Research Article

A study of serum Gamma Glutamyl Transferase activity to assess severity in Myocardial Infarction

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Abstract

Background: Myocardial infarction is commonly due to occlusion of the coronary artery following rupture of a vulnerable atherosclerotic plaque which can cause damage or death (infarction) of heart muscle. Gamma-Glutamyl transferase is an important enzyme in the maintenance of the steady-state concentration of glutathione both inside cells and in the extracellular fluids. Several studies demonstrated that an increase in serum GGT activity can be used as a marker for increased oxidative stress in humans. It has been shown that GGT activity is directly related to the oxidative events, and plays an important role in the evolution of atheromatous plaque.

Material and Methods: Fifty patients diagnosed as having myocardial infarction and 50 age and sex matched normal healthy control were studied after taking their written consent. The blood samples were collected under aseptic conditions in plain vacutainer for GGT & CK-MB estimation. GGT estimation was done by Carboxy Substrate Method while CK-MB estimation done by immuno inhibition method on Fully Automated Biochemistry Analyzer Miura 300.

Results: Mean serum GGT levels in patients with MI were 32.82 ± 13.99 U/L and in controls 12.4 ± 7.49 U/L. While CK-MB level in patient with MI was 70.52 ± 34.31 U/L and in controls 22.14 ± 7.29 U/L. Both these values are statistically significant (p<0.001).

Conclusion: Serum GGT has got a significant association with severity of acute myocardial infarction and it correlates with CK-MB level. Elevation of GGT activity in the present study shows a positive association with cardiovascular mortality. GGT can be diagnostic as well as prognostic marker for valuable adjunct in stratifying patient risk and in assessing the severity of disease.

Keywords: Gamma Glutamyl Transferase (GGT), Myocardial Infarction, Oxidative Stress

1. Introduction

Myocardial infarction is commonly due to occlusion of the coronary artery following rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids and white blood cells in the wall of an artery. The resulting ischaemia and oxygen shortage, if left untreated for a sufficient period of time, can cause damage or death (infarction) of heart muscle cell.

Glutathione (glutamyl-cysteinyl-glycine, GSH) is the most abundant nonprotein thiol in most cells. As a substrate for the glutathione peroxidases and glutathione S-transferases, GSH plays fundamental roles in protection against oxidative stress and in detoxification/metabolism of endogenous and exogenous compounds, including carcinogens and drugs.
addition, GSH also plays roles in cell cycle regulation, cell signaling, and apoptosis. Gamma Glutamyl transpeptidase (GGT), a glycosylated protein that is partially embedded into the outer surface of the plasma membrane, catalyzes the transfer of the gamma glutamyl moiety from glutathione or glutathione-conjugates to acceptors like amino acids and dipeptides. By breaking down extracellular GSH into its constitutive amino acids, GGT provides cysteine, the rate-limiting amino acid, for GSH de novo synthesis. As such, GGT is critical for maintaining GSH and cysteine homeostasis, and its deficiency results in oxidative stress and cellular susceptibility to oxidant injury. In addition, GGT also catalyzes the metabolism of endogenous compounds such as leukotriene C4 and xenobiotics after their conjugation with GSH. In this reaction, GSH conjugates are cleaved by GGT into g-glutamyl group and cysteinylglycine-conjugates; the latter are further cleaved by peptidases until their final conversion to mercapturic acids and excretion into urine. Therefore, GGT plays critical roles in antioxidant defense, detoxification, and inflammatory processes. Gamma Glutamyl transpeptidase (GGT) catalyzes the transfer of the glutamyl moiety from glutathione, and glutathione S-conjugate to acceptors to form another amide or to water to produce free glutamate. Functionally, GGT plays important roles in glutathione homeostasis and mercapturic acid metabolism. The expression of GGT is increased as an adaptive response upon exposure of oxidative stress2.

Recent studies have provided evidence that the enzyme gamma glutamyl transferase (GGT), normally found in serum, is often accumulated within the plaque environment in substantial amounts, and that this activity is a potential source of a variety of prooxidant species. GGT appearing in plaque tissue may originate from the serum enzyme, which in fact associates with the circulating lipoprotein fractions2.

Gamma-Glutamyl transpeptidase is an essential enzyme in the maintenance of the steady-state concentration of glutathione both inside cells and in the extracellular fluids. The enzyme activity is used diagnostically, but abnormal activity is a likely contributor in the pathology of diseases in which an oxidative component is involved3. Several studies demonstrated that an increase in serum GGT activity can be used as a marker for increased oxidative stress in humans. It has been shown that GGT activity is directly related to the oxidative events, and plays an important role in the evolution of atheromatous Plaque4.

GGT is becoming an important addition to the multimarker approach to cardiovascular risk evaluation. It should be considered a valuable adjunct in stratifying patient risk and in assessing the aggressiveness of appropriate treatment, with hopes of preventing unnecessary cardiac events and deaths in future years5.

The prevalence of GGT is high in patients with CHF. Furthermore, GGT is positively associated with CHF severity and with long-term outcome in both men and women. GGT elevation seems to be largely a reflection of overall disease burden6.

2. Material and Method

The present study was conducted to estimate serum levels of GGT & its correlation with CK-MB in patients with myocardial infarction at the clinical chemistry laboratory of biochemistry department of S.S.G. Hospital and Medical College, Baroda.

Fifty patients of 25-65 years of age group diagnosed as having Myocardial Infarction forms the test group. Fifty age and sex matched healthy volunteers form the control group.

Subjects with uncontrolled diabetes mellitus, chronic alchoholics, smokers, patients on hypolipidemic drugs( i.e. statin ) were excluded from the study.

The study groups were selected from ICCU and Wards of Medicine Department of S.S.G. Hospital and Medical College, Baroda. They were diagnosed by clinical signs and symptoms with other supportive investigations like ECG.

After obtaining written consent from the subject 5ml of blood samples were collected under aseptic precautions in plain vacutainers.

A detailed history including personal data, present complaints, past history, family history and treatment history was taken followed by a physical examination.

Serum GGT activity and all other parameter i.e. CK-MB and Lipid Profile data were obtained within a same day from antecubital vein blood samples after overnight fasting, according to the usual clinical laboratory procedures.
Estimation of GGT was done by carboxy substrate method using kit from Crest Biosystems on fully automated biochemistry analyzer Miura-300.

Reference range: < 9 U/L (males)
< 14 U/L (females)

CK-MB estimation done to correlate it with GGT in MI patients.
Estimation of CK-MB by immuno inhibition method on fully automated biochemistry analyzer MIURA 300.
Lipid Profile estimation was done to correlate it with GGT in MI patients.
Estimation of Serum Cholesterol by CHOD-POD method, serum triglyceride by GPO-PAP method, LDL and HDL by Direct enzymatic method on fully automated biochemistry analyzer.

3. Result and Data Analysis
Fifty patients of MI & Fifty age & sex matched healthy controls were analyzed for study. Statistical analysis was done by using t-test to find out significance of difference between two groups and correlation coefficient to find out statistical correlation between two variables and its significance. Interpretation was done according to p-values as follows:

- $p < 0.05$ was considered significant
- $p \geq 0.05$ was considered nonsignificant

Estimation of serum GGT was carried out in patients as well as in controls.

Table 1: Shows symptoms in patients of Myocardial Infarction

| Presenting Complaints | No. of Cases | Percentage (%) |
|-----------------------|--------------|----------------|
| Chest Pain            | 50           | 100            |
| Perspiration          | 31           | 62             |
| Palpitation           | 36           | 72             |
| Dyspnoea              | 45           | 90             |
| Pedal edema           | 28           | 56             |

Table 1 shows chest pain was the most common presenting symptom being present in all 50 patients with MI.

Table 2: Serum GGT levels in patients of MI and in controls

| Serum GGT (U/L) | Cases | No. | Mean | SD  | p-value  |
|----------------|-------|-----|------|-----|----------|
| Patients       | 50    | 32.82 | 13.99 |     | P <0.001 |
| Controls       | 50    | 12.4 | 7.49 |     |          |

Table 2 Shows the mean serum GGT levels in patients and controls which were observed to be 32.82 ± 13.99 U/L and 12.4 ± 7.49 U/L respectively. This value is statistically significant ($p = <0.001$) calculated by statistical software MedCalc version 11.5.1.

Table 3A: Serum GGT level in both sex in MI

| Sex Distribution of GGT level | Male | Female |
|-------------------------------|------|--------|
| Mean GGT level (in U/L)       | 40.48 ± 14.30 U/L | 25.12 ± 8.23 U/L |

Table 3A shows mean level of GGT is higher in males as compared to females in patient of Myocardial Infarction.
Table 3B: Positive Family History in patients with MI

|                  | Total Number of cases | Number of Cases with positive Family History | Percentage (%) | P value |
|------------------|-----------------------|----------------------------------------------|----------------|---------|
| Patients of MI   | 50                    | 27                                           | 54             | P< 0.001|
| Controls         | 50                    | 7                                            | 14             |         |

Table 3B shows family history of Myocardial Infarction was positive in 54% patients as compared to 14% which is highly significant.

Table 4: Serum CK-MB level in in patients of MI and in controls

| Serum CK-MB level (U/L) | Cases | No.  | Mean  | SD    | p-value     |
|-------------------------|-------|------|-------|-------|-------------|
| Patients                | 50    | 70.52| 34.31 |      | P <0.001    |
| Controls                | 50    | 22.14| 7.29  |      |             |

Table 4 Shows the mean serum CK-MB levels in patients and controls which were observed to be 70.52 ± 34.31 U/L and 22.14 ± 7.29 U/L respectively. This value is statistically significant (p= <0.001) calculated by statistical software MedCalc version 11.5.1.

Table 5: Correlation of Serum GGT and CK-MB in patients of Myocardial Infarction:

| Correlation Coefficient | Significance level | N  |
|-------------------------|--------------------|----|
| 0.9429                  | P <0.001           | 50 |

The table 5 show positive and significant correlation between serum GGT and CK-MB in present study.

Figure 1: Correlation between GGT & CK-MB level
Table 6 Study of Lipid Profile in patients of MI and controls

| TEST PARAMETER | Patients | Controls |
|----------------|----------|----------|
|                | Mean     | SD       | Mean     | SD       |
| Total Cholesterol (mg/dl) | 185.48   | 25.92    | 150.70   | 15.45    |
| Triglyceride (mg/dl) | 145.76   | 53.06    | 109.64   | 42.05    |
| HDL (mg/dl)      | 37.22    | 4.29     | 46.58    | 5.97     |
| LDL (mg/dl)      | 107.84   | 27.96    | 82.48    | 16.49    |
| VLDL (mg/dl)     | 29.14    | 12.57    | 22.16    | 8.16     |

Table 6 shows High levels of Cholesterol and Low density Lipoprotein, Triglycerides while Low level of High Density Lipoprotein associated with Myocardial Infarction.

Table 7: Correlation of Serum GGT and LDL-C in patients of Myocardial Infarction:

| Correlation Coefficient | Significance level | N  |
|-------------------------|--------------------|----|
| 0.3207                  | 0.0022             | 50 |

The table 7 show moderately positive correlation between serum GGT and LDL-C in present study.

4. Discussion

Coronary heart disease is the leading cause of death in most industrialized countries, and its importance as a major public health problem is increasing in developing countries. Cardiology epidemiology has recently highlighted a clear link between serum gamma glutamyl transferase (GGT) and risk for infarction, stroke and cardiovascular death.

Increase of GGT in all cause mortality, considered a biomarker for “oxidative stress” associated with glutathione metabolism and possibly a “proatherogenic” marker because of its indirect relationship in the biochemical steps with low density lipoprotein cholesterol oxidation.

The proposed mechanism of GGT was to catalyzes the initial step in the extracellular degradation of antioxidant glutathione, which results in the amino acids cysteine and glycine. The reactive thiol of cysteinyl–glycine generates superoxide anion radicles and hydrogen peroxide through its interaction with free iron. These GGT mediated reactions have
been shown to catalyze oxidation of low density lipoprotein, which may contribute to oxidative events influencing plaque evolution and rupture.

4.1 Sex Distribution of GGT in patients with myocardial infarction

In the present study the mean value of serum GGT was seen to be elevated more in male than female. (40.48 ± 14.30 U/L in Male as compared to 25.12 ± 8.23 U/L in females) The study of Elifriede Ruttman et al an epidemiological investigation in a cohort of 163944 Austian adults, proposed that, in both men and women, high GGT was significantly (p<0.001) associated with mortality from CVD. In male, subgroups analysis showed that high GGT was positively associated with incident fatal events of congestive heart failure (p<0.001) and ischemic stroke (p<0.001). So the present study correlates with this study.

4.2 GGT in case and control groups of Myocardial Infarction

In the present study serum GGT level was observed in myocardial infarction was 32.82 ± 13.99 U/L and in control serum GGT were observed was 12.4 ± 7.49 U/L which shows significant statistical difference (p<0.001)

The study by Goya Wannamethee et al found that in middle aged British men GGT was elevated with significant increase in all cause mortality largely due to an excess of deaths from myocardial infarction mortality. In the present study, we also found high level of GGT in myocardial infarction patients.

Serum GGT activity is affected by genetic and environmental factors, with heritability estimated at 0.52. The study by Michele Emdin et al. found positive correlation of GGT with Total Cholesterol, Triglyceride and LDL Cholesterol. In the present study we also found positive correlation of GGT with Lipid profile. Positive correlation of Lipid profile with GGT in MI and positive Family history suggest that GGT level in Myocardial Infarction is affected by genetic and environmental factors.

5. Conclusion

- There was male predominance in the occurrence of myocardial infarction.
- Serum GGT has got a significant association with severity of myocardial infarction.
- The present study shows moderately positive correlation between increased gamma glutamyl transferase and CK-MB levels.
- Elevation of GGT activity in the present study shows a positive association with cardiovascular mortality. GGT is becoming a prognostic marker for valuable adjunct in stratifying patient risk and in assessing the severity of disease.
- High levels of Cholesterol, Low density Lipoprotein and while Low level of High Density Liprotein associated with Myocardial Infarction.
- Positive correlation of Lipid profile with GGT in MI and positive Family history suggest that GGT level in Myocardial Infarction is affected by genetic and environmental factors.

References

1. Weiner, Longo, Fauci, Kasper, Hauser, Jameson et al. Harrision’s Principles of Internal Medicine. 18th Edition. Birmingham: McGraw-Hill companies; 2011
2. A. Paolicchi, M. Franzini, M. Emdin, C. Passino and A. Pompella. The Potential Roles of Gamma-Glutamyltransferase Activity in the Progression of Atherosclerosis and Cardiovascular Diseases. Vascular Disease Prevention. 2006;3(3):1-6.
3. Hongqiao Zhang and Henry Jay Forman. Red in Translation: Redox Regulation of g- Glutamyl Transpeptidase. American Journal of Respiratory Cell and Molecular Biology. 2009;41: 509-515.
4. Sabri Demircan, Mustafa Yazici, Kenan Durna, Fethi Kilicaslan, Serdar Demir, Mesut Pinar, Okan Gulel, The Importance of Gamma-Glutamyltransferase Activity in Patients with Coronary Artery Disease. Clin. Cardiol. 2009; 32(4):220–225.
5. Jennifer E. Mason, RN, Rodman D. Starke, John E. Van Kirk. Gamma-Glutamyl Transferase: A Novel Cardiovascular Risk Bio Marker. *Preventive cardiology*. 2010;13:36-41.

6. Matthias Frick and Hanno Ulmer Gerhard Poelzl, Christian Eberl, Helene Achrainer, Jakob Doerler, Otmar Pachinger. Prevalence and Prognostic Significance of Elevated g-Glutamyltransferase in Chronic heart failure. *Circulation Heart Failure*. 2009;2:294-302.

7. Jousilahti P, Vartiainen E, Pekkanen J, Tuomilehto J, Sundvall J, Puska P. Serum Cholesterol Distribution and coronary heart disease risk. *American Jornal of Heart Association*, *Circulation*. 1998;97:1087-1994.

8. Michele Edmin, Claudio Passino, Maria Franzini, Aldo Paolicchi, Alfonso Pompella. Gamma Glutamyl Transferase and Pathogenesis of cardiovascular disease. *Future Cardiol*. 2007;3(3):263-270.

9. Elfriede Ruttmann, Larry J. Brant, Hans Concin, Gunter Diem, Kilian Rapp, Hanno Ulmer et al. Gamma Glutamyl Transferase as a risk factor for cardiovascular disease mortality, an epidemiological investigation in a cohort of 163 944 Austrian Adults. *American Journal of Heart Association*, *Circulation*. 2005;112:2130-2137.

10. Goya Wannamethee, Shah Ebrahim, and A. Gerald Shaper. Gamma Glutamyl Transferase: Determinants and Association with mortality from Ischemic Heart disease and all causes. *American Jornal of Epidemiology*. 1995;142(7):699-708.

11. Michele Emdin, Alfonso Pompella and Aldo Paolicchi. Gamma-Glutamyltransferase, Atherosclerosis, and Cardiovascular Disease: Triggering Oxidative Stress Within the Plaque. *Circulation*. 2005;112:2078-2080.