Original Research Article

Evaluation of serum gamma-glutamyl transferase and highly sensitive C-reactive protein as biomarkers of oxidative stress and inflammation in type 2 diabetes mellitus patients with good and poor glycemic control

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ABSTRACT

Background: Diabetes mellitus comprises a group of common metabolic disorders which share common phenotype of hyperglycaemia. Oxidative stress and inflammation are associated with poor glycaemic control and further pathogenesis and complications of diabetes mellitus. This study investigated for correlation of good and poor glycaemic control with these factors.

Methods: Subjects selected for the study were divide into three groups, group I control (n=35), group II type 2 diabetes mellitus patients with good glycaemic control (n=35) and group III type 2 diabetes mellitus patients with poor glycaemic control (n=35). Patients complete blood count, random blood sugar, HbA1c, HsCRP and GGT were investigated. These parameters were statistically analysed for correlation between HbA1c with GGT and HbA1c with hsCRP.

Results: The mean HbA1c in group I, II and III were found to be 5.17%, 6.54% and 9.23% respectively. It was statistically significant (p=0.01), as according to the criteria defined for study recruitment. Furthermore, mean GGT and hsCRP levels were evaluated; a statistically significant difference in mean GGT levels as well as hsCRP of three different groups were obtained with a p value of 0.02. Correlation between HbA1c and hsCRP was found to positive (R2=0.17, p=0.03). When GGT was compared with HbA1c across the groups, there was a statistically significant correlation (R2 = 0.09, p=0.03).

Conclusions: Present study established a positive correlation between HbA1c and GGT, HbA1c and hsCRP, indicating increasing oxidative stress and inflammation in patients with poor glycaemic control.

Keywords: GGT, HbA1c, HsCRP, Inflammation, Oxidative stress, Type 2 diabetes

INTRODUCTION

Diabetes mellitus is one of the mounting diseases posing a medical challenge worldwide.[1] It is notably pervasive in developing countries and India being one of them, accounts for the predominant diabetic population with an estimate of 42 million which is, 6% of the adult population.[2] Although pathologically type 2 diabetes results from genetic predisposition, previous studies have unveiled that, modifiable risk factors such as rapidly changing lifestyle, obesity and physical inactivity are the main non-genetic determinants of the disease.[3]

Research has clearly stipulated that; high glycemic index is associated with an increased risk of complications of diabetes and its stringent control is the primary requisite for the prevention of complications. Furthermore, it is said that on an average diabetes mellitus reduces life expectancy by at least 5-10 years which can be contributed to both the modifiable risk factors and complications.[4] Absolute perception of the pathogenesis, identifying high risk patients and preventing long-term complications has been the major goals of research in diabetes. Also, recent cumulative evidences propound that, with poor glycemic control, oxidative stress and inflammation emerge as key components leading to progression of diabetes and its complications.[5]

In this perspective, appreciable amount of investigations and data advocate that highly sensitive C-reactive protein (hsCRP) and gamma glutamyl transferase (GGT) are two physiological markers, linked with subsequent development of complications of diabetes. Highly sensitive C-Reactive Protein is an acute phase protein produced in the liver which increases in chronic inflammation. Serum GGT is a cell surface protein which has antioxidant property and catabolizes extracellular glutathione.[6,7]

Present research intends to study the serum GGT and hsCRP level in type 2 diabetic patients and correlate between good and poor glycemic controls and inflammatory markers.

METHODS

An institution-based longitudinal, prospective observational study was conducted for over a period of 22 months from October 2015 to September 2017 in the departments of the general medicine unit. Ethical clearance of the study was obtained from ethics committee so as to allow data collection. Data was collected on a pre-designed proforma which included-detailed history, systemic examination, complete blood count, random blood sugar, HbA1c, HsCRP and GGT.

Inclusion criteria

The subjects selected for study were grouped as follows:

- Group I: Control group (n=35) This group consists of age and sex matched healthy subjects. They are free from any ailment which could affect the parameters under study. They are not on any medication. They are taken from general population.
- Group II: Type 2 DM patients with good glycemic control (n=35) This group consists of patients with type 2 DM with duration less than 8 years, HbA1c level less than 7%. They are on life style modifications and oral hypoglycemic drugs and free from clinical evidence of any complication of diabetes mellitus.

- Group III: Type 2 DM patients with poor glycemic control (n=35). This group consists of patients with type 2 DM with duration more than 8 years, HbA1c level more than 7.1%. They are on life style modifications, oral hypoglycemic drugs, insulin or combination of all three and associated with one or more micro-vascular or macro-vascular complication of diabetes mellitus.

Exclusion criteria

- Type 1: diabetes mellitus
- All alcoholics, patients with known liver or gastrointestinal diseases, acute coronary syndrome
- Patients on corticosteroids, ATT drugs, antiepileptic drugs, methotrexate, amiodarone, tamoxifen or other hepatotoxic drugs
- Any chronic infection like tuberculosis and inflammatory diseases like sarcoidosis etc.
- Hemolytic anemia.

Based on previous studies and admission in our hospital, for confidence level of 95%, with margin of error of around 5%, sample size taken for the study was calculated to be 81. A total of 105 individuals were taken as study participants.

Statistical analysis

In the statistical analysis of present study, continuous variables were presented as mean for parametric data and median if the data was non-parametric or skewed. Student t test was applied for calculation of statistical significance whenever the data followed normative distribution. Mann Whitney test was applied whenever data followed non normative distribution. Categorical variables were expressed as frequencies and percentages. Nominal categorical data between the groups was compared using chi-square test or fisher's exact test as appropriate. P <0.05 was taken to indicate a statistically significant difference. Minitab version 17 was used for computation of statistics.

RESULTS

The study population in present study was divided into three groups: group I - control group (n=35), group II-type 2 diabetes mellitus patient with good glycemic control (n=35) and group III-type 2 diabetes mellitus patient with poor glycemic control (n=35). There were 19 males and 16 females in group I, 18 males and 15 females in group II, and 17 males and 18 females in group III (Figure 1). There were 18 patients in age group of 40-49 years, 30 patients in age group of 50-59 years, 26 patients in age group of 60-69 years, 23 patients in age group of 70-79, and 8 patients in age group of 80-89 years (Figure 2). There was no statistically significant difference between the groups and hence they were comparable in demographic parameters.
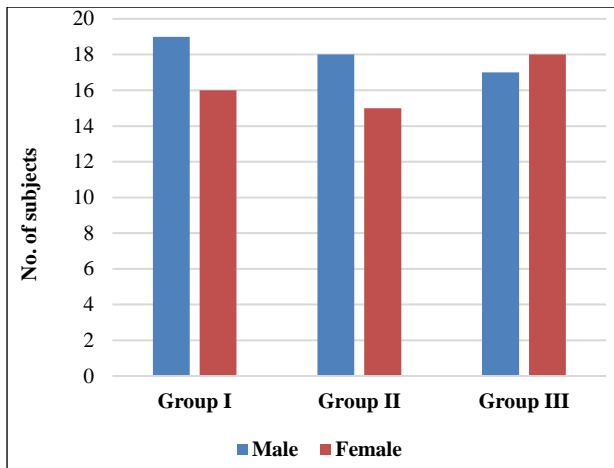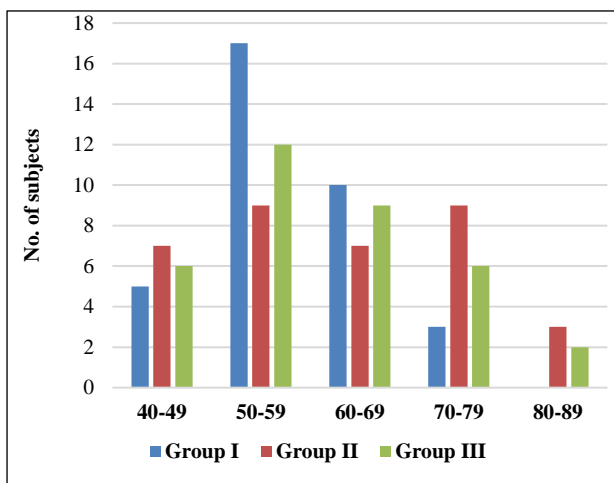
Figure 1: Gender profile.

Figure 2: Age profile.

Table 1: Mean HbA1c in study groups.

Groups	Mean	P -value
Group I	5.17±1.2	
Group II	6.54±0.8	0.01
Group III	9.23±0.7	

On comparison of the mean BMI in three groups, there was a statistically significant (p=0.04) difference. Mean BMI in group I was 21.4 kg/m², in group II was 26.2 kg/m², in group III was 28.3kg/m². There was no significant (p=0.62) difference in the BP recordings of all the three groups. The mean HbA1c in group I, II and III were found to be 5.17%, 6.54% and 9.23% respectively. It was statistically significant (p=0.01), as according to the criteria defined for study recruitment (Table 1).

Other parameters such as total cholesterol, triglycerides, serum creatinine and estimated glomerular filtration rate were also assessed. Total cholesterol in group I was found to be 144 mg/dL, in group II was 182 mg/dL, in group III was 236 mg/dL There was statistically significant (p=0.01) difference. Triglycerides levels in

group I was 132 mg/dL, in group II was 192 mg/dL and in group III was 251 mg/dL, depicting a statistically significant (p=0.01) differences among the three groups. Serum creatinine was highest in group III with the mean value of 1.49 mg/dL, moderately higher value in group II 1.1 mg/dL, and least in group I 0.67 mg/dL, which again was found to be statistically significant (p=0.01). Glomerular filtration rate when estimated was found to be highest in group I with mean value of 122.4 ml/minute, 102.67 ml/minute in group II and least in group III 80.71 ml/minute. Statistically significant (p=0.04) difference was found in the three groups.

Furthermore, mean GGT and hsCRP levels were evaluated in all the three groups. There was a statistically significant mean GGT levels in the three different groups with a p value of 0.02. The mean GGT levels were found in group I, II and III are 17.14 U/L, 25.17 U/L and 28.84 U/L respectively (Table 2).

Table 2: Mean GGT in study groups.

Groups	Mean	P -value
Group I	17.14±2.3	
Group II	25.17±1.8	0.02
Group III	28.84±2.1	

The mean hsCRP level in group I was 1.96 mg/L, in Group II was 2.22 mg/L, in Group III was 2.86 mg/L. There was a statistically significant level of hsCRP among the three different groups with a p value of 0.02 (Table 3).

Table 3: Mean highly sensitive quantification of C-reactive protein (HsCRP) in study groups.

Groups	Mean (mg/L)	P -value
Group I	1.96±0.3	
Group II	2.22±0.2	0.02
Group III	2.86±0.2	

As one of the objectives of present study states, hsCRP levels were assessed in the study population, mean HbA1c at hsCRP <3 mg/L was 6.8±0.42 and mean HbA1c at hsCRP >3 mg/L was 8.1±0.71.There was a statistically significant difference between HbA1c levels at hsCRP <3mg/L and >3mg/L (p=0.03) (Figure 3).

Correlation between HbA1c and hsCRP was found to positive ($R^2 = 0.17$, p= 0.03) (Figure 4).

Various other parameters such as blood pressure, BMI, LDL (low density lipoprotein) and serum creatinine were assessed for correlation with hsCRP, no statistically significant correlation was found. When GGT was compared with HbA1c as across the groups, there was a statistically significant correlation found ($R^2=0.09$, p=0.03) (Figure 5). Also, when correlation was assessed

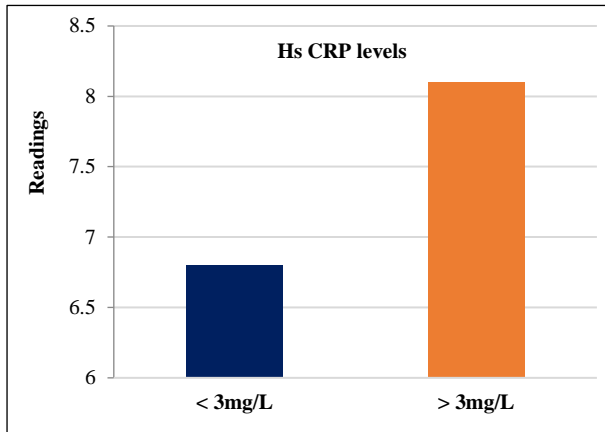for hsCRP with GGT, a positive result was obtained (Figure 6).
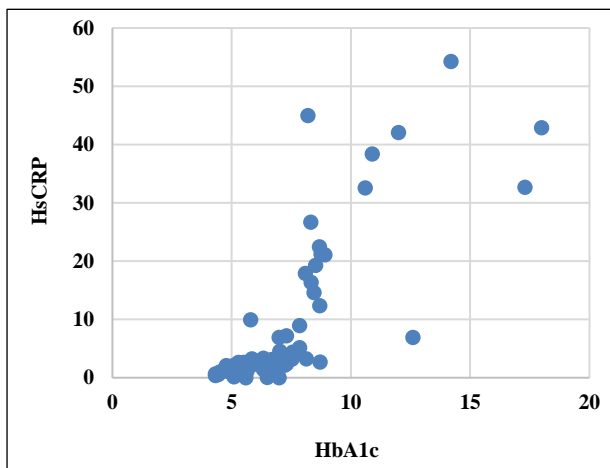

Figure 3: Correlation of HbA1c with hsCRP.
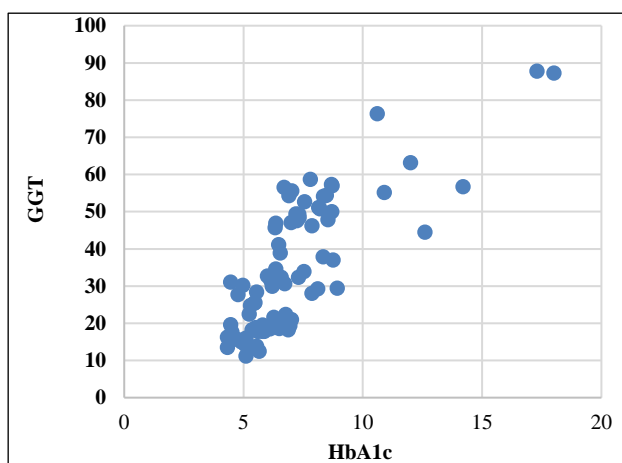

Figure 4: Correlation of hsCRP with HbA1c.

Figure 5: Correlation of HbA1c with GGT.

DISCUSSION

This longitudinal, prospective study was conducted on healthy population and diabetic population divided into three groups: group I - control group (n=35), group II- type 2 diabetes mellitus patient with good glycemic control (n=35), group III - type 2 diabetes mellitus patient with poor glycemic control (n=35). Age and gender profiles were matched, and demographic parameters of these groups were comparable.
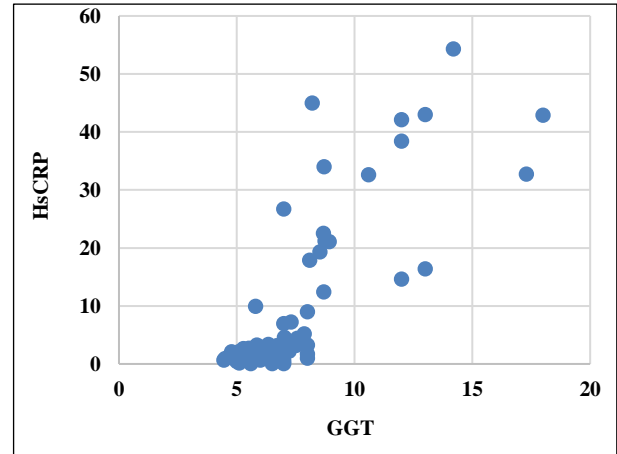

Figure 6: Correlation of GGT with hsCRP.

Present study showed significant difference between mean GGT of study groups. Mean GGT was higher in subsequent groups (group III > group II > group I). This indicates poor the glycemic control, higher will be the oxidative stress which reflects in higher mean GGT in different study groups.

Results of present study was comparable with study by Gohel MG et al, ($R2 = 0.79$, p=0.001).[8] When trend of GGT was compared with HbA1c as across the group, there was statistically significant correlation ($R2=0.47$, p=0.03) across the study groups. Hence, higher the HbA1c, higher was GGT. This further strengthens the hypothesis that poor the glycemic control, higher the oxidative stress and they share mutual linear relationship. Similar observation was found in the study of Khan DA et al, ($R2 = 0.3$, p=0.001).[9]

Association between GGT and BMI and GGT and waist circumference (WC) was found to be statistically significant (BMI, $R2=0.51$; WC, $R2=0.35$; p=0.01). Clinical studies suggest that oxidative stress plays a major role in the pathogenesis of obesity and its complications. Hence the association between GGT and BMI/ obesity. Similar study conducted by Das AK et al, (BMI, $R2 = 0.58$; WC, $R2 = 0.47$; p=0.02) portrayed significant association.[10]

Study by Cheung et al, have emphasized, role of GGT in the pathogenesis of hypertension.[11] They found GGT as an independent predictor of new-onset hypertension ($R2 = 0.38$, p=0.01). In another research project by Jung CH et al, involving 10,988 participants, GGT showed strong positive correlations ($R2=0.5$, p=0.01) with systolic blood pressure and diastolic blood pressure.[12] Similarly present

study as well depicted statistically significant correlation (R2 = 0.43, p=0.01).

In the pathogenesis of atherosclerosis low density lipoprotein plays crucial role, whose oxidation is catalyzed by GGT. This possibly explains the linear relation between the GGT and total cholesterol; hence correlation for the same was analyzed.

Present study found statistically significant correlation (R2 = 0.42, p=0.01) among the two parameters. In a study by Emiroglu MY et al, they found GGT strongly associated with LDL-C in causing IHD (R2 = 0.51, p=0.001).[13]

In a study by Zhang JX et al, they found serum GGT is strongly associated with the increased uric acid concentrations between GGT and uric acid at higher quartiles.[14] Correspondingly in present study, there was a statistically significant correlation (R2=0.26, p=0.04). Study by Koenig G et al, also emphasised the similar relation between GGT and uric acid (R2=0.31, p=0.01).[15]

Accordingly, serum gamma-glutamyl transferase (GGT), being a marker of oxidative stress, has shown to be linked with diabetes mellitus in some population-based studies.

Apart from this it is also known to be elevated in a condition such as non-alcoholic fatty liver disease, which is also assumed to cause hepatic insulin resistance and result in hyperinsulinaemia or systemic insulin resistance. Therefore, GGT could be utilized as a marker of insulin resistance in the pathogenesis of diabetes.[8]

Currently there is a strong evidence to recommend that GGT is also a marker of oxidative stress along with fatty liver. Experimental studies have reported that GGT has a central role in the maintenance of intracellular antioxidant defenses through its mediation of extracellular glutathione transport into various types of cells. They primarily maintain intracellular concentrations of glutathione (GSH), a crucial antioxidant mechanism for the cell, found on the outer side of the cell membrane.[16]

In present study there was a statistically significant difference between HbA1c levels at hsCRP <3 mg/L and >3 mg/L (p=0.01). Similar observations were made in studies of Sarinnapakorn V et al, (p=0.001), Tutuncu Y et al, (p=0.02) and Joshi MD et al, (p=0.01).[17,18]

Furthermore, observation by Joshi et al, found that distribution of hsCRP in the diabetic population was skewed, with a mean of 4.33 mg/L and a median of 2.53 mg/L. Greater part of diabetic population i.e., about 42% had higher (>3 mg/dL) hsCRP level.[19]

In present study, there was statistically significant difference found between mean hsCRP levels among study groups. Similar observation was made by Gohel et al, study.[8] The hsCRP is a protein of an acute phase

secreted by the liver as well as by other tissues in response to any inflammatory condition. hsCRP has pro-inflammatory activity and considered one of the most important pro-atherosclerotic mediators.[20]

Current study also assessed for correlation between hsCRP with blood pressure and hsCRP with BMI, and both were found to have poor correlation. Similar study conducted by Sarinnapakorn V et al, also found poor correlation for both.[17]

Additionally, correlation coefficient of hsCRP with LDL-C and hsCRP with serum creatinine were found to be 0.14 and 0.13 respectively, indicating weak correlation. In a study by Sarinnapakorn V et al, they found similar penurious correlation between hsCRP with LDL-C and hsCRP with serum creatinine.[17]

Low-grade inflammation is characteristic of the metabolic syndrome. In a study by Sigdel M et al, they found that as number of the components of by Sigdel M et al, increased, there was a linear increase in hsCRP levels in whole study population (p <0.001), diabetic subjects (p t<0.001), as well as in controls (p t<0.001).[21]

In present study, there was statically significant (p=0.01) difference between hsCRP levels between patients with diabetic associated with metabolic syndrome in comparison to diabetic without Metabolic syndrome. Similar observation was made by Sigdel M et al, (p=0.04) and Joshi et al, (p=0.01).[18,21]

Kollathody S et al, found, higher the number of components of metabolic syndrome, higher was the hs-CRP levels.[22] Study by Ridker PM et al, recommended, owing to the fact that the inflammation and central obesity are the key players for developing insulin resistance, hsCRP could be used as a defining marker of Sigdel M et al, in the near future.[23]

In present study, serum levels of GGT and hsCRP were positively correlated. Our findings show that serum GGT activity and hsCRP level were significantly increased in patients with type 2 diabetes mellitus compared to healthy control.

Studies have pointed out that GGT could be the expression of subclinical inflammation which also contributes to the development of type 2 DM and insulin-resistant state. Research also shows rise in levels of hsCRP and GGT in diabetic subjects and their significant association which might be a result of inflammation and oxidative stress in diabetes mellitus.

In a study by Khan DA et al, they found that diabetic patients had significantly elevated median of HbA1c, hsCRP and GGT as compared to controls by Sharma R et al, also emphasized similar findings.[9,24] Thus, various studies have pointed connection between glycemic control and inflammation marked by hsCRP. As diabetes

is state of inflammation which is linked to various intracellular events, pro-inflammatory markers are raised in diabetics. Poor the control of glycemia, higher is the inflammation.

Present study established a positive correlation between HbA1c and GGT, HbA1c and hsCRP, indicating increasing oxidative stress and inflammation in patients with poor glycemic control.

Higher the level of HbA1c and GGT, stronger was the correlation between them, additionally an hsCRP level was found to be higher in diabetic patients with metabolic syndrome than without. Hence, there was positive correlation between GGT and hsCRP in diabetes mellitus indicating linear relation between oxidative stress and inflammation.

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