IJBPAS, March, 2014, 3(3): 280-288

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

γ-GLUTAMYLTRANSFERASE EXPRESSIONS IN INDIAN TYPE2 DIABETIC PATIENTS

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ABSTRACT

Elevated activities of serum enzyme, γ-glutamyl transferase (GGT) has been associated with obesity and oxidative stress, GGT predicts the Type 2 diabetes mellitus (T2DM). Our aim is to establish whether relationships exist between either adiposity or IR and hepatic enzymes in a group of T2DM in Indian population. Study group's follows: 20 Healthy subjects, 30 newly detected patients, and 43 type 2 diabetics on treatment with combination of metformin & pioglitazone and 30 pioglitazone alone treated. In all subjects blood sugar was estimated and anti diabetic activity criterion was taken for the diagnosis. In the same subjects fasting and post-prandial γ-glutamyltransferase activity was estimated and levels were compared with γ-glutamyltransferase activity in healthy and treated subjects. γ-glutamyltransferase levels were significantly positive relationships with type 2 diabetes and oxidative stress, which are reduced elevated levels of GGT in treatment with pioglitazone alone and combination with metformin. Hepatic enzyme γ-glutamyltransferase activities which is predict the development of T2DM in Indian population were controlled to normal by pioglitazone alone and combination with metformin treatment.

Keywords: γ-glutamyltransferase, Type2 Diabetes, HbA1c, Blood Glucose

INTRODUCTION

Serum γ-glutamyltransferase (GGT) levels were raised in obese individuals and a particularly strong association with central obesity has been described. The elevated GGT levels are a marker for visceral fat, and specifically for hepatic steatosis (fatty liver) and that hepatic steatosis leads to hepatic insulin resistance, increase the Serum γ - glutamyl transferase is widely used as a marker of alcohol induced liver diseases **[1, 2]**. It is known, however, that GGT levels raise, alcohol consumption, with age **[3],** obesity **[4-7]**, and established diabetes **[8]**. A strong linear association between GGT and waist-to-hip ratio has been described **[9]**, and there is evidence linking raised GGT levels with other cardiovascular risk factors, including physical inactivity, hypertension, and dyslipidemia **[7]**. The association of high levels of GGT with moderate obesity in non drinking men has been attributed to obesity; related hepatic steatosis **[10]**.There was a strong association between serum GGT and hepatic steatosis as determined by ultrasonography **[11]**. Type 2 diabetes is the major clinical manifestation of long-term insulin resistance **[12]**. Elevation of GGT in oxidative stress **[13]**, hyperglcemea **[14]** and hepatic inflammation **[15]**. Hence, this is whether elevated GGT levels predict type 2 diabetes. We have examined the association between serum GGT levels and risk of type 2 diabetes in a Indian population at the all age groups followed for over a three months.

MATERIALS AND METHODS

L-g-glutamyl-3-carboxy-4-nitroanilide, glycylglycyne, Tris Hcl from Hi-Media Ltd, Mumbai, India., Glucose kit from Excel Diagnostics, hyderabad, India.,Pioglitazone from Nacto pvt ltd Hyd.,Pioglit-15 from Sun Pharma. pvt ltd Sikkim.,Pioglit-MF from Sun Pharma. pvt ltd Sikkim.

Study Design Follows

Group1: Twenty non-diabetic subjects (control).

Group2: Thirty newly diagnosed type 2 diabetes without treatment.

Group3: Thirty diabetic patients on Pioglitazone alone treatment for three months

Group4: Forty two diabetic patients on oral antidiabetic combination treatment for three months (Pioglitazone and metformin).

Before starting study their age, sex and BMI are recorded.

All patients were recruited at the department of general medicine, Mahatma Gandhi memorial Hospital, Warangal, Andhra Pradesh, India from March 2008 to April 2010. All subjects were attending general health check up at our outpatient department

(Diabetic Care Programme) in MGM Hospital. Subjects were excluded if they had chronic gastrointestinal diseases associated with chronic pancreatitis, history of any malignant disease, history of alcohol abuse, kidney or liver failure and other diseases affecting carbohydrate metabolism. Fasting as well as post-prandial blood samples were collected from all subjects and fasting and post-prandial serum glucose **[16]** and GGT levels (Szasz) and fasting lipid profile levels **[17-20]** were estimated. The study was approved by institutional ethics committee (University College of Pharmaceutical Sciences Kakatiya University, Warangal) and informed consent was obtained from each subject.

Statistical Analysis

All variables are expressed as mean ± SD. Group differences of continuous variables were compared using ANOVA followed by Student - Newman Keuls post hoc test. For all analyses, a P value < 0.05 was considered to be statistically significant. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA).

RESULTS

γ-glutamyltransferase levels in fasting and postprandial states in different groups were compared (Healthy, newly detected, pioglitazone alone treated and combination drug treated). **Table 1** represents the clinical characteristics of all group subjects γ-glutamyltransferase levels fasting and postprandial in oral antidiabetic (combination) drugs (17.3±1.5&20.1±1.3U/l) and pioglitazone alone treated groups showed significantly lowered (22.4±2.2 & 32.7±2.1U/l) than newly diagnosed type-2 diabetic patients (47.0±2.4and 62.1±4.2U/l). Statistical significant levels of γ-glutamyltransferase in different groups represented in **Table 1**. The results of present study indicates that fasting γ-glutamyltransferase levels in different groups were statistically significant ($P<0.05$). The significant relation between γ-glutamyltransferase and blood glucose and glutathione was observed in type 2 diabetic patients ($p<0.001$). Type 2 diabetes with HbA1c >12% showed significantly ($p<0.031$) higher γ-glutamyltransferase activity (mean±SD; 62.1±4.2U/l) and then healthy ((HbA1c<7% and γ-glutamyltransferase activity (19.2±1.2U/I, respectively)). Variations in γ-glutamyltransferase activity for 3 months treatment in type 2 diabetic patients showed, a significant reduced the HbA1c ($p<0.001$), similarly fasting and post-prandial. γ-glutamyltransferase levels and Triglycerides, LDL&VLDL in pioglitazone and combination treated groups but increased the Glutathione and HDL levels ($P <0.001$),

DISCUSSION

The present study results reveals that Gamma GT levels were increased in newly detected type 2 diabetic patients irrespective of treatment status; the increased was more pronounced in untreated diabetic patients and decreased with clinical improvement under the pioglitazone treatment, pioglitazone & metformin combination treatment. Gamma GT levels were increased depending on the clinical severity of the disease. Our results were support of **[21]**, reported that similar relationship between elevated GGT levels and insulin resistance in type 2 diabetes has been described.

In the present study GGT levels were increased in type 2 diabetic patients due to oxidative stress associated development of type 2 diabetes and hepatic inflammation. These findings were support of future investment of **[22]** reported that increased GGT activity may reflect not only hepatic oxidative stress (mediated by fat accumulation inside hepatocytes) but also systemic oxidative stress. Oxidative stress has been associated with insulin resistance in several studies **[23, 24]**. Reactive oxygen species can activate transcription factors, such as nuclear factor-kB and activating Protein-1 **[25, 26]**, participants in important inflammatory signaling pathways recently implicated in the pathogenesis of insulin resistance.

GGT could be a marker of hepatocyte damage or hepatic dysfunction. Liver disease is a condition associated with elevated concentrations of GGT **[27]**. Serum GGT activity might be a reflection of the chronic inflammation associated with low levels of anti-inflammatory hormones present in obesity (*e.g.* adiponectin) or with the reduced effectiveness of insulin as a modulator of cytokine action **[28]**. Indeed adiponectin has been found to be inversely related to GGT and predicts GGT activity independently of insulin resistance **[29]**. Serum GGT activity has been shown to predict concentrations of inflammatory markers such as C-reactive protein and fibrinogen **[30]** and is strongly associated with C-reactive protein independently of sex, obesity, and alcohol and smoking habits **[31]**.

The present study results showed GGT activity was decreased with Pioglitazone, pioglitazone & metformin combination treatment, because of antioxidant and anti inflammatory activity of pioglitazone and metfomin. These findings were support of **[32]**, Metformin inhibits proinflammatory responses and NuclearFactor-B in Human Vascular Wall Cells and antioxidant activity **[33]**. Several reports have demonstrated an anti-inflammatory action of the specific ligands of PPARγ Activators of PPAR γ inhibit the generation of pro-inflammatory cytokines such as interleukin-1β (IL- 1β), tumor necrosis factor-α (TNF- α) and interleukin-6 **[34]**. PPAR γ agonists increase HDL particles to inhibit dyslipidemea and antioxidant activivity **[35]**.

In the present study results indicate that serum GGT activity is positively associated with hyperglycemia. All these studies and our present findings stress the importance of pioglitazone and metformin combination therapy.

CONCLUSIONS

Indian ethnic group's shows increased Post-prandial γ-glutamyltransferase levels were significantly higher in type 2 diabetic group when compared to normal controls. This is a contradictory finding that serum γ-glutamyltransferase activity in patients with type-2 diabetes mellitus relatively positive with Oxidative stress, HbA1c, blood glucose and lipid profile levels, which were completely controlled to normal by pioglitazon alone and combination with metformin treatment but increased the HDL levels.

ACKNOWLEDGEMENTS

The first author is thankful to University grants commission (UGC), New Delhi, India for providing Rajiv Gandhi National Junior Research Fellowship.

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Table 1: Clinical Data & Characteristics of Subjects Participating in the Study

Parameter/Groups	Healthy volunteers	Newly-detected Diabetes	Pioglitazone alone Treated	Oral Antidaibetic (combination)
Age(years)	25.85±2.4	44.12±12.1	44.12±12.1	49.9±10.6
Sex(M/F)	20(12/08)	30(17/13)	30(17/13)	43(25/18)
BMI(Kg/m2)	21.5±2.3	26.3±3.3	24.3±3.3	23.5±2.3
Fasting glucose (mg %)	96.5±6.4	174.3±5.4	112.8±8.3[a]	92.4±4.8[c]
Post-prandial glucose (mg %)	113.5±9.3	223.5±6.7	126.4±7.1[c]	108.3±5.6[c]
Fasting GGT activity (U/l)	14.5±.1.1	47.0±2.4	22.4±2.2 [b]	17.3±1.5[c]
Post-prandial GGT activity (U/l)	19.2±1.2	62.1±4.2	32.7±2.1[b]	20.1±1.3[c]
Glutathione (nmol)	55.1±4.2	22.4±1.8	35.4±2.1[a]	43.1±3.8[c]
HbA1c (%)	4.7±0.31%	14.3±1.50%	7.3±1.11%[b]	5.8±0.56%[c]
Total cholesterol (mg%)	125.4±11.3	234.5±15.1	141.2±11.7[c]	128.6±9.8[c]
Triglycerides (mg%)	108.3±8.6	206.7±19.4	131.6±12.2[b]	112.7±8.6[c]
VLDL(mg%)	21.66±2.7	41.34±3.3	26.32±2.1[b]	22.54±1.8[b]
LDL (mg%)	52.54±4.7	161.76±14.2	75.08±5.9[c]	61.96±4.1[c]
HDL (mg%)	51.2±3.8	31.4±2.7	39.8±2.8[b]	44.1±3.7[b]

(Data Were Mean ± SD; [a]$p<0.05$, [b] $p<0.01$, [c] $p<0.001$ vs. Diabetic Patients and Healthy Volunteers).