Role of Alanine Transaminase and Transient Elastography in Categorising Nonalcoholic Fatty Liver Disease Subgroups

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ABSTRACT

Objective: To investigate the association of alanine transaminase (ALT) with transient elastography grades to define various nonalcoholic fatty liver disease (NAFLD) groups for disease status.

Study Design: Cross-sectional, descriptive study.

Place and Duration of the Study: Gastroenterology Outpatient Clinic, Ziauddin Hospital, from January to December 2022.

Methodology: This study included 194 NAFLD patients. Demographic data, body mass index, enzymes, and transient elastography (TE) findings were recorded. NAFLD patients were categorised as nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH), steatofibrosis (significant fibrosis F2-F3 with normal ALT), and cirrhosis using TE grades.

Results: The median age of the patients was 44 [IQR 18.25] years; 146 (75.3%) were males. Out of 194 NAFLD patients, 21 (10.8%) were NAFL, 116 (59.8%) were NASH, 14 (7.2%) showed steatofibrosis, and 43 (22.2%) were cirrhotic. On transient elastography, the majority were with S3 steatosis (n=107, 55.2%) and 59 (30.2%) had F0-F1 fibrosis. There was a statistically significant difference in the mean rank of age, ALT, AST, and GGT levels within 4 groups of NAFLD ($p < 0.001$). Most of the patients with all the stages of fibrosis had increased ALT levels ($p=0.034$).

Conclusion: This study concluded that a combination of ALT levels and transient elastography findings could be considered for differentiating uncomplicated steatosis from NASH, steatofibrosis, and cirrhosis, hence limiting the use of liver biopsy. This may prove a reliable way to measure the severity of the disease.

Key Words: *Nonalcoholic fatty liver disease, Cirrhosis, Transient elastography.*

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a significantly growing disease worldwide. It represents a spectrum of disorders ranging from simple steatosis and non-alcoholic steatohepatitis (NASH) to advanced liver fibrosis and cirrhosis, with its complications including hepatocellular carcinoma. Diagnosis of NAFLD is supported by the clinical history, various laboratory tests, and imaging techniques. Though liver biopsy is a gold standard for a definitive diagnosis, as it is an invasive procedure associated with complications and low patient compliance, it is not preferred by clinicians as the first line of diagnosis.

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Recently, vibration-controlled transient elastography (VCTE) has emerged as a noninvasive tool to measure hepatic liver stiffness and fat deposition. This technique is easy to perform, has high accuracy, is free from complications, and is easily available in outpatient settings. The European Association for the Study of Liver (EASL), the American Association for the Study of Liver Diseases (AASLD), and the Asian Pacific Association for the Study of Liver (APASL) have recommended the use of transient elastography for liver stiffness measurement in NAFLD fibrosis and liver biopsy is desired only in inconclusive results.[1-3] Liver enzymes have been considered predictive markers of NAFLD.[4] ALT has proved to be a more specific marker than other liver enzymes for hepatic injury. However, several researchers have observed inconsistent results where some demonstrated high ALT levels in NASH whereas others observed normal ALT levels in biopsy-prove NASH and low ALT levels with fibrosis.[5-7]

Currently, there are no serological or noninvasive tests that can distinguish NASH from bland steatosis. There remains a dire need to establish an assessment plan to distinguish simple steatosis, NASH, and significant fibrosis with normal ALT (stea-

tofibrosis) and cirrhosis in the most cost-effective and non-invasive manner.

In this study, the association of liver enzymes with various NAFLD groups was investigated and the combination of ALT levels and transient elastography findings was identified as a marker of disease status.

METHODOLOGY

This cross-sectional study was conducted at the Gastroenterology outpatient clinic at Ziauddin Hospital, Clifton, Karachi. After Institutional Review Board (IRB) approval (Reference code: 44101021ASPAT), non-probability purposive sampling was used to identify NAFLD patients from January to December 2022. The sample size was calculated by using open Epi version 22, online software keeping a 95% confidence level with absolute precision of 6%. The calculated value of 135 was determined by the prevalence of 14.8%.[8] The sample size was augmented to 194.

Informed consent was taken from all the participants. Primary screening by ultrasound was performed on all patients ≥18 years to diagnose the fatty liver. Chronic alcoholics, pregnant females, and patients with secondary causes of hepatic steatosis or fibrosis, such as Wilson's disease, autoimmune liver disease, hepatitis B and C positive, gastric bypass surgery, drug-induced hepatic steatosis and patients with decompensated liver disease were excluded.

Demographic history including age, gender, weight, height, and BMI were recorded. LFTs including ALT, AST, ALP, and GGT were performed. Transient elastography was performed by a Fibrotouch device (HISKY Medical Technologies). The degree of steatosis was measured by the controlled attenuation parameter (CAP) as S0 (normal: <5% liver fat) = up to 240 dB/m; S1 (mild steatosis 5-33%) = 241 to 260 dB/m; S2 (moderate steatosis 34- 66%) = 261 to 290 dB/m; and S3 (severe steatosis >66%) >290 dB/m. Fibrosis was staged as F0-F1 = up to, 7.4 KPa, F2 = 7.5 -10 KPa, F3 = 10.1-14 KPa and F4 = >14 KPa.[9] ALT cut-off level for NASH was taken as 35 IU/L for males and 25 IU/L for females. Based on the transient elastography findings and ALT levels, NAFLD patients were grouped as nonalcoholic fatty liver (NAFL steatosis, fibrosis F0-F1, ALT normal), nonalcoholic steatohepatitis (NASH steatosis; fibrosis F0-F3, ALT increased), steatofibrosis (SF steatosis, fibrosis F2-F3, ALT normal), and cirrhosis (steatosis, fibrosis F4, ALT variable).

The statistical packages of Social Sciences version 23 (SPSS 23) were used to analyse the data. Quantitative data were tested for normality using the Kolmogorov-Smirnov test, assuming normality at $p > 0.05$. The descriptive statistics were performed to calculate the median [IQR] (interquartile range) for all continuous variables and frequency and percentage were recorded for the categorical variables. Kruskal- Wallis H test was used to see the statistical difference between the mean rank of continuous variables within four groups of NAFLD. The post-hoc independent sample test was used to determine the significance of the differences between the mean ranks of all pairs of groups. Chi-square test was used to see the association of categorical variables with various NAFLD groups and to see the association of elevated serum ALT levels with elastography findings. A p-value <0.05 was considered significant.

RESULTS

This study included 194 patients with NAFLD. The median age of the patients was 44 [18.25] years. There were 146 (75.3%) males and 48 (24.7%) females. Out of 194 NAFLD patients, 21 (10.8%) were NAFL, 116 (59.8%) were NASH, 14 (7.2%) showed steatofibrosis and, 43 (22.2%) were cirrhotic. Transient elastography showed that 107 (55.2%) patients were with S3 steatosis and 59 (30.2%) with F0-F1 fibrosis (Table I).

Table I: Baseline characteristics of NAFLD patients.

Characteristics	Total (n = 194) Median [IQR] / Frequency (%)
Age	44 [18.25]
Gender	
Male	146 (75.3)
Female	48 (24.7)
BMI	28.1 [4.9]
NAFL	21 (10.8)
NASH	116 (59.8)
Steatofibrosis	14 (7.2)
Cirrhosis	43 (22.2)
ALT	55 [26]
AST	35 [13]
ALP	64 [23]
GGT	44 [29]
Steatosis	
S1	34 (17.5)
S2	53 (27.3)
S3	107 (55.2)
Fibrosis	
F0-F1	59 (30.4)
F2	45 (23.2)
F3	47 (24.2)
F4	43 (22.2)

Data are shown as numbers (percentage) or median [IQR] (interquartile range). BMI: Body mass index; NAFL: Nonalcoholic fatty liver; NASH: Non-alcoholic steatohepatitis; ALT: Alanine amino-transferase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transpeptidase.

Kruskal Wallis H test showed a statistically significant difference in mean rank of age, ALT, AST, and GGT levels within 4 groups of NAFLD ($p < 0.001$) (Table II). Chi-square showed a significant association between fibrosis and NAFLD groups ($p < 0.001$). A significant association of serum ALT levels with fibrosis ($p=0.034$) was also seen where most of the patients with all the stages of fibrosis had increased ALT levels (Table III).

DISCUSSION

NAFLD has become one of the main reasons for referrals to hepatology services worldwide. Only a few studies are available from the South Asian regions which have correlated liver enzymes with fibroscan findings. To the best of the authors' knowledge, this is the first study where the ALT values and elastography reports of NAFLD patients were combined for grouping into NAFL, NASH, steatofibrosis, and cirrhosis.

Table II: Association of different variables with NAFLD groups.

Variable	NAFL (n=21) Mean Rank/ Frequency (%)	NASH (n=116) Mean Rank/ Frequency (%)	SF (n=14) Mean Rank/ Frequency (%)	Cirrhosis (n=43) Mean Rank/ Frequency (%)	Chi-square	df	p-value
Age	116.02	80.28	128.82	124.70	27.660	3	<0.001*
Gender							
Male	14 (9.6)	91 (62.3)	11 (7.5)	30 (20.5)	2.245	3	0.523**
Female	7 (14.6)	25 (52.1)	3 (27.1)	13 (27.1)			
BMI	102.48	89.55	105.18	114.02	6.479	3	0.90*
ALT	21.62	121.34	29.32	92.43	80.289	3	<0.001*
AST	46.05	106.13	57.18	112.49	30.682	3	<0.001*
ALP	80.26	102.92	94.36	92.33	3.470	3	0.325*
GGT	43.19	101.28	58.89	126.40	38.195	3	<0.001*
Steatosis							0.53**
S1	2 (5.9)	22 (64.7)	3 (8.8)	7 (20.6)	5.111	6	
S2	5 (9.4)	35 (66.0)	5 (9.4)	8 (15.1)			
S3	14 (13.1)	59 (55.1)	6 (5.6)	28 (26.2)			
Fibrosis							
F0-F1	21 (35.6)	38 (64.4)	0 (0.0)	0 (0.0)	253.2	9	
F2	0 (0.0)	36 (80.0)	9 (20.0)	0 (0.0)			<0.001**
F3	0 (0.0)	42 (89.4)	5 (10.6)	0(0.0)			
F4				43 (100)			

*Data are shown as numbers (percentage) or mean rank. *Kruskal-Wallis H test. **Chi-square. df: Degree of freedom; Level of significance at <0.05. BMI: Body mass index; NAFL: Nonalcoholic fatty liver; NASH: Nonalcoholic steatohepatitis; ALT: Alanine amino-transferase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transpeptidase.*

Table III: Liver fibrosis and steatosis of NAFLD patients stratified by serum ALT levels.

Variables	Serum ALT		Total	p-value
	Normal (n = 45)	Increased (n = 149)		
Steatosis				
S1	10 (29.4)	24 (70.6)	34	0.622*
S2	11 (20.8)	42 (79.2)	53	
S3	24 (22.4)	83 (77.6)	107	
Fibrosis				
F0-F1	20 (33.9)	39 (66.1)	59	0.034*
F2	7 (15.6)	38 (84.4)	45	
F3	6 (12.8)	41 (87.2)	47	
F4	12 (27.9)	31 (72.1)	43	

*Data are shown as numbers (percentage). *Chi-square; Significant level <0.05. ALT: Alanine amino-transferase.*

In this study, NAFLD was more predominant in males than females (75.3 *vs*. 24.7%) which is in line with the other studies.[8,10-13] A recent meta-analysis also observed a higher prevalence of NAFLD in males as compared to females (39.7 *vs*. 25.6%).[14] The possible explanation might be the role of female hormones, especially in premenopausal women on hormone replacement therapy that has been known to provide a protective effect against the disease.[15] In this series, the majority of the patients with NAFLD had NASH (59.8%) which corresponded to other studies in the Western and Asian countries where the diagnosis was confirmed by histology.[10,16-18] These studies showed that the use of this blend of elastography and ALT was in agreement with the histological diagnosis.[10,16-18] This study was also in agreement with other studies where the majority of patients with fatty liver (NAFL) had F0-F1 fibrosis.[19-21] However, a subset of patients with fatty liver was defined who had significant fibrosis and normal ALT (steatofibrosis). Moreover, a significant association of liver stiffness within NAFLD groups was also observed.

This study is in line with the studies done by Thong *et al.* and Bandana *et al.* who found a significant association between raised ALT levels and liver stiffness. They noted significantly increased ALT in advanced fibrosis whereas this study noted that the majority of the stages of fibrosis had increased ALT levels.[13,20] As ALT is directly involved in pyruvate metabolism, hence inflammation and injury to liver cells release the enzyme in the circulation leading to raised serum ALT which can be considered a determinant of NAFLD.

This study observed significant differences in the mean rank of liver enzymes with various groups of NAFLD which revealed that liver enzymes could be used as reliable diagnostic markers of the disease groups when combined with the elastography reports. Nevertheless, it was attempted to provide a simple, easy, noninvasive way to stratify NAFLD patients into four groups. This might help in identifying the severity of the disease, facilitating an early treatment plan, preventing complications, and reducing disease mortality. Further studies will be required to assess other non-invasive

tests as predictors of NAFLD. The limitation of the study was that these results could not be compared with a liver biopsy as a gold standard. However, this sample is representative, with evidence of similarities with results published by other researchers.

CONCLUSION

A combination of ALT levels and transient elastography findings could be considered for differentiating bland steatosis from NASH, steatofibrosis, and cirrhosis, hence limiting the use of liver biopsy. This could prove a reliable way to measure the severity of the disease in the future.

DISCLOSURE:
This study was part of a PhD thesis titled Role of Alanine Transaminase and Transient Elastography in Categorising Nonalcoholic Fatty Liver Disease Subgroups.

ETHICAL APPROVAL:
The study was approved by the Institutional Research Board of Ziauddin University (Reference code: 44101021ASPAT).

PATIENTS' CONSENT:
Informed, written consent was taken prior to the research.

COMPETING INTEREST:
The authors did not declare any conflict of interest.

AUTHORS' CONTRIBUTION:
AS, ZA, TM: Designed the study.
ZA: Performed examination of the patients.
AS: Collected the data, did literature search, analysis and manuscript writing.
ZA, TM, AK: Did literature search, supervised and directed the study.
All authors read and approved the study for publication.

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