Serum Gamma Glutamyl Transferase levels in Obese South Indian adults with reference to atherogenic lipid risk factors and lipid peroxides

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ABSTRACT

Background: Obesity has reached epidemic proportions especially in South India. In recent years, Waist circumference (WC), which reflects central obesity, has been regarded as an independent predictor of risk. Generation of free radicals occurs in central obesity and depletes intracellular glutathione, thereby inducing the production and release of Gamma Glutamyl Transferase [GGT] into circulation. High GGT levels are known to be associated with Type 2 Diabetes mellitus, hypertension and related vascular complications. Hence, this study was planned with a view to exploring the nexus among these components of metabolic syndrome and serum GGT levels, especially as the characteristic biochemical events are not fully understood and addressed yet.

Materials and methods: In this prospective study we included 41 obese and 39 non-obese adults who were non-smokers and non-alcoholics, between the ages of 20 and 40 years. Lipid profile, Malondialdehyde (MDA), Fasting Plasma Glucose (FPG), blood pressure (BP) and GGT activity were measured in both the groups. Data were compared with un-paired student t-test. Pearson’s correlation was used to find the association of serum GGT levels with other variables in obese individuals.

Results: Atherogenic dyslipidemia was documented in obese individuals which was associated with significantly elevated levels of serum GGT (p<0.001). High levels of serum GGT were also associated significantly with increase in BP (p<0.001), Lipid peroxidation [(serum MDA), (p<0.05)] and atherogenic lipid ratios [(TC/HDL, LDL/HDL) (p<0.001)].

Conclusions: Elevated serum GGT levels are associated with the components of metabolic syndrome and high degree of lipid peroxidation in obese South Indian adults.

KEYWORDS: Obesity, GGT, Atherosclerosis, Lipid profile, Oxidative stress.
INTRODUCTION

Gamma Glutamyl transferase [GGT] also known as Gamma Glutamyl Transpeptidase has long been used in the diagnosis of hepato-biliary diseases, primarily fatty liver and cholestasis. It is also an excellent diagnostic marker of alcoholic liver disease [1]. GGT is expressed in the liver, kidney, cerebrovascular endothelium and pericytes and is an enzyme responsible for the extracellular catabolism of antioxidant glutathione and acts as a pro-oxidant in the extracellular space. Elevated serum GGT levels may be a reflection of high degree of oxidative stress and oxidative stress is known to be associated with central obesity. Serum Malondialdehyde (MDA) is a well known marker of degree of lipid peroxidation in vivo. [2]. High levels of GGT have also been found to be associated with various atherosclerotic risk factors such as Diabetes mellitus, hyperlipidemia, and hypertension, independent of alcohol consumption and hepatic dysfunction. Moreover, certain documented evidences are available suggesting a direct link between increased GGT activity and occurrence or progression of atherosclerosis [3-6].

Obesity has reached epidemic proportions in India in the 21st century with morbid obesity affecting 5% of the Indian population. Obesity when unchecked does lead to a host of lifestyle diseases [7]. The root cause of all diseases like Diabetes mellitus, hypertension, coronary artery disease and stroke has been traced to obesity [8-10].

Waist circumference (WC) which reflects central obesity is an independent and better predictor of risk compared to body mass index and waist hip ratio. Males with WC more than 40 inches and females more than 35 inches are known to be at a higher risk. A high WC is known to be associated with an increased risk of type 2 Diabetes mellitus (Type 2 DM), dyslipidemia, hypertension and coronary vascular disease. There are ethnic and age related differences in body fat distribution that modify the predictive validity of WC as a surrogate marker of abdominal fat [11, 12]. With the increasing association among obesity, hypertension, fatty liver and atherosclerosis in the background, we proposed this study to compare the various risk factors, as related to obesity, in the light of GGT levels, especially in South Indian population, where there is a paucity of published reports.

Aim and objectives

1. To explore the association of GGT levels with fasting plasma glucose, waist circumference, blood pressure, lipid peroxidation, lipid profile and lipid ratios in the obese south Indian population.
2. To compare the above mentioned parameters with non-obese age and gender matched healthy control group.

MATERIALS AND METHODS

Sample size: In this prospective study, we included 41 obese individuals (cases) and 39 healthy non-obese (controls) adults who were non-smokers and non-alcoholics, between the ages of 20 and 40 years.

Exclusion criteria [for both the groups]: Subjects with liver diseases like alcoholic liver disease, cholestasis and hepatitis were excluded from the study. People who were on anti epileptics and other drugs were also excluded from the study. A proper informed consent was obtained from all the subjects. This was a prospective case control study conducted in the Department of Biochemistry at a tertiary centre with equipped clinical laboratory facilities.

Study Parameters: Waist circumference was measured using standard measuring tape at the level of the upper hip bone with the tape being snug without compressing on the skin. Blood pressure was measured by standard sphygmomanometer (Manual) in supine posture; while the lipid profile and GGT were estimated with the auto analyzer [Hitachi-902 chemistry analyzer] using procedures approved by International Federation of Clinical Chemistry and Laboratory Medicine [IFCC]. Five ml of fasting (post-absorptive) venous blood sample was collected from the subjects and the following parameters were estimated.

- Fasting plasma glucose: glucose oxidase and peroxidase method.
- GGT: Carboxy substrate method
- Total cholesterol: cholesterol esterase/oxidase method
- Triacyl glycerol: Lipase-glycerol kinase method.
LDL cholesterol: is calculated by Friedwald’s equation
HDL cholesterol: polyanion precipitation method
VLDL was calculated by the formula $\text{VLDL} = \frac{\text{TAGs}}{5}$
Serum MDA by OxiSelect™ Thiobarbituric acid reactive species (TBARS) Assay Kit for MDA Quantitation by colorimetry.

**Statistical analysis:** Data were expressed as mean +/- SD; an un-paired student t-test was used to compare the data. For exploring the association between serum GGT levels and other variables Pearson’s correlation was used. A $p$ value <0.05 was considered as significant for all statistical purposes. SPSS version 17 for Windows was used for all statistical analysis. (SPSS Inc., Chicago, USA).

**RESULTS**

The age and gender distribution of the study population is depicted in Table 1. There was no significant difference in the mean age and gender of participants between the two groups. All physiological and biochemical parameters were expressed as mean±SD; results were compared by using un-paired Student t-test.

**Table 1: Age and gender distribution of the study population**

<table>
<thead>
<tr>
<th>variables</th>
<th>Cases (n=41)</th>
<th>Controls (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (58.53%)</td>
<td>21 (53.84%)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (41.46%)</td>
<td>18 (46.15%)</td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>28 (68.29%)</td>
<td>26 (66.67%)</td>
</tr>
<tr>
<td>30-40</td>
<td>13 (31.70%)</td>
<td>13 (33.34%)</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of Age, blood pressure and waist circumference between the cases and controls.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=41) [Mean ± SD]</th>
<th>Controls (n=39) [Mean ± SD]</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.8 ± 4.82</td>
<td>33.8 ± 6.06</td>
<td>0.09</td>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>145±14.81</td>
<td>120±2.79</td>
<td>0.0006*</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>93.4 ±10.24</td>
<td>79.7 ± 2.12</td>
<td>0.0004*</td>
</tr>
<tr>
<td>WC (inches)</td>
<td>45.6 ± 7.15</td>
<td>33.3 ±3.99</td>
<td>0.0006*</td>
</tr>
</tbody>
</table>

SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, WC = Waist Circumference, SD = Standard Deviation, *$p$ value <0.001.

Waist circumference, systolic and diastolic blood pressures were significantly high in obese subjects compared to the control group with $p$ value < 0.001. But there was no significant difference in the mean ages of the participants between the two groups. (Table 2).
Table 3: Comparison of serum lipids between the cases and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=41) [Mean ± SD]</th>
<th>Control (n=39) [Mean ± SD]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>215.1 ±35.22</td>
<td>164 ±48.21</td>
<td>0.0004†</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dL)</td>
<td>166.1 ± 63.98</td>
<td>112 ±52.24</td>
<td>0.021*</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)</td>
<td>139 ±31.04</td>
<td>100.4 ±40.71</td>
<td>0.0004†</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>31.6 ±5.90</td>
<td>41 ±8.50</td>
<td>0.0008†</td>
</tr>
<tr>
<td>VLDL Cholesterol (mg/dL)</td>
<td>45.8 ±14.03</td>
<td>22.4 ±10.45</td>
<td>0.0006†</td>
</tr>
</tbody>
</table>

LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, HDL= High Density Lipoprotein. *p value <0.05, † p value < 0.001.

Table-3 shows that total cholesterol, triacylglycerols and LDL cholesterol levels were significantly high in cases as compared to controls; while HDL-cholesterol levels were significantly low in cases. Alteration in the fasting lipid profile indicates pro-atherogenic dyslipidemia in obese subjects.

Table 4: Comparison of lipid ratios between the cases and controls.

<table>
<thead>
<tr>
<th>Lipid Ratios</th>
<th>Cases (n=41) [Mean ± SD]</th>
<th>Controls (n=39) [Mean ± SD]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC/HDL</td>
<td>8.2 ±2.4</td>
<td>5.11 ± 1.04</td>
<td>0.0004*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>4.5±1.25</td>
<td>2.5±1.35</td>
<td>0.0007*</td>
</tr>
</tbody>
</table>

TC= Total Cholesterol, LDL=Low Density Lipoprotein, VLDL=Very Low Density Lipoprotein, HDL= High Density Lipoprotein. *p value < 0.001.

Table-4 shows that the lipid ratios were significantly higher in obese individuals as compared to controls. Alterations in the lipid ratios support pro-atherogenic dyslipidemia in obese subjects.

Table 5: Comparison of serum GGT, MDA and Fasting Plasma Glucose levels between the cases and controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=41) [Mean ± SD]</th>
<th>Control (n=39) [Mean ± SD]</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GGT (IU/L)</td>
<td>33.3±25.50</td>
<td>19.7±7.7</td>
<td>0.002*</td>
</tr>
<tr>
<td>Serum MDA (µmol/L)</td>
<td>2.19 ± 2.2</td>
<td>1.58 ± 1.6</td>
<td>0.003*</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>86.5 ± 14.7</td>
<td>81.5 ± 11.4</td>
<td>0.08</td>
</tr>
</tbody>
</table>

GGT = Gamma glutamyl transferase, FPG = Fasting Plasma Glucose, MDA=Malondialdehyde. *p value < 0.05.
Table 5 depicts GGT levels in cases which were significant. \( p \text{ value} = 0.002 \). Serum MDA levels were significantly high in obese individuals compared to the control group \( p \text{ value} = 0.03 \). But there was no statistically significant difference in Fasting Plasma Glucose between the two groups.

**Table 6: Correlation of serum GGT levels with other variables in cases**

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>WC</th>
<th>SBP</th>
<th>DBP</th>
<th>FPG</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GGT</td>
<td>( r )</td>
<td>0.62</td>
<td>0.54</td>
<td>0.45</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>( p )</td>
<td>0.000*</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

WC = Waist Circumference (Inches), SBP = Systolic Blood Pressure (mmHg), DBP = Diastolic Blood Pressure (mmHg), FPG = Fasting Plasma Glucose (mg/dl), MDA = Malondialdehyde (µmol/L), GGT = Gamma Glutamyl Transferase (IU/L). \*\( p \text{ value} < 0.05 \).

**Table 7: Correlation of serum GGT levels with serum lipids in cases.**

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>TC</th>
<th>LDL</th>
<th>TAG</th>
<th>VLDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum GGT</td>
<td>( r )</td>
<td>0.69</td>
<td>0.64</td>
<td>0.71</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>( p )</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

TC= Total Cholesterol (mg/dl), LDL=Low Density Lipoprotein (mg/dl), VLDL=Very Low Density Lipoprotein (mg/dl), HDL= High Density Lipoprotein (mg/dl), GGT = Gamma Glutamyl Transferase (IU/L). \*\( p \text{ value} < 0.05 \).

Tables 6 and 7 shows the results of Pearson’s correlation analysis between the serum GGT levels and other variables. Statistically significant positive correlation was observed when we correlated serum GGT levels with all the variables, with the exception of FPG levels. A significant negative correlation was observed with serum HDL levels.

**DISCUSSION**

In the present study, levels of serum GGT were measured in obese subjects (cases) and compared with that of healthy non-obese control population who were life time non-alcoholics. Pukka et al., in their study showed that GGT levels rise with age in both sexes [13]. In this study, the mean age of the cases was not significantly different from that of controls (Table 1). Alcoholics and the subjects with age >60 years in both sexes were excluded from our study. The major difference between the two groups was the waist circumference which was significantly higher among the cases. It is known that GGT has a protective effect in maintaining appropriate intracellular glutathione levels, which is a powerful antioxidant. Therefore, it is possible that the generation of free radicals, which can occur in central obesity, may deplete intracellular glutathione and thus induce the activity of GGT into the circulation. Oxidative stress with the attendant low-grade inflammation has been implicated in a number of pathological conditions, including aging, atherosclerosis and Diabetes mellitus [14-18].

It is known that GGT catalyzes the oxidation of LDL and conversion of LDL to oxidised LDL, a process involved in the pathogenesis of atherosclerosis [19, 20]. The study demonstrates that dyslipidemia, high systolic and diastolic blood pressure in obese individuals are associated with elevated levels of GGT- a putative marker of sub-clinical atherosclerosis. Although there was no significant difference in FPG levels between the two groups, the previous studies have confirmed elevated GGT levels in Type 2 DM cases [4, 5]. This depicts the fact that elevated GGT levels with accompanying dyslipidemia and hypertension could be used as diagnostic predictors, independent of pronounced elevation in FPG. Our study clearly demonstrates low HDL, high LDL, high total cholesterol/HDL ratio and high LDL/HDL ratio in cases when compared to the control group. Moreover, we also documented significant positive correlation of serum GGT with the components of metabolic syndrome. Estimation of serum GGT levels can thus help us to assess the risk of impending complications like hypertension, atherosclerosis, Type 2 DM and coronary artery disease in obese individuals. Elevation of serum GGT activity is a risk factor for myocardial infarction and stroke [21-23].

Hence, necessary non-pharmacological interventions can be initiated in these subjects that include lifestyle and dietary modifications. High serum GGT is associated with oxidative stress [13-18]. Even in this study we documented a high degree of lipid peroxidation in obese individuals. Supplementation of anti-oxidants will definitely help in reducing the risk of various complications related to atherosclerosis and Type 2 DM. GGT estimation can be a useful and cost-effective screening procedure especially in rural population to assess the risk of developing various complications related to atherosclerosis. Hence, GGT could be a simple, sensitive and reliable diagnostic tool that can be included in the routine armamentarium of investigations pertaining to the individuals on treatment for hypertension, Type 2 DM and coronary artery disease.

This study clearly demonstrates that documentation of high serum GGT levels in individuals may not be attributed only to alcoholism [1] but, it could serve as an indicator of higher oxidative stress, inflammation and fatty liver in obese individuals. Therefore, GGT could function as an early predictor and reliable marker of sub-clinical atherosclerosis and its complications. In routine practice sophisticated techniques such as echocardiography, colour doppler and other radiological imaging modalities are utilized to detect vascular abnormalities resulting from atherosclerosis. However, the modalities are hampered by lack of availability in primary and secondary health care centres, besides the cost factor. Morbidity associated with vascular changes still goes undetected, especially in view of the lower socio-economic status of the population. Estimation of GGT levels in serum could reflect a pragmatic approach. However, further studies on a larger population would provide a better insight.

**CONCLUSION**

We conclude that there is a significant association of elevated serum GGT levels with the anthropometric, physiological and biochemical components of metabolic syndrome namely; WC, blood pressure, impaired FPG, atherogenic dyslipidemia. High serum GGT is also associated with a higher degree of lipid peroxidation in individuals with central obesity. GGT in serum could thus be used as a screening test especially in primary and secondary health care centres to assess the risk of developing various complications related to central obesity.

**IMPLICATIONS & SCOPE FOR FUTURE STUDY**

There is a paucity of published data in this field, on South Indian population, where there is a rising incidence of morbid obesity related complications especially diabetes mellitus,
atherosclerosis and coronary artery disease. Documentation of high serum GGT levels could help us to advocate pharmacological and non-pharmacological interventions to prevent the further complications in individuals with central obesity. Lifestyle and dietary modifications, in addition to supplementation of anti-oxidants, could reduce the incidence of various complications related to atherosclerosis and Type 2 DM. However; further studies on a larger population are needed in South India on direct association of GGT with the degree of atherosclerotic process. The effects of lifestyle changes and supplementation of anti-oxidants on serum GGT can also be explored.

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