ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 03, pp. 28558-28562, March, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

SERUM GAMMA GLUTAMYL TRANSFERASE AND MAGNESIUM AS MARKERS OF OXIDATIVE STRESS IN TYPE2 DIABETIC PATIENTS

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ARTICLE INFO

Article History:
Received 19th December, 2015
Received in revised form
07th January, 2016
Accepted 23rd February, 2016
Published online 31st March, 2016

Key words:

Diabetes mellitus, Oxidative stress, Gamma glutamyl transferase, Magnesium, Albumin.

ABSTRACT

Background and Aims: Oxidative stress and adverse metabolic changes are key components of type2 diabetes mellitus. Gamma glutamyl transferase (GGT) enzyme maintains the GSH-cysteine homeostasis as well as detoxifies the toxic molecules thereby lower oxidative stress in the body. This enzyme is associated with albumin which is also a vital antioxidant molecule. Magnesium is required for the action of various glucose metabolizing enzymes and thus maintains glucose homeostasis as well as affects insulin secretion and sensitivity via modulation of oxidative stress. The purpose of the present study was to elucidate the role of serum GGT, magnesium and albumin in relation to hyperglycemia in type 2 diabetic patients.

Method and Results: The study comprised of 50 diagnosed cases of type 2 diabetes mellitus and 30 age and sex matched healthy controls. Subjects were selected after applying appropriate inclusion and exclusion criterion. Written informed consent was obtained from all the subjects to draw their fasting blood samples under sterilized conditions. Diabetic patients were segregated into two Groups : Group 1 (fasting blood glucose levels 130-200mg/dl) and Group 2 (fasting blood glucose levels >200mg/dl). Serum GGT, magnesium, albumin and fasting blood glucose were estimated and data was statistically analyzed. Serum GGT activity was significantly high ($p<0.05$) and serum magnesium and albumin levels were significantly low ($p<0.05$) in type2 diabetic patients as compared to controls. A significant negative coefficient of correlation ($p<0.05$) was observed between Group 2 patients and their GGT activity.

Conclusions: Increased serum GGT activity and low Mg and albumin levels are markers of high oxidative stress and metabolic disturbances in type 2diabetes. Body may try to compensate increased oxidative stress by preserving GSH by lowering of GGT activity in a state of increased hyperglycemia.

Citation: Ritu Sharma and Mridula Mahajan, 2016. "Serum gamma glutamyl transferase and magnesium as markers of oxidative stress in type2 diabetic patients", *International Journal of Current Research,* 8, (03), 28558-28562.

INTRODUCTION

The incidence of type 2 Diabetes is increasing at an alarming pace worldwide. Oxidative stress and metabolic disturbances are integral components in the pathogenesis of this disease. Several biomarkers such as C-reactive protein, C-peptide, IL-6, TNF alpha are known to play a role in inflammatory process of both type 1 and type2 diabetes mellitus. Hence, evaluation of oxidative stress and associated metabolic changes is highly important to control the advanced complications of this disease. Gamma glutamyl transferase (GGT) is a cell surface protein (enzyme) which is responsible for the extracellular catabolism of GSH (an important antioxidant molecule).

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This hydrolysis of GSH produces cysteinyl-glyceine and other thiol compounds which generate superoxide anions through its interaction with free iron and thus aggravates oxidative stress in the body (Paolicchi *et al*., 1999). Such type of environment adversely affects beta cell function, insulin secretion and increase the risk of CAD in such patients. GGT itself is a pro-atherogenic molecule and has been reported to occur in atherosclerotic plaques (Paolicchi *et al*., 2004). In few population studies, elevated GGT levels have been reported in diabetic as well as in patients suffering from cardiovascular disease (Onat *et al*., 2012; DEmircan *et al*., 2009; Ko *et al*., 2015). GGT in serum is carried primarily with lipoproteins and albumin. Oxidative stress conditions also affect albumin levels as its structure gets modified and looses its important metabolic functions. Moreover, it is an important circulating antioxidant molecule linked with GGT in serum (Bourdon *et al*., 1999).

Oxidative stress environment causes variations in the levels of important minerals playing a vital role in maintaining the healthy state of beta cells. Magnesium acts as a cofactor for many enzymes required for glucose metabolism by utilizing high energy bonds (Saris *et al*., 2000). Furthermore, Mg also serves as an antioxidant molecule (Olatunji and Soladoye, 2007). Previous studies have reported low serum magnesium levels in type 2 diabetic patients in different populations (Rao and Shariff, 2015; Song *et al*., 2004). This mineral affects insulin secretion adversely under conditions of oxidative stress. All these three molecules i.e. GGT, Mg and albumin seems to contribute in the pathogenesis of type2 diabetes mellitus via modulation of oxidative stress. Incidence of type2 diabetes mellitus is relatively high in Indian Punjabi population owing to life style and probably genetic factors. Variations in especially GGT activity could give significant information of disease pathogenesis and risk of its advanced complications. It would be interesting to study the role of GGT in this population as marker of oxidative stress in patients suffering from type2 diabetes mellitus. Hence, the present study was aimed to investigate the role of serum GGT, magnesium and albumin in relation to hyperglycemia in type 2 diabetic patients belongining to North West Indian Punjabi population.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Govt. Medical College, Amritsar India. 50 diagnosed cases of type2 diabetes were recruited from the Medicine department of Guru Nanak Dev Hospital (Attached hospital of Govt Medical College, Amritsar). Only those patients were included who were free from any major renal complications, acute episode of CAD, hepatic and thyroid dysfunction, chronic infection, Rheumatoid arthritis, past/present history of smoking and chronic alcoholism. Mean blood urea and serum creatinine levels in diabetic patients were 32±6mg/dl and 1.0±0.6mg/dl respectively. These patients visited the clinical Biochemistry laboratory of Guru Nanak Dev Hospital, Amritsar for their routine investigations. They were referred by the Medicine department of the Hospital. Only those cases were selected who had their fasting blood sugar levels > 130mg/dl.

Type 2 diabetics were segregated into two Groups: Group 1(fasting blood glucose 130-200mg/dl); Group2 (more than 200mg/dl). 30 age and sex matched normal healthy subjects were taken as controls from the general population. Written informed consent was obtained from all the subjects to obtain their blood samples. All the subjects were screened for fasting blood glucose levels, gamma-glutamyl transferase activity, serum magnesium and serum albumin levels. Gamma-glutamyl transferase was estimated by IFCC kinetic method procuring kit from ERBA diagnostic Mannheim Gmbh. Briefly as per the protocol adopted, GGT in serum catalyzes the transfer of the glutamyl group from the substrate gamma-glutamyl-3-carboxy-4-nitroanilide to glycylglycine forming glutamyl glyclglycine and 5-amino-2-nitrobenzoate, the rate of formation of which is proportional to the activity of GGT (IU/L) in serum measured at 420nm. Serum magnesium was estimated by calmagite method (Crest Biosystems, Goa). Briefly serum magnesium combines with calmagite in an alkaline medium to form red coloured complex, intensity of which was directly proportional to the concentration of magnesium in serum sample at 510nm. Serum albumin by BCG dye method (ERBA, Mannheim Gmbh) and fasting blood glucose by Glucose oxidase method.

Stasitical Analysis

Student's test was applied. Pearson coefficient of correlation was calculated and significance was accepted at p value=.05

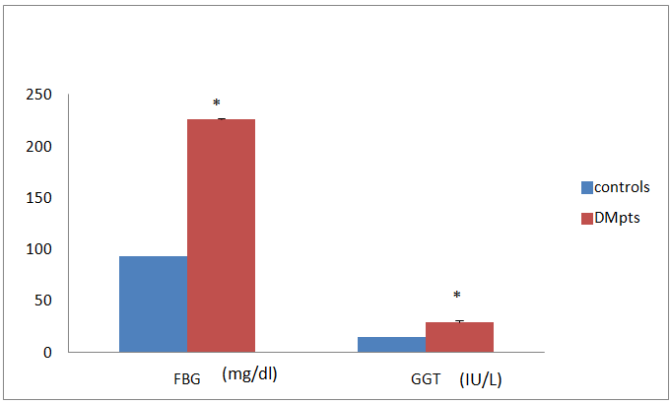
RESULTS

50 diagnosed cases of type 2 diabetes were selected and studied who were referred to Clinical Biochemistry laboratory for their routine investigations. They were screened for fasting blood glucose levels, serum GGT, magnesium and albumin, considering that variations in these parameters creat stressful conditions in human body and may play a role in the pathogenesis of diabetes mellitus. Serum GGT activity was significantly increased (*P*=.05) in type 2 diabetic patients as compared to normal subjects (Fig 1) whereas serum magnesium and albumin levels were significantly low (*P*=.05) in these patients (Fig 2). These results indicate that there is prevalence of increased oxidative stress associated with disturbed glutathione homeostasis (reflected by increased GGT activity) and reduction in activity of magnesium dependent glucose metabolizing enzymes due to low serum Mg levels. In the present study almost 100% of diabetic patients had clear cut increased GGT activity and low Mg levels as compared to controls and no overlapping was observed in this respect. However, little overlapping was observed in serum albumin levels of diabetic and normal individuals. No significant difference (p>0.05) was observed in GGT activity between males and females (data not shown), hence no further stratification of subjects were done pertaining to gender.

Next, we were interested in investigating if the fasting blood glucose levels bear any relationship with GGT, magnesium or albumin levels. Therefore, type2 diabetics were further classified into two Groups; Group 1 (fasting blood glucose levels: 130-200mg/dl) and Group 2(more than 200mg/dl). Diabetic patients having their fasting blood glucose levels more than 200mg/dl had significantly low (*P*=.05) serum GGT activity as compared to patients having fasting blood glucose levels less than 200mg/dl (Table 1). A Significant negative (r=-0.434; *P*=0.034) coefficient of correlation was observed between fasting blood glucose levels and serum GGT activity. However no such association was observed with respect to magnesium and albumin levels which were all together low in patients as compared to controls.

DISCUSSION

Type 2 diabetes mellitus is a threat to human health because of increasing morbidity and mortality associated with advanced complications of this disease. Oxidative stress is a major factor which plays an important role in the pathogenesis of type2 diabetes mellitus. Gamma glutamyl transferase enzyme is a key enzyme in glutathione metabolism and detoxification and any variations in its activity may serve a good indicator of the status of GSH-cysteine homeostasis in the human body.



*P=.05: Significant difference in serum GGT activity was observed when type 2 diabetic patients were compared with normal healthy subjects

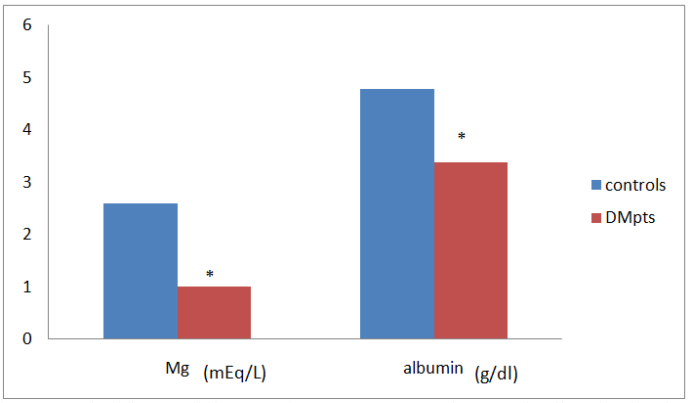
Fig. 1. Fasting Blood glucose levels and serum GGT activity in type 2 diabetic patients in comparison to controls

Table 1. Effect of hyperglycaemia on serum GGT, Magnesium and albumin levels in type 2 diabetic patients

Fasting blood glucose levels (mg/dl) ⇩	Serum GGT (IU/L)	Serum magnesium (mEq/L)	Serum Albumin (g/dl)
GROUP 1 (131-200mg/dl) (n=28)	30.0±7.56	0.98±0.18	3.92±0.57
GROUP 2 (Above 200mg/dl) (n=22)	22.9±13.3*	1.02±0.12	3.73±0.84

*P=.05: Significant difference in serum GGT activity between Group 1 and Group2 diabetic patients

A Significant negative (r=-0.434; P=.034) coefficient of correlation was observed between fasting blood glucose levels and serum GGT activity in Group 2 diabetic patients.



*p=.05: Significant difference in serum magnesium and albumin levels between type 2 diabetic patients and normal healthy subjects

Fig. 2. Serum Magnesium and albumin levels in type2 diabetic patients in comparison to controls

The maintenance of good concentration of GSH is highly essential to combat the increased oxidative stress. Hence we were interested to study the status of GGT, Mg and serum albumin in type 2 diabetic patients in comparison to normal subjects. Serum GGT activity was observed to be significantly increased in diabetic patients than controls. This increased GGT activity indicates the increased degradation of GSH which in turns is suggestive of high oxidative stress conditions. Moreover, the degradative products of GSH such as cysteine and other thiol compounds promote atherosclerosis by increasing LDL oxidation which is itself a risk factor for CAD (Sen *et al*., 2009). The information derived from GGT estimation seems to be highly vital as it tells us about the extent of oxidative stress as well as indicate the increased risk of advanced complications of diabetes such as risk of CAD. Furthermore, increased oxidative stress promotes beta cell dysfunction and reduces insulin secretion. It seems that increased catabolism of GSH by increased GGT activity and concomitant increase in LDL oxidation generates the favourable conditions for development of diabetes and its advanced complications. These results support the findings of other workers who reported increased GGT activity in diabetic patients as compared to controls in other populations (Gohel *et al*., 2013; Knubchavhandani *et al*., 2014). Moreover, other lines of evidence support a relationship between raised GGT and metabolic syndrome (Lee *et al*., 2007). Thus higher levels of GGT are accompanied by more insulin resistance and increased risk of type 2diabetes.

Serum GGT activity was significantly low in those diabetic patients who had their fasting blood glucose levels more than 200mg/dl than those having levels less than 200mg/dl. A significant negative coefficient of correlation was observed between fasting blood glucose levels and GGT activity in Group 2 diabetic patients (Table 1). This was an interesting finding. It is well known that persistent hyperglycemia generates oxidative stress. It may be possible that relatively high fasting blood glucose levels (as observed in Group 2 diabetics) act as a trigger to depress the GGT activity as a compensatory mechanism to combat the increased oxidative stress by preserving the GSH levels. It may be possible that with further severity of hyperglycaemia in the state of uncontrolled diabetes, this compensation becomes inadequate and GGT activity changes, however the trend cannot be predicted as it needs a separate follow-up study. It is important to mention here that despite the possibility of compensation/ decompensation mechanism, GGT activity might remain high in diabetics as compared to healthy subjects (Fig. 1.)

In diabetic patients, hyperglycaemia via several mechanisms such as glucose autooxidation, imbalance in the amount of reduced and oxidized coenzyme forms leads to biochemical sequel which promotes oxidative stress (Bonnefont-Rousselot, 2002; Robertson and Harmon, 2004). This also causes oxidative protein damage. Human serum albumin is a potent circulating antioxidant molecule which performs diverse functions pertaining to carbohydrate and lipid metabolism. GGT in serum is carried with albumin and lipoproteins (Whitfield, 2001). In the present study, serum albumin levels were relatively low in type2 diabetics as compared to normal subjects but no significant difference was observed in Group 1 and Group2 diabetic patients. In the state of hyperglycaemia, both oxidative stress and glycation of proteins occurs. Both these events tend to modify albumin. Also, in this disease, albumin excretes in the urine, severity of which depends upon the degree of nephropathy. It is pertinent to mention here that we did not include diabetics with evident nephropathy but excretion of this protein even in small concentrations cannot be ruled out in this disease because of the possible occurrence of hidden mild to moderate kidney dysfunction in this disease. In

diabetics, protein breakdown also takes place due to hormonal and metabolic events. All these factors tend to lower serum albumin levels and increases oxidative stress and aggravate the pathogenesis as human serum albumin is a very important circulating antioxidant molecule (Bourdon *et al*., 1999) Poor nutritional state and adverse metabolic changes are a cause and effect of type2 diabetes mellitus. Magnesium is one of the essential minerals and is important in maintaining glucose and insulin homeostasis. We observed significantly low serum Mg levels in diabetic patients as compared to controls (Fig 2). Low magnesium levels in type2 diabetes have also been reported by other workers (Rao and Shariff, 2015; Song *et al*., 2004; Pham *et al*., 2005). Meta-analysis of eight studies reported a significant inverse association between magnesium intake and risk of type2 diabetes (Dong *et al*., 2011). Diminished levels of magnesium decreases tyrosine kinase activity at insulin receptor and increases intracellular calcium levels leading to impairment in insulin signaling. Low serum magnesium levels could be due to enhanced renal excretion in diabetics. Hypomagnesaemia has been linked with albuminuria (Rao and Shariff, 2015). Some studies showed that hyperglycaemia contributes to low magnesium levels by causing depression in the net tubular reabsorption of magnesium (McCarty, 2005). Magnesium has been reported to possess antioxidant property. Serum magnesium levels are inversely associated with systemic inflammation markers (Kim *et al*., 2010).

Conclusions

Our findings suggest that variations in serum GGT and magnesium contribute significantly in the pathogenesis of type 2 diabetes mellitus by promoting oxidative stress and metabolic disturbances.

Limitations

The present study was conducted on small number of patients, but the results clearly showed the importance of gamma-glutamyl transferase and magnesium in type 2 diabetic patients as marker of oxidative stress and advance diabetic complications. In order to firmly establish the association of relatively low GGT with higher fasting blood glucose levels (Group 2 patients), a follow-up study is required which could also justify the hypothesis of compensatory mechanism to preserve GSH levels in the state of higher degree of hyperglycaemia.

Acknowledgements

We are grateful to all the subjects who participated in this study for their cooperation. Help rendered by the technical staff of Clinical Biochemistry laboratory is highly acknowledged.

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