Gamma Glutamyltransferase as a Biomarker for Acute Coronary Syndrome

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ABSTRACT :

BACKGROUND:

GGT (gamma- glutamyl transferase) enzyme is found on the surface of various cells and plays a role in the catabolism of glutathione which is known as one of the major anti oxidants,

OBJECTIVE:

Of this study : was to emphasize the changed GGT values as anew biochemical marker for acute coronary syndrome

METHOD:

Type of study is a case – control study. The study population: We enrolled 50 patients with acute coronary syndrome who had been admitted to ccu unit in Merjan Teaching hospital, measurement of serum gamma- glutamyl transferase level using gamma- glutamyl transferase was measured by enzymatic method using the Abbott Architect C16000 autoanalyser and compared to the serum gamma- glutamyl transferase level of the 50 control group , the period of the study is 6 months. Both groups will under go an exclusion criteria for all causes that may lead to a high gamma-glutamyl transferase level mainly hepatobiliary diseases , alcohol history, diabetes mellitus , and drugs that affect the gamma- glutamyl transferase level .

RESULT:

Of the study: showed that gamma- glutamyl transferase level is elevated in all patients with documented acute coronary syndrome after exclusion of other factors which cause elevation of gamma- glutamyl transferase in comparism with the control .

CONCLUSION & RECOMMENDATION:

This study depicts association of gamma- glutamyl transferase with acute coronary syndrome in population from Babylon governorate & Larger community based studies are needed to establish the role of gamma- glutamyl transferase in development of the commonly mentioned risk factors of acute coronary syndrome. We recommend the use of GGT as a biomarker within the 4 days events of acute coronary syndrome .

KEY WORDS: glutamyl transferase , acute coronary syndrome, risk factors

INTRODUCTION:

Currently, several molecules have demonstrated their value in improving the diagnosis and prognostic classification of patients with acute myocardial infarction[1,2]

The limitations of AST as a biomarker were recognized due to its lack of specificity for myocardial tissue. One year later, Wroblewski proposed the use of lactate dehydrogenase (LDH) in the diagnosis of AMI[3]. The 1960s marked the beginning of creatine kinase (CK) as a better biomarker as it was demonstrated to be more cardiac-specific and clinically useful due to its kinetics after AMI [4]

In the following years the development of new laboratory techniques was essential to describe the CK-MB isoenzyme as the molecule that showed the highest diagnostic accuracy [5]. & demonstration of typical rising and falling of CK, CK-MB, LDH, or AST activities along with clinical and electrocardiographic features [6,7]. Gamma-glutamyl transferase (GGT) is a enzymatic based liver function test (LFT). At first, was called GGTP (gamma-glutamyl transpeptidase), it was believed that it is the most sensitive LFT for detection of alcohol toxicity. GGT is not exclusive to the liver but also found in other tissues, as the kidneys , lung, pancreas, and vascular endothelium, besides the extracellular fluid , and as albumin carrier molecules .The early function of GGT that was observed is that its activity was mainly in tissues with a transport function, as the renal and biliary

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system. So accordingly the GGT played a major role in the transport of amino acids, through a sequence of reactions forming a "gamma-glutamyl cycle. Longitudinal and cross-sectional investigational studies since 1990 have associated GGT with an increase in all-cause mortality, as well as chronic heart disease events such as congestive heart failure and components of the metabolic syndrome (abnormal body mass index and levels of high-density lipoprotein cholesterol, glucose, triglycerides, and systolic and diastolic blood pressure). In the upper reference range, GGT was found to be an independent biomarker of the metabolic syndrome, with a 20% per GGT quartile trend rise. Additionally, GGT was positively correlated with an 18% per quartile risk of cardiovascular events and a 26% per quartile increased risk of all-cause mortality. Furthermore, it may be considered a biomarker for "oxidative stress" associated with glutathione metabolism and possibly a "proatherogenic" marker because of its indirect relationship in the biochemical steps to low-density lipoprotein cholesterol oxidation. 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 years[8,9,10,11] In the upper reference range, GGT was found to be an independent biomarker of the metabolic syndrome, with a 20% per GGT quartile trend rise. Additionally, GGT was positively correlated with an 18% per quartile risk of cardiovascular events and a 26% per quartile increased risk of all-cause mortality. Further- more, it may be considered a biomarker for ''oxidative stress'' associated with glutathione metabolism and possibly a ''proatherogenic'' marker because of its indirect relationship in the biochemical steps to low-density lipoprotein cholesterol oxidation. [12]. Normal function of GGT : One of the early observations about GGT was that its activity was greatest in tissues with a transport function, such as the kidneys and in the biliary system.. [8,13] GGT as a Protection Against Oxidative Stress : Glutathione plays an important role in protecting cells against oxidants that are produced during normal metabolism. If oxidative stress increases, then so will the requirement for reduced glutathione, and conversely if glutathione is not available then the effects of oxidative stress will be greater [14]. Investigation of the role of GGT in the mechanism of cardiac diseases will be helpful in developing preventive strategies and treatment methods. Although CAD is one of the most common types of heart disease, it is difficult to predict the risk of CAD and intervene at an early stage. GGT has been confirmed to play a role in the occurrence and progression of CAD, especially in prognosis judgment.[15] GSH (glutathion) is a tripeptide comprised of three amino acids: gamma-glutamic acid, L-cysteine and L-glycine. Its primary biological function is to act as a nonenzymatic reducing agent to help keep cysteine thiol side chains in a reduced state on the surface of proteins. GSH also prevents oxidative stress in most cells and helps trap free radicals that can damage DNA and RNA. The physiological role of GGT is to initiate the hydrolysis of extracellular GSH by cleaving the gamma-glutamyl amide bond of the tripeptide to cysteine and other thiol compounds, which are known to promote LDL oxidation by reducing Fe (III) to redox-active Fe(II) [16]. Recently, catalytically active GGT has been found within atherosclerotic coronary plaques from autopsy studies and surgical endoarterectomies [17] GGT has been considered to play a central role in the formation of the fibrous cap, apoptosis of cellular elements of the lesion, plaque erosion and rupture, enhanced platelet aggregation and thrombosis(18) Growing body of data points out that GGT, an enzyme responsible for the extracellular catabolism of antioxidant glutathione, may directly take part in atherogenesis [19,20] Hence, there are two probable explanations for the association between serum GGT and cardiovascular risk: either GGT derives in part from atheromatous plaques, which would be more common and diffuse in patients with adverse cardiovascular risk profiles, or GGT is associated with the risk factors even before the plaques are entirely developed. ,serum GGT forms complexes with lipoproteins thus suggesting that the intense GGT activity within atheromatous lesions, co localized with oxidized low density lipoprotein, may derive from the accumulation of low density lipoprotein-associated GGT within the arterial wall and -lipoprotein-associated GGT activity increases with total serum GGT activity, supporting the hypothesis that increasing levels of serum GGT may be linked to an augmented influx of GGT-carrying lipoproteins into the plaque Based on current available data , recently postulated that an increase in serum concentration of GGT, even within its laboratory reference intervals regarded as physiologically normal, is a promising biomarker for cardiovascular risk[21,22,23] .

Significant relationship between serum GGT activity and atherosclerotic process has raised the question about whether serum GGT levels can aid detection of individuals at high risk for future cardiovascular events. Although widely used as a diagnostic tool for hepatobiliary disorders and alcohol abuse in clinical practice, compelling epidemiological evidence suggests that serum GGT may emerge as a potential biochemical risk indicator of cardiovascular morbidity and mortality. Several population-based studies have documented powerful cross-sectional associations between serum GGT concentrations and certain cardiovascular risk factors, irrespective of alcohol consumption [24,25,26,27] To be a unique biomarker for cardiac and metabolic risk evaluation, GGT must meet certain stringent characteristics,(28,29, 30) It must measure a single specific entity, either physiologic or pathologic, and offer additional information over presently used determinant . It must also add to the clinical assessment of a specific problem and correlate with known cardiovascular disease risk factors.[31] Demographically , it must be applicable to both men and women of differing ages and varying ethnicities. It must be easily standardized, with both a high sensitivity and specificity, and have automated testing readily avail- able in most regions. GGT enzyme analysis has been available for many years, meets all of these strict measures, and thus would appear to pass accepted criteria as defined by Vasan [32] as a biomarker for increased cardiovascular risk. GGT levels have also been confirmed to be an independent predictor of early mortality in STEMI patients without previously known diabetes who underwent mechanical revascularization Therefore Coronary artery disease associated with increment of a variety of biomarkers , in this study we are trying to evaluate a new biomarker , GGT , to assess its usefulness and accuracy in the diagnosis of acute coronary syndrome and whether can be used efficiently instead of the available biomarkers , especially serum troponin.

PATIENTS & METHODS :

A short term case - control study was conducted on (50) patients with acute coronary syndrome (ACS) who were admitted to the cardiac care unit (CCU) at Merjan teaching hospital within a period of 6 months (from first of September 2016 to first of March 2017) .The patients were excluded from the study if they are : diabetic , alcoholic , pregnant , hepatobiliary diseases , any active malignancy , and any history of drugs that may affect the level of serum GGT .

The diagnosis of acute coronary syndrome was established by the patients clinical symptoms , Electrocardiography (ECG) changes, ECHO study findings and cardiac biochemical markers specifically the serum troponin level (rising its level suggest acute myocardial infarction) Detailed history and examination for each patient were done and accordingly all the patients with history of diabetes , hepatobiliary diseases , alcoholics , or on drugs that may affect the serum GGT level have been excluded . Most of the patient who enrolled in the study were hypertensive and smokers , but their percentage was not taken in consideration in this study as it would not affect the result of comparison (serum GGT vs. serum Troponin).Control group of (50) healthy adult , gender and age matched , who attended to the hospital for reasons other than medical issues and within the same period of time , underwent the same exclusion criteria of the case group

Blood sampling and laboratory methods : A venous blood sample of 3 ml size ,was drawn from the patient at the date of admission to CCU , collected into gel tube ,serum then separated by centrifugation

GGT calculated manually by enzymatic method using the Abbott Architect C16000 autoanalyser and compared to the serum gamma- glutamyl transferase level of the 50 control group . Statistical analysis was carried out using SPSS version 20. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups when study variable was normally distributed. Mann-Whitney test was used to compare means between two groups when study variable was none normally distributed. Pearson's chi square (X^2) was used to find the association between categorical variables. A *p*-value of $\leq$ 0.05 was considered as significant.

RESULTS :

In this study, case group of (50) patients were enrolled, the age ranged from 36 to 91 years old (mean ± SD (62.82 ± 13.03)), 28 cases were male and 22 cases were females versus a control group of (50) persons with mean of age of (67.72 ± 14.13), no significant difference on age and gender ratio between case and control group, p value $\leq$ 0.075 was insignificant

Table 1: The mean differences of age by study group.

Variable	Study group	N	Mean ± SD	t-test	P-value
Age (years)	Acute coronary syndrome	50	62.82 ± 13.03	-1.802	0.075
	Control group	50	67.72 ± 14.13		

Table 2 : Association between gender and study variables.

Study variable	Study group		χ^2	P-value
	Acute coronary syndrome	Control group		
Gender				
Male	28 (56.0)	22 (44.0)	1.44	0.23
Female	22 (44.0)	28 (56.0)		
Total	50 (100.0)	50 (100.0)		

The results of the distribution of patients with acute coronary syndrome according to serum gamma glutamyl transferase level. (44%) of (50) cases of the patients with ACS presented with high level of the serum GGT level in a comparison with the serum troponin level between the case and the control group , all the cases were positive troponin whereas the control group shows negative troponin level.(as seen in table 3)

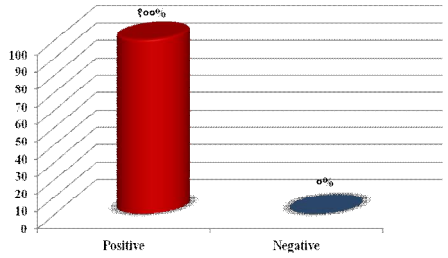

Figure 1: Shows the distribution of patients with acute coronary syndrome according to troponin level.

The major corner of this study is the result of the mean GGT level in the patients with ACS in comparison to the mean GGT level in control group without ACS As significant difference in the mean GGT was present, (Z = - 4.591, P= <0.001*) Mean GGT level is higher in the case group than the control group without risk factors.

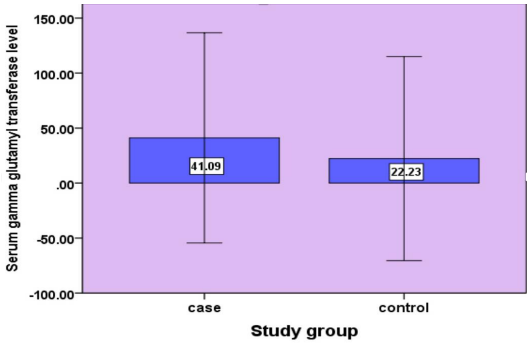

Figure 2: Mean differences of serum GGT level by study groups.

Table 3 shows the sensitivity, specificity and overall accuracy of SGGT findings in comparison to troponin finding regarding acute coronary syndrome. The sensitivity of SGGT to detect acute coronary syndrome was (44%) that mean the high level of SGGT (> 30 IU/L) was able to detect (44%) of patients with acute coronary syndrome correctly meanwhile; its specificity was (88%) that

SGGT findings versus troponin finding		Troponin finding		Total
		Positive (Acute coronary syndrome)	Negative (control group)	
SGGT findings	High	22	6	28
	Normal (0-30 IU/L)	28	44	72
	Total	50	50	100

mean normal level of SGGT was able to detect approximately (88%) of persons free from acute coronary syndrome correctly. So the overall out put is SGGT level in diagnosing ACS :
Sensitivity = 44% Specificity = 88%
Overall accuracy = 66% in diagnosing ACS .

Table 4: Shows the Association between serum GGT level and study group ,

Study variable	Study group		χ^2	P-value	Odds ratio	95% CI
	Acute coronary syndrome	Control group				
SGGT High Normal (0-30 IU/L) Total	 22 (44.0) 28 (56.0) 50 (100.0)	 6 (12.0) 44 (88.0) 50 (100.0)	12.698	<0.001*	5.762	2.079-15.971

P value <0.05 was significant , where SGGT acts as a risk factor besides being a viable biomarker for ACS and showed a significant odd ratio of (5.762), making patients with higher SGGT (5 times) more likely to be diagnosed as having acute coronary events, With P – value of (<0.001)

DISCUSSION:

In the present study, levels of serum GGT were measured in ACS patients (cases) and compared with that of control subjects who were life time non-alcoholics. We observed higher values of GGT among ACS group (post. the acute event , within the first 24 hours) Several studies have shown that circulating concentration of GGT were higher in patients with ACS than in those with healthy control subjects. One of the first studies that support this relation, where GGT levels were first associated with cardiovascular disease and all-cause mortality in a British Regional Heart Study by Wannamethee [33] reported in October of 1995. This study evaluated 7613 British men over 11.5 years in England, Wales, and Scotland. The study plan included personal history questionnaires, history and physical exams, and laboratory screening for GGT, total cholesterol, high-density lipoprotein cholesterol, and non-fasting glucose. Increasing GGT levels were strongly associated with all-cause mortality, particularly in patients with ischemic heart disease. A lesser correlation was seen in relation to blood pressure, heart rate, and cigarette smoking. This goes against the finding of our study in that (There was no correlation with acute cardiac events) Another study that showed no correlation to acute vascular events against what our current study shows , is the cross-sectional and longitudinal study reported by *Ruttmann* [34,35] ,in 2005 involved the Vorarberg Health Monitoring and Promotion Program in western Austria, with the participation of 163,944 adults and GGT evaluated as a risk factor for cardiovascular mortality. Another major study that support our finding of GGT as significant biomarker in ACS and on exploring the link between GGT activity and risk factors conducted by Meisinger et al concluded that serum GGT was a strong predictor of acute coronary events in apparently healthy men, Lawton JS in his study mentioned that with regard to CAD there exist a significant sex differences between men and women [35,36]. Here in our study population also we noted a significant difference in the proportion of males and females affecting ACS, as the cases were predominately males, suggesting that males were

affected more than females Also The significant male predominance observed in the present study is in line with other studies done by El- Menyar et al(36 ,)and Noureddine et al.[37,38,39] Several studies have shown that circulating concentration of GGT were higher in patients with ACS than in those with healthy control subjects. [40,41] A study by *Ergen et al*. About GGT ,calcium, and phosphorus level in acute coronary syndrome Also found significantly higher GGT level , even in normal reference range , in short term mortality in patients with ACS. (P <0.001) , goes with the current study results. An eloquent study by Drs. *Paolicchi* and *Emdin* [42] at the University of Pisa in 2004 specifically identified GGT in coronary atheroma removed at the time of surgical atherectomy. The enzymatically active GGT identification in the plaque was done by an azo-coupling reaction using gamma-glutamyl-4-methoxy-2-naphthylamide as a substrate for GGT activity, stained with fast garnet GBC as the chromogen. They felt the ‘‘pathogenic mechanism proposed for the role of GGT should be considered independent, complementary, and synergistic to conventional determinates. There is also evidence that atherosclerotic plaques contain GGT activity, revealed by many studies [43] The most recent and supporting study to our findings, took place in 29 October 2015 [44]. Where they evaluated the clinical utility of GGT activity in predicting high troponin levels in patients with acute coronary syndrome (ACS) admitted to the emergency department with chest pain. A total of 200 troponin-positive and 203 troponin-negative patients were classified into groups 1 and 2, respectively. γ-Glutamyl transferase activity was significantly higher in group 1 (44 ± 34 U/L) compared with group 2 (31 ± 26 U/L, *P* = .001). GGT activity cutoff >25.5 (our GGT cutoff was > 30.) Showed 62% sensitivity And 61% specificity

In predicting troponin positivity. Logistic regression analysis demonstrated a significant predictive value of GGT for troponin positivity. Comparing to our current study where the results showed SGGT level in diagnosing ACS: cut of > 30 U/L

Sensitivity = 44% Specificity = 88% Overall accuracy = 66% in diagnosing ACS. Reaching end results of Spearman rank correlation analysis showed a moderately strong relationship between GGT activity and troponin positivity. Considering the predictive value of high GGT activity for troponin positivity, GGT activity may complement other diagnostic biomarkers for predicting troponin positivity in patients having ACS admitted with chest pain.

Although coronary angiography is currently the “gold standard” for diagnostic assessment of atherosclerotic lesions within coronary vessels, it is costly and invasive, and does carry a small risk of complication. Increasing evidence showed that among the non-invasive tests, GGT is emerging as an interesting cardiovascular risk marker for CAD, and GGT assay had shown acceptable diagnostic accuracy in our study also [45,46]. In multi-speciality hospitals, techniques such as echocardiography, colour doppler and coronary angiography are utilized to detect vascular abnormalities resulting from atherosclerosis. Due to lack of availability of these sophisticated techniques in primary (PHCs) and secondary health care centres, morbidity associated with atherosclerotic changes still goes undetected, especially in view of the lower socio-economic status of the population. In this scenario, as an easily available screening test, GGT could serve as an early predictor and trustworthy marker of sub-clinical atherosclerosis and its complications. According to a prospective study on 6997 subjects, aged 40-59 years with no history of CAD or diabetes mellitus, and which was followed up for a period of 24 years revealed that the elevated GGT was significantly related with the increased risk of fatal CAD events and mortality, which was independent of the traditional CAD risk factors. [47] Another major study on exploring the link between GGT activity and risk factors conducted by Meisinger et al concluded that serum GGT was a strong predictor of acute coronary events in apparently healthy men, independent of other risk factors for cardiovascular disease [48]

CONCLUSION:

GGT is a unique biomarker in the continuum of cardiovascular disease risk. GGT is thus a potentially valuable addition to the growing list of clinically available tests useful as adjuvant diagnosing test in acute coronary syndrome after exclusion of all other causes of high GGT especially alcohol and drug history.

Limitation of the Study : The study lacked the follow-up analysis of future cardiovascular events and mortality, which mean that the prognostic value of GGT level was not evaluated. Patient’s credibility about their history of alcohol drinking , as we depend on their story regarding alcohol consumption , putting in mind the great impact of alcohol on the level of GGT and how it may affect the results of the study There was no specific time after the acute event when the

serum GGT had been collected; all samples were collected within the first hours of admission to CCU, regardless the time of onset of the acute event.

Recommendation : Measuring serum GGT level at the admission of patient with suspected acute coronary syndrome will aid in supporting the diagnosis.

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