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A Study to Correlate AST/ALT Ratio and GGT Levels in Patients with Non-Alcoholic Fatty liver Disease

Authors
Dr Swetha B[1*], Dr. Vinod George Thykadavil[2]
[1]Junior Resident, [2]Professor
[1,2]Department of Biochemistry, St John's Medical College, Bangalore
*Corresponding Author
Dr Swetha B
John Nagar, Bangalore, India
Email: *swethabheema516@gmail.com*

Abstract
Non-alcoholic fatty live disease is a condition defined by excessive fat accumulation in the form of triglycerides in the liver (> 5% of hepatocytes). There is a need for noninvasive biomarkers to detect progression of liver damage in NAFLD. . As of today, limited studies exist on usage of liver enzymes as non-invasive biomarkers in NAFLD.
Material & Method: *This original research study included total of forty-one (41) cases and forty (40) controls were selected for the study. Patients with Ultrasound diagnosed fatty liver disease with no history of alcohol consumption referred to phlebotomy for liver function test.AST, ALT and GGT estimated by Modified IFCC with P5P method. Data analysis was done by applying R software*
Result: *The mean value of serum AST, ALT and GGT were elevated in non-alcoholic fatty liver disease compared to controls. Serum AST/ALT ratio decreased and GGT values were increased in cases compared to controls. Independent student T test was used to analyze the data set for serum AST/ALT ratio and GGT amongst cases and the p value obtained was statistically significant.*
Conclusion: *As increased level of liver enzymes could indicate severity of steatohepatitis and fibrosis. There is poor correlation between AST/ALT ration and GGT in NAFLD. So along with liver enzymes other noninvasive biomarkers (TNF α, IL 6, IL 8) are useful to know the extent of liver injury in NAFLD patients.*
Keywords: *NAFLD, AST, ALT, GGT, steatosis, NASH (Non alcoholic steatohepatitis).*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is now more common than alcoholic liver disease owing to the rapid rise in the prevalence of obesity,[1] and NAFLD is the most common cause of abnormal liver function tests.[2]

Ludwig first described patients who had histological features identical to those of alcoholic hepatitis, but who had no history of alcohol abuse[3]. NAFLD (Non-alcoholic fatty liver disease) is a condition defined by excessive fat accumulation in the form of visible intracellular triglycerides (steatosis) in the liver (> 5% of hepatocytes histologically). Hepatic steatosis unrelated to excessive alcohol consumption or daily ingestion of less than 20g ethanol is termed as non-alcoholic fatty liver disease (NAFLD)[4] or in patients consuming less than 30 g

(three units) of alcohol per day for men and less than 20 g (two units) of alcohol per day for women.

Liver biopsy, has been recommended for confirming its diagnosis and for providing prognostic information[5]. But biopsy is an invasive and inconvenient procedure for the patients. So, there is a need for non-invasive biomarkers to detect progression of liver damage in NAFLD. As of today, limited studies exist on usage of liver enzymes as non-invasive biomarkers in NAFLD. AST, ALT and GGT are few such enzymes which can be used as biomarkers.

The objectives of this study is to estimate and compare the serum aspartate transaminase (AST), alanine transaminase (ALT) and γ-Glutamyl transferase (GGT) levels in apparently healthy controls and patients diagnosed with non-alcoholic fatty liver disease (NAFLD)and to estimate the strength of correlation between AST / ALT ratio and GGT levels in non-alcoholic fatty liver disease (NAFLD).

Material and Methods

This was a case control, retrospective study. The source of data was, ultrasound diagnosed non-alcoholic fatty liver subjects referred to phlebotomy for liver function tests in St John's Medical College Hospital between October 2016 and September 2017, as per the inclusion and exclusion criteria.

All Adults (18-60yrs) with Non-alcoholic fatty liver disease diagnosed by abdominal ultrasound will be included. The controls were apparently healthy, age and sex matched individuals aged between 18 – 60 years attending the executive health check-up.

A total of 41 males (23 cases and 18 controls) and 38 Females (17 cases and 21 controls) are taken for the study.

Inclusion criteria for Cases: All Adults (18-60yrs) with Non-alcoholic fatty liver disease diagnosed by abdominal ultrasound were included. Alcoholism ruled out by patient history. For Controls: Age and sex matched individuals attending the health plan / executive health check-up were included.

Exclusion criteria for cases and controls includes Patients with associated gallbladder diseases (obstructed gall stones, cholecystitis, cholangiocarcinoma), viral hepatitis, drug induced hepatitis. Patients on hepatotoxic drugs (Anti tubercular drugs, anti-epileptics, sedatives). Hepatocellular carcinoma and other carcinomas.

Blood samples were collected by venepuncture with aseptic precautions. Blood samples for estimation of AST, ALT and GGT were collected in serum vacutainers. Serum samples were checked for sample integrity for HIL (haemolysis, icterus, lipemia) effects, kept in the lab at 25^0 C were separated within 2 hours and analysed within 4 hours of sample collection. Analysis of AST, ALT & GGT performed on SIEMENS DIMENSION EXL automated analyzer in clinical chemistry system using fresh venous blood sample by IFCC with P5P method.

Evaluation of the data was carried out statistically by applying Independent Student 't' test for comparisons. 'p' value was calculated. 'p' value less than 0.05 was considered as significant.

Results

The mean values of AST,ALT and GGT were 26.94±10.02, 31.94±9.08 and 37.94±11.9 (mean ± SD) respectively in cases and 23.5±6.52, 23.54±6.01 and 29.85±11.43in controls respectively in the females summarized in the Table(1)

The mean values of AST,ALT and GGT were 35.35±17.4, 35.1±16.0 and 46.4±19.4 (mean ± SD) respectively in cases and 20.61±7.4, 28±9.57 and 31.88±10.18 in controls respectively in the males summarized in the Table(2)

The mean value of AST/ALT ratio was 0.93±0.4 and 1.02±0.2 in cases and controls respectively in females. The mean value of AST/ALT ratio was 0.82±0.51 and 0.826 ± 0.3 in cases and controls respectively in males.

Figures 1,2,3 and 4show the graphical representation of difference in the AST/ALT ratio and GGT values in cases and controls between female and male subjects.

In Females, the correlation between AST/ALT ratio and GGT gave a Pearson correlation of 0.19 and a p

value <0.0001, which shows poor correlation between the two parameters as shown in Table (3).
In Males, the correlation between AST/ALT ratio and GGT gave a Pearson correlation of 0.13 and a p value <0.0001, which shows poor correlation between the two parameters as shown in Table (4)

Table 1: MEAN, SD of AST, ALT, AST/ALT ratio and GGT in Females

Mean Values of AST, ALT, GGT in Females				
	AST U/L	**ALT U/L**	**AST/ALT**	**GGT U/L**
Controls	23.5± 6.52	23.54±6.01	1.02±0.24	29.85±11.43
Case	26.94±10.02	31.94±9.08	0.93±0.49	37.94±11.93

Table *2* : MEAN, SD of AST, ALT, AST/ALT ratio and GGT in Males

Mean Values of AST, ALT, GGT in Men				
	AST U/L	ALT U/L	AST/ALT	GGT U/L
Controls	20.61±7.4	28±9.57	0.77±0.3	31.88±10.18
Case	35.35±17.4	35.1±16.0	1.17±0.51	46.4±19.4

Figure 1: AST/ALT ratio in Females

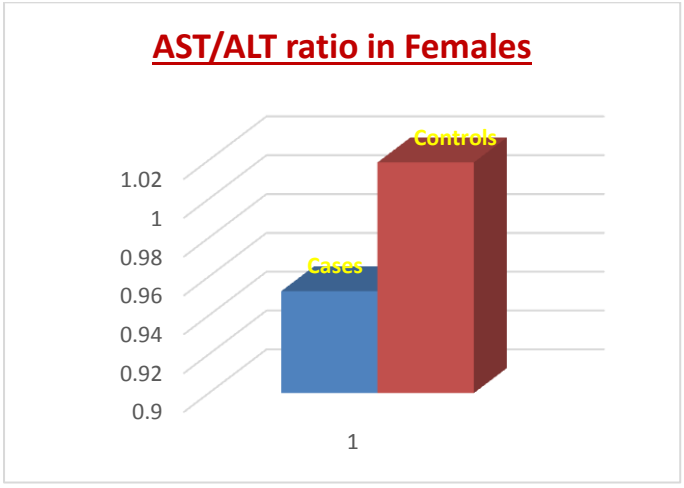


Figure 2: AST/ALT ratio in Males

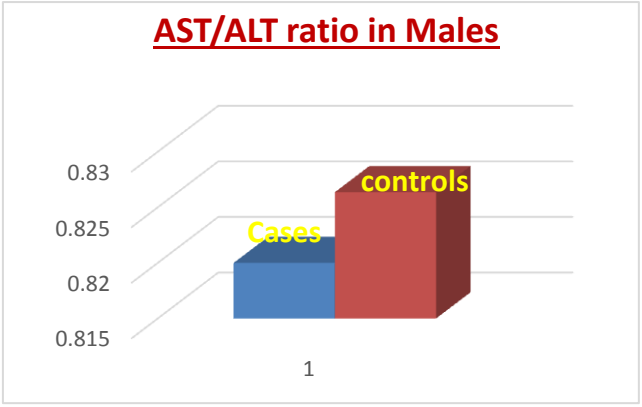


Figure 3: GGT in Females:

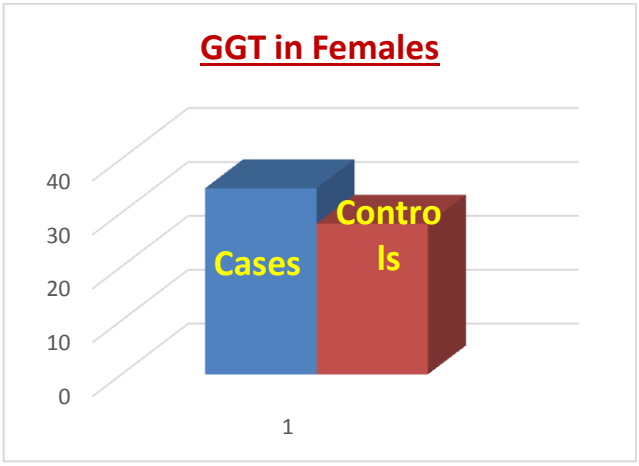


Figure 4: **GGT in Males**

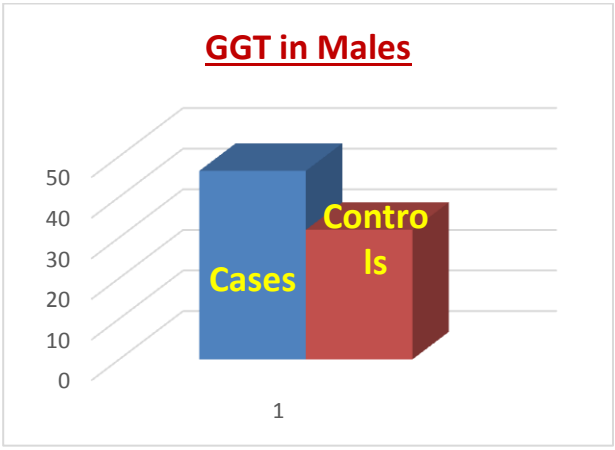


Table 3 : Correlation of AST/ALT ratio and GGT values in Females

	Pearson correlation (r value)	**Significance (P value)**
AST/ALT ratio vs GGT values	0.19*	< 0.0001**
* Poor Correlation (r value- nearer to zero) * * Strongly significant (P value: < 0.05)		

Table 4 *:* Correlation of AST/ALT ratio and GGT values in Males

	Pearson correlation (r value)	**Significance (P value)**
AST/ALT ratio vs GGT values	0.13*	< 0.0001**
* Poor Correlation (r value- nearer to zero) * * Strongly significant (P value: < 0.05)		

Figure 5 & 6 showing correlation between AST/ALT ratio and GGT in females and males

Figure 5: Correlation between AST/ALT ratio and GGT values in Women

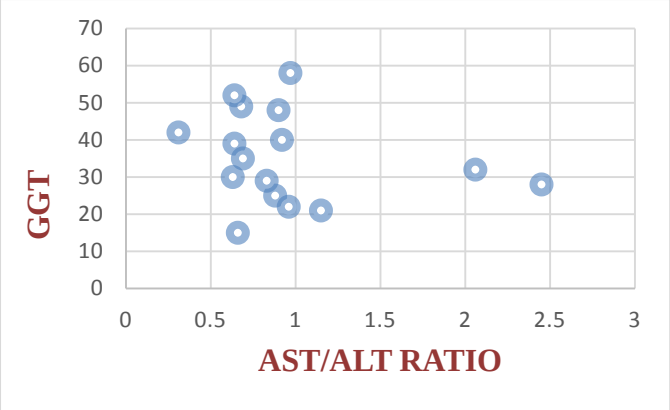
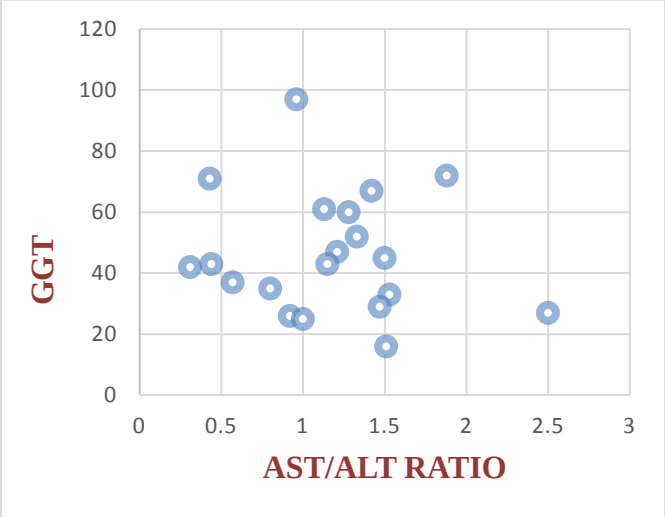


Figure 6: Correlation between AST/ALT ratio and GGT values in MALES



There was significant increase in mean values of AST, ALT and GGT values in cases than in controls both in females and males and decrease in AST/ALT ratio in cases than in controls in females and males. The correlation observed between AST/ALT ratio and GGT values was poor in both Men and Women.

Discussion

NAFLD is a major global public health problem, but there is insufficient evidence to justify screening for NAFLD in the general population.[6] Liver biopsy remains the gold standard for characterizing liver histology in NAFLD, but it is invasive, expensive and carries some morbidity and very rare mortality risk. Thus, it should be performed in those who would benefit the most from diagnostic, therapeutic guidance, and prognostic perspectives.WGO global guidelines suggest that metabolic syndrome, diabetes, elevated ALT and AST are independent predictors for progression and mortality in NAFLD[7] ALT, GGT, AST are markers of liver injury and may be useful surrogate measures of NAFLD reflect hepatocellular damage. NAFLD and NASH have been reported to be most common causes of chronically elevated liver enzymes and is often the tipping point for further diagnostic evaluation.

The presence of a significant increase in AST, ALT and GGT values in NAFLD patients (both male and female) compared to controls and decrease in AST/ALT ratio in NAFLD cases compared to controls. Only 30% of our study subjects with NAFLD had ALT above laboratory reference intervals (>40 U/L). Also, 55% of our study subjects with NAFLD had GGT above laboratory reference intervals limit (>30 U/L.)The correlation between GGT and AST/ALT ratio in NAFLD cases shown poor (r =0.19 & 0.13 in females and males) and p value (0.0001) is statistically significant.

Our findings are thus in total agreement with those of Debmalyasanyal et al[7] studied on Profile of liver enzymes in non-alcoholic fatty liver disease in patients with impaired glucose tolerance and newly detected untreated type 2 diabetes concluded that diabetic subjects with NAFLD had significantly higher ALT, AST, GGT and significantly lower AST: ALT ratio in comparison with diabetic subjects without NAFLD. The results of our study also support previous studies by López-Amador et al[8], G. Vernon, A. Baranova & Z.M Younossi[9]noted that liver function enzyme elevation, particularly alanine aminotransferase (ALT), is often the first sign of NAFLD, an increase of one to three times its normal. ALT level elevation seen in 72.5% of NAFLD patients with a higher level relative to AST level (25% patients). And concluded that elevation in ALT in NAFLD patients levels were higher than AST levels. These findings are also in partial agreement with those of Banderas et al.[10], Dixon et al.[11] they concluded that serum concentrations of GGT, AST, ALT, and insulin were elevated in the NASH patients and found that ALT was an independent predictor for the presence of NASH.

Huseyn et al[12] concluded that increased serum concentrations of GGT and ALT in patients with NASH suggest that pre-existing oxidative stress plays a role in the development and progression of both NASH and endothelial dysfunction**.** Kerner et al.[13] showed that high serum ALT concentrations were correlated with high C-reactive protein levels. The authors concluded that hepatic inflammation as a result of hepatic steatosis might be a potential contributor to low-grade systemic inflammation seen in patients with NASH. Sorbi et al[14] were the first authors to specifically perform study on the AST / ALT ratio. They evaluated 70 patients with NASH, matched with patients with alcoholic hepatitis, and observed that in NASH average index was 0.9, statistically different from the average of 2.6 observed in alcoholic hepatitis. Conclude that ratio below 1 suggests NASH and above 2 is highly suggestive of alcohol consumption. Kunde *et al.*[15] found a significant increase ALT values in biopsy-proven NAFLD with the application of the new standard. The sensitivity and specificity for NASH were 42% and 80% (ALT > 30 U/L) compared with 74% and 42% (ALT > 19 U/L) and concluded that significant increase in the prevalence of fatty liver and portal fibrosis and steatosis is detected in subjects with elevated ALT levels.

In NAFLD/NASH, aminotransferase levels may be elevated two to four times over the upper limit of normal[16]. However, in the absence of advanced disease, routine liver function tests are either normal or typically show only mild elevations in aminotransferase levels. In contrast to our results Adams et al[17] reported that aminotransferase levels fall over time as hepatic steatosis and inflammation improve. Aminotransferase levels do not correlate with the degree of fibrosis. To know the extent of hepatic inflammation there is a need for other non-invasive biochemical markers.

oxidative stress, lipid peroxidation and activity of microbiota can lead to an increase in mitochondrial permeability and caspase activation. At the tissue level, this leads to steatosis, and death by apoptosis and necrosis. An increase in interleukin (IL)-6 and transforming growth factor (TGF)-beta levels leads to trans glutamination, which may induce formation of Mallory-Denk bodies in the cells. An increase in TGF-beta, IL8 and RANTES levels result in the recruitment of neutrophils, leading to cellular inflammation. An increase in TGF-beta levels activates stellate cells and leads to collagen secretion as well as a reduction in extracellular matrix degradation. This phenomenon ultimately results in histological fibrosis. Fas LFas ligand; TNF Tumour necrosis factor

Conclusions

Rise of AST, ALT reflects oxidative stress, cell injury and steatosis in NAFLD.GGT acts as a mediator in the transportation of extracellular glutathione into most types of cells. Production of free radicals leads to reduction in glutathione and induces GGT to protect glutathione levels. This study shown a poor correlation between AST/ALT ratio and GGT levels in NAFLD patients and p value (0.0001) is statistically significant. So along with liver enzymes other noninvasive biomarkers (TNF α, IL 6, IL 8) are useful to know the extent of liver injury in NAFLD patients. So that it is useful in detection of complications of NAFLD (cirrhosis & hepatocellular carcinoma) as early as possible.

Conflict of interest- None
Source of funding - Nil

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