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Research Article
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SERUM GAMMA GLUTAMYL TRANSFERASE AS A MARKER OF OXIDATIVE STRESS IN TYPE 2 DIABETIC RETINOPATHY

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ABSTRACT

Background: *Diabetic retinopathy is a disorder causing microangiopathy in the retina. It is a common cause of visual impairment, affecting type 1 diabetics almost three times more than type 2 diabetics. Oxidative stress is said to be one of the pathophysiological causes of diabetic retinopathy. Of late serum gamma-glutamyltransferase is being considered an early marker of oxidative stress and microvascular complications.* ***Objective:*** *Therefore this study was undertaken to investigate whether serum gamma-glutamyltransferase is a useful marker of oxidative stress in type 2 diabetic retinopathy.* ***Materials and methods:*** *90 participants took part in the study. Thirty served as controls, 30 were type 2 diabetics without retinopathy and 30 were type 2 diabetics with retinopathy. Unhemolysed blood samples were collected in the fasting state and analysed for malondialdehyde, gamma-glutamyl transferase, glycated haemoglobin (HbA1c), and lipidprofile.* ***Results:*** *All the parameters were significantly elevated in diabetics when compared to controls, being more pronounced in diabetics with retinopathy ($p<0.0001$). There was a strong positive correlation between serum malondialdehyde levels and gamma-glutamyltransferase within the reference range in patients with diabetic retinopathy when compared to diabetics without retinopathy.* ***Conclusion:*** *This study shows that serum gamma-glutamyltransferase can be used as a marker of oxidative stress and microvascular complications.*

KEY WORDS

GGT, free radicals, diabetic retinopathy.

INTRODUCTION

Diabetic retinopathy (DR) is a chronic complication of diabetes mellitus, which is a common cause of preventable blindness [1].It is due to damage to the microvasculature of the retina because of chronic hyperglycemia [1]. This complication is directly related to the duration of diabetes mellitus, hyperglycemia, dyslipidemia, hypertension, proteinuria, pregnancy, socioeconomic status and obesity [2-4]. Diabetes mellitus is the leading cause of blindness between the ages of 20 – 74 years in the United States [5]. India has seen an epidemic rise in the incidence of diabetes and DR is fast becoming a common cause of visual impairment in the affected population. Blindness in DR is due to progressive retinopathy and clinically significant macular edema. Early diagnosis of diabetes mellitus (DM) and prompt glycemic control can prevent or delay this complication [6].

The prevalence of DR varies in type 1 and type 2 DM. In a EURODIAB IDDM complications study which included subjects attending 31 European diabetes centres, the prevalence of DR was between 25 – 60% in type 1 diabetics [6]. Asian Young Diabetes Research

(ASDIAB) study investigated 724 young diabetics for DR. They were between 12 – 40 years of age, with diabetes <12 months duration, in 7 centres of 4 Asian countries. They noted that DR prevalence was least among Indians (5.3%) compared to other ethnic groups like Malay's (10%) and Chinese (15.1%). The low prevalence in Indians was partly explained by the higher levels of fasting C- peptide and also glucagon stimulated C- peptide [7]. A study done at a diabetic centre in South India on a cohort of 6792 type 2 diabetic patients revealed a DR prevalence of 34.1%. The retinal screening was done using ophthalmoscopy and retinal photographs. Out of this 30.8% had non proliferative DR, 3.4% had proliferative DR and 6.4% suffered from diabetic macular edema (DME) [8]. The Chennai Urban Rural Epidemiology (CURES) Eye Study, which was a population based study showed the prevalence of DR to be about 17.6% in diabetic patients. The prevalence was low in newly diagnosed diabetic patients (5.1%) compared to chronic diabetics (20.8%) [9]. In Andhra Pradesh Eye Disease Study (APEDS) of self reported diabetics, the prevalence of DR was 22.4% [10].

Abnormalities in several biochemical pathways are proposed that link hyperglycemia to the microvascular complications in the retina. They are polyol accumulation, oxidative stress, advanced glycation end products (AGEs) and protein kinase C activation [1].

The retina has the highest oxygen uptake and glucose oxidation compared to other tissues. It also has a higher concentration of polyunsaturated fatty acids. This renders the retina more susceptible to oxidative stress and lipid peroxidation due to glucose toxicity in hyperglycemia. Malondialdehyde, a product of lipid peroxidation is a well defined marker of oxidative stress [11]. Gamma-glutamyltransferase (GGT) has been recognised as a marker of liver disease and alcohol intake. But recent research has found an association between serum GGT and oxidative stress. In the CARDIA (Coronary Artery Risk Development in Young Adults) study diabetic and hypertensive adults showed a strong correlation between serum GGT levels and microalbuminuria [12]. The study has shown that serum GGT maybe a marker of oxidative stress even when the confounding effects of alcohol are accounted for. An inverse relation between plant foods like vitamin C, folate, β carotene and fibre with serum GGT levels was found in this study. Another study showed that serum GGT level was inversely related to antioxidants like glutathione and glutathione reductase in diabetics with elevated lipid profile revealing decreased antioxidant defences in this population [13]. Similarly dyslipidemia also contributes to the pathology of DR via the mechanism of oxidative stress [14]. Randomised controlled trials and observational studies have indicated that glycated haemoglobin (HbA1c) is a good predictor of microvascular complications [15, 16].

In this study serum malondialdehyde (MDA), GGT, HbA1c and lipid profile levels were compared in diabetics with retinopathy, in diabetics without retinopathy and in healthy controls in a rural population.

MATERIAL AND METHODS

The study was undertaken at Dr Pinnamaneni Siddhartha Institute of Medical Sciences and Research Foundation (Dr PSIMS & RF), Chinnaoutapalli after obtaining institutional ethical committee clearance from the head of the institute and informed consent from the patients. This was a cross sectional case control study. Type 2 diabetic patients aged between 35-50 years attending the Ophthalmology outpatient services were the subjects in this study. Thirty healthy age and sex matched controls (group 1), 30 type 2 diabetics without retinopathy (group 2) and 30 type 2 diabetics with retinopathy (group 3), were compared on the following blood parameters viz; malondialdehyde (MDA), GGT, HbA1c, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). Five ml of blood was collected after an overnight fast of 12 hours and analysed for the above mentioned parameters. Except for MDA which was done manually by the thiobarbituric acid method, the rest of the parameters were analysed on a fully automated clinical chemistry analyser, Randox Daytona using Randox kits. Serum low density lipoprotein was estimated by the Friedewald's formula. An EDTA vacutainer was used to collect the blood sample for HbA1c. For all other tests unhemolysed serum was used. Participants were diagnosed as diabetics when their fasting serum

glucose was ≥ 126 mg/dl (American Diabetic Association criteria). Retinopathy was diagnosed based on fundus examination of both eyes under mydriasis using an ophthalmoscope by an ophthalmologist. Age and sex matched controls included in the study were those with fasting serum glucose of <100 mg/dl, without a past history of DM. Subjects were excluded if they had type 1 DM, had renal or cardiovascular complications, cerebrovascular accidents, hypertension, patients with acute or chronic inflammatory conditions, those consuming alcohol or smokers.

Statistics: The EXCEL software package was used for data entry and analysis. Values are expressed as mean ± standard deviation (s.d). The differences between the means was calculated by student't' test and a 'p' value of 0.05 or less was considered statistically significant. Relation between two variables was appreciated by correlation.

RESULTS AND DISCUSSION

Table1. Shows serum MDA levels were significantly increased in group 2 and group 3 patients when compared to group 1 and this rise was more in group 3 who were diabetics with retinopathy ($p < 0.0001$). Serum GGT levels were also elevated in diabetics with a marked increase seen in diabetics with retinopathy ($p < 0.0001$). Glycated haemoglobin (HbA1c) that indicates the mean blood glucose in the previous 8-10 weeks, was higher in the participants with diabetic retinopathy than without retinopathy ($p<0.0001$). Similarly **Table 2.** Shows total cholesterol, serum triglycerides and LDL were markedly elevated in diabetics with retinopathy ($p<0.0001$) except for high density lipoprotein (HDL), which was low; **Figure 1 and 2** show the strong positive correlation between MDA and GGT levels in group 3 and 2 respectively. This was more pronounced in group 3. **Figure 3** shows a positive correlation between GGT and serum triglycerides in group 3. Though there was a positive correlation between GGT and other parameters except HDL, it was not significant. Serum GGT and HDL showed a negative correlation.

Table 1: Blood levels of various parameters in the different groups

Participants	MDA (nmol/ml)	GGT (I.U/l)	Glycated haemoglobin(HbA1c)	'p' value
Controls(n=30)	4.76±0.7	28±4.3	4.64±0.4	<0.0001 a,b
Diabetics without retinopathy((n=30)	7.05±1.1	47.1±4.5	7.15±0.5	<0.0001 a,c
Diabetics with retinopathy(n=30)	10.4±1.2	56.8±4.4	9.9±0.5	<0.0001 b,c

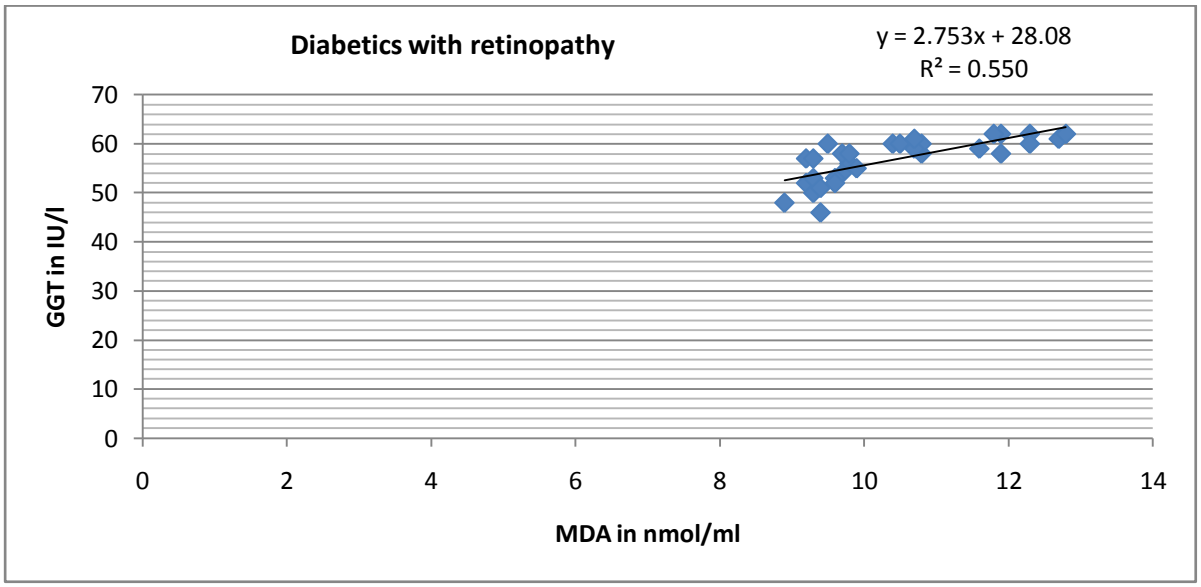
All values expressed as mean ± standard deviation. 'p' value 0.05 or less is significant.
a = 'p'value between controls and DM without retinopathy
b = 'p'value between controls and DM with retinopathy.
c = 'p' value between DM without and with retinopathy.

Table 2: Lipid profile in the different groups

Participants	HDL-C (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C mg/dl	'p' value
Controls (n=30)	57.5±8.4	153.4±2.4	70.9±9.9	78±22.5	<0.0001 a,b
Diabetics without retinopathy(n=30)	46.6±6.5	257.2±50.8	123.2±29.7	185±51.1	<0.0001 a,c
Diabetics with retinopathy(n=30)	35.8±2.8	325±39.3	192±26.4	254.5±38.1	<0.0001 b,c

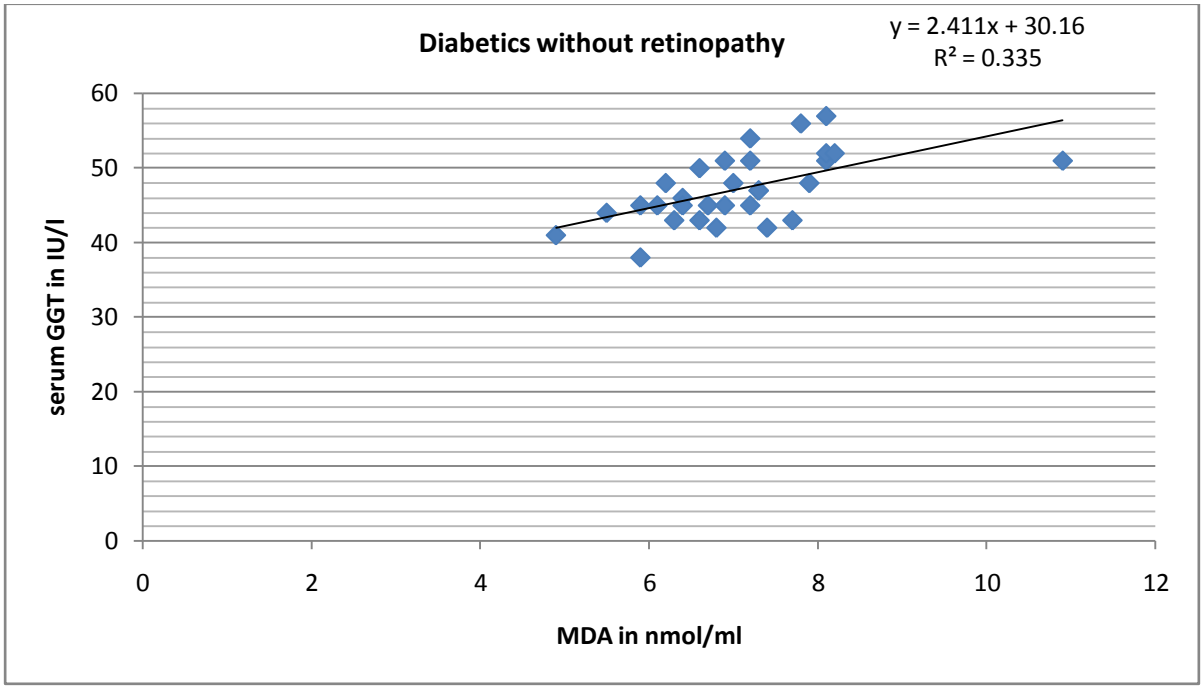
All values expressed as mean ± standard deviation. 'p' value 0.05 or less is significant.
a = 'p'value between controls and DM without retinopathy
b = 'p'value between controls and DM with retinopathy.
c = 'p' value between DM without and with retinopathy.

Figure 1: Correlation between GGT and MDA in diabetics with retinopathy



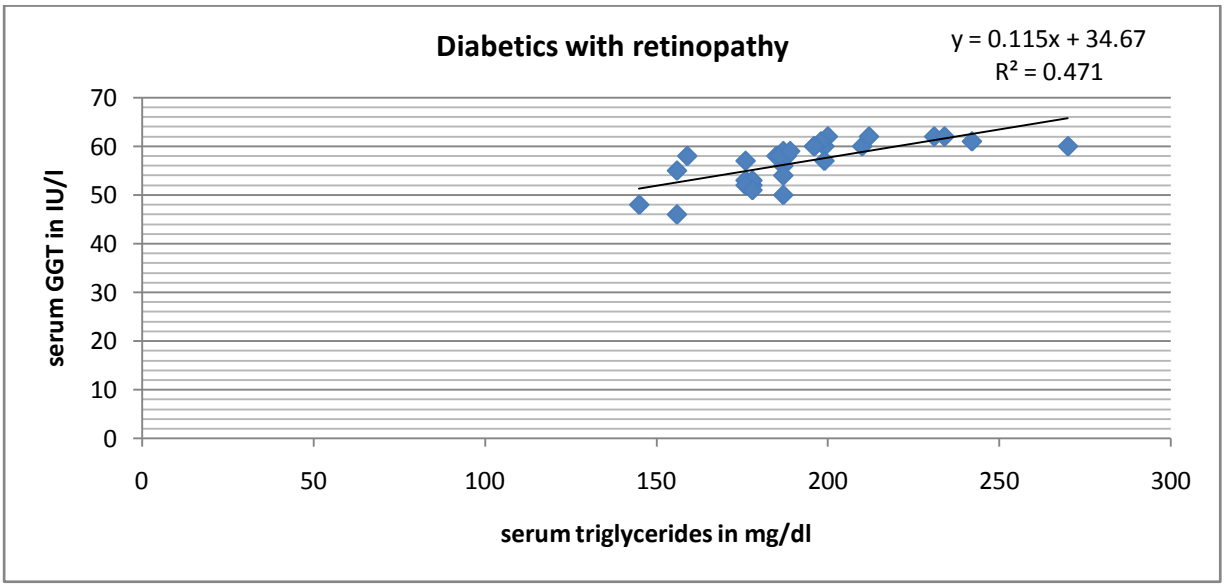
Strong positive correlation between gamma-glutamyltransferase (GGT) and malondialdehyde (MDA) in diabetics with retinopathy.

Figure 2: Correlation between GGT and MDA in diabetics without retinopathy.



Strong positive correlation between serum gamma-glutamyltransferase(GGT) and malondialdehyde(MDA) in diabetics without retinopathy.

Figure 3: Correlation between GGT and serum triglycerides.



Strong positive correlation between serum gamma glutamyltransferase (GGT) and serum triglycerides.

In the present study a significant rise in serum MDA levels were seen in diabetic patients and the difference was more pronounced in patients with diabetic retinopathy. This is in concordance with studies done by Hong Zhi Pan et al and El- Mesallamy who got similar results [11, 17]. Retina is rich in polyunsaturated fatty acids and consumes a lot of oxygen and glucose relative to any other tissue. This renders it susceptible to oxidative stress. Reactive oxygen species (ROS) results from glucose auto-oxidation, protein glycation, increased entry into polyol pathway and prostanoid formation. The increased ROS leads to increased production of vascular endothelial growth factor that leads to neovascularisation and ischemia in the diabetic retina. The increased oxidation and ischemia lead to increased lipid peroxidation products like MDA, which are angiogenic [11].

Several studies have shown cellular GGT to be a marker of oxidative stress whose serum values are elevated along with serum MDA levels [18-20]. Cellular GGT is a membrane bound enzyme that transfers the glutamyl moiety of glutathione to acceptors. Its main function is to make cysteine available for synthesis of glutathione within the cell, thus preventing oxidative stress. Recent experimental studies indicate that cellular GGT also has a pro oxidant potential. This effect occurs when GGT is expressed in the presence of iron or other transition metals. The cysteinylglycine product of GGT action has a strong ability to reduce ferric ions to ferrous which again promotes free radical production. Lee et al have found a positive correlation of serum and cellular GGT levels in their study [21].This relationship has been found to be strong even within normal serum GGT levels. The present study also shows a strong positive correlation between serum MDA and GGT levels in diabetics more so in diabetics with retinopathy, indicating significant oxidative stress.

The present study showed a significant increase in TC, LDL and TGL except serum HDL in diabetics when compared to controls and the rise was more pronounced in patients with diabetic retinopathy. This is similar to the findings of Sharma et al and Lyon et al [14,22]. Dyslipidemia is common in diabetics more so in poorly controlled diabetes. It has been postulated that hyperlipidemia leads to elevation of blood viscosity and alterations in the fibrinolytic system causing hard exudate formation. There may also be incorporation of serum triglycerides into the cell membrane, leading to changes in membrane fluidity and leakage of plasma into retina. This results

in hemorrhage and edema in the retina [23,24]. Chew et al have found that reduction of raised serum lipids may help prevent retinal hard exudate formation and loss of vision [25].Gupta et al have observed that oral atorvastatin therapy in patients with type 2 diabetes with dyslipidemia reduces the severity of hard exudates and subfoveal migration of lipids in clinically significant macular edema thus preventing worsening of visual acuity in patients with DR [26].

Glycated hemoglobin (HbA1c) was significantly elevated in diabetics with retinopathy when compared with diabetics without retinopathy in the present study. Duration of diabetes and inadequate glycemic control are directly proportional to HbA1c levels. This is similar to the findings of Ozmen et al and Kareem et al [2,27]. Glycated haemoglobin (HbA1c) is due to post translational changes in the haemoglobin molecule and their levels correlate well with the plasma glucose values over 8 – 10 weeks. Ronal et al in their study found that HbA1c has a high oxygen affinity, which leads to tissue hypoxia causing micro and macroangiopathy [28]. Sabanayagan et al in their study found that with an HbA1c cut-off of 7%, the prevalence of retinopathy below this point was 7.2% and above this was 35.4% and the sensitivity was 55.6% and specificity was 85% [29].

CONCLUSION

The present study has shown that oxidative stress and hyperlipidemia are some of the factors associated with diabetic retinopathy. Poor glycemic control as reflected by increased HbA1c causes worsening of retinopathy. Serum GGT is a useful marker for studying oxidative stress like serum MDA. Elevations in serum GGT within the normal range in conditions of oxidative stress have been reported in several studies. It can be used as a surrogate marker of microvascular complications in diabetes mellitus. Further studies using a larger sample size will be required to validate the results.

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