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Correlation Between Serum Alanine Aminotransferase (ALT) Levels and Ultrasonographic Grading of Hepatic Steatosis

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ABSTRACT

Non-alcoholic Fatty Liver Disease (NAFLD), commonly known as "Fatty Liver," is characterized by the absence of lobular inflammation and macro vesicular changes caused by inflammation (steatosis). It encompasses two subgroups: Steatosis and Non-Alcoholic Steatohepatitis, both falling under the umbrella of NAFLD. Ultrasonography is commonly used to assess the grade and severity of the disease, while Alanine Transaminase (ALT) levels in the blood serve as biomarkers for its diagnosis. The study aimed to explore the correlation between ALT levels and the grades of fatty liver as detected through ultrasound. A cross-sectional study was conducted at the Diagnostic Centre of Combined Military Hospital, Lahore, from April 2023 to June 2023. A total of 100 participants undergoing abdominal ultrasound and suspected of having fatty liver were selected using a convenience sampling technique. ALT levels were measured for all participants. Among the 100 participants. 21% did not exhibit fatty liver, with 71.4% having normal ALT levels, 19% having ALT levels between 41–60 U/L, and 9.5% with ALT levels greater than 80 U/L. Grade 1 fatty liver was identified in 32 patients, with 34% having normal ALT, 62.5% between 41–60 U/L, and 3.1% over 80 U/L. Grade 2 fatty liver was seen in 26% of patients, among which 26.9% had ALT levels between 41–60 U/L, and 73.1% between 60–80 U/L. Grade 3 fatty liver was observed in 21% of patients, with 9.5% having ALT levels between 60–80 U/L, and 90.5% with ALT greater than 80 U/L. conclusion: This study demonstrates a direct relationship between ALT levels and the severity of fatty liver disease, suggesting that ALT may serve as a potential biomarker for assessing disease progression.

Keywords: Alanine transaminase, Non-alcoholic Fatty Liver Disease, Steatosis, Fatty Liver, Ultrasound

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver conditions worldwide, affecting approximately 25% of the global population, with rising incidence due to increasing rates of obesity and metabolic syndrome (Younossi *et al*., 2016: Brunt *et al.*, 1999). While the exact cause of NAFLD is unknown, factors such as insulin resistance, inflammatory cytokines, oxidative stress, and steatosis may contribute to its development (Wilkins *et al.*, 2013). Alanine aminotransferase (ALT), a key biomarker of liver health, leaks into the bloodstream after hepatocellular injury and has a plasma half-life of approximately 42 hours. While ALT elevations often indicate liver damage, it is not liver-specific, as skeletal or cardiac muscle injury can also increase circulating ALT (Thakur *et al.*, 2024). Previously called serum glutamate pyruvate transaminase, ALT is involved in the alanine cycle, converting alanine and α-ketoglutarate into pyruvate and glutamate. Primarily found in the liver, ALT is also present in the kidney, skeletal muscle, and myocardium. Elevated ALT levels can result from conditions like hepatitis, biliary duct injury, heart failure, or myopathy (Washington & Van Hoosier, 2012). Additionally, even normal-range liver markers may be linked to age-related impairments in older heart failure patients (Fisher *et al.*, 2015). Liver ultrasonography (US) can help predict metabolic abnormalities in NAFLD, including liver histology, portal hypertension, cardiovascular risk, and hepatocellular carcinoma (Ballestri *et al*., 2015). It detects hepatic steatosis, which is associated with atherosclerosis, and can identify fatty changes as small as 20%. While not diagnostic, US shows signs like hyperechoic hepatic echoes, hepatorenal hyper echogenicity, blurred veins, and thick subcutaneous tissue (Khov *et al.*, 2014). US reliably detects moderate to severe fatty liver and is preferred for screening due to its affordability, safety, and accessibility (Hernaez *et al.*, 2011). NAFLD is associated with increased hepatic inflammation, fibrosis, type 2 diabetes, and elevated mortality rates (Targher *et al.*, 2021). It is an independent predictor of cardiovascular disease (CVD) and correlates with other conditions, including chronic kidney disease, metabolic syndrome, and obesity (Bang & Cho, 2015; Lu *et al.*, 2013). NAFLD can be divided into two types: nonalcoholic fatty liver (NAFLD) and nonalcoholic steatohepatitis (NASH). NASH may progress to fibrosis, cirrhosis, and hepatocellular carcinoma. The prevalence of NAFLD is estimated to be 34% and is increasing due to metabolic syndrome, obesity, diabetes, insulin

resistance, and hypertension. Early diagnosis is challenging as patients often remain asymptomatic in the initial stages. Screening measures include monitoring fasting liver transaminases, glucose, and lipid profiles in children over 10 who are overweight. However, ALT and AST levels often appear normal in adults and children with NAFLD, making them unreliable diagnostic markers. While liver biopsy is useful in suspected NASH cases, it carries risks. Liver ultrasonography (USG) is a safer, cheaper, and non-invasive first-line tool for clinical and epidemiological settings, with lipid metabolism derangements and non-esterified fatty acids correlating with fatty liver in overweight individuals (Khov *et al.,* 2014). The Ultrasonographic Fatty Liver Indicator (US-FLI) strongly correlates with metabolic abnormalities and histological features of nonalcoholic steatohepatitis (NASH), making it a useful tool to identify patients who may benefit from a liver biopsy (Ballestri *et al.*, 2012). Research has identified predictive sonographic indicators for NASH, such as reduced echo amplitude, splenomegaly, and focal fat sparing, which distinguish NASH from simple steatosis. Controlled attenuation parameter (CAP) devices can quantify steatosis grades but are less accessible compared to USG, which is limited in sensitivity for steatosis below 20% (Shannon *et al.*, 2011) (Shannon *et al*., 2011). NAFLD in children can lead to cirrhosis and metabolic complications, potentially predicting type 2 diabetes. Severe fatty liver grades on ultrasound are linked to elevated ALT, while mild grades show no significant changes (Buffie & Pamer, 2013). ALT alone is unreliable for assessing fibrosis, with a threshold of 81 U/L offering moderate sensitivity and specificity (Thong & Quynh, 2021). Lowering the ALT threshold to 23 IU/L improved NAFLD diagnosis (Martin-Rodriguez *et al.*, 2017). Some NAFLD and NASH patients have normal ALT levels, highlighting the need for better markers (X. Ma *et al.*, 2020). ALT is useful for liver injury detection, but NAFLD remains the main cause of elevated ALT in U.S. adults (Kim *et al.*, 2008). Elevated ALT in obese women showed poor diagnostic utility for NASH or IPF, raising healthcare cost concerns (Kunde *et al.*, 2005).

NAFLD is characterized by excess liver fat unrelated to alcohol consumption and ranges from simple steatosis to NASH and advanced fibrosis, with a global prevalence of 20–30% (Younossi *et al.*, 2016). Ultrasonography is a cost-effective, non-invasive tool for detecting and grading fatty liver (grades 0–3), based on echogenicity, with increased brightness indicating higher fat content (Chalasani *et al.*, 2012). ALT, primarily found in the liver, is widely used to assess liver function, with several studies showing its correlation with fatty liver grades on ultrasonography (Abangah *et al.*, 2014; Hernaez *et al.*, 2011)

MATERIALS AND METHODS

Study Design and Setting: This is a cross-sectional study that was conducted at the Combined Military Hospital, Lahore

Inclusion criteria: The age range for this study was 18 years and above, including both males and females. Patients undergoing abdominal ultrasonography and serum ALT testing for routine or diagnostic purposes were included.

Exclusion criteria: Pregnant females were excluded from this study.

Duration: 4 months

Sample Size: 100 patients

Sampling Technique: Convenience sampling technique

Sample Selection Criteria

Inclusion Criteria: All female and male Patients aged between 18-60 years. Most commonly the 35–50-year-old group in men and the over 65 years old group in women

Symptomatic patient: The patient has cirrhosis, hepatitis, and surgery with liver

Exclusion Criteria: All pregnant females

Ethical Considerations

The research adhered to the guidelines established by the ethical committee of the University of Lahore, ensuring the protection of participants' rights.

• Written informed consent was obtained from all participants.

• Strict confidentiality measures were implemented for all collected information and data.

• Participants' identities remained anonymous throughout the study.

• Subjects were assured that there were no drawbacks or risks associated with the study procedures.

• Participants were informed of their freedom to withdraw from the study at any point.

• Any known risks associated with the research were disclosed to the participants.

Data Collection Procedure

All participants were surveyed for variables such as age, and all the mentioned variables for each patient were documented and preserved in their respective Dual Case Record forms (CRFs).

For each participant, the following data were collected:

* Via biochemical assay, Serum ALT levels were measured.

* Grading of fatty liver, categorized as Grade I, through ultrasonography

* Il, or Ill, was categorized by a trained radiologist using given criteria.

* Body Mass Index (BMI) calculated by weight/height(m^2)

As per WHO criteria, patients were categorized into underweight, normal, overweight, and obese

* Patients' cholesterol levels were assessed through their lipid profiles

* Diabetic profile was assessed through the patient's medical records and history
* Alcohol intake profile, collected through patient history

Statistical Analysis

The correlation between serum ALT levels and grades of fatty liver was assessed using Spearman's correlation. Multivariable regression analysis was performed to adjust for potential confounders. A p-value <0.05 was considered statistically significant

This study was carried out at CMH Lahore Medical College following approval from an ethical review committee. Patients who met the inclusion/exclusion criteria were enrolled after obtaining written consent, and data were collected using data collection sheets.

Patients presenting symptoms such as abdominal pain, nausea, loss of appetite, or weight loss were examined. Liver scans were typically performed using a sector or curvilinear transducer with a frequency of 2- 5 MHz. Patients were positioned supine and instructed to take deep breaths. Scanning was conducted in both longitudinal and transverse planes, exploring intercostal, subcostal, and subxiphoid views. To avoid gas and shadowing, the probe was angled beneath the ribs, while attention was paid to observing a homogeneous, smooth, coarse echotexture. Fatty liver was identified by posterior attenuation, an enlarged liver, and a homogeneous echotexture, with echogenicity either equal to or minimally exceeding that of the renal cortex or spleen. Data collection was performed according to the variables outlined in a questionnaire or proforma.

Data Analysis

Data was collected during the allocated period. Statistical package SPSS version 26 was used for the evaluation of the data and compilation of results. Then the results were presented in the form of graphs, tables, and charts. Qualitative data was displayed in the form of frequencies and percentages, such as male and female. Quantitative data was displayed in the form of mean and standard deviation; p-value less than 0.05 was considered significant.

RESULTS

The study comprised 100 participants. Among them, 45% were male and 55% were female. The majority reported symptoms like abdominal pain (76%) and fatigue (78%). Most patients were non-alcoholic (94%) and non-diabetic (66%).(Table:1). In terms of liver conditions, 20% had cirrhosis, and 21% had a history of hepatitis. (Table:1). The BMI distribution showed 37% overweight, 41% normal weight, 14% obese, and 8% underweight individuals. (Table:3). Ultrasound results showed that 55% of participants had a smooth liver echotexture, 28% irregular, and 17% coarse. (Table:4). ALT levels varied: 26% had levels between 0-41 U/L, 31% between 41-60 U/L, 22% between 60-80 U/L, and 21% had levels greater than 80 U/L. (Figure.1 Table:5)

Cholesterol profile shows that out 100 patients 77% patients have normal cholesterol levels while 23% patients have increased cholesterol levels. (Table:6). A correlation between ultrasound fatty liver grading and ALT levels showed that as the grade increased, ALT levels rose significantly. Grade 3 patients (21%) had the highest ALT levels, with 90.5% having ALT > 80 U/L. A strong positive correlation (Spearman r = 0.814, p < 0.001) was observed between ALT levels and fatty liver severity. (Figure.2 Table:7)

Table. 1. Univariate analysis

Variables	**Response**	**n/ %**
Gender	Male	45
	Female	55
Abdominal pain	Yes	76
	No	24
Alcholic	Yes	6
	No	94
Diabetic	Yes	34
	No	66
History of hepatitis	Yes	21
	No	79
Fatigue	Yes	78
	No	22
Blood pressure	Normal	72
	High	28
Cirrhosis	Present	20
	Absent	80
Total		

Table. 2. show the demographic data of patients. 24% of patients fall in the first age group, 56% in the second and 20% in the 3rd.

(years)		
	Frequency	Percent
18-30	24	24.0
30-60	56	56.0
above 60	20	20.0
Total	100	100.0

Table. 3. shows that 37% of the included patients were overweight, 8% underweight, 41% had normal weight, 14% were obese.

BMI (kg/m^2)		
	Frequency	Percent
overweight	37	37.0
underweight	8	8.0
normal	41	41.0
obese	14	14.0
Total	100	100.0

Table. 4. shows that 55% of the patients had smooth liver echotexture, 28% had irregular,17% had coarse echotexture.

liver echotexture		
	Frequency	Percent
Smooth	55	55.0
irregular	28	28.0
coarse	17	17.0
Total	100	100.0

Table. 5. shows 26% had normal ALT levels, 31% had ALT levels between 41-60U\L, 22%(60-80U\L), 21%(>80 U\L)

ALT levels		
	Frequency	Percent
0-41 U/L	26	26.0
41-60 U/L	31	31.0
60-80 U/L	22	22.0
>80 U/L	21	21.0
Total	100	100.0

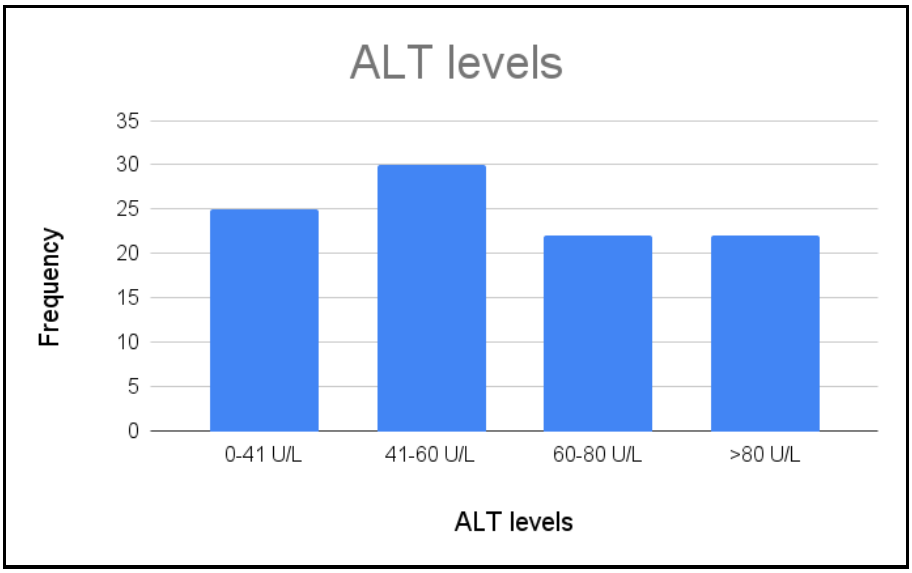

Figure. 1. shows the ALT levels in the patients.

Table. 6. shows 77% patients had normal cholesterol level and 23% had increased level of cholesterol.

cholesterol level		
	Frequency	Percent
normal	77	77.0
increased	23	23.0
Total	100	100.0

Table. 7. shows that out of total 100 patients 21 were normal out of which 71.4% had normal ALT levels 19% had ALT levels between 41-60 UL and only 9.5% patients had ALT levels greater than 80UL. 32 patients were identified as having grade 1 fatty liver. With 34 percent falling in grade 1, 62.5 %, 3.1%, 0.0% falling in normal, moderate and high ALT levels respectively. 26 patients fell in the fatty liver grade 2 category 26.9% had ALT levels between 41-60U/L,73.1 % had ALTs falling in 60-80U/L. 21 patients only fell in the grade 3 level of fatty liver with 9.5% having ALT levels between 60-80U/L and 90.5% had ALT levels greater than 80U/L

ALT levels * USG findings Cross tabulation									
		ALT levels				Total	Chi-Square	p-value	Spearman correlation
		0-41 U/L	41-60 U/L	60-80 U/L	>80 U/L				
normal	% within USG findings	(15)71.4%	(4)19%	(0)0%	(2)9.5%	100.00%	151.498	<0.001	0.814
Grade1	% within USG findings	(11)34.4%	(20)62.5%	(1)3.1%	(0)0.00%	(32)100%			
Grade2	% within USG findings	(0)0.0%	(7)26.9%	(19)73.1%	(0)0.00%	(26)100%			
Grade3	% within USG findings	(0)0.00	0.00%	(2)9.5%	(19)90.5%	(21)100%			
Total	% within USG findings	(26)26%	(31)31%	(22)22%	(21)21%	(100)100%			

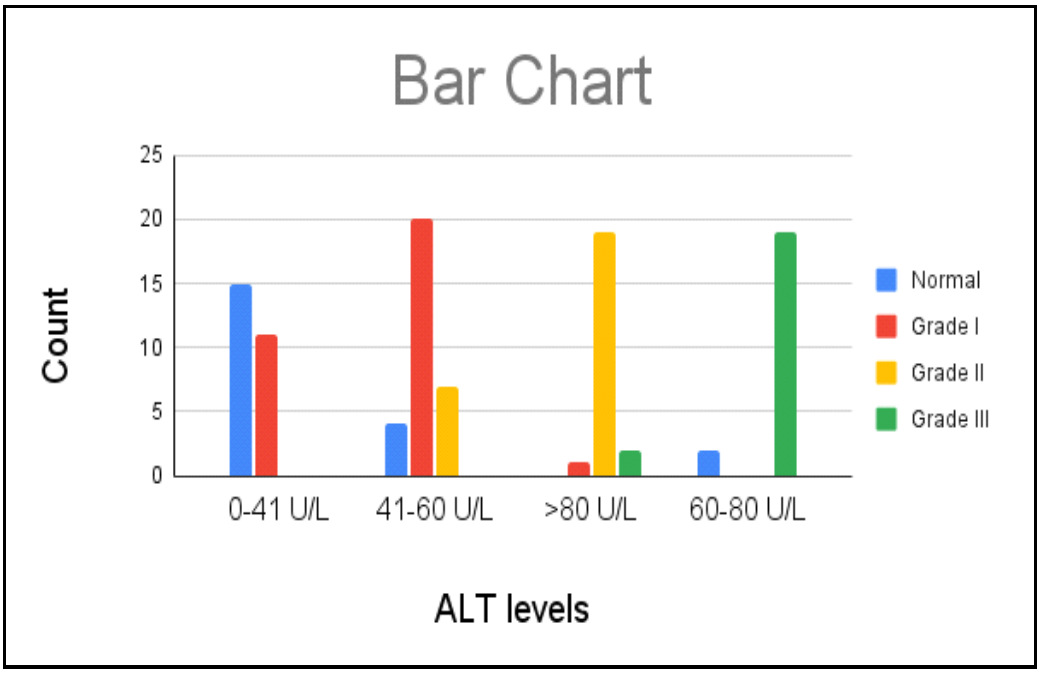

Figure. 2. shows patients ALT levels and the grade of fatty liver they fell in. The graph above shows the correlation of serum ALT levels with grades of fatty liver disease.

DISCUSSION

Hepatic steatosis stands as the most prevalent liver ailment globally. Recent research highlights non-alcoholic fatty liver disease (NAFLD) as a primary contributor to metabolic syndrome, with potential progression to cirrhosis or liver failure in cases of non-alcoholic steatohepatitis (NASH). However, diagnosing progressive NASH leading to cirrhosis or hepatocellular carcinoma poses challenges.

Existing imaging modalities and laboratory tests often fall short in diagnosing NASH definitively, with liver biopsy remaining the sole reliable option. Nevertheless, due to cost-effectiveness, risks, sampling variability, and consent criteria, liver biopsy may not

be feasible in all cases. Recent findings indicate a significant statistical relationship between ultrasonography (USG) and steatosis grades, as well as fibrosis stages and USG. According to a study, non-invasive tools like liver ultrasonography (US) can predict metabolic abnormalities, including liver histology, portal hypertension, cardiovascular disease risk, and early hepatocellular carcinoma detection and treatment in NAFLD patients. Ultrasonography is a valuable imaging modality in the assessment of hepatic steatosis, a condition that is frequently associated with atherosclerosis in the carotid and coronary arteries. It can identify fatty liver changes with an accuracy that allows detection of steatosis as subtle as 20% of liver fat content (Ballestri *et al.*, 2015). While histology remains the gold standard for diagnosing non-alcoholic fatty liver disease (NAFLD), imaging techniques, particularly ultrasonography, offer practical advantages in clinical settings. Although imaging is not the primary tool for screening or diagnosing NAFLD, bedside ultrasound serves as a non-invasive and readily accessible option that can reveal key sonographic features. These features include hyperechoic hepatic echoes, which indicate increased liver fat content, and hyperechoic hepatorenal structures, which further suggest the presence of hepatic steatosis. Additionally, blurring of the portal or hepatic veins and thickened subcutaneous tissue are also observed, indicating underlying liver changes. Sonographic signs such as image attenuation, diffuse echogenicity, heterogeneous liver parenchyma, and hepatomegaly can further enhance the diagnostic value of ultrasound in detecting liver abnormalities (Khov *et al.*, 2014) From a clinical standpoint, ultrasonography is particularly effective in identifying moderate to severe forms of fatty liver, providing a reliable screening method. It holds distinct advantages over more invasive procedures, offering a non-invasive, cost-effective, and safe alternative that can be performed widely in clinical practice. The affordability and accessibility of ultrasound, combined with its ability to detect significant fatty liver changes, make it an ideal first-line tool in the screening of NAFLD. Its role in the early detection of hepatic steatosis and its association with atherosclerotic cardiovascular diseases underscores its importance in broader metabolic risk assessments (Hernaez *et al.*, 2011).

However, the correlation between aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and liver disease severity remains unclear. ALT levels alone do not reliably identify fibrosis stages or steatosis grades. Elevated AST and ALT levels are observed in patients with mild to severe fatty liver disease. This study aims to explore the correlation between fatty liver grades and blood ALT levels, seeking to determine its viability as a diagnostic biomarker. This cross-sectional study included reports from 100 patients undergoing abdominal ultrasound for suspected fatty liver disease, with tests conducted to measure blood alanine transaminase (ALT) levels. In most cases, ALT levels increased with the progression of fatty liver grade, and patients with ALT levels exceeding 100 U/L often exhibited liver scarring or cirrhosis. Rising ALT levels may serve as biomarkers for assessing fatty liver grade and indicating severe liver damage. According to our research, analysis of gender susceptibility indicates that women are more likely to develop NASH with elevated AST or ALT levels, though it does not definitively indicate NASH. Conversely, males are more predisposed to definite NASH. In 2022, Ayesha Kamran *et al*. studied 138 asymptomatic individuals with fatty liver, graded as mild, moderate, or severe via USG. Severe grades correlated with elevated serum ALT, while mild grades did not show significant changes, highlighting the need for grading to identify NAFLD/NASH cases (Thong & Quynh, 2021) cautioned against relying solely on ALT levels for severity assessment, as ALT levels often misclassify patients with advanced fibrosis or cirrhosis. A threshold of 81 U/L was associated with a sensitivity of 53% and specificity of 67% for advanced fibrosis (Thong & Quynh, 2021). Similarly, (Martin-Rodriguez *et al.*, 2017) showed that lowering the ALT threshold to 23 IU/L improved diagnostic accuracy, successfully identifying over 90% of NAFLD cases .(Y. Ma *et al.*, 2020) found that 25% of NAFLD and 19% of NASH patients exhibited normal ALT levels, emphasizing the need for better diagnostic markers beyond ALT (X. Ma *et al.*, 2020). W. Ray Kim *et al*. demonstrated that serum ALT levels rise with hepatocellular injury, making ALT a valuable marker for detecting liver disease. NAFLD is the most common cause of abnormal ALT levels in U.S. adults, potentially affecting up to 3% of the population, with elevated ALT levels correlating to NAFLD severity (Kim *et al.*, 2008). Sachin S. Kunde studied the impact of a new ALT range in 233 obese women, finding increased prevalence of fatty liver and idiopathic pulmonary fibrosis (IPF) with elevated ALT levels. However, ALT showed poor diagnostic utility for NASH or IPF, raising concerns about healthcare costs if the new range is adopted (Kunde *et al.*, 2005). A systematic review and meta-analysis by (Ahn & Kang, 2018) showed a positive correlation between serum ALT levels and fatty liver severity assessed via ultrasonography. Similarly, (Abangah *et al.*, 2014) found a significant correlation between ALT levels and fatty liver grade in a large Chinese cohort with NAFLD. However, (Fracanzani *et al.*, 2008) reported a weak correlation between ALT levels and hepatic steatosis in Italian patients with NAFLD (Bedossa & Poynard, 1996). ALT's utility as a sole marker for fatty liver assessment has limitations, as its levels can be influenced by factors like age, gender, BMI, and coexisting liver diseases. Additionally, ALT may not always reflect liver inflammation or fibrosis, critical aspects of NAFLD progression (Hamaguchi *et al.*, 2007). To improve fatty liver disease assessment, researchers have explored combining ALT with other non-invasive markers, such as imaging techniques, serum

biomarkers, and clinical risk scores, to predict advanced liver fibrosis and identify individuals at higher risk of progression (Goudarzi *et al.*, 2024; Zheng *et al.*, 2023) found a significant correlation between ultrasonographic findings and histological outcomes in 109 NAFLD patients, with a notable association between liver enzyme levels (ALT, AST, and ALP) and ultrasonographic results (Zheng *et al.*, 2023). studied the relationship between metabolic syndrome and ALT levels in NAFLD, using ultrasound imaging for fatty liver diagnosis and Adult Treatment Panel criteria for metabolic syndrome (Fracanzani *et al.*, 2008) Screening patients for unsuspected liver disease using ALT and AST has been questioned, as the set thresholds lack sensitivity and specificity for diagnosing NASH in NAFLD patients. Imaging studies such as USG, CT, and MRI have limitations in differentiating NASH from isolated fatty liver, particularly below 33% steatosis. Liver biopsy remains essential for diagnosing suspected liver diseases, including NAFLD, despite challenges in interpretation, especially for NASH and steatohepatitis. Past studies often omitted variables like BMI, diabetes history, and cholesterol levels, which are crucial factors assessed in this research. Correlation coefficients helped guide clinicians in the evaluation of potential biomarkers or risk factors in disease progression. According to the data, as the severity of fatty liver increases, ALT levels also tend to rise along with it. But this is nit considered a strong correlation, which implies that ALT alone is not a definitive indicator of grades in fatty liver. Patients with fatty liver, particularly in the early stages, may have normal ALT levels, and elevated ALT can also result from non-hepatic diseases. The above findings support the use of ALT in association with metabolic parameters rather than in isolation. A multifactorial approach considering BMI, lipid profile, and diabetic status may enhance the clinical assessment of NAFLD. Correlation coefficients help identify the strength and direction of associations between variables, which can guide clinicians in evaluating potential biomarkers or risk factors in disease progression

CONCLUSION

The levels of ALT in the bloodstream correlate directly with the severity of fatty liver. As the fatty liver becomes more severe, ALT levels typically rise, making them useful biomarkers for assessing the corresponding grade.

RECOMMENDATION

It is important to increase the patient population to have better results. For this study, a sample size of 100 was utilized, which is relatively small given the prevalence of fatty liver disease. In future research, employing probability sampling techniques would be advisable to mitigate the risk of obtaining false results.

Limitation

This study has several limitations. Firstly, the use of a convenience sampling technique may introduce selection bias and limit the generalizability of the findings to a broader population. Secondly, while adequate for preliminary insights, the sample size of 100 patients may not be large enough to detect more subtle associations or variations among subgroups. Additionally, the cross-sectional nature of the study prevents any assessment of causality or temporal relationships between variables. The reliance on patient self-report for some clinical history elements, such as alcohol consumption or previous hepatitis, may also be subject to recall bias. Moreover, the study was conducted at a single center, which may not reflect patients' demographic and clinical characteristics in other regions or healthcare settings. Finally, liver ultrasound findings were subject to operator dependency, which could affect the consistency and accuracy of the results.

AUTHORS' CONTRIBUTION

R.R. and U.B.Z. were responsible for the conception, study design, data collection, analysis, and drafting of the manuscript. M.N. and H.H. contributed to data acquisition, preliminary data interpretation, and critical revisions of the manuscript. M.A., M.AS., and F.A. assisted with the literature review and manuscript formatting. K.S., A.F., and N.H. were involved in data entry, reference organization, and proofreading.

CONFLICTS OF INTEREST

There was no conflict of interest

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