of Gastroenterology*. 2005; 11(45): p. 7109-7117.
 14. Emdin M, Passino C, Donato L, Paolicchi A, Pompella A. Serum gammaglutamyltransferase as a risk factor of ischemic stroke might be independent of alcohol consumption. *Stroke*. 2002; 33: p. 1163-1164.
 15. Emdin M, Pompella A, Paolicchi A. Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease triggering oxidative stress within the plaque. *Circulation*. 2005; 112: p. 2078-2080.
 16. Lee DH, Jacobs DR, Gross M, Kiefe CI, Roseman J, Lewis CE, et al. Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the CARDIA study. *Clinical Chemistry*. 2003; 49(8): p. 1358-1366.
 17. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and metaanalysis. *Diabetes Care*. 2009; 32: p. 741–750.
 18. Meisinger C, Löwel H, Heier M, Schneider A, Thorand B. Serum gammaglutamyltransferase and risk of type 2 diabetes mellitus in men and women from the general population. *Journal of Internal Medicine*. 2005; 258: p. 527-535.
 19. Lee DH, Ha MH, Kim JH, Christiani DC, Gross MD, Steffes M, et al. Gamma-glutamyltransferase and diabetes- a 4 year follow-up study. *Diabetologia*. 2003; 46: p. 359-364.
 20. Shaw L, Stromme J, London J, Theodorsen L. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for gamma-glutamyltransferase. *J. Clin. Chem. Clin. Biochem*. 1983; 21: p. 633-646.
 21. Neese J, Duncan P, Bayse D, al. e. Development and Evaluation of a Hexokinase/Glucose-6-phosphate Dehydrogenase Procedure for Use as a National Glucose Reference Method. HEW Publication No. (CDC) 77-8330. HEW. USPHS, Centers for Disease Control. 1976..
 22. Jeppsson , Jan-Olof. , al e. Approved IFCC Reference Method for the Measurement of HbA1c in Human Blood. IFCC Scientific Working Group on HbA1c Standardisation and Network of Reference Laboratories for HbA1c. *Clin Chem Lab Med*. 2002.; 40(1): p. 78-89.
 23. Lawlor D, Sattar N, Smith G. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. *Am J Epidemiol*. 2005; 161(11): p. 1081-8.
 24. Kang Y, Min H, Son S, Kim I, Kim Y. The association of serum gamma glutamyltransferase with components of the metabolic syndrome in the Korean adults. *Diabetes Res Clin Pract*. 2007; 77(2): p. 306-13.
 25. Iqbal A, Khoja A, Iftikhar U, Mehjabeen. A Comparison Of The Effects Of Gamma Glutamyltransferase On

Age And Obesity Among Normal, Hypertensive And Type 2 Diabetics. *Biomedica*. 2009; 25: p. 123-27.

26. Meisinger C, Doring A, Schneider A, Lowel H, Group KS. Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population. *Atherosclerosis*. 2006; 189(2): p. 297-302.
27. Gohel MG, Chacko AN. Serum GGT activity and hsCRP level in patients with type 2 diabetes mellitus with good and poor glycemic control: An evidence linking oxidative stress, inflammation and glycemic control. *Journal of Diabetes & Metabolic Disorders*. 2013; 12(56).
28. Dyck P, Kratz K, Karnes J, Litchy W, Klein R, Pach J, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy and nephropathy in a population based cohort study. *Neurology*. 1993; 43: p 817-24.
29. Ikeda Y, Fujii J, Taniguchi N, Meister A. Expression of an active glycosylated human gamma-glutamyl transpeptidase mutant that lacks a membrane anchor domain. *Proc Natl Acad Sci USA*. 1995; 92: p. 126–130.
30. Moussa S. Oxidative stress in diabetes mellitus. *Rom J Biophys*. 2008; 18(3): p. 225–236.
31. Karp D, Shimooku K, Lipsky P. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. *J Biol Chem*. 2001; 276: p. 3798–3804.