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Association of Gamma – Glutamyl Transpeptidase to HDL-C ratio with Severity of Non – Alcoholic Fatty Liver Disease

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Abstract:

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) is a growing health concern globally. The Gamma-glutamyl transpeptidase to HDL-C ratio (GGT/HDL-C) has been proposed as a potential marker of oxidative stress and liver dysfunction. **Objective:** To evaluate the diagnostic role of the GGT/HDL-C ratio in NAFLD and its correlation with severity of hepatic steatosis. **Methodology:** A case-control study including 100 subjects (50 NAFLD, 50 controls) was conducted. liver function, and lipid profile parameters were analyzed. GGT/HDL-C ratio was calculated and its diagnostic accuracy assessed using ROC analysis. **Results:** GGT/HDL-C ratio was significantly elevated in NAFLD subjects (2.51±3.44 vs

0.64±0.34, p<0.001). The ratio increased with NAFLD severity and showed a strong correlation with AST (r=0.533), ALT (r=0.578), and TG (r=0.310). ROC analysis showed an AUC of 0.797, with a sensitivity 60% and specificity 82%. **Conclusion:** GGT/HDL-C ratio is a promising, cost-effective marker that correlates with NAFLD severity and may enhance early diagnosis.

Keywords: Non-Alcoholic Fatty Liver Disease (NAFLD), Gamma-glutamyl transpeptidase (GGT), High-density lipoprotein- Cholesterol (HDL-C)

Introduction:

The incidence of NAFLD (Non-alcoholic Fatty Liver Disease), a major chronic liver disease, has increased in many countries with improper or a lack of proper diet, stress, and a sedentary lifestyle (1). The prevalence of non-Alcoholic Fatty Liver Disease globally is approx.25% and the prevalence in India is about 9-32% (2). NAFLD is significantly increased with the high oxidative stress and proinflammatory status which is due to excess accumulation of fatty acids inducing high rates of β-oxidation increasing the ROS production in the mitochondria. This may cause cellular damage, oxidative stress, activation of Kupffer cells, and pro-inflammatory pathways (8). GGT is a cell membrane enzyme primarily found in hepatocytes and biliary epithelial cells. It plays important role in maintaining adequate intracellular levels of glutathione by continuous hydrolysis and resynthesis inside the cell. Glutathione acts as a major antioxidant in scavenging the free radicals produced during normal metabolism. In oxidative stress, the requirement of reduced glutathione is increased which is overcome by increased synthesis by GGT. The levels of serum GGT raises in conditions like dyslipidemia occurs in liver dysfunction, alcohol intake and drugs overdosage leads to oxidative stress in the cell and increases the GGT enzyme activity. Hence GGT has a physiological role in counteracting the oxidative stress (9). HDL-C has strong properties of antioxidative and anti-inflammatory functions, and its decreased levels are associated with IR, dyslipidaemia, obesity, and atherogenic indices. According to the study, impaired anti-inflammatory and anti-oxidative function

of HDL-C might be related to NAFLD severity and hence, it can be speculated that GGT/HDL-C ratio can be a predictive factor associated with NAFLD (10).

NAFLD represents a wide spectrum of liver conditions ranging from steatosis to steatohepatitis and cirrhosis. GGT is an enzyme involved in oxidative stress regulation, while HDL-C is known for its antioxidative and anti-inflammatory roles. The GGT/HDL-C ratio may reflect oxidative stress burden and liver injury severity. This study evaluates the association between GGT/HDL-C ratio and NAFLD severity.

Material and Method:

The case-control study was conducted in the Department of Biochemistry and Department of Medicine at HIMSR and associated HAHC hospital**,** New Delhi. A total of 100 subjects were included in the study among them 50 were NAFLD subjects and 50 were clinically healthy controls. Institutional ethical clearance (EC/NEW/INST/2020/961) was obtained from the Hamdard Institute of Medical Science and Research, Delhi before performing the study. Informed written consent was obtained from the patients and healthy subjects. Patients with NAFLD diagnosed/confirmed by Ultrasound aged more than 18 years, both males and females, were included in the study. The patients were excluded from the study after taking their medical history for any of the following reasons: Alcohol intake or smoking habits, pregnant women, drug abuse, consumption of steatosis-causing drugs, viral hepatitis and other comorbidities or with any known cause for their increased liver enzymes. Clinically healthy control subjects without any known disease or comorbidities were included. liver function tests, lipid profile, and ultrasonography were performed. GGT/HDL-C ratio was analyzed. 3ml blood intravenous samples were taken after 8-12 hr fasting for biochemical parameters (LFT, lipid profile, and fasting blood glucose levels) and collected in an appropriate vacutainer. (Sodium fluoride vial for fasting glucose and others in plain serum separator tube). All biochemical parameters were analyzed on a fully automated clinical chemistry analyzer (Beckman Coulter AU 480). Levels GGT/HDL-C

ratio was calculated from measured biochemical parameters in NAFLD subjects and control groups.

Statistical analysis: Analysis was performed using SPSS version 28.0 software. The obtained data was assessed for its normality by applying the test of normality (Shapiro-Wilk's test and Kolmogorov-Smirnov test). The variables are presented as mean ± SD and median (interquartile range) for parametric and non-parametric data, respectively. Comparison between groups was analyzed by Student t- test/Mann-Whitney U test for continuous variables and chi-square test for categorical variables. Receiver Operating Characteristics (ROC) curves analysis was performed. Values of $p < 0.05$ were considered statistically significant.

Result:

The study included 50 NAFLD patients and 50 healthy control subjects. The age and sex distribution of all participants are given in "Table 1". Out of 50 healthy subjects,44% were male and 56% were female, and in the NAFLD group, 46% were male and 54% were female. There was no significant difference found between the mean age group of NAFLD subjects (49.1±14.16) and the control group (48.52±13.3).

Table 1. Age and sex distribution of the study population.

	NAFLD (n=50)	**Control (n=50)**
Gender	Male - 23 (46%) Female - 27 (54%)	Male - 22 (44%) Female- 28 (56%)

The biochemical parameters of NAFLD patients and healthy controls are given in "**Table 2"**. Serum triglycerides (TG) and VLDL levels were significantly higher in NAFLD

patients compared to controls ($p < 0.001$). Serum HDL-C levels were significantly lower in NAFLD compared to the control group ($p = 0.004$).

Table 2: Biochemical profile of the study population

Variable	NAFLD	Control	p-value
AST (IU/L)	37 (39)	26 (15)	0.001*
ALT (IU/L)	33.5(52.2)	27(25.25)	0.001*
ALP (IU/L)	33.5(52.55)	98.5(47.25)	0.215*
TC (mg/dl)	168.24 ±50.5	167.94 ±32.97	0.956#
TG (mg/dl)	176.24 ±77.3	106.64 ±36.65	<0.001#
HDL-C(mg/dl)	41.9±20.6	48.1±12.7	0.004#
LDL-C(mg/dl)	99.8±37.38	101.5±28.7	0.692 #
VLDL (mg/dl)	36.50±17.36	21.3±6.62	<0.001#

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL, very low-density lipoprotein. Continuous variables were presented as mean ± SD or median (interquartile range). The statistical significance of differences between the control and NAFLD group was analyzed by Student's t-test (#) and the Mann-Whitney U test (*).

The GGT/HDL-C ratio levels in NAFLD patients and control groups are given in **Table 3.** The ratio of GGT/HDL-C was significantly higher in the NAFLD group (2.51±3.44) and had a p-value <0.001 compared to the control group (0.64±0.34).

Table 3 GGT/HDL-C ratio in NAFLD and control group

Groups	GGT/HDL-C Ratio (Mean ± SD)

NAFLD	2.51 ± 3.44
Control	0.64 ± 0.34

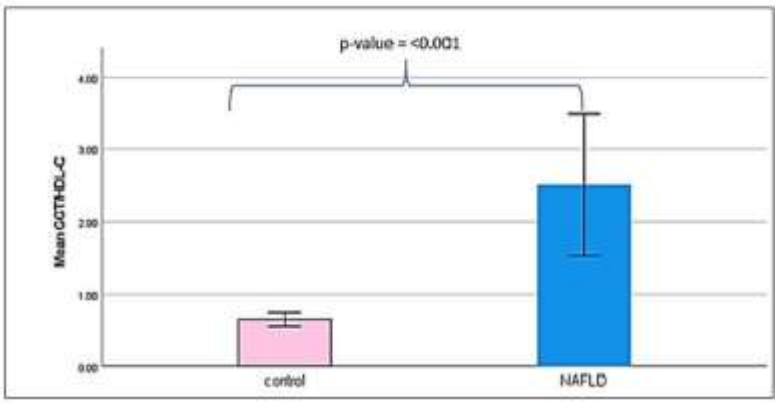


Figure 1 shows the GGT/HDL-C ratio in both groups.

The NAFLD group was further classified into 3 grades based on the degree of steatosis illustrated in the ultrasound: grade 1 (mild), grade 2 (moderate), and grade 3 (severe). The GGT/HDL-C ratio showed a significantly increasing trend with an increase in grades and severity of hepatic steatosis in **Table 4.**

Table 4. Levels of GGT/HDL-C ratio in different NAFLD grades

Parameter	GRADE 1	GRADE 2	GRADE 3
GGT/HDL-C (Mean ± SD)	1.41 ± 1.00	3.55 ±1.54	7.10±4.10

*****Data is expressed as mean ± SD.*

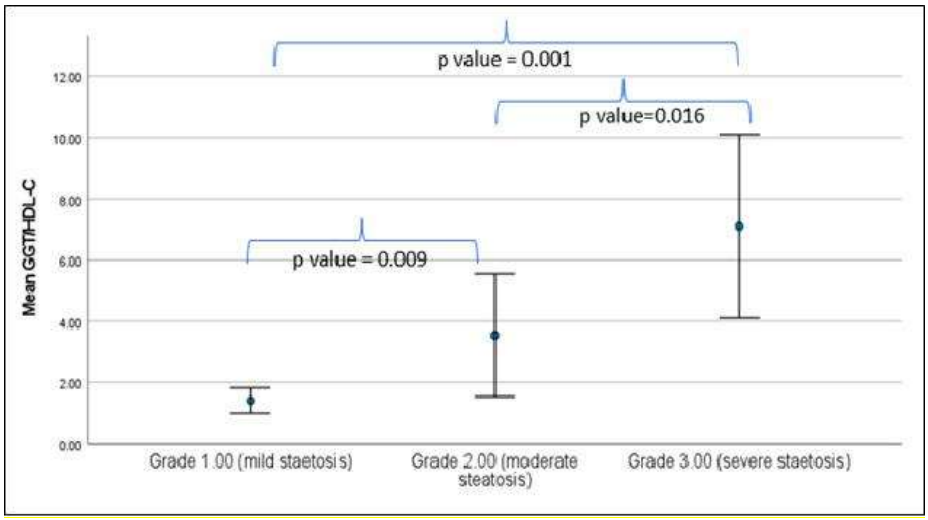
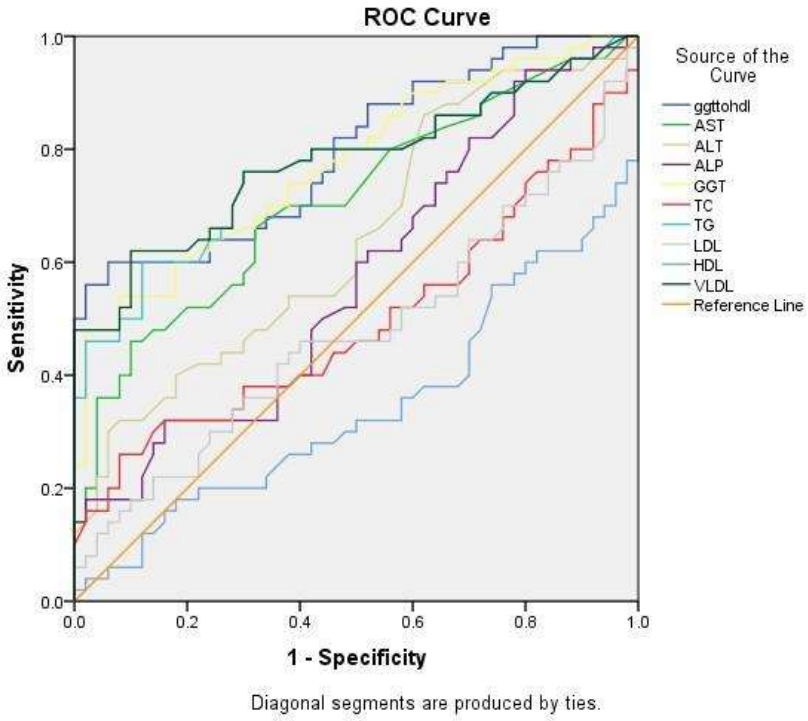


Figure 2. Levels of GGT/HDL-C ratio in different NAFLD grades

Figure 3 revealed that the Area under the curve (AUC) and optimum cut-off value were obtained to compare the diagnostic efficacy with other parameters. The AUC of the GGT/HDL-C ratio obtained was 0.797, which was significantly higher than other test variables. Thus, this ratio may have good diagnostic efficacy with 60% and 82% of sensitivity and specificity, respectively, at an optimum cut-off value of 1.0084. Compared to the GGT/HDL-C ratio, GGT and HDL-C individually have much lower AUC (0.643 & 0.669, respectively) and are not suitable from the NAFLD diagnostic point of view**.**



Correlation analysis of GGT/HDL-C with other variables in NAFLD subjects: GGT/HDL-C was seen to be negatively correlated with BMI and positively correlated with WC, SBP, and DBP. However, no statistically significant correlation was seen between GGT/HDL-C and these variables in. The GGT/HDL-C ratio was seen to be positively correlated with liver enzyme panel (AST, ALT, GGT, and ALP). A statistically significant positive correlation was seen between GGT/HDL-C and the liver enzyme panel. There was a significant negative correlation of GGT/HDL-C with HDL-C and a significant positive correlation of GGT/HDL-C with TG. The correlation of the GGT/HDL-C ratio with other variables was not significant "Table 5"

Table 5. Spearman correlation analysis of GGT/HDL-C with other parameters

Variable	r value	p-value
AST	0.533	<0.001

ALT	0.578	<0.001
ALP	0.342	0.015
GGT	0.893	<0.001
TC	-0.006	0.961
TG	0.310	0.028
LDL	0.171	0.238
HDL	-0.260	0.006
VLDL	0.148	0.250

Discussion: In this hospital-based case-control study, NAFLD was found to be significantly associated with GGT/HDL-C ratio, and also with some of the baseline characteristics (biochemical) of the study population. ROC curve of the GGT/HDL-C ratio was obtained and GGT/HDL-C ratio AUC was higher than the other variables. For the NAFLD group, ALT, AST, GGT, TG, HDL-C, and VLDL differed significantly compared to the control group, as shown in Table 2 of the current study. The observed association of NAFLD with these baseline characteristics is supported by the reported evidence of linked risk factors of NAFLD with dyslipidemia, diabetes, obesity, and metabolic syndrome (6).

The other examined variable in this study was GGT/HDL-C ratio, which was also found to be significantly high in the NAFLD group compared to the control group (**Table 3**). In our study, GGT/HDL-C showed a positive correlation with liver enzymes (ALT, AST, and ALP) and with triglycerides, and a significant negative correlation with HDL-C in the NAFLD group. Furthermore, this study also showed a significant stepwise increase in GGT/HDL-C levels with the increase of NAFLD grade or hepatic steatosis, which implies that this parameter may also be able to predict NASH, which is one of the further complications of NAFLD (7).

GGT is the plasma membrane-bound enzyme that maintains the homeostasis of glutathione and plays a key role in reducing the effects of oxidative stress. Previous studies

have reported that an increase of GGT in circulation appears to be linked to hepatic steatosis (8). The mechanism proposed is that excess fat in the liver may exacerbate oxidative stress, resulting in excessive GSH consumption and compensatory increase in GGT production. Finally, increased GGT production may be due to a low-grade hepatic inflammation caused by hepatic steatosis, and it may act as an oxidative marker (9). The findings of these previous studies support our outcome of GGT/HDL-C being significantly higher with an increase of hepatic steatosis shown in "**Fig 1**". Earlier evidence suggests that GGT has a very close association or elevates along with transaminases in fatty liver and in other liver disorders (10). Similar findings were seen in this study.

Dyslipidaemia is one of the risk factors in NAFLD, where hypertriglyceridemia and low HDL levels are the common features. The higher the TG level with more increase of TG-rich lipoproteins (VLDL), the greater the CETP-mediated transfer of CE from HDL to VLDL in exchange for TG, resulting in TG-rich small, dense HDL that are catabolized more quickly, resulting in low HDL-C levels (11). These findings add to our results of GGT/HDL-C ratio being positively correlated with TG in NAFLD.

Therefore, from the findings of this study, it can be considered that the GGT/HDL-C ratio may play a role in predicting the occurrence of NAFLD. Besides, these parameters have a relatively low cost and can be easily performed along with LFT and lipid profile in a routine clinical biochemistry laboratory. However, further studies need to be conducted on a larger sample size with multicentre analysis, and with other associated metabolic risk factors, which are the limitations of this study.

Conclusion:

The GGT/HDL-C ratio is significantly elevated in NAFLD and increases with disease severity. It correlates well with liver enzymes and lipid abnormalities, offering a cost-effective, accessible marker for NAFLD screening and monitoring.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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