journal homepage: https://jablm.acclmp.com/

Journal of Applied Biochemistry & Laboratory Medicine (2024) 05 (01):8-14

Original Article

A Cross-Sectional Analysis of The Use of Different Ratios Of Hepatic Enzymes As Potential Markers For Non-Alcoholic Steatotic Hepatitis (NASH)

Sen Susruta[1], Pathak Indrani[2], Karmakar Minakshi[3], Bhattacharjee Nivedita[4], Choudhuri Sharmistha[5]*

[1]Director, Department of Lab Medicine, CK-Birla Hospitals-CMRI (The Calcutta Medical Research Institute) & BM Birla Heart Research Centre, Kolkata-700027, India

[2]Supervisor Pathology, CK-Birla Hospitals-CMRI (The Calcutta Medical Research Institute) & BM Birla Heart Research Centre, Kolkata-700027, India

[3]Consultant Pathologist, CK-Birla Hospitals-CMRI (The Calcutta Medical Research Institute) & BM Birla Heart Research Centre, Kolkata-700027, India

[4]Associate Consultant Microbiologist, CK-Birla Hospitals-CMRI (The Calcutta Medical Research Institute) & BM Birla Heart Research Centre, Kolkata-700027, India

[5]Assistant Professor, Dept. of Biochemistry, RG Kar Medical College, Kolkata-700004.

Keywords:
NAFLD, NASH, AST/GGT ratio, ALT/GGT ratio, AST/ALT ratio, ROC curve, Cut off values.

ABSTRACT

Background:
Non-alcoholic steatohepatitis is becoming the major hepatic disorder among the world population due to various factors. Along with radiological markers, biochemical markers play a crucial role in its early diagnosis and management. In this context, ratios of different metabolic parameters of the liver play a better diagnostic role than the individual ones. The importance of the hepatic enzyme ratio of AST/ALT has been reported in several earlier studies as indicators of hepatic metabolic parameters, but very few studies have been conducted to establish the association of ALP/GGT and AST/GGT ratios with the degree of steatosis in NASH.

Aims and Objectives:
The present study was conducted to find out the importance of AST/GGT and ALT/GGT ratios as diagnostic markers of NASH in comparison to the other markers.

Methodology:
The present study was performed using the data of NASH patients obtained from the hospital records following the ethical guidelines. 35 cases and 42 control data were included. Direct bilirubin was ascertained as an indicator of steatosis and AST, ALT, GGT and ALP were chosen as the markers of hepatic metabolic markers. All these parameters were measured using fully automated biochemical analysers. Ratio of AST/GGT, ALT/GGT, and AST/ALT were determined and their cut off values were ascertained using the ROC curve analysis. The median values of each study parameter was compared using the Mann-Whitney test and their association with the degree of steatosis in the case group was tested by using Spearman's correlation analysis.

Results:
All study parameters including the ratios of AST/GGT, ALT/GGT, and AST/ALT were significantly raised from the control subjects (P <.005 for all). The correlation study revealed a strong but significant negative association of the AST/GGT and ALT/GGT ratio with the direct bilirubin, the direct biomarker of steatosis (r = -.405 and -.422 respectively with P = .01). AST/ALT ratio did not show such correlation. ROC curve analysis showed the cut off values of AST/GGT and ALT/GGT ratios to be 1.21 and 1.31 respectively with an optimum degree of sensitivity and specificity of 91% and 67%, and 88% and 79%.

Conclusion:
Results of the present study suggest that the ratio of biochemical parameters of the liver are much better markers for assessing the steatotic hepatic damage than the individual markers. Among these ratios, the present study found the ratio of AST/GGT and ALT/GGT to be most robust and useful in the context of their sensitivity and specificity. The present study also suggests that multiple ratios should be used for a better prediction and analysing the hepatocellular damage in NASH..

*Correspondence:
Dr. Sharmistha Choudhuri
Assistant Professor, Dept. of Biochemistry, RG Kar Medical College, Kolkata-700004
Email: sharmistha_choudhuri@yahoo.in

INTRODUCTION:

Non-alcoholic fatty liver disease (NAFLD) is a broad spectrum of liver disorders which ranges from benign fat accumulation in hepatocytes to non-alcoholic steatotic hepatitis (NASH) leading to the final stage of cirrhosis and end-stage liver disease if untreated [1, 2]. Development of NASH from NAFLD can be best explained by the multiple parallel hit hypothesis that states that NASH develops due to a cross-talk among different organ systems including the adipose tissues and intestine[3, 4]. The whole process involves fat deposition in the liver, oxidative stress, infiltration of hepatocytes by cytokines and macrophages, and changes in the Kupffer cells that finally evolve into inflammatory steatosis, fibrosis, cirrhosis, and an end-stage liver disease[5]. With the rapid increase in NAFLD cases worldwide, the prevalence of NASH cases is also increasing rapidly among the world population. NAFLD is now becoming commoner among the population due to rising obesity and is prevailing at present as the commonest cause for abnormal liver function tests. Although it was the most prevalent disorder in Western countries till the recent past, the present data suggest that it is playing an important role in worldwide hepatic disorders now and its worldwide prevalence is 15-40% which is still increasing [6]. It has been well documented till now that insulin resistance, obesity, diabetes mellitus, and dyslipidemia have a strong relationship with NAFLD, but the definite pathogenesis that lead to its progression to NASH is still being explored.

The definitive diagnosis of NAFLD depends on the ultrasonographic (USG) findings of hepatic steatosis or liver biopsy[7]. For obvious reasons, the performance of USG is more feasible than the liver biopsy. However, as many patients with NAFLD are symptomless, many diagnoses follow incidental findings of mildly raised ALT levels which is comparatively higher than the rise in AST. But, with the progress of the disease from NAFLD to NASH, ALT levels typically start falling with an increase in AST sometimes due to progressive fibrosis. However, neither the single value of AST or ALT is a sufficiently good predictor of the disease progress in NAFLD[8]. Among the biochemical markers, the AST/ALT ratio has been a good indicator for assessing fat deposition in the liver. Studies have indicated that a ratio of -.640 could successfully predict the NAFLD with 95% sensitivity[9]. In contrast, some other studies found that the gamma-glutamyl transferase (GGT) was the most important predictor of NFFLD and NASH with no predictive significance of AST and ALT[8].

Although the AST/ALT ratio has been used in several studies to predict various degrees of deposition of fat in the liver in NAFLD and NASH, the ratio of AST/GGT, or ALT/GGT has been not explored significantly for assessing or predicting fat deposition in the liver in the spectrum of these disorders. The present study hypothesized that along with the AST/ALT ratio, the other two ratios mentioned above may also play important roles in assessing and predicting lipid deposition in the liver in the NAFLD. With these aims and objectives the present study was designed.

METHODOLOGY:

The present retrospective study was conducted by analyzing the data collected from the case records of patients suffering from NASH attending a tertiary care hospital in a metropolitan city. The data were collected from the lab reports and patient history sheets after obtaining permissions from the lab authority and complying with their ethics committee guidelines for a period of one year i.e. from January 2023 to January 2024. 35 cases of complete datasets from the NASH patients were selected. The definitive diagnosis of NASH was done by ultrasonography and the biochemical assays were performed using fully automated biochemical analyzer Cobas 6000 from Roche Diagnostics. The coefficient of variation (CV) for all tests were below 10% and the parameters tested in the lab were all accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL). 42 control data were selected from persons attending with non hepatic disorders and did not suffer from any disease that could affect their liver functions. The control data were selected in a way that they are matched for age and sex with the case group.

Statistical analysis:

All data were tested initially for their distribution pattern, i.e whether they follow a normal distribution pattern or not by using the Smirnov Kolmogorov Test. The data were found to follow a non-parametric distribution ($P > 0.05$). Accordingly, the Mann Whitney test was performed to assess the difference in test parameters between the case and control groups and Spearman's correlation analysis was performed to assess the correlation strength between the study variables among the case group. For all statistical analyses, the P value was considered to be significant at $P<.05$ for a 95% confidence interval. All statistical analyses were performed using the SPSS software version 22.0 for Windows 10.

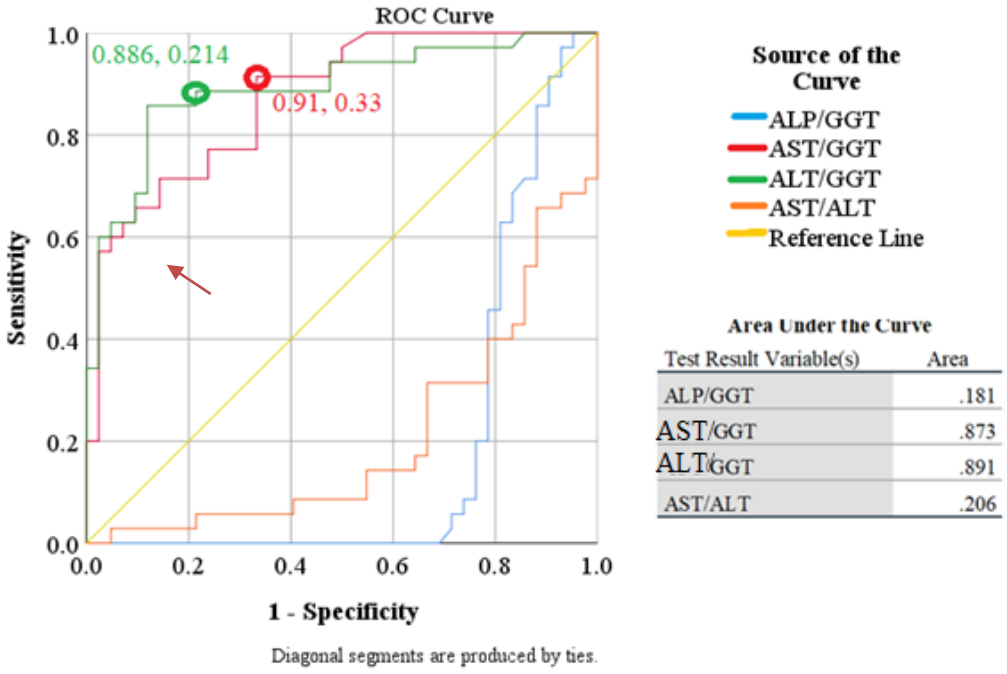
RESULTS:
After analyzing the obtained data the output of their results was depicted in the following tables:

Table 1: Mann-Whitney test showing the differences in study parameters between the case and control group:

Tests	GROUP	N	Mean Rank	Mann-Whitney U	Z Value	P value
Total Bilirubin	Control	42	22.04	22.50	-7.30	<.005
	Test	35	59.36			
	Total	77				
Direct Bilirubin	Control	42	22.36	36.00	-7.25	<.005
	Test	35	58.97			
	Total	77				
AST	Control	42	21.93	18.00	-7.33	<.005
	Test	35	59.49			
	Total	77				
ALT	Control	42	21.81	13.00	-7.38	<.005
	Test	35	59.63			
	Total	77				
GGT	Control	42	27.25	241.50	-5.04	<.005
	Test	35	53.10			
	Total	77				
ALP	Control	42	29.62	341.00	-4.03	<.005
	Test	35	50.26			
	Total	77				
ALP/GGT	Control	42	50.17		-4.79	<.005
	Test	35	25.60	266.00		
	Total	77				
AST/GGT	Control	42	25.95		-5.60	<.005
	Test	35	54.65	187.00		
	Total	77				
ALT/GGT	Control	42	25.32		-5.87	<.005
	Test	35	55.41	160.50		
	Total	77				
AST/ALT	Control	42	49.28		-4.42	<.005
	Test	35	26.65	303.00		
	Total	77				

Results from Table 1 reveal that although all parameters are significantly raised in the case group, the rise in ALT and AST are highest, with Z scores of -7.38 and -7.33 respectively. When the ratio between the test parameters are considered, it is found that ALT/GGT is the highest (Z = -5.87) followed by the AST/GTT ratio (Z = -5.60). The receiver operator characteristic curve (ROC) analyses were performed to determine the suitable cut-off values for the ratio between the study parameters (Figure 1). Results showed that the AST/GGT and ALT/GGT ratios represent the highest area under the curve of 0.873 and 0.891 respectively. These two ratios also indicate the cut-off values of 1.21 and 1.31 respectively with an optimum sensitivity and false positivity (1-specificity) of 0.91, 0.33, and 0.88, 0.21 respectively.

Figure 1: Receiver operator characteristic curve for determining the cut off values for different ratios for diagnosis of NASH



Area Under the Curve

Test Result Variable(s)	Area
ALP/GGT	.181
AST/GGT	.873
ALT/GGT	.891
AST/ALT	.206

In the present study, we assessed the strength of the association of these ratios with the steatosis in NASH patients by carrying out a correlation study. For this, we did Spearman's correlation analysis for data distributed in a non-parametric pattern. In this study, we correlated the values of these ratios signifying the metabolic derangement with the values of direct bilirubin for assessing the degree of steatosis in the liver induced by NASH. The results of this correlation analysis is shown in Table 2:

Table 2: Spearman correlation analysis results between the enzyme ratios and the bilirubin levels in the case group:

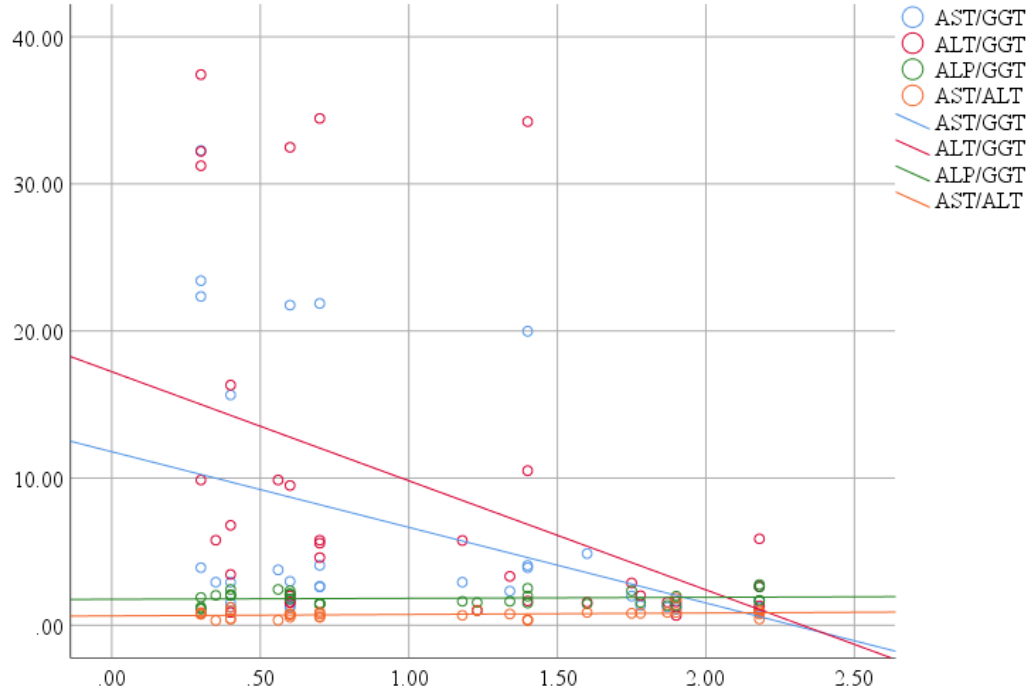
Spearman's Correlation study.

		BT	BD	ALP/GGT	AST/GGT	ALT/GGT	AST/ALT
Total Bilirubin (BT)	Pearson Correlation	1	.812**	.160	-.380*	-.365*	.095
	Sig. (2-tailed)		.000	.357	.024	.031	.586
	N	35	35	35	35	35	35
Direct Bilirubin (BD)	Pearson Correlation	.812**	1	.103	-.405*	-.422*	.223
	Sig. (2-tailed)	.000		.558	.016	.012	.198
	N	35	35	35	35	35	35
ALP/GGT	Pearson Correlation	.160	.103	1	-.187	-.128	-.123
	Sig. (2-tailed)	.357	.558		.281	.463	.483
	N	35	35	35	35	35	35
AST/GGT	Pearson Correlation	-.380*	-.405*	-.187	1	.951**	-.035
	Sig. (2-tailed)	.024	.016	.281		.000	.843
	N	35	35	35	35	35	35
ALT/GGT	Pearson Correlation	-.365*	-.422*	-.128	.951**	1	-.141
	Sig. (2-tailed)	.031	.012	.463	.000		.419
	N	35	35	35	35	35	35
AST/ALT	Pearson Correlation	.095	.223	-.123	-.035	-.141	1
	Sig. (2-tailed)	.586	.198	.483	.843	.419	
	N	35	35	35	35	35	35

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Results of the correlation study showed that the ratios of AST/GGT and ALT/GGT were most well correlated to the direct bilirubin showing a correlation coefficient of -.40 and -.42 respectively with P values <.05 for both. These strong correlations have been illustrated in Figure 2 using the scatter diagram.

Figure 2: Scatterplot showing the association of selected study parameters with Direct bilirubin level.



DISCUSSION:

NASH is one of the most unfavourable outcomes of NAFLD. Approximately 90% of NAFLD are benign and associated with mild steatosis that does not lead to any serious hepatocellular injury or inflammation resulting in cirrhosis and end stage liver disorder[10-13]. Only 10% to 30% of the NAFLD progress to marked inflammatory injury to hepatocytes that lead to a higher grade of inflammation and steatohepatitis followed by cirrhosis and end stage liver disease and cirrhosis[14, 15]. This stage is known as NASH and needs aggressive management for survival as this is closely associated with future developments of hepatocellular carcinoma and cardiovascular disorders. An early and definitive diagnosis is the first and foremost factor leading to successfully managing this disorder. Along with a determining role of USG, the biochemical parameters play crucial roles in the early diagnosis and detection of NASH patients. Patients having the risk factors of metabolic syndrome are particularly vulnerable to progress to NASH and more than 90% of the NASH patients have at least one biochemical feature of metabolic syndrome[16]. It has also been suggested that different ratios of hepatic enzyme parameters are better indicators of the implicated damage of the hepatocytes in NASH rather than a single parameter[9]. Reports on the AST/ALT ratio are already available from several studies[9, 17, 18]. Hence, in the present study, we searched for more such ratios related to liver function that could be used as useful markers for the diagnosis of NASH. For that, we selected the AST/GGT and ALT/GGT as GGT has been stated to be a useful marker for NASH in several studies already[8, 19] and was stated to be 83% sensitive and 69% specific whereas AST and ALT did not show any predictive value for NASH[19]. In our present study, we found significantly higher levels of AST/GGT and ALT/GGT ratio in the case group (Table 1). Although, the AST/ALT ratio showed significantly higher level in the case group, but it had a lower Z (Table 1) value than the other two, signifying that the difference of AST/GGT and ALT/GGT ratios in the case group were greater than those in the control subjects in comparison to the AST/ALT ratio. These case control comparison results (Mann-Whitney test in Table 1) were further strengthened by the findings of correlation analyses as shown in Table 2.

The Spearman's correlation analysis (Table 2) and the scatter plot (Fig 2) showed a significant negative correlation of the AST/GGT and ALT/GGT ratios with the direct bilirubin (correlation coefficient 'r' = -0.405 and -0.422; P = .016 and .012 respectively) indicating their significant inverse
relationship with the degree of steatosis. In contrast to the above two ratios, the AST/ALT ratio did not show any type of association with the direct bilirubin in our study (r = 0.223, P = .198). Thus, the results of Mann-Whitney test and correlation study in the present study asserted the importance of the AST/GGT and ALT/GGT ratios in comprehending the degree of hepatocyte damage and steatosis in NASH.

After ascertaining the role of these ratios in relating the hepatocyte damage to the metabolic derangement in the liver, we proceeded to determine the cut off value for these ratios which could help in an early diagnosis of the NASH cases based on these ratios. For this we used the ROC curve analysis using the values of these ratios in cases and control subjects (Fig 1). The ROC curve analysis showed that the AUC for both ALT/GGT and AST/GGT (0.891 and 0.873 respectively) represented a wide range of data from the cases and control and so could optimally be used for cut off value determination. The cut off values for the ALT/GGT and AST/GGT ratios were found to be 1.31 and 1.21 with sensitivity of 88% and 91% and specificity of 70% and 67% respectively. These data substantiated that both of these cut-off values showed statistical validity and can be used as robust markers for the degree of steatosis in NASH patients. However, as found in several studies that multiple parameters should be used for more definitive diagnosis, we also suggest that both of these ratios should be used simultaneously and when possible with the other ratios like the AST/ALT ratio as a more comprehensive and holistic approach for an early and definitive diagnosis of NASH.

The major limitation of the present study was that it was based on a cross-sectional data analysis. A better approach will be definitely to analyze these ratios using a vertical study design where these ratios can be followed up in the same set of cohorts through a longer period involving the largest spectrum of the disease as possible.

REFERENCES:

1.Fang YL, Chen H, Wang CL, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model". World J Gastroenterol. 2018;24(27):2974-83Available from: https://www.ncbi.nlm.nih.gov/pubmed/30038464.

2.Farrell GC, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. Nat Rev Gastroenterol Hepatol. 2013;10(5):307-18Available from: https://www.ncbi.nlm.nih.gov/pubmed/23458891.

3.Vonghia L, Francque S. Cross talk of the immune system in the adipose tissue and the liver in non-alcoholic steatohepatitis: Pathology and beyond. World J Hepatol. 2015;7(15):1905-12Available from: https://www.ncbi.nlm.nih.gov/pubmed/26244065.

4.Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. J Hepatol. 2018;68(2):280-95Available from: https://www.ncbi.nlm.nih.gov/pubmed/29154964.

5.Magee N, Zou A, Zhang Y. Pathogenesis of Non-alcoholic Steatohepatitis: Interactions between Liver Parenchymal and Nonparenchymal Cells. Biomed Res Int. 2016;2016:5170402Available from: https://www.ncbi.nlm.nih.gov/pubmed/27822476.

6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of non-alcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84Available from: https://www.ncbi.nlm.nih.gov/pubmed/26707365.

7. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology. 2012;142(7):1592-609Available from: https://www.ncbi.nlm.nih.gov/pubmed/22656328.

8.Alam S, Noor EASM, Chowdhury ZR, Alam M, Kabir J. Non-alcoholic steatohepatitis in non-alcoholic fatty liver disease patients of Bangladesh. World J Hepatol. 2013;5(5):281-7Available from: https://www.ncbi.nlm.nih.gov/pubmed/23717739.

9.Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. Frontline Gastroenterol. 2014;5(3):211-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/25018867.

10.Teli MR, James OF, Burt AD, Bennett MK, Day CP. The natural history of non-alcoholic fatty liver: a follow-up study. Hepatology. 1995;22(6):1714-9Available from: https://www.ncbi.nlm.nih.gov/pubmed/7489979.

11.Matteoni CA, Younossi ZM, Gramlich T, et al. Non alcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–19.

12.Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen TI, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut. 2004;53(5):750-5Available from: https://www.ncbi.nlm.nih.gov/pubmed/15082596.

13.Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology. 2006;44(4):865-73Available from: https://www.ncbi.nlm.nih.gov/pubmed/17006923.

14.Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology. 1990;12(5):1106-10Available from: https://www.ncbi.nlm.nih.gov/pubmed/2227807.

15.Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140(1):124-31Available from: https://www.ncbi.nlm.nih.gov/pubmed/20858492.

16.Dixon JB, Bhathal PS, O'Brien PE. Non-alcoholic fatty liver disease: predictors of non-alcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121(1):91-100Available from: https://www.ncbi.nlm.nih.gov/pubmed/11438497.

17.Marchesini G, Moscatiello S, Di Domizio S, Forlani G. Obesity-associated liver disease. J Clin Endocrinol Metab. 2008;93(11 Suppl 1):S74-80Available from: https://www.ncbi.nlm.nih.gov/pubmed/18987273.

18.Sorbi D, Boynton J, Lindor KD. The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating non-alcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol. 1999;94(4):1018-22Available from: https://www.ncbi.nlm.nih.gov/pubmed/10201476.

19.Tahan V, Canbakan B, Balci H, Dane F, Akin H, Can G, et al. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk. Hepatogastroenterology. 2008;55(85):1433-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/18795706.

ACKNOWLEDGEMENT: Nil
CONFLICT OF INTEREST: Nil