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Serum Gamma Glutamyl Transferase is a Potential Biomarker for Isolated Systolic Hypertension

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Abstract

Background: *Oxidative stress plays a key role in the development of Isolated systolic hypertension (ISH) commonly associated with type2 diabetes mellitus. Gamma glutamyl transferase (GGT) enzyme maintains the GSH-cysteine homeostasis and detoxifies whereas magnesium maintains glucose homeostasis and hence both these molecules lowers oxidative stress.*

Objectives: *The aim of the present study was to elucidate the relationship of serum GGT and magnesium with isolated systolic hypertension associated with type 2 diabetes mellitus.*

Methods: *60 diagnosed cases of type 2 diabetes mellitus were segregated into two Groups: Group1 comprised patients having uncontrolled ISH and Group2 included patients with controlled generalized hypertension. Control group (n=40) comprised of non-diabetic subjects divided on the basis of presence or absence of ISH. Serum lipid profile, GGT, magnesium, albumin and fasting blood glucose were estimated. Data was statistically analyzed using t-test and chi-square. P<0.05 was considered as statistically significant.*

Results: *Serum GGT activity was markedly high (p<0.05) in type2 diabetic patients having ISH (Group 1) as compared to Group 2 patients while serum magnesium and albumin levels did not differ significantly (p>0.05). Similar results were also observed in control group. Serum Mg and albumin levels were low in type2 diabetic patients as compared to control group with or without ISH. A significant positive correlation (p<0.05) was observed between increased GGT and systolic blood pressure in both patient and control subjects. Lipid profile of patients and control group did not show significant difference.*

Conclusions: *Serum GGT may act as potential biomarker for isolated systolic hypertension and can be used for its diagnosis and prognosis.*

Keywords: *Type 2 Diabetes mellitus, isolated systolic hypertension, oxidative stress, gamma glutamyl transferase, magnesium.*

Introduction

Isolated systolic hypertension (ISH) has been changed from a benign condition to the major cardiovascular risk factor. Occurrence of isolated systolic hypertension is highly common in type diabetics especially in the elderly. Approximately 30-60% of diabetics are observed to be hypertensive. Oxidative stress is a key role-player in the pathogenesis of hypertension. Furthermore, poor glycemic control promotes oxidative stress

and atherosclerosis which further makes the management of isolated systolic hypertension more difficult. Hence, control of oxidative stress and hyperglycaemia is highly important to check the development of advanced coronary events. Various studies have shown the role of nitric oxide, hydrogen peroxide, superoxide dismutase and glutathione peroxidase in generalized hypertension. Gamma glutamyl transferase (GGT) is a cell surface protein (enzyme) which is responsible for the extracellular catabolism of GSH (an important antioxidant molecule). This hydrolysis of GSH produces cysteinyl-glyceine and other thiol compounds which generate superoxide anions through its interaction with free iron and thus promotes foam cell formation and aggravates oxidative stress in the body[(1)]. Such type of environment promotes atherosclerosis, adversely affects beta cell function, insulin secretion and increase the risk of acute coronary event in such patients. GGT itself is a pro-atherogenic molecule and has been reported to occur in atherosclerotic plaques[(2)]. Elevated serum GGT has been reported as an independent predictor of long term mortality in acute myocardial infarction patients[(3)]. In few population studies, elevated GGT levels have been reported in diabetic as well as in patients suffering from cardiovascular disease and generalized hypertension[(4-6)]. GGT in serum is carried primarily with lipoproteins and albumin. Albumin itself is an important circulating antioxidant molecule linked with GGT in serum[(7)].

It is quite evident that oxidative stress is involved in the pathogenesis of hypertension and isolated systolic hypertension category is no exception except that we need to study it more. Antioxidants may have beneficial effect on lowering the risk of isolated systolic hypertension. Studies have shown that vit C, E, beta carotene and zinc supplementation resulted in significant reduction in systolic blood pressure while the diastolic pressure was not affected appreciably. The DASH diet has recommended the use of fruits, vegetables, high fibre and low fat dairy products to lower the isolated systolic hypertension[(8)]. Magnesium is an important mineral which serves as an antioxidant molecule as well as a cofactor of several glucose metabolizing enzymes. Low serum magnesium levels have been reported in diabetics as well as in cases of generalized hypertension. Low magnesium levels level leads to altered phosphorylation of Insulin receptor and hence promotes peripheral Insulin resistance in type2 diabetes mellitus[(9-13)]. Hence with proper glucose metabolism, the oxidative stress will be low and as a result the risk of cardiovascular diseases decreases.

In the present study, we were interested to investigate the role of GGT and magnesium in isolated systolic hypertensive cases associated with type2 diabetes mellitus in Indian Punjabi population having high incidence of diabetes and hypertension. To be more definites, we included a group of apparently healthy subjects with and without isolated systolic hypertension. However, these subjects were free from type2 diabetes mellitus.

Material and Methods

The study was conducted in the Department of Biochemistry, Govt. Medical College, Amritsar India. 60 diagnosed cases of type2 diabetes were recruited from the Medicine department of Guru Nanak Dev Hospital (Attached hospital of Govt Medical College, Amritsar). As per our objective, we were interested to screen them for the presence of isolated systolic hypertension. Out of 60 diabetic patients, 40 had isolated systolic hypertension which was uncontrolled despite on strict medication (Group1). This information was self reported and was also verified from their files and 20 had controlled generalized hypertension i.e. they had both raised systolic and diastolic blood pressure. Their mean systolic blood pressure of Group 1 patients was 160±10mm of Hg. All the patients were on requisite treatment. 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 of Group 1 patients were 50±6mg/dl and 2.0±0.6mg/dl respectively. Mean age of these patients was 50±12 yrs. 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. A total of 40 age and sex matched apparently healthy subjects were selected from the general population. 24 subjects had isolated systolic hypertension (mean systolic pressure; 150±12mm of Hg) and 16 subjects had no hypertension. However all these subjects were free from type2 diabetes mellitus. 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 albumin levels. The history of isolated systolic hypertension was self reported. Gamma-glutamyl transferase was estimated by IFCC kinetic method procuring kit from ERBA diagnostic Mannheim Gmbh. Briefly as per the protocol, 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 was estimated by BCG dye method (ERBA, Mannheim Gmbh) and fasting blood glucose by Glucose oxidase method. All the data was analyzed with Student't test. Significance was accepted at p value=.05

Results

In the present study, approximately 67% of type2 diabetics and 60% of apparently normal subjects (without diabetes mellitus and other disorders) had isolated systolic hypertension (Table 1). Isolated systolic hypertension is a matter of high concern these days because of its association with other abnormalities such as diabetes mellitus and difficult management. Oxidative stress is playing a key role in the pathogenesis of hypertension and we were particularly interested in Isolated systolic hypertension.

Serum GGT, Mg and albumin levels of type2 diabetic with uncontrolled isolated systolic hypertension (Group 1) were compared with those having controlled generalized hypertension (Group 2; Table 2). Serum GGT activity was markedly increased ($p<0.05$) in patients suffering from isolated systolic hypertension (Group1) as compared to Group2 patients. A positive correlation was observed between GGT and systolic pressure in these patients (Group 1). However, there was no significant difference ($p>0.05$) in serum Mg and albumin levels in Group1 and Group2 patients.

Next we were interested to see if GGT activity varies in apparently normal subjects with and without systolic hypertension (Table 3). This would give a clear picture of GGT as a potential biomarker in this respect. Serum GGT activity was highly increased ($p<0.05$) in subjects with isolated systolic hypertension as compared to subjects who were healthy having no hypertension. However the levels were relatively low than that observed in Group 1 and Group 2 patients. Similar to patient Group 1, a significant ($p<0.05$) positive correlation was observed between GGT and systolic pressure in subjects suffering from isolated systolic hypetension. However, no significant difference ($p>0.05$) was observed in serum Mg and albumin levels. It is important to mention here that although magnesium and albumin levels did not differ significantly in subjects with and without isolated systolic hypertension, the levels were altogether

low in patients than control subjects with increased systolic blood pressure (Fig1), suggesting that the combination of type2 diabetes and isolated systolic hypertension is markedly associated with increased oxidative stress. No significant difference in lipid profile of patients and control group (with/without ISH) was observed. Also, in the present study, we did not observed any significant difference in serum GGT between males and females and hence no stratification of subjects were done in this respect (data not shown)

Table1: Percentage of subjects with isolated systolic hypertension

Subjects	With Isolated systolic hypertension
Type 2 diabetic patients (n=60)	67%
Normal Subjects (n=40)	60%

Table 2: Serum GGT, Magnesium and albumin levels in Isolated systolic hypertensive patients

Patients (n=60) ⇩	Serum GGT (IU/L)	Serum magnesium (mEq/L)	Serum Albumin (g/dl)
GROUP 1 (Type2 diabetics with Isolated systolic hypertension) (n=40)	**40.0±7.56**	**0.98±0.18**	**3.92±0.57**
GROUP 2 (Type2 diabetics without Isolated systolic hypertension) (n=20)	**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 patients.
**p>0.05 (NS).

A Significant positive (r=0.48; p<0.05) correlation was observed between GGT and systolic blood pressure.

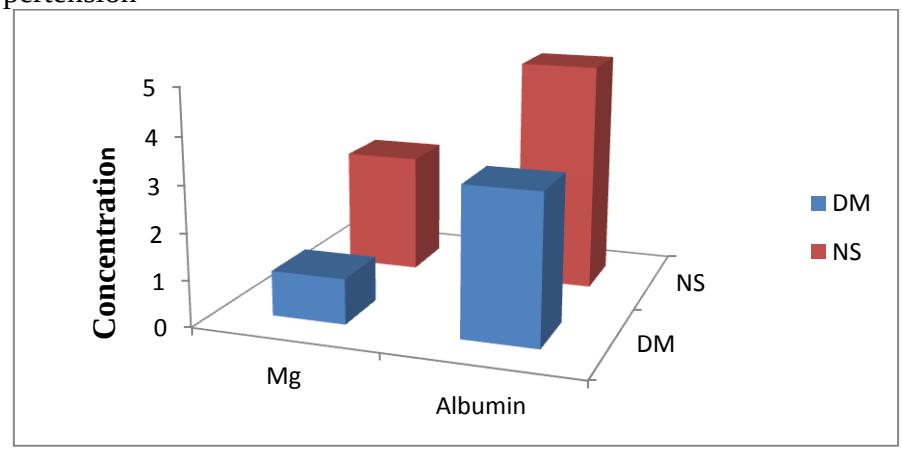
Table 3: Serum GGT, Magnesium and albumin levels in apparently healthy subjects with Isolated systolic hypertension (Control group)

Healthy Subjects (n=40) ⇩	Serum GGT (IU/L)	Serum magnesium (mEq/L)	Serum Albumin (g/dl)
Isolated systolic hypertension positive (n=24)	**16.0±5.36**	**2.6.±0.20**	**4.94±1.66**
No Isolated systolic hypertension (n=16)	**9.9±4.3***	**2.8±0.16****	**4.83±1.84****

**P*=.05: Significantly increased serum GGT activity was observed in normal subjects with isolated systolic hypertension.
**p>0.05(NS)

A Significant positive (r=0.52; p<0.05) correlation was observed between GGT and isolateds systolic pressure.

Fig 1: Serum Magnesium and albumin levels in type2 diabetic patients (Group 1) and control subjects with isolated systolic hypertension



P<0.05: Significant difference in serum Mg and albumin levels between diabetic patients and non-diabetic subjects with isolated systolic hypertension
DM: Diabetes mellitus; NS: Normal subjects

Discussion

Isolated systolic hypertension is a very common finding associated with type2 diabetes mellitus these days and oxidative stress has been observed to be an important role player. This worsens the diseased state and also increases the risk of acute coronary syndromes. Gamma glutamyl transferase enzyme is a key enzyme in glutathione metabolism and detoxification of toxic metabolites and any variations in its activity may serve as a good indicator of the status of GSH-cysteine homeostasis in the human body. The maintenance of good concentration of GSH, removal of toxic metabolites and optimal glucose homeostasis is highly essential to lower the increased oxidative stress in the body. Hence we were interested to study the role of especially GGT and Mg in the pathogenesis of isolated systolic hypertension in type 2 diabetic patients as well as in apparently healthy subjects having increased systolic pressure but no diabetes mellitus. Serum GGT activity was observed to be significantly increased in type2 diabetic patients with uncontrolled isolated systolic hypertension(Group 1) as compared to Group 2 patients. Raised serum 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[14]. These results support the findings of other workers who reported increased GGT activity in hypetensives and in diabetic patients as compared to controls [15,16]. It is important to mention here that these studies included subjects having generalized hypertension and not the isolated systolic hypertension. Moreover, other lines of evidence support a relationship between raised GGT and metabolic syndrome which also comprises of hypertension and type2 diabetes mellitus as its core components[17]. Increased GGT activity was positively correlated with increased systolic blood pressure in Group 1 diabetic patients. Serum GGT activity was also observed to be markedly raised in non –diabetic controls having isolated systolic hypertension as compared to controls with no hypertension, although the relative activity was lower than that in patients. A strong correlation of GGT with ISH clearly indicates that elevated GGT estimation has great potential to act as biomarker to predict the risk of ISH. Hyperglycaemia via several mechanisms such as glucose auto-oxidation, imbalance in the amount of reduced and oxidized coenzyme promotes oxidative stress and may increase the stiffness in arteries particularly resulting in increased systolic blood pressure[18,19].

Poor nutritional state along with adverse metabolic changes are associated with increased oxidative stress which could further aggravate isolated systolic hypertension confounded with type2 diabetes mellitus. Magnesium is important in maintaining glucose and insulin secretion. We observed significantly low serum Mg levels in diabetic patients with or without systolic hypertension as compared to non-diabetic controls with and without systolic hypertension (Table 2,3; Fig 1). Similar results were observed in case of serum albumin levels. It is pertinent to mention here that magnesium and albumin was not observed to be specifically related to raised systolic blood pressure but certainly their levels are indicative of diseased pathology. Low serum magnesium levels in hypertensive and in type2 diabetes have also been reported by other workers [8,13,20]. A significant inverse association between magnesium intake and risk of type2 diabetes have been reported in a Meta-analysis of eight studies [21]. Low magnesium levels decreases tyrosine kinase activity at insulin receptor and increases intracellular calcium levels causing impairment in insulin signalling[10]. Low serum magnesium levels could be due to enhanced renal excretion in diabetics. Hypomagnesaemia has been linked with increased excretion of albumin in urine[13]. It has been reported that hyperglycaemia causes low magnesium levels by depressing the net tubular reabsorption of magnesium[22]. Magnesium has

been reported to be inversely associated with systemic inflammation markers[23].

Our study emphasized on a very important clinical condition i.e. isolated systolic hypertension. It is encountered in the literature that sometimes the traditional parameters such as SOD, catalase etc fail to differentiate patients from controls and the important information gets missed. Based on our findings, it is advocated that GGT can serve as a potential biochemical marker for ISH. The major limitation of the present study is the small number of subjects; however the results encourage conducting a study on a larger population.

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Abbreviations

GGT: Gamma glutamyl transferase
ISH: Isolated systolic hypertension
Mg: Magnesium
GSH: Glutathione
DM: Diabetes Mellitus
CAD: Coronary artery disease