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STUDY OF ASSOCIATION BETWEEN SERUM GAMMA GLUTAMYL TRANSFERASE AND ACUTE ISCHEMIC NON-EMBOLIC STROKE

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ABSTRACT

Introduction: Gamma glutamyl transferase is an important enzyme present on cellular membrane in considerable amounts and plays a role in intracellular ingress of amino acids and peptides in the form of gamma glutamyl peptides. Glutathione is its most important substrate. Gamma-glutamyl transferase (GGT) plays key roles in glutathione homeostasis by breaking down extracellular glutathione and providing cysteine, the rate-limiting substrate, for intracellular de novo synthesis of glutathione.

Aims: To study the association between serum gamma glutamyl transferase and acute ischemic non-embolic stroke and the association between serum gamma glutamyl transferase and distribution of cerebral infarction areas.

Materials and Methods: It is a single centre, hospital based, case control study. This Study was conducted from 1st March 2020 to 31st August 2021 at Department of N.R.S. Medical College and Hospital, Kolkata (Department of GENERAL MEDICINE – Ward and Out Patient department).

Result: In our study, the distribution of mean serum Total Cholesterol, Triglycerides and LDL Cholesterol between cases and controls was statistically significant ($p < 0.0001$ for all of the three parameters). However, the distribution of mean serum HDL cholesterol between cases and controls

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was not statistically significant (p = 0.3651). In Stroke Survivors, the serum GGT level (Mean ± SD) of patients was 64.4737 ± 19.2897 IU/lt. In Stroke Non-survivors, the serum GGT level (Mean ± SD) of patients was 67.8333 ± 16.5471 IU/lt. Distribution of serum GGT level between Stroke Survivors and Non-survivors was not statistically significant (p-value = 0.5899).

Conclusion: we concluded that. it should be interpreted cautiously as increased serum GGT might reflect an elevated oxidative stress that is only transient at the onset of stroke. Hence further studies, particularly prospective cohort study with large sample size, can help to establish GGT as a predictor for acute ischemic stroke.

Keywords: Diabetes, Gamma-glutamyl transferase, Oxidative and Stroke.

INTRODUCTION

Among all neurological diseases of adult life, cerebrovascular accidents (stroke) clearly rank the first in frequency and importance. Stroke is the second leading cause of death and a major cause of disability worldwide [1]. The mortality rate of stroke in the acute phase is as high as 20% and it remains higher for several years after the acute event in stroke patients than in the general population [2]. Because of its increased mortality and morbidity, stroke entails a high socio-economic burden.

Early identification of individual at risk could be of help in primary prevention strategies. Hypertension, diabetes mellitus, smoking, obesity, dyslipidemia are known modifiable risk factors of stroke. Gamma glutamyl transferase (GGT) is a newly proposed and not well known marker for early identification of individuals at risk of stroke.

Gamma glutamyl transferase (GGT) mediates intracellular intake of extracellular glutathione which is an important component of antioxidant mechanisms. Glutathione is produced during normal metabolic processes and plays an important role in the protection of cells against oxidative stress [3].

Gamma glutamyl transferase is an important enzyme present on cellular membrane in considerable amounts and plays a role in intracellular ingress of amino acids and peptides in the form of gamma glutamyl peptides. Glutathione is its most important substrate. Gamma-glutamyl transferase

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(GGT) plays key roles in glutathione homeostasis by breaking down extracellular glutathione and providing cysteine, the rate-limiting substrate, for intracellular de novo synthesis of glutathione [4].

Glutathione is one of the most important antioxidant in our body that maintains normal redox status of our body. Increased oxidative stress in our body enhances requirement for glutathione. In conditions giving rise to cellular stress, intracellular glutathione levels decrease. Decreased intracellular glutathione levels induce formation of GGT enzyme so as to maintain pre-existing levels. So, in order to maintain adequate levels of intracellular glutathione, so as to combat intracellular stress effectively, there is proportionate increase in GGT levels in our body. However, increased levels of GGT also causes catabolism of extracellular glutathione and during this process there is formation of reactive oxygen species like superoxides and peroxides. Hence, increased levels of GGT produces pro-oxidant reactants that catalyse LDL peroxidation and atheroma formation, which is the first step of atherosclerotic plaque formation. Till now, the mechanism of the relationship between cardiovascular and cerebrovascular risk factors and GGT level is not fully known.

GGT has been used for years as an index of hepatic dysfunction and marker of alcohol use. In population based studies, after exclusion of alcohol consumption, a positive correlation has been demonstrated between higher GGT levels and advanced age, male gender, increases in body mass index (BMI), smoking, sedentary life style, hypertension, tachycardia, hyperglycemia, increased LDL-cholesterol, and decreased HDL-cholesterol levels, hypertriglyceridemia, menopause, and oral contraceptive use [5].

Recently, serum GGT level was shown to be a predictive factor for cardiovascular diseases and blood GGT activity is an independent biomarker of coronary artery calcification. Several studies have shown that atherosclerosis is associated with raised serum GGT levels, which suggests that GGT can be used as an early marker of atherosclerosis. The Framingham offspring study was one of the first epidemiological studies to test the association between GGT levels and the risk of cardiovascular diseases (CVD). The study also revealed that GGT might predict the metabolic and cardiovascular risks associated with the onset of metabolic syndrome, incident CVD, and indicate whether the condition is fatal.

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Studies with small sample sizes have shown a positive correlation between increased infarct area and elevated GGT levels in patients with acute ischemic strokes [6]. However, GGT has not yet been used as a tool to predict the risk of strokes in clinical practice, for which more evidence is needed.

MATERIALS AND METHODS

Study area: The present work was conducted in the N.R.S. Medical College and Hospital, Kolkata (Department of GENERAL MEDICINE – Ward and Out Patient department).

Study period: Study was done from 1st March 2020 to 31st August 2021 through one and a half year period.

Sample size: Total 100 individuals with 50 cases and 50 controls, both male and female, (calculated using 'Formula for Differences in Means' and Confidence interval of 95% and Power of 80%) were included in the study.

Sample design: Patients with Acute ischemic non-embolic stroke presenting in General Medicine ward of NRS Medical College were selected as cases. Patients with non-cardiovascular and non-cerebrovascular disease, presenting in general medicine OPD in NRS MEDICAL COLLEGE with other complaints, were selected as controls. Matching was done between cases and controls for age, sex, diabetes mellitus, hypertension, and dyslipidemia.

Study design: It is a single centre, hospital based, case control study.

Inclusion criteria:

1. Cases: Patients presenting in General Medicine ward of NRS MEDICAL COLLEGE AND HOSPITAL with clinical features and CT Scan Brain suggestive of Acute Ischemic non-embolic stroke.
2. Controls: Individuals not known to have any cardiovascular or cerebrovascular disorders, who consulted to general medicine OPD or get admitted in General Medicine ward for other reasons (after proper matching and eliminating possible confounding factors as age, sex, diabetes mellitus, hypertension, and dyslipidemia).

Exclusion criteria:

1. History of chronic liver disease

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2. History of renal disease
3. Cardio Embolic stroke
4. Known endocrine and autoimmune diseases other than diabetes
5. Alcoholics
6. Smokers
7. Those who had undergone surgical interventions related to coronary, carotid, or extremity arteries
8. Past history of major cardiovascular and cerebrovascular events in the such as coronary artery disease (CAD), congestive heart failure (CHF), valvular heart disease, atrial fibrillation, past history of cerebrovascular accidents, and myocardial infarction.

Statistical Analysis:

Data collected was entered in Microsoft Excel spread sheet and was analysed using SPSS (IBM SPSS Statistics for Windows), Version 26.0. Data has been summarised as mean and standard deviation for numerical variables, and count and percentages for categorical variables. In statistical analysis, Student's t-test, independent samples t-test, chi-square test, and Kruskal-Wallis test were performed. The significance level was evaluated at $p < 0.05$.

RESULT AND DISCUSSION

This study is a Single-centre, Hospital based, Case control study conducted in N.R.S. Medical College and Hospital Kolkata (at General Medicine Ward and Out-Patient department) and the study was done from 1st March 2020 to 31st August 2021, i.e., through one and half year period. The study was conducted to determine the role of GGT as a predictor for risk of developing acute ischemic stroke in susceptible individuals and as an indicator of outcome in patients with acute ischemic stroke.

In our study, the male to female ratio was 1:1.56. In Case group, 31(62%) patients were Female and 19(38%) patients were Male. In Control group, 30(60%) patients were Female and 20(40%) patients were Male. The association of Sex with Outcome was not statistically significant (p=0.8375).

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Gurbuzer N. et al. (2014) in a similar study did not find a significant difference between groups regarding gender distribution (p= 0.273). [7]

In our study, the mean Age of the cases was 64.3000 ± 7.5266 years with a range of 48 to 74 years. The mean Age of the controls was 57.8600 ± 8.4782 years. The distribution of mean age between cases and controls was statistically significant (p<0.0001).

Gurbuzer N. et al. (2014) did not find a significant difference between groups regarding their mean ages [7]. However, **Yao et al.** found significant difference between groups regarding age with an average age of 65 ± 13.2 years among AIS patients [10]. **Singh et al.** Found that younger age group individuals were more in control group than cases and this difference reached statistical significance (p = 0.002)[8].

In our study, 37 (74%) cases and 40 (80%) controls were Hypertensive and 13 (26%) cases and 10 (20%) controls were Non-Hypertensive (Normotensive). 24 (48%) cases and 25 (50%) controls were Diabetic and 26 (52%) cases and 25 (50%) controls were Non-Diabetic. 28 (56%) cases and 26 (52%) controls were having Dyslipidemia and 22 (44%) cases and 24 (48%) controls were Not having Dyslipidemia. 44 (88%) cases and 37 (74%) controls were Obese (BMI ≤ 25 kg/m^2) and 6 (12%) cases and 13 (26%) controls were Non-Obese (BMI > 25 kg/m^2). Thus the prevalence of hypertension, diabetes, dyslipidemia and obesity were comparable in cases and controls. This ensures proper matching of confounding factors in case and control groups.

Gurbuzer N. et al. also reported that the frequencies of hypertension, diabetes mellitus and dyslipidemia in cases and controls were comparable (88.3% versus 88.6%, 38.3% versus 38.6% and 36.6% versus 40.9% respectively) [7].

In our study, the distribution of mean BMI between cases and controls was not statistically significant (p=0.3548). However, the distribution of mean SBP and DBP between cases and controls was statistically significant (p = 0.0007 and p = 0.0005 respectively). Also, the distribution of mean FBS and PPBS between cases and controls was statistically significant (p = 0.0003 and p < 0.0001 respectively).

In our study, the distribution of mean serum Total Cholesterol, Triglycerides and LDL Cholesterol between cases and controls was statistically significant (p < 0.0001 for all of the three parameters).

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However, the distribution of mean serum HDL cholesterol between cases and controls was not statistically significant (p = 0.3651).

In our study, the distribution of serum GGT levels between cases and controls was statistically significant (p < 0.0001). We found that patients with acute ischemic non-embolic stroke had a significantly high serum GGT levels when compared to the controls (65.28 ± 18.7232 IU/lt versus 27.58 ± 20.2376 IU/lt, p < 0.0001). Also an association is established between serum GGT with acute ischemic non-embolic stroke with Odds ratio = 11.3846, Relative Risk = 3.5472, 95% CI = 4.4568 to 29.0814, p < 0.0001. These findings are in accordance with most of the data published worldwide.

Korantzopoulos P et al. also reported that GGT levels were significantly higher in stroke patients compared to controls [6]. **Gurbuzer N. et al.** found that the mean GGT level in the AIS group was significantly higher relative to the control group (23.3 ± 11.8 versus 15.0 ± 5.7 IU/lt; p < 0.0001; cut-off value for GGT was calculated as 26.4 IU/lt) [7]. Singh et al. found the mean serum GGT level was significantly increased in cases (54.95 ± 20.54 versus 32.14 ± 5.07 IU/lt; p< 0.0001) [8]. **Vaid et al. (2018)** conducted a study based at Amritsar, India and observed a statistically significant difference in serum GGT levels between cases and controls (51.74 ± 22.19 versus 17.99 ± 3.8 IU/lt; P < 0.001) [9].

In our study, out of the 50 cases of acute ischemic non-embolic stroke, 3(6%) patients presented with ACA territory infarct, 25(50%) patients presented with MCA territory infarct, 8(16%) patients presented with Posterior cerebral circulation infarct(PCI) and 14(28%) patients presented with lacunar infarct (classification is based on the arterial territory involved). Of these, the mean serum GGT level was maximum for MCA territory infarcts followed by Posterior Cerebral circulation infarcts (PCI) and minimum for Lacunar infarcts. The mean serum GGT level was significantly higher in the MCA and PCA territory infarcts than in the ACA territory infarct or lacunar infarct.

In our study, the distribution of serum GGT level with sex and age group among cases was not statistically significant.

In our study, the distribution of serum GGT level with Hypertension and BMI among cases was not statistically significant (p = 0.5765 and p = 0.2983 respectively). However, the distribution of

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serum GGT level with Diabetes mellitus (71.0417 ± 16.3490 IU/lt versus 59.9615 ± 19.1963 IU/lt, p = 0.0336) and with Dyslipidemia (70.5714 ± 16.8468 IU/lt versus 58.5455 ± 18.8287 IU/lt, p = 0.0214) among cases was statistically significant.

Gurbuzer N. et al. could not demonstrate statistically significant relationship of serum GGT with diabetes mellitus, but found a significant positive relationship of GGT with hypertension (p = 0.124 and 0.028, respectively) [7].Yao et al. found a positive association of GGT with BMI (p < 0.001), but could not demonstrate statistically significant relationship of GGT with hypertension or diabetes mellitus [10]. Singh et al. found that the serum GGT level was significantly higher in cases with diabetes mellitus, hypertension and dyslipidemia (p-value <0.0001 in all) [8].

In our study, the distribution of serum GGT level with serum HDL cholesterol among cases was statistically significant. The mean serum GGT level was higher in the group of cases with HDL cholesterol < 40 mg/dl than those with serum HDL cholesterol > 40 mg/dl (70.8750 ± 17.6807 IU/lt versus 60.1154 ± 18.1666 IU/lt, p = 0.0393). The distribution of serum GGT level with serum LDL cholesterol among cases was not statistically significant.

Yao et al. found statistically significant relationship of GGT with serum LDL cholesterol and triglycerides levels, but could not demonstrate significant relationship with HDL cholesterol levels [10]. **Gurbuzer N. et al.** demonstrated significant increased GGT levels in the subgroup with higher LDL cholesterol and triglyceride levels among cases with AIS [7].

In our study, out of 50 patients with acute ischemic non-embolic stroke, 12 (24%) patients died during their course of hospital stay and the remaining 38 (76%) patients were discharged from the hospital. The mean serum GGT level was higher among the patients who died as compared to the patients who were discharged, but it was not statistically significant (67.8333 ± 16.5471 IU/lt versus 64.4737 ± 19.2897 IU/lt, p = 0.5899).

Yao et al. found that the mortality rate of the patients in the highest GGT quantile group were significantly higher than those of the other three groups. The mean GGT level in patients who died was also significantly higher than that of the survived patients (37.0 ± 26.8 versus 29.0 ± 21.5 IU/lt; p = 0.012) [10].

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Majority of the findings of our study are in accordance with most of the previous studies published worldwide, except a few. From the above discussion it is evident that serum GGT level is associated with increased risk for acute ischemic non-embolic stroke and is associated with other risk factors for cerebrovascular disease as diabetes mellitus and dyslipidemia. And also there is association of serum GGT level with the arterial territory involved in acute ischemic non-embolic stroke. However we could not find a significant association of serum GGT level with mortality in patients with acute ischemic non-embolic stroke.

CONCLUSION

- Traditionally, serum GGT is seen as a marker of alcohol use and liver dysfunction. Of late, serum GGT was established as a marker of cardiovascular disease.
- This study showed that serum GGT level was significantly higher in acute ischemic non-embolic stroke patients compared to an age – sex matched non-stroke patients and validates the association between serum GGT and acute ischemic non-embolic stroke.
- Also serum GGT level was significantly higher in sub-group of stroke patients with other risk factors like diabetes mellitus and dyslipidemia. Therefore, it is worth to say that measuring GGT level for estimating the risk of stroke, particularly in patients with established risk factors, is a cheap, convenient and effective method.
- However, it should be interpreted cautiously as increased serum GGT might reflect an elevated oxidative stress that is only transient at the onset of stroke. Hence further studies, particularly prospective cohort study with large sample size, can help to establish GGT as a predictor for acute ischemic stroke.

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Table 1: Distribution of Serum GGT level (IU/lt) among Cases and Controls

	Number	Mean	SD	Minimum	Maximum	Median	p-value
GGT in cases	50	65.2800	18.7232	18.0000	99.0000	64.5000	< 0.0001
GGT in controls	50	27.5800	20.2376	8.0000	70.0000	22.0000	

Table 2: Determination of Association between Serum GGT level (IU/lt) and Acute Ischemic Non-embolic Stroke

	Cases (With Acute ischemic non-embolic stroke)	**Controls** (Without Acute ischemic non-embolic stroke)	**Total**
Positive GGT	40	13	53

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(>50 IU/lt)			
Negative GGT (< 50 IU/lt)	10	37	47
Total	50	50	100

Table 3: Distribution of serum GGT level (IU/lt) between Survivors and Non-Survivors in Case group (patients with Acute Ischemic Non-embolic Stroke)

Serum GGT level (IU/lt)	Stroke Survivors (N = 38)	Stroke Non-survivors (N = 12)	P-value
Mean	64.4737	67.8333	0.5899
SD	19.2897	16.5471	

Table 4: Comparison of Conventional Risk Factors for Acute Ischemic Non embolic Stroke (Confounding Variables) between Cases and Controls (Univariate Analysis)

	Variable	Present/Absent	Case	Control	Total	Statistical Significance
1.	Age (years)	NA	64.30 ± 7.5266	57.86 ± 8.4782	61.08 ± 8.6391	p-value: 0.0001
2.	Hypertension	Yes	37	40	77	a. O. R.: 0.7115 b. p-value: 0.4769 c. 95% CI: 0.2785 to 1.8176
		No	13	10	23	
3.	Diabetes Mellitus	Yes	24	25	49	a. OR: 0.9231 b. p-value: 0.8415 c. 95% CI: 0.4213 to 2.0224
		No	26	25	51	
4.	Dyslipidemia	Yes	28	26	54	a. OR: 1.1748 b. p-value: 0.6883 c. 95% CI: 0.5347 to 2.5813
		No	22	24	46	

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5.	Obesity	Yes	44	37	81	a. OR: 2.5766 b. p-value: 0.0806 c. 95% CI: 0.8913 to 7.4486
		No	6	13	19	