ORIGINAL RESEARCH

Utility of Gamma- Glutamyl Transferase (GGT) and AST/ALT ratio as a diagnostic tool for Alcoholic liver disease: A Cross Sectional Study

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ABSTRACT

Background: Alcoholic liver disease (ALD) is predominant form of chronic liver disease reported up-till now. Gamma glutamyl transferase(GGT) and AST/ALT ratio are routinely used as diagnostic tool in Alcoholic liver disease. However, no single laboratory marker is definitely helpful in detecting alcohol related injuries. There is always a need of combining different markers so as to be sensitive and specific enough to detect alcoholic liver disease. Hence, present study conducted to find role of GGT and AST:ALT ratio in isolation as well as in combination in diagnosing alcoholic liver disease. **Method:** It is cross sectional study conducted in tertiary care hospital. ALD patients diagnosed using case definition – on the basis of 'CAGE questionnaire, clinical evaluation and fatty liver on Ultrasonography' were enrolled and their serum GGT and AST:ALT levels were measured. **Result:** among 110 enrolled participants, Independently GGT has sensitivity of 96.4% and specificity of 92.7% and that of AST/ALT ratio is 87.3% and 90%. However, it was observed that Sensitivity of tests was highest (100%) when done concordantly and specificity of tests was highest (98.2%) when done discordantly as opposed to GGT and AST/ALT ratio done separately. **Conclusion:** GGT & AST/ALT used concordantly can be best utilized as screening test, however for definitive diagnosis both tests used discordantly are more useful.

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INTRODUCTION

Alcoholic liver disease is spectrum of liver injuries that are related to alcohol abuse.[1] It is one of the reasons for morbidity and mortality globally. These are predominantly associated with excessive drinking over long period. However, heavy drinking for shorter period may also be associated with alcoholic liver disease.[2,3] Many countries recognize the serious public health problem caused by the harmful use of alcohol and have taken steps to prevent the health and social burden and treat those in need of care. But clearly much more needs to be done to reduce the loss of life suffering associated with harmful alcohol use.

Gamma Glutamyl transferase (GGT) is traditional biochemical marker associated with alcoholic liver diseases though it may also be elevated in pancreatic diseases, renal failure , myocardial infarction, diabetes mellitus and chronic obstructive pulmonary disease (COPD).[4,5,6,7] Further, Serum Glutamic Pyruvate Transaminase known as Alanine Transaminase (ALT), is found in high concentration in liver and to a lesser extent in other organs like heart, kidney, skeletal muscle, lung and pancreas.[8] Also, Serum Glutamic Oxaloacetic Transaminase also known as aspartate aminotransferase occurs in large amount in liver, renal, cardiac, and skeletal muscles. AST to ALT ratio is also used as marker of the alcoholic liver diseases.[9] AST:ALT ratio of >1 is associated with alcoholic liver disease.

However, No single laboratory marker is definitely helpful in detecting alcohol related injuries. There is always a need of combining different markers so as to be sensitive and specific enough to detect alcoholic liver disease. Hence, present study conducted to find role of GGT and AST:ALT ratio in isolation as well as in combination among alcoholic liver disease.

MATERIAL AND METHODS

The present study is a cross-sectional study conducted in a tertiary care teaching institute under Department of Biochemistry. Patients attending Medicine OPD were approached and those diagnosed with alcoholic liver disease as per operational definition were identified. Operational definition for alcoholic liver disease was considered as follows-

- History of alcohol abuse (assessed by CAGE questionnaire) * and
- Clinical evaluation by physician for sign & symptoms of hepatitis and
- Fatty liver on ultra-sonography examination

*CAGE questionnaire is a 4-point scale with responses as 'yes' or 'no' is a useful screening tool for identifying potential alcohol abuse developed by Dr John A Ewing. Any two 'yes' responses were considered positive.

Further, the patients requiring Indoor patient care (IPD) or Intensive Care Unit Care (ICU) were excluded from the study. All the eligible candidate aged 30-50 years complying with the operational definition were informed about the process and conduct of the study. Informed consent was taken from those who show willingness to participate. Case record form (CRF) was developed to capture information on socio-demography and alcohol abuse (by CAGE questionnaire). Also, 5 ml of venous blood sample was collected under aseptic precautions in plain bulb. Serum was separated by centrifugation at 3000 rpm for 15 min and estimation was performed within 2 hours of sample collection. Aspartate and Alanine aminotransferase (AST & ALT) were estimated by Modified IFCC Method, while Gamma glutamyl transferase (GGT) – was estimated by carboxy substrate method.

Data was analyzed for diagnostic utility of GGT and AST/ALT ratio individually as well as in combination. Receiver Operating Characteristic (ROC) curve and Area Under Curve (AUC) were be plotted to estimate cut off value.

SAMPLE SIZE

While reviewing literature on the topic, we found related study where difference in mean of GGT among alcoholic liver disease and non-alcoholic liver disease was 32.6 IU/L. Sample size was estimated using Open EPI Version 3.0. At confidence interval of 95%, sample size was calculated as 110.

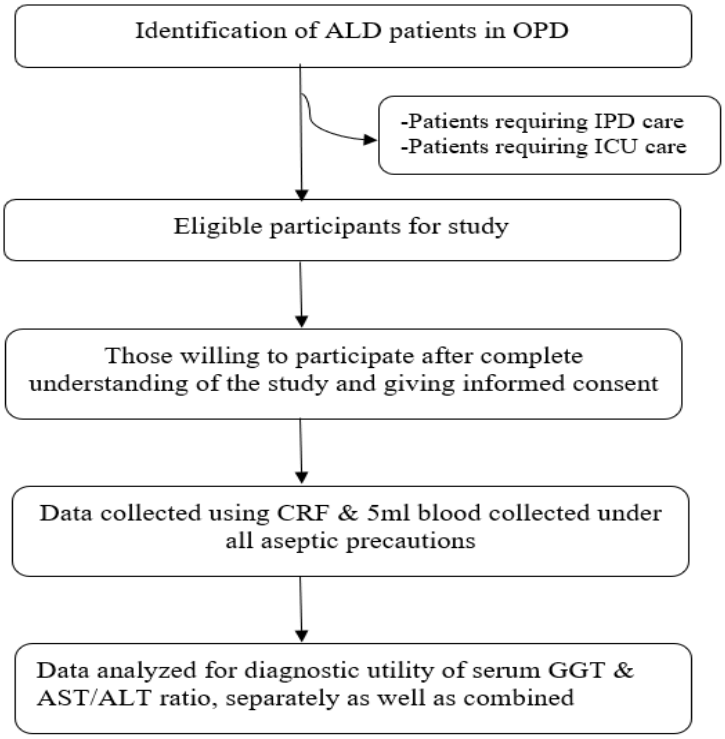

Figure 1: Flowchart depicting methodology

ETHICAL CONSIDERATIONS

Ethical approval was sought from Institutional Ethics Committee (IEC) {Reg. No. ECR/1033/Inst/MH/2018/46/20/06/2020} before the initiation of study. Participants were informed about the purpose and conduct of the study. An information sheet was provided to the participants for better understanding. A written informed consent was taken from the participants before subjecting them Case Record Form (CRF).

OBSERVATIONS & RESULTS

We enrolled 110 participants andanalyzed the data using SPSS version 23.0. The results of the study are discussed under following headings-

A. Socio-demographic profile of participants

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B. Clinical Profile of patients with Alcoholic liver disease
C. Laboratory Tests as diagnostic tool for alcoholic liver disease

A. Socio-demographic profile of participants

All the enrolled 110 participants were male. Mean age of participants was 41.91 ± 7.76 and 42.31 ± 9.6 among Patients with Alcoholic liver disease and Non-diseased individuals respectively. Mean years of schooling among Patients with Alcoholic liver disease and Non-diseased individuals was observed to be 6.42 ± 4.47 and 8.20 ±4.77 respectively. Important socio-demographic details of all participants are depicted in table no.1.

Chi square test was applied to observe whether there is any difference between the study groups. We observed no significant difference (p-value >0.05) among participants in terms of age, education, occupation, marital status and socio-economic status.

Table No. 1: Socio-demographic profile of participants

Parameters		Patients with Alcoholic liver disease	Non-diseased individuals	Chi Square test*
Age Groups	30-35 yrs	12 (21.8%)	15 (27.28%)	p-value= 0.47
	35-40 yrs	18(32.7%)	12 (21.82%)	
	40-50 yrs	25 (45.5%)	28 (50.9%)	
Education	Illiterate	9 (16.4%)	8 (14.5%)	p-value= 0.24
	Primary School	11 (20%)	8 (14.5%)	
	Middle School	28 (50.9%)	22 (40%)	
	High School	4 (7.3%)	11 (20%)	
	Graduation & above	3 (5.5%)	6 (10.9%)	
Occupation	Unskilled Worker	35 (63.7%)	27 (49.1%)	p-value= 0.26
	Semi-skilled Worker	6 (10.9%)	5 (9.1%)	
	Skilled Worker	12 (21.8%)	17 (30.9%)	
	Shopkeeper	2 (3.6%)	6 (10.9%)	
Marital Status	Married	41 (74.5%)	40 (72.5%)	p-value= 0.83
	Un-Married	14 (25.5%)	15 (27.5%)	
Socio-economic status**	Lower Class	2 (3.6%)	4 (7.3%)	p-value= 0.07
	Lower-Middle Class	32 (58.2%)	18 (32.7%)	
	Middle Class	14 (25.5%)	21 (38.2%)	
	Upper Middle	5 (9.1%)	11 (20%)	
	Upper Class	2 (3.6%)	1 (1.8%)	

*Chi square test p value is considered statistically significant when < 0.05
**Calculated using Modified BG Prasad Classification for Socio-economic Class

B. Clinical Profile of patients with Alcoholic liver disease

While enrolling patients for alcoholic liver disease confirmation of diagnosis was based on following criteria-History of alcohol abuse (assessed by CAGE questionnaire), Clinical evaluation for symptoms & signs and Fatty liver on ultra-sonography examination CAGE score value of 2 or more was considered positive for alcohol abuse. CAGE scores of the participants are graphically presented as a pie chart in figure no.1

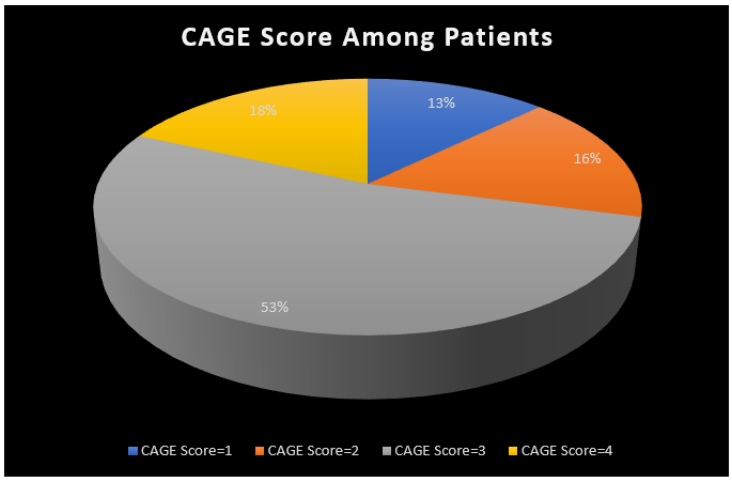

Figure No.1: Pie chart depicting CAGE score found among Alcoholic Liver Disease Patients

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Common symptoms and signs observed among alcoholic liver disease patients were jaundice, fatigue, fever, loss of appetite, nausea, vomiting, abdominal tenderness and weight loss. It is graphically presented as bar chart in figure no.2

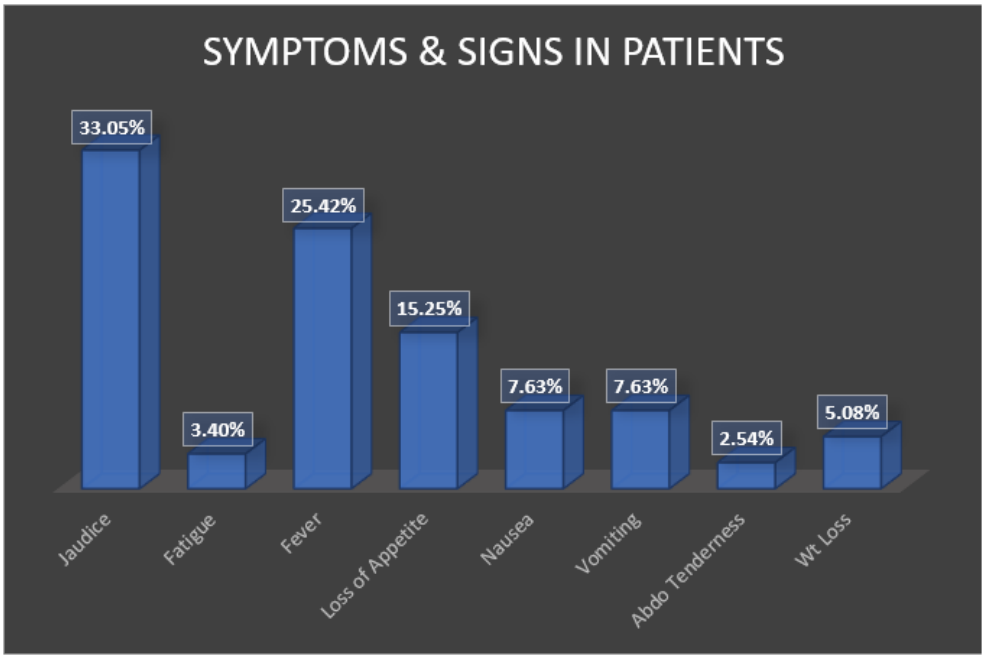

Figure No.2: Bar diagram of different symptoms & signs observed among Alcoholic Liver Disease Patients

C. Laboratory Tests as diagnostic tool for alcoholic liver disease

To understand diagnostic utility of tests we measured serum GGT and serum AST:ALT ratio. Mean score for GGT was 96.66 ± 31.36 IU/L & 25.30 ± 12.89 IU/L among patients with alcoholic liver disease and non-disease individuals respectively. Also, mean of AST:ALT ratio was 1.77 ± 0.87 and 0.71± 0.28 among patients with alcoholic liver disease and non-disease individuals respectively. Test results analysed are further discussed under following headings-

1. Diagnostic utility of GGT
2. Diagnostic utility of AST:ALT ratio
3. Diagnostic utility of concordant testing
4. Diagnostic utility of discordant testing
5. Correlation between serum GGT & AST:ALT ratio

1. Diagnostic utility of GGT

Independent t test performed on mean serum GGT values between alcoholic liver disease group and non-diseased group. The value is found to be significantly higher (p value<0.01) among alcoholic liver diseased group. These findings are depicted in table no.2. Chi square test performed on findings of GGT test results shows that there is significant different test results (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals. GGT test findings are depicted in table no.3. Also bar chart of these findings is depicted in figure no.3

Table No.2: Mean difference in serum GGT test results in both study groups

		Disease status	Mean±S.D.	Independent t test
Serum GGT		Alcoholic liver disease	96.66 ± 31.36	p-value < 0.01
		Non-diseased	25.30 ± 12.89	

Table No.3: Serum GGT test results in both study groups

Test		Patients with Alcoholic liver disease	Non-diseased individuals	Chi square test p-value
Serum GGT	Positive	53 (96.4%)	4 (7.3%)	<0.01
	Negative	2 (3.6%)	51 (92.7%)	

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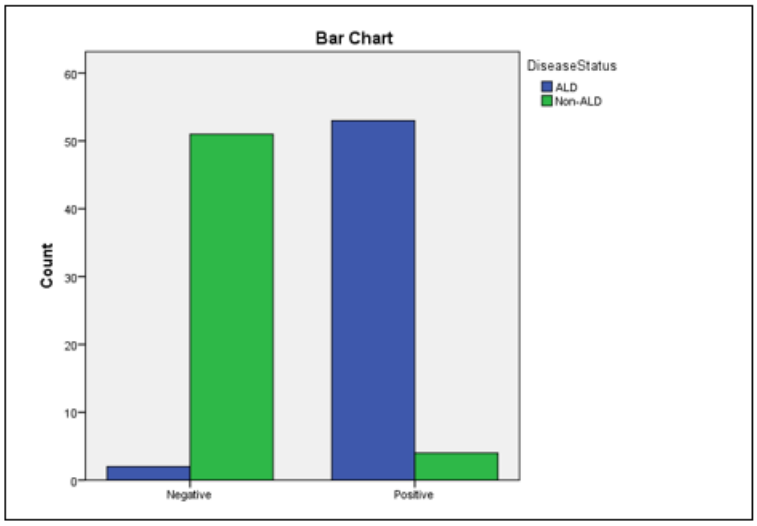

Figure no.3: Bar chart for GGT Test results

ROC curve plotted for GGT values shows that -for a sensitivity of 96.4% and specificity of 92.7% cut off values is 50.85 U/L and area under cover is 0.987. ROC Curve for serum GGT values is depicted in figure no.4.

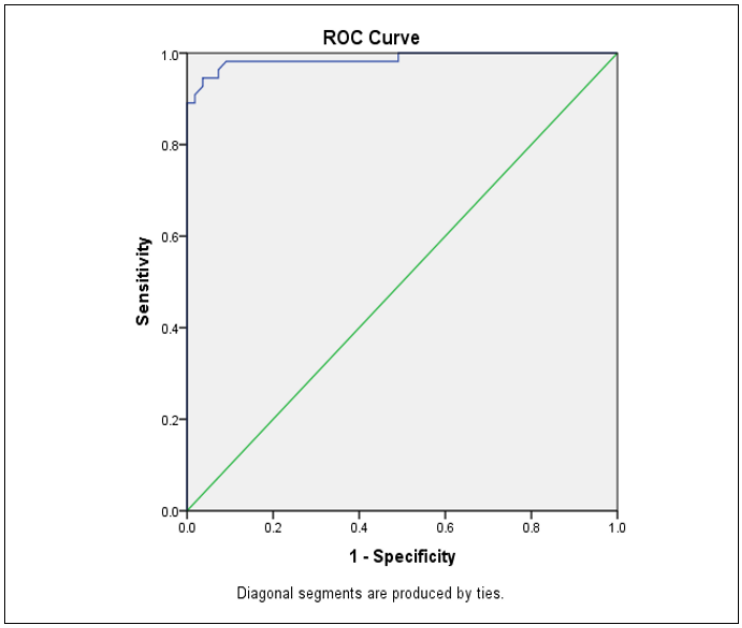

Figure No. 4: ROC Curve for GGT as a diagnostic tool for Alcoholic liver disease

2. Diagnostic utility of AST:ALT ratio

Independent t test performed on mean serum AST & ALT values between alcoholic liver disease group and non-diseased group. The value is found to be significantly higher (p value<0.01) among alcoholic liver diseased group. These findings are depicted in table no.4. Chi-square test performed on findings of AST:ALT ratio shows that there are significant different test results (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals. AST:ALT ratio findings are depicted in table no.5. Also, bar chart of these findings is depicted in figure no.5

Table No.4: AST: ALT ratio test results in both study groups

	Disease status	Mean±S.D.	Independent t test
Serum AST	Alcoholic liver disease	122.15 ± 89.10	p-value < 0.01
	Non-diseased	27.14 ± 24.95	
Serum ALT	Alcoholic liver disease	84.52 ± 78.52	p-value < 0.01
	Non-diseased	40.39 ± 29.10	

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Table No.5 AST: ALT ratio test results in both study groups

Tests				Chi square p-value
AST:ALT ratio	Positive	48 (87.3%)	6 (10.9%)	<0.01
	Negative	7 (12.7%)	49 (89.1%)	

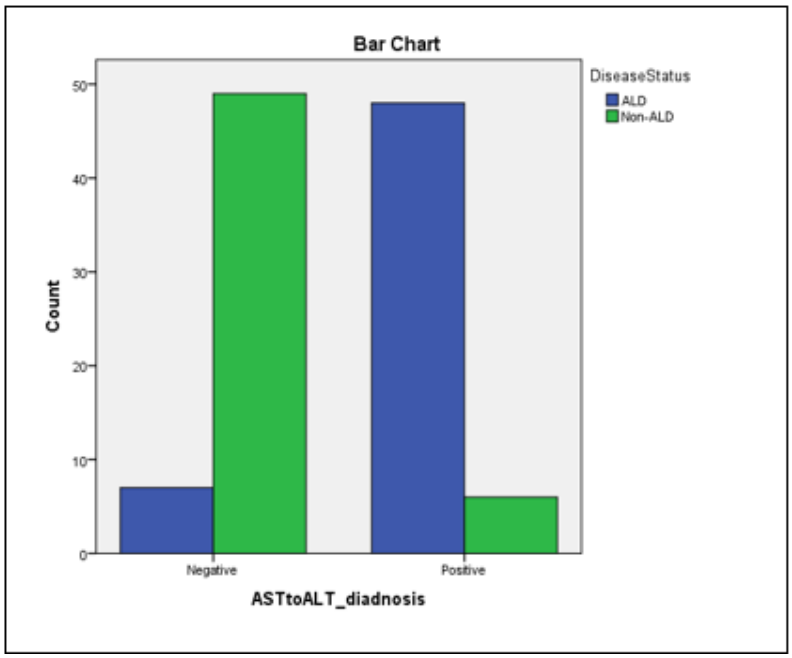

Figure no.5: Bar chart for AST:ALT ratio test results

ROC curve plotted for AST: ALT ratio values shows that -for a sensitivity of 87.3% and specificity of 90% cut off values is 1.025 and area under cover is 0.904. ROC Curve for serum AST: ALT ratio values is depicted in figure no.6.

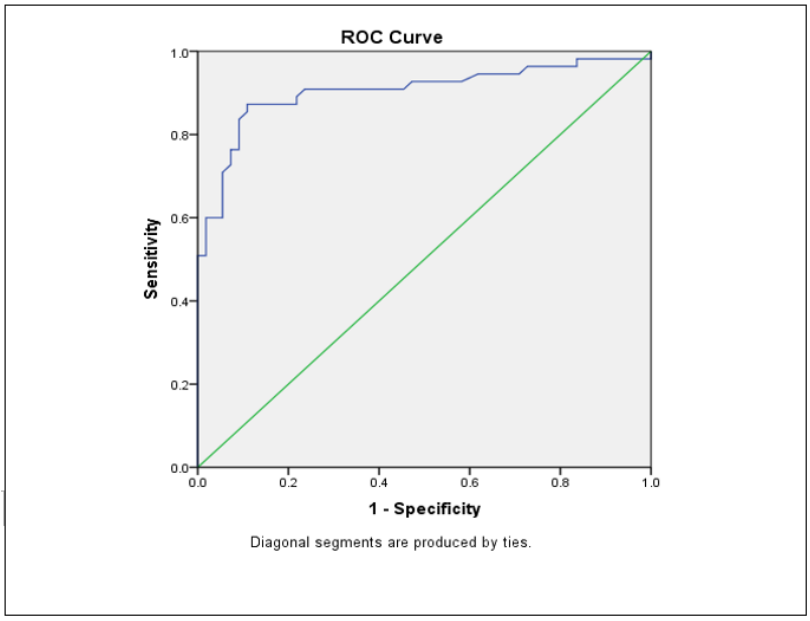

Figure No. 6: ROC Curve for AST: ALT ratio as a diagnostic tool for Alcoholic liver disease

3. Diagnostic utility of concordant testing

Chi square test performed on findings of GGT test and AST:ALT ratio- Concordance, results shows that there is significant different test results (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals. Sensitivity and specificity of concordant testing was 83.6% and 98.2% respectively. GGT test and AST:ALT ratio- Concordance test findings are depicted in table no.6. Also bar chart of these findings is depicted in figure no.7.

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Table No.6 GGT test and AST: ALT ratio- Concordance test results in both study groups

Tests		Patients with Alcoholic liver disease	Non-diseased individuals	Chi square p-value
GGT & AST:ALT ratio - Concordance	Positive	46 (83.6%)	1 (1.8%)	<0.01
	Negative	9 (16.4%)	54 (98.2%)	

*Concordance- Final test results were labelled as positive for alcoholic liver disease only when both GGT AND AST:ALT ratio test results were positive

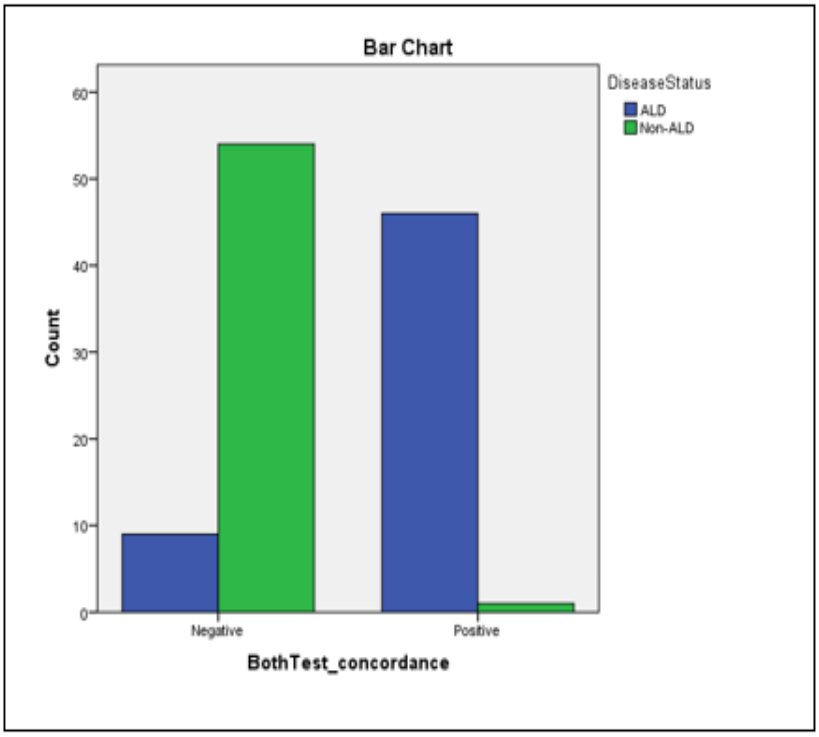

Figure No. 7: Bar chart for GGT test and AST:ALT ratio- Concordance test results

4. Diagnostic utility of discordant testing

Chi square test performed on findings of GGT test and AST:ALT ratio- Discordance, results shows that there is significant different test result (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals. Sensitivity and specificity of Discordant testing was 100% and 83.6% respectively. GGT test and AST:ALT ratio- Discordance test findings are depicted in table no.7. Also, bar chart of these findings is depicted in figure no.8.

Table No.7: GGT test and AST: ALT ratio- Concordance test results in both study groups

Tests		Patients with Alcoholic liver disease	Non-diseased individuals	Chi square p-value
GGT & AST:ALT ratio - Discordance	Positive	55 (100%)	9 (16.4%)	<0.01
	Negative	0	46 (83.6%)	

Discordance- Final test results were labelled as positive for alcoholic liver disease when either GGT OR AST:ALT ratio test results were positive

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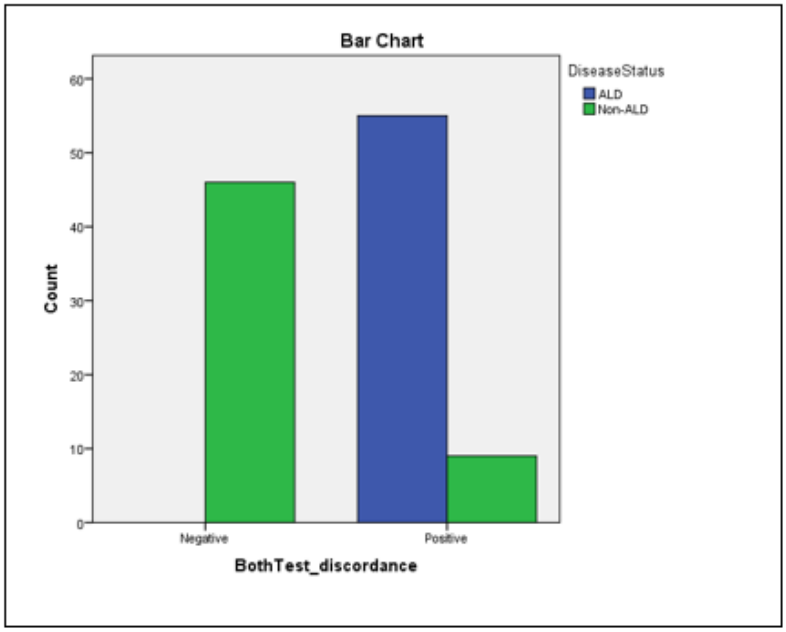

Figure No. 8: Bar chart for GGT test and AST: ALT ratio- Discordance test results

5. Correlation between serum GGT & AST:ALT ratio

Pearson correlation coefficient of +0.49 suggests that there is strong linear relationship between serum GGT and AST: ALT ratio as presented in table no. 8 and graphically represented in figure no.9

Table No. 8: Correlation between serum GGT and AST: ALT ratio

Pearson Correlation	0.49
P value	<0.01
Sample size	110

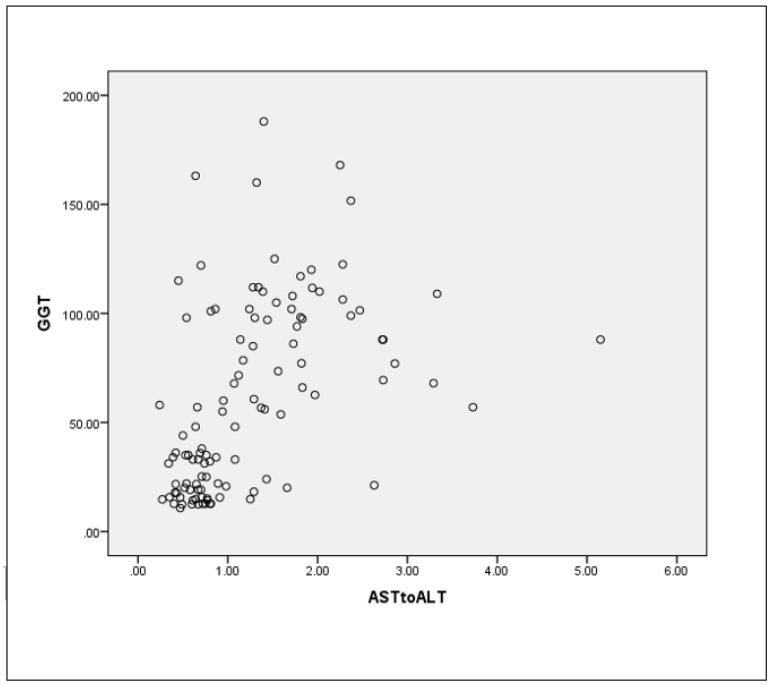

Figure No. 9: Graphical presentation of Correlation between serum GGT and AST:ALT ratio

DISCUSSION

In the present study, the mean values of Serum GGT, AST and ALT values in alcoholic liver disease group were 96.66±31.36, 122.15±89.10 and 84.52±78.52 as compared to 25.30±12.89, 27.14±24.95 and 40.39±29.10. These values are significantly higher in cases of alcoholic liver disease when compared with non-diseased group with a significant p value <0.01.

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Similar results were also found in a study carried out by singh*et al.* in which a raised value of all 3 parameters was seen in cases of Alcoholic liver disease.[10] Similar results were established in a study carried out by Rizal *et al.* where the mean GGT levels in cases of Alcoholic liver disease was 98.32 U/L.[11]Various other studies conducted by Cushier A *et al.*, Das *et al.* and Brandl *et al.* have shown significantly increased values of GGT, AST and ALT in their studies pertaining to alcoholic liver diseases.[12,13,14]

In present study the AST: ALT Ratio in Alcoholic liver disease and non-diseased was found to be 1.77 ± 0.87 and 0.71± 0.28, which shows that the AST: ALT ratio is significantly increased in cases of ALD. In a study conducted by Parmar *et al.* in 2016, the AST: ALT ratio was found to be around 2.12.[15] Study by Shikha Rizal *et al.* also showed a raised AST: ALT Ratio in cases of Alcoholic liver disease.[11] In a similar study by Hyder *et al.*, where serum GGT, AST, ALT and AST: ALT ratio were established among Alcoholic liver disease cases, there was a significant rise in AST: ALT ratio and serum GGT levels, which is in accordance to our study.[8]The sensitivity and specificity by receiver operating characteristic curve (ROC) plotted for AST: ALT ratio values shows that for a sensitivity of 87.3% and specificity of 90% cut off values is 1.025 and area under curve (AUC) is 0.904. Also, ROC curve plotted for GGT values shows that -for a sensitivity of 96.4% and specificity of 92.7% cut off values is 50.85 U/L and area under cover is 0.987.

Similar findings were evidenced in a study by Junling wang *et al.* sensitivity, specificity, and area under ROC for AST/ALT ratio and GGT diagnosis of Alcoholic liver disease, were reported to be 75.29%, 94.34%, and 0.826 and 80.23%, 79.25%, and 0.815 respectively.[16]

In present study, Concordant GGT & AST/ALT ratio and discordant GGT & AST/ALT ratio were calculated to understand how these tests adds to the diagnosis of alcoholic liver disease together. GGT test and AST:ALT ratio-Concordance, results shows that there is significant different test results (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals with sensitivity and specificity of concordant testing 83.6% and 98.2% respectively. Similarly, GGT test and AST:ALT ratio-Discordance, results shows that there is significant different test result (p-value <0.01) between Patients with Alcoholic liver disease and non-diseased individuals with sensitivity and specificity of discordant testing was 100% and 83.6% respectively. Further studies in this direction need to be conducted as we could not found study with such combination.

In our study, sensitivity and specificity of Discordant testing was 100% and 83.6% respectively. GGT test and AST: ALT ratio, which suggests that 100 % and 83.2 % patients were diagnosed as cases of ALD when GGT and AST: ALT ratio was found to be positive respectively.

CONCLUSION

Both the tests serum GGT & AST/ALT ratio can be effectively used concordantly or discordantly depending on purpose of the program. When purpose of program is screening for ALD both tests can be used concordantly, however when purpose is definitive diagnosis of ALD then both tests used discordantly are more useful.

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