ORIGINAL ARTICLE

Association of Serum Gamma-Glutamyl Transferase and C-Reactive Protein as a Biomarkers of Oxidative Stress in Patients of Type 2 Diabetes Mellitus

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ABSTRACT

Objective: To compare Serum gamma-glutamyl transferase and serum C-reactive protein as biomarker of oxidative stress in patients of type 2 Diabetes Mellitus.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: The study was conducted at Armed Forces Institute of Pathology, department of Chemical Pathology and Endocrinology Rawalpindi. The duration of study was 6 months i.e., 17 Nov 2021 – 17 May 2022 after approval from Institutional Review Board FC-CHP21-12/Read-IRB/22/846.

Materials and Methods: An analytical, cross-sectional research was carried out at Armed Forces Institute of Pathology Rawalpindi. An overall 300 diabetic patients were included between ages of 45 – 65 years. Group I had 100 nondiabetic individuals of 45 – 65 years of age with HbA1c < 5.7 %. Group II and III included 100 patients each of DM of matched age with HbA1c 6.5 – 7 % and greater than 7 % respectively, without any other chronic disease. Serum gamma-glutamyl transferase, HbA1c, Serum C-Reactive Protein were analyzed. Moreover, some more biochemical investigations such as serum liver enzymes were measured to rule out any liver disease. One-way ANOVA was followed up by post-hoc Tukey analysis for intergroup comparison.

Results: Mean serum gamma-glutamyl transferase levels were markedly increased in group III patients followed by group II and normal in group I. The mean of serum gamma-glutamyl transferase in group I was (9.38$\pm$4.05U/l), group II (34.27$\pm$15.07 U/l) and group III (47.08$\pm$20.56 U/l). The mean of Serum C-Reactive Protein in group I was (11$\pm$6.02 mg/l), group II (62.07$\pm$ 26.94 mg/l) and group III (107.73$\pm$57.03 mg/l). Pearson correlation revealed prominent positive correlation between HbA1c, serum gamma-glutamyl transferase and Serum C-Reactive Protein with r value of serum gamma-glutamyl transferase (0.838367) and Serum C-Reactive Protein (0.684722). One-way ANOVA and post-hoc Tukey analysis had *p* value of < 0.05 which was statistically significant.

Conclusion: Serum gamma-glutamyl transferase is better marker of oxidative stress in patients of type 2 diabetes mellitus as compared to Serum C-Reactive Protein. The r value of serum gamma-glutamyl transferase is (0.838367) and Serum C-Reactive Protein is (0.684722) indicating strong positive correlation of serum gamma-glutamyl transferase with HbA1c. Therefore, Serum gamma-glutamyl transferase can be used for the prevention and monitoring of complications of type 2 Diabetes Mellitus.

Key Words: *Armed Forces Institute of Pathology (AFIP), C Reactive Protein (CRP), Diabetes Mellitus (DM), Gamma Glutamyl Transferase (GGT), Institutional review board (IRB).*

Introduction

Diabetes Mellitus (DM) is a collective term for several metabolic conditions that all exhibit the hyperglycemia phenotype. The disease's most recognizable symptoms and long-term problems are caused by hyperglycemia, which also serves as the disease's definition. The main objectives of diabetes mellitus research have been to comprehend the pathophysiology and prevent long-term consequences. Unquestionably, one of the most difficult health issues of the twenty-first century is diabetes. Recent studies have established role of inflammation and oxidative stress in pathophysiology of complications of DM. [1,3]

Serum GGT is a cell-surface enzyme that contributes to glutathione's extracellular degradation (GSH). The

enzyme is produced in numerous tissues, although most of the Serum GGT are synthesized in the liver.[2] The role of Serum GGT in controlling extracellular glutathione (GSH) transport system serves as basis of intracellular antioxidant defenses. Several pathogenic diseases, including aging, carcinogenesis, inflammation, reperfusion injury and atherosclerosis relate to oxidative stress. Additionally, oxidative stress might also be a contributing factor to the development and pathophysiology of diabetes.[4,5] The majority of research examines the part oxidative stress plays in the development of cardiovascular problems in diabetic individuals.[3] In addition, increased levels of Serum GGT is linked with increased body weight and a liver disease called nonalcoholic fatty liver disease "NAFLD". It is caused when excessive fat is deposited in a liver. NAFLD is believed to be main contributory factor responsible for hepatic insulin resistance and ultimately development of hyperinsulinemia and systemic insulin resistance.

Thus, Serum GGT may reflect metabolic changes and may serve as diagnostic for the syndrome of insulin resistance. Several potential explanations explain the link between elevated Serum GGT levels and glycemic control in patients with type 2 diabetes with excellent and poor control.[5,18] In the pathophysiology of diabetes, Serum GGT may therefore play the role of the insulin resistance syndrome marker. Moreover, Serum GGT may leak into the serum due to regular cellular turnover and cellular stressors. There are multiple putative reasons for Serum GGT leakage, including oxidative stress, protein degradation, glycosylation, and endothelial cell injury. Thus, elevated Serum GGT levels may identify individuals with a minimal but constant increase in oxidative and other cellular stress.[16,17] Serum GGT is an upcoming biomarker of oxidative stress monitoring in disease progression from very beginning. The rationale of study is to evaluate Serum GGT as an early marker of oxidative stress in patients of type 2 Diabetes Mellitus. The main objective of this study is to compare Serum Gamma-Glutamyl Transferase and Serum C-Reactive Protein as biomarker of oxidative stress in patients of type 2 Diabetes Mellitus.

Materials and Methods

An analytical, cross-sectional research was carried out at Armed Forces Institute of Pathology Rawalpindi. The duration of study was 6 months. An overall 300 diabetic patients were included between ages of 45 – 65 years. Nonprobability convenient sampling technique was used. The study started after the approval of Ethical Review Committee i.e., 17 Nov 2021 – 17 May 2022 (FC-CHP21-12/Read-IRB/22/846.) Group I had 100 nondiabetic individuals of 45 – 65 years of age with HbA1c < 5.7 %. Group II and III included 100 patients each of DM of matched age with HbA1c 6.5 – 7 % and greater than 7 % respectively, without any other chronic disease.

We excluded patients with deranged liver enzymes and chronic disorders, as they could interfere with our results by falsely altering the concentration of Serum GGT. The patients taking hepatotoxic drugs were also excluded.

All eligible participants in this study were informed of the study's goals. Detailed history was taken in endocrine clinic department of chemical pathology and endocrinology AFIP. Group I included 100 nondiabetics individuals with 45 – 65 years of age with HbA1c < 5.7 %. Group II and III included 100 patients each of DM of matched age with HbA1c 6.5 – 7 % and greater than 7 % respectively, without any other chronic disease. Detailed history was followed up by review of patient's past medical reports from Laboratory Information Management System (LIMS). For participation in research groups and venipuncture, informed written agreement was obtained from all study participants. The emphasis was placed on the voluntary nature of participation in this study. During a standardized interview, the questions also focused on sociodemographic variables and the characteristics in the background diabetes has (length and type of DM, mode of treatment used for DM, and any complications). In addition, each participant got a comprehensive, standardized medical checkup, which included blood collection. Under fasting conditions, 5 ml of each participant's venous blood was collected using a disposable vacutainer equipment (Plain and EDTA). Serum and plasma were separated within a half-hour and kept at 2-8°C for analysis of Serum CRP and Plasma HbA1c. Samples were analyzed in multiple batches.

Glycosylated hemoglobin (HbA1c) was analyzed by Turbidimetric Inhibition Immunoassay (TINIA)

method on Sebia Capillary Octa-3. Carboxy substrate kinetic method was used to determine the activity of Serum GGT on Chemistry Analyzer Advia 1800.

One-way ANOVA was conducted among three group for comparison of means. Post-hoc Tukey analysis was used to compare the intergroup mean. Pearson linear correlation was used to study correlation between HbA1c, Serum GGT and Serum CRP. Statistically speaking, p-value of < 0.05 and r value of > 0.75 was considered as significant.

Results

Mean of serum GGT were strikingly increased in the patients of group III followed by group II and normal in group I. The mean of Serum GGT in group I was (9.38$\pm$4.05U/l), group II (34.27$\pm$15.07 U/l) and group III (47.08$\pm$20.56 U/l). The mean of Serum CRP in group I was (11$\pm$6.02 mg/l), group II (62.07$\pm$ 26.94 mg/l) and group III (107.73$\pm$57.03 mg/l). Pearson correlation revealed prominent positive correlation among HbA1c, Serum GGT and Serum CRP with r value of Serum GGT (0.838367) and Serum CRP (0.684722). One-way ANOVA and post-hoc Tukey analysis was conducted to compare the mean. Statistically speaking, p-value of < 0.05 was significant.

Table I: Mean Serum GGT and Serum CRP among Study groups (N = 300)

Group		Serum GGTT U/l	Serum CRP mg/l
I	(HbA1c < 5.7 %)	9.38$\pm$4.05	11$\pm$6.02
II	(HbA1c 6.5 – 7 %)	34.27$\pm$15.07	62.07$\pm$ 26.94
III	(HbA1c > 7%)	47.08$\pm$20.56	107.73$\pm$57.03

Table II: Comparison of Serum GGT among Groups (N = 300)

Groups	Comparision	Mean Difference	p-Value
Group I (HbA1c < 5.7 %)	Group II (HbA1c 6.5 – 7 %)	119$\pm$80	< 0.001*
Group I (HbA1c < 5.7 %)	Group III (HbA1c > 7%)	199$\pm$99	< 0.001 *
Group II (HbA1c 6.5 – 7 %)	Group III (HbA1c > 7%)	145$\pm$70	< 0.001 *

*p value < 0.05 considered as Statistically Significant

Table III : Comparison of Serum CRP among Groups (N = 300)

Group	Comparison	Mean Difference	p-Value
Group I (HbA1c < 5.7 %)	Group II (HbA1c 6.5 – 7 %)	150$\pm$90	< 0.001*
Group I (HbA1c < 5.7 %)	Group III (HbA1c > 7%)	250$\pm$110	< 0.001 *
Group II (HbA1c 6.5 – 7 %)	Group III (HbA1c > 7%)	190$\pm$95	< 0.001 *

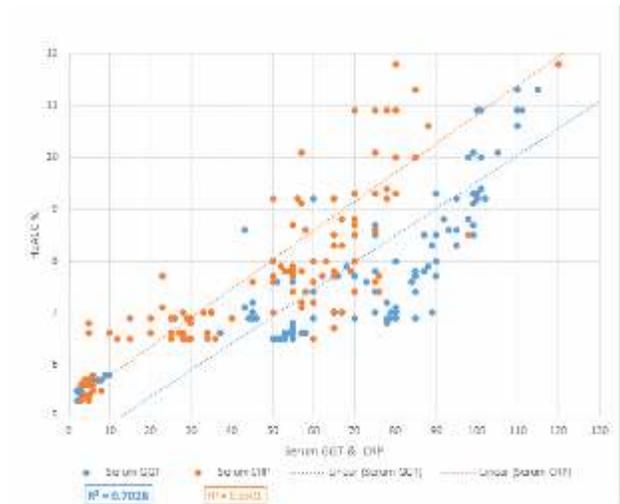*p value < 0.05 considered as Statistically Significant

Figure 1: Pearson Correlation Scatterplot of Serum GGT & Serum CRP (N = 300)

Discussion

Patients of type 2 DM having poor glycemic control have considerably higher concentrations of Serum GGT and HbA1c compared to healthy individuals and those with tightly regulated glycemic control. In addition, we found a substantial positive linear correlation between Serum GGT and HbA1C. These results imply a correlation between oxidative stress (as evidenced by a higher serum GGT) and glycemic management with type 2 diabetic patients and associated comorbidities. This shows that oxidative stress and chronic inflammation have a major role in the pathogenesis of type 2 diabetes.[19-21]

Mean of Serum GGT was lowest in control group i.e individuals with normal glycemic control (HbA1c < 5.7 %) and significantly increased in group II (HbA1c 6.5 – 7 %) and Group III (HbA1c > 7 %). The mean of Serum GGT in group I was (9.38 U/l), group II (34.27 U/l) and group III (47.08 U/l). r value of Serum GGT was (0.838367) indicating positive correlation between Serum GGT and HbA1c. A serum GGT elevation may be indicative of nonalcoholic fatty liver disease, which is characterized by an excessive accumulation of fat in the liver. It is believed that a fatty liver results into hepatic insulin resistance leading to hyperinsulinemia.[22]

Serum CRP is a well-known marker of inflammation, however its role in oxidative stress secondary to type 2 diabetes mellitus has not been established. The mean of Serum CRP in group I was (11 mg/l), group II (62.07 mg/l) and group III (107.73 mg/l). However,

Pearson correlation revealed insignificant correlation of Serum CRP and HbA1c with r value of (0.684722).

There are other studies supporting our results and findings. R Sharma et al. demonstrate a strong correlation of Serum hsCRP and Serum GGT in diabetic with poor glycemic control, which may be due to oxidative stress and inflammation in diabetes.[9]

The findings of Thamer C et al. and Andre P et al. seconds a correlation between high serum GGT, uncontrolled diabetes and metabolic syndrome. Elevated levels of GGT are associated with increased insulin resistance, an increased risk of developing type 2 diabetes, and inadequate glycemic control. Moreover, Serum GGT may leak into the serum due to regular cellular turnover and cellular stressors. There are multiple putative reasons for Serum GGT leakage, including oxidative stress, protein degradation, glycosylation, and endothelial cell injury.[23,24]

Marchesini G et al. and Silventoinen K et al. determined that the close affiliation of serum GGT activity with other metabolic disorders related to DM, such as atherosclerosis, cardiovascular diseases, and dyslipidemia. Increased oxidative stress, fatty liver and insulin resistance may be responsible for the pathogenesis of disease increased activity of serum GGT. Increasing evidence suggests that Serum GGT is a measure of both fatty liver and oxidative stress. [12,13] Study revealed that Serum GGT plays a crucial role in the maintenance of intracellular antioxidant defenses by mediating the transport of extracellular glutathione into most cell types. It is an enzyme generally found on the outside of the cell membrane whose major job is to maintain intracellular concentrations of glutathione (GSH), the cell's most important antioxidant defense. Growth in Serum GGT activity may be a reaction to oxidative stress, allowing for an increase in the GSH precursors' movement into cells.[25]

Our research had numerous limitations. First, this was a cross-sectional study that reports no causal effect. In addition, serum GGT levels in the follow-up were not included in the analysis. In addition, some confounding variables, such as fasting insulin concentration and markers other than Serum CRP that can assess the inflammatory status of the patient in correlation with Serum GGT, could not be included in this study.

Conclusion

It can be concluded that serum GGT is a better and more specific marker of oxidative stress in patients of type 2 DM as compared to serum CRP. Serum GGT can be used for prevention and monitoring of complications of type 2 Diabetes Mellitus.

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DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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