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Relationship between Liver Function Tests Levels with Degree of FibroScan Test in non-alcoholic Fatty Liver Disease

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ABSTRACT

Background and Objectives: Non-Alcoholic Fatty Liver Disease (NAFLD) occurs when liver fat content exceeds 5-10%. The initial stage is simple fatty liver, which can progress to alcoholic steatohepatitis and ultimately lead to cirrhosis of the liver. The first step in treatment is a weight loss diet.

Methods: In this study, patients with non-alcoholic fatty liver disease who had undergone all necessary tests to rule out other causes of liver involvement, such as viral and autoimmune hepatitis and Wilson's disease, were evaluated. These patients were approved by a gastroenterologist and underwent a FibroScan over a six-month period to assess their condition. The initial checklist included demographic information (height and weight), blood pressure, history of alcohol consumption, and liver enzyme levels.

Results: Among 86 participants, 25 (29.1%) had Grade 0 fatty liver, 39 (54.7%) had Grade 1, 14 (11.6%) had Grade 2, and 8 (4.7%) had Grade 3 fatty liver. Additionally, 8 patients had anemia, 3 (2.5%) had elevated bilirubin levels, 3 (2.5%) had iron deficiency, and only 1 patient had liver issues related to an autoimmune problem or specific disease. There was no significant relationship between the FibroScan score and enzyme levels in any gender.

Conclusion: The prevalence of non-alcoholic fatty liver disease is higher in women than in men, and liver enzymes do not accurately reflect the degree of liver fibrosis. It is recommended that imaging methods, especially FibroScan, be used instead of routine enzyme level measurements to assess liver tissue conditions.

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Abbreviations

NAFLD, Non-alcoholic Fatty Liver Disease; NASH, Non-alcoholic steatohepatitis; AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; TG, Triglyceride; Chol, Cholesterol; HDL, High Density Lipoprotein; FLI, Fatty Liver Index; BMI, Body Mass Index; HCC, Hepatocellular Carcinoma; GGT, Gamma-glutamyl transpeptidase; TE, Transient Elastography; LFT, Liver Function Tests; APRI, AST to Platelet ratio index

Introduction

Liver Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease that ranges from simple steatosis and non-alcoholic steatohepatitis (NASH) to cirrhosis and hepatocellular carcinoma (HCC) (1, 2). One of the most significant reasons for liver grafting in the United States is the prevalence of HCC, which increased by 11.5 times from 2002 to 2016 (3). The global deaths attributed to NAFLD have increased by 80.2%, amounting to 0.17 million deaths in the past 30 years, as of 2019 (4). In most cases, non-alcoholic fatty liver disease is a benign, non-progressive disease in the form of simple steatosis, which is usually asymptomatic and diagnosed by chance during imaging studies or while investigating the cause of increased liver enzymes (5). If untreated, this condition can eventually lead to liver cancer and death. This disease is characterized by increased levels of triglycerides, certain liver enzymes, inflammatory biomarkers, and liver steatosis levels (6). There is a strong relationship between liver function tests, lipid profiles, and FibroScan results and the diagnosis of NAFLD (7). Non-alcoholic fatty liver usually appears after metabolic syndrome disorders such as obesity, insulin resistance, hypertension, dyslipidemia, and impaired fat metabolism, increasing the risk of death due to cardiovascular disease (8). Obesity and insulin resistance increase lipolysis in fatty tissue, leading to a flow of free fatty acids to the liver, which contributes to increased inflammation in the liver (9). NASH is a more aggressive form of non-alcoholic fatty liver characterized by steatosis, liver damage, and lobular inflammation, with or without a fibrosis pattern. The prevalence of non-alcoholic fatty liver has been reported differently depending on the diagnostic method used in various regions of the world (10). About 30 to 40 percent of patients with cirrhosis either receive a liver transplant or die from complications related to liver disease (11). Therefore, it is crucial to distinguish patients with NASH from those with simple steatosis to guide treatment and assess potential risks. Liver biopsy is the reference standard procedure; however, it is invasive and may have complications. Additionally, a liver biopsy only samples a small portion of the liver, which may lead to sampling error. Due to the high prevalence of non-alcoholic fatty liver in the population and the low percentage of individuals with NASH, liver biopsy may not be suitable for many patients.

Therefore, reliable non-invasive tests are needed to detect or rule out advanced fibrosis to reduce the need for liver biopsy (12). The fatty liver index (FLI) is a simple and accurate predictor of liver steatosis in the general population. FLI is an algorithm based on body mass index (BMI), waist circumference, triglycerides, and GGT for diagnosing liver steatosis. The algorithm was designed to investigate the development of the fatty liver index, which can vary between zero and 100. An FLI of less than 30 rules out fatty liver, while an FLI of 60 or greater confirms fatty liver (13). Chronic liver disease is one of the main causes of mortality and morbidity in European countries. In addition to viral infections, NAFLD is known as one of the most important causes of chronic liver disease in the Western world. A significant proportion of NAFLD patients progress to cirrhosis and HCC, particularly if it is associated with other factors such as alcohol abuse or hepatitis infection. Given that many patients with NAFLD have normal liver enzymes and even normal ultrasound results, it is very important to identify these patients who are at risk of cirrhosis (14). NAFLD is usually asymptomatic, so diagnosis typically begins with abnormal findings in liver enzymes or the detection of steatosis on imaging. If abnormal liver function tests (LFTs) are present, they typically manifest as a brief increase in ALT or AST levels. However, about 80% of patients have normal ALT levels, and even when they are elevated, as fibrosis progresses to cirrhosis, ALT levels may decrease while AST levels may increase (15).

ALT values are not associated with histological findings and do not help in diagnosing NAFLD or determining disease severity. Therefore, they are considered inadequate parameters for both doctors and patients, and the testing process can be time-consuming. As a result, researchers are seeking non-invasive and accurate methods to evaluate liver fibrosis. Among these methods, transient elastography (TE) is one of the most promising options (16). Transient elastography can reliably assess the extent of liver fat accumulation and the mechanical properties of the liver related to tissue stiffness and fibrosis (17).

The advantages of TE include quick availability of results, a painless procedure, a completion time of approximately three minutes per patient, and ease of administration. Given the strong correlation between liver enzyme levels and the severity of NAFLD, identified through FibroScan, it may be possible to avoid unnecessary and costly fibroscans. In this study, we aim to compare the results of FibroScan with other non-invasive methods, such as measuring serum levels of liver enzymes, in an effort to find a more accurate and faster approach for diagnosing non-alcoholic fatty liver disease (18).

Materials and Methods

In this study, 86 patients with NAFLD who underwent FibroScan were included. For all patients, the initial checklist included demographic information, height, weight, blood pressure, history of alcohol consumption, and liver enzyme levels. Additionally, blood sugar tests, lipid profiles, ferritin levels, and complete blood counts (CBC) were performed for all patients, all conducted at the Zanjan Central Laboratory of Haft-e-Tir.

Increased liver enzymes were defined as ALT levels exceeding 19 U/L in women and 31 U/L in men, up to five times the normal range, after excluding other causes of elevated liver enzymes, such as viral and autoimmune hepatitis, Wilson's disease, and alpha-1 antitrypsin deficiency. All patients underwent FibroScan by the same gastroenterologist using the same machine, and their fatty liver severity was assessed blinded, without knowledge of the patient's ultrasound report.

It is important to note that patients were enrolled in the study from the outset, with a final diagnosis made using the FibroScan and after ruling out other causes of fatty liver. Additionally, enzyme levels were rechecked for all participants. The METAVIR scoring system was used to evaluate liver fibrosis:

F0 means no fibrosis can be detected.
F1 is equivalent to fibrosis with expansion of portal zones.
F2 is equivalent to fibrosis with expansion of most portal zones and occasional bridging.
F3 is equivalent to fibrosis with expansion of most portal zones, marked bridging, and occasional nodules.

It should be noted that the indicator of the FibroScan examination is based on the criteria for diagnosis and treatment (1, 5, 6, 8-10). Patients were assured that all their information would remain confidential. In addition, patients were referred only on the basis of indicators for further interventions for FibroScan. The limitations of the project included patient dissatisfaction, costs, and lack of easy access to the FibroScan center. The collected information was analyzed using SPSS statistical software. The degree of agreement between the severity of fatty liver was determined using the chi-square test. The p-value for analysis was 0.05.

Results

In this study, 86 patients participated. Table 1 shows that the average BMI in patients is higher than normal, and overweight individuals have been seen in most patients.

Table 1.Frequency and average demographic variables of participants and underlying disease and different FibroScan grades.

Variable		Frequency	mean ± SD
Male		31 (36%)	
Female		55 (64%)	
Total patients BMI (kg/m^2)			28.24 ± 1.41
BMI	20 >	3 (3.4%)	
	20-25	17 (19.7%)	
	25<	66 (76/9%)	
CBC	Normal	78 (90.7%)	
	Anemia	8 (9.3%)	
Iron profile	Normal	83 (96.5%)	
	Iron deficiency	3 (3.5%)	
Autoimmune hepatitis	yes	1 (1.2%)	
Wilson disease Viral hepatitis	no	85 (98.8%)	
Grade of Fibrosis	F0	25 (29.1%)	
	F1	39 (54.7%)	
	F2	14 (11.6%)	
	F3	8 (4.7%)	

As can be seen in Table 1, Grade 0 fatty liver was present in 25 patients, Grade 1 in 39 patients, Grade 2 in 14 patients, and Grade 3 in 8 patients. Among the patients studied, 8 had anemia, 3 had an increase in bilirubin, 3 had iron deficiency, and only 1 had liver problems related to an immune issue or specific disease. The average height of the participants was 153 ± 8.39 cm, and the average weight was 66.13 ± 10.04 kg.

Table 2. Average serum metabolic indices and liver enzymes.

Variable		mean ± SD	Frequency
ALT (IU/L)		66.18 ± 14.75	
AST (IU/L)		54.74 ± 8.92	
ALP (IU/L)		285.81 ± 19.35	
Bili D	Normal		83 (96.5%)
	Increased		3 (3.5%)
Cholesterol ($^{mg}/_{dl}$)		235.69 ± 29.84	
Triglyceride ($^{mg}/_{dl}$)		199.43 ± 31.76	
HDL ($^{mg}/_{dl}$)		42.40 ± 4.46	
HbA_1C (%)		5.71 ± 0.41	
FBS ($^{mg}/_{dl}$)		102.62 ± 14.49	

ALT, Alanine Transaminase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; Bili D, Direct Bilirubin; HDL, High Density Lipoprotein; HbA1C, ; FBS, Fasting Blood Sugar

In Table 2, the levels of AST and ALT enzymes can be seen to be slightly higher on average than normal baseline levels. Additionally, the level of HDL is at the lower limit of the normal range, while TG and cholesterol levels are higher than the upper limit of the normal range. The average fasting blood sugar (FBS) level is within the normal range. The average levels of cholesterol, TG, HDL, and FBS were 235.69 ± 29.84, 199.43 ± 31.76, 42.40 ± 4.46, and 102.62 ± 14.49 mg/dl, respectively. The average levels of ALT, AST, and ALP were 66.18 ± 14.75, 54.74 ± 8.92, and 285.81 ± 19.35, respectively.

Disscussion

In this study, 86 patients participated; 31 (36%) were male, and 55 (64%) were female. Grade 0 fatty liver was present in 25 patients, Grade 1 in 39 patients, Grade 2 in 14 patients, and Grade 3 in 8 patients. Among the patients studied, 8 had anemia, 3 had an increase in bilirubin, 3 had iron deficiency, and only one had liver problems related to an immune issue or specific disease. A significant relationship was not found between fibroScan levels and enzyme levels in either gender.

In a study conducted by Tatsumi and his colleagues in 2015 in Japan, patients with non-alcoholic fatty liver were involved, aiming to assess the accuracy of different non-invasive diagnostic methods. A total of 119 cases of chronic liver disease and cirrhosis were entered into the study. The results showed that the biopsy findings did not correlate with enzyme levels in determining the stage of the disease, although changes in enzyme levels indicated liver cirrhosis. This study used hyaluronic acid, collagen type 4, and the AST to Platelet Ratio Index (APRI). Additionally, the study found that the data from Fibroscan matched the liver biopsy results, which serves as a good predictor for disease prognosis (19).

Table 3. The relationship between liver enzyme levels and FibroScan grade and gender.

Gender	Enzymes	FibroScan grade				P-value
		F0	F1	F2	F3	
Male	AST	52.77±9.1	59.64±8.32	51.2±10.91		0.313
	ALT	68.55±15.24	57.05±11.93	66.4±16.71		0.117
	ALP	305.22±10.9	307.41±11.1	310.2±10.8		0.880
Female	AST	52.93±8.76	53.8±8.31	51.44±5.59	61.25±11.84	0.312
	ALT	69.18±14.46	67.43±14.68	63.00±16.49	82.00±4.54	0.224
	ALP	275.43±11.40	272.56±10.69	275.2±10.15	274.00±10.86	0.430

AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase

According to Table 3, there is no significant relationship between the degree of fibro scan and the level of liver enzymes in any gender ($p>0.05$). This means that as liver tissue fibrosis deteriorates, the level of liver enzymes in the blood does not change proportionately. This lack of relationship was seen in both genders.

As noted in the above study, Fibroscan is consistent with liver biopsy results, and since biopsy findings were not significantly associated with increased liver enzyme levels, grading of liver fibrosis based solely on enzyme levels is inappropriate. Our study also found similar results, showing no relationship between Fibroscan results and liver enzyme levels in different genders.

In the study conducted by Razavizade and his colleagues in 2012, 245 cases of NAFLD were evaluated based on ultrasound findings, and the levels of liver enzymes (ALP, ALT, AST), cholesterol, and HDL were measured. The average age of these patients was 41.6 years. According to the ultrasound findings, the patients were divided into two categories: moderate and severe fatty liver. The results showed no significant difference in enzyme levels between the moderate and severe groups (20). These findings align with our study and indicate that the degree of FibroScan cannot predict the increase in liver enzymes. In other words, elevated liver enzyme levels in the serum do not accurately reflect the stage of liver fibrosis and cannot serve as a reliable indicator of the deterioration of the condition.

In the meta-analysis conducted by Musso and his colleagues in 2011 in Italy, a total of 32 studies were selected from the 4,185 articles reviewed, based on the study criteria to assess the accuracy of non-invasive methods for diagnosing NAFLD. The results showed that among the liver enzymes, GGT is more specific for measuring extrahepatic complications, such as vascular damage, while ALT is more specific for liver damage. Together with FibroScan, these enzymes provide reasonable accuracy in the diagnosis of NAFLD, although they are still not as accurate as a liver biopsy and are not recommended for use individually (21). The results of this study differ from those of our own. This discrepancy appears to be due to the differences in study design: our study is cross-sectional, while the aforementioned study is a review, which may complicate the evaluation of the accuracy of the methods used in the articles. Additionally, the aforementioned study stated that ALT is specific to liver damage but did not address whether the level of this enzyme is directly related to the degree of FibroScan.

In another study conducted by Wong and his colleagues in 2010 in China, they compared the diagnostic accuracy of FibroScan and liver biopsy. The study involved 246 patients with non-alcoholic fatty liver, with an average body mass index (BMI) of 27.3. Elastography could not be performed in two cases where the BMI was greater than 35. The results indicated that FibroScan provides diagnostic power comparable to that of liver biopsy, although this accuracy decreases in cases of advanced fatty liver. Considering the painful, dangerous, and invasive nature of liver biopsy, along with the high probability of false negatives, the authors proposed FibroScan as an appropriate screening method (16). Unlike our study, the aforementioned study did not examine whether serum liver enzyme levels are good predictors for determining the degree of fibrosis in non-alcoholic fatty liver. However, our study investigated this issue separately by gender.

In the study conducted by Degos and colleagues in 2010 in France at 23 educational centers, a total of 1,307 patients with liver fibrosis were enrolled. The study aimed to compare non-invasive diagnostic methods for advanced liver fibrosis and assess the accuracy of liver enzymes versus FibroScan. The average age of the patients in this study was 47.2 years, and their average BMI was 23.8, with 69 percent being male. The results indicated that the detection strength of FibroScan was significantly higher than that of liver biomarkers; however, there was no significant difference between the two tests for advanced liver fibrosis (22). These results differ from those of our study, as most of our patients were women. This discrepancy may be attributed to genetic and environmental factors at the study site, where women in Zanjan are more likely to have fatty liver.

In a study conducted by Yoneda and colleagues in 2008 in Japan, they examined non-invasive methods for diagnosing liver fibrosis in patients with NAFLD. The study included 97 patients with NAFLD, and evaluated hyaluronic acid, type 4 collagen, and FibroScan. The results indicated a strong and direct correlation between liver stiffness and increased levels of these biomarkers (23). These findings are contrary to those of our study, with the difference attributed to the enzymes measured; in our study, we assessed conventional liver enzymes. Given that all gastrointestinal guidelines list functional liver enzymes as the first line for diagnosing liver disease, the results of our study may have greater clinical applicability than those of the aforementioned study.

In a study conducted by Imajo *et al*., they first determined the degree of liver tissue involvement using elastography. Subsequently, the microscopic slides of 120 patients with NASH were examined separately and independently by two experienced pathologists, who statistically analyzed the relationship between the severity of histological criteria and the levels of aminotransferase enzymes. The results showed a strong statistical relationship between the histological indicators and serum levels of aminotransferase enzymes. However, there was no correlation between the elastography results and the laboratory or histological findings (24). The diagnostic agreement between the two pathologists in reporting various related lesions for all examined indicators was very good ($p < 0.01$). The results of this study align with those of our own.

In the study conducted by Yoneda and colleagues, 65 patients were examined. The degree of fatty liver was determined using elastography, and enzyme levels were measured from the first to the sixth month after diagnosis. The results indicated that liver enzyme levels increased during the first six months, but began to decline after six months as the disease progressed. This trend was also observed in uric acid levels. The study showed that there was no significant relationship between liver enzyme levels, uric acid, and fatty liver grade (25). The results of the study align with our findings. While fibroelastography indicated an increase in the degree of fatty liver throughout the study, enzyme levels began to decline after some time.

In a study conducted by Abenavoli *et al*., 90 patients with non-alcoholic fatty liver, as confirmed by elastography, were examined. The serum levels of enzymes AST, ALT, TG, and cholesterol were measured. The height and weight of the patients were assessed for BMI calculation. There was no relationship between the AST levels measured in patients and the results of elastography. The average AST level was not significantly related to the results of liver elastography. In this study, the increase in triglyceride and cholesterol levels was not significantly associated with the degree of non-alcoholic fatty liver. However, the relationship between the increase in average BMI and non-alcoholic fatty liver was significant (26). The results of this study confirm our findings.

In a study conducted by Layal Al Danaf and colleagues in 2022, the authors demonstrated that ultrasound alone is not sufficient for assessing the advancement of liver disease. Furthermore, the high positive correlation of the AST/ALT ratio, APRI, and Fib-4 scores with fibrosis stages in patients with NAFLD suggests that they could be clinically used in combination with FibroScan to predict significant fibrosis and cirrhosis, thereby avoiding the need for liver biopsy. This offers benefits to patients, including cost savings and less invasive procedures (27).

Conclusion

According to the results obtained in our study, the prevalence of non-alcoholic fatty liver is higher in women than in men, and liver enzymes cannot accurately detect the degree of liver fibrosis. The present study indicates that enzyme levels in patients with fatty liver are not suitable for determining the increasing or decreasing severity of the disease; therefore, accurate imaging should be utilized. It is recommended to use imaging methods, especially FibroScan, to assess the condition of liver tissue in these patients instead of routine measurements of these enzymes. Additionally, due to genetic and environmental factors in Zanjan, it is advisable to screen women for fatty liver more frequently than men in the community.

Declaration

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Authors' contributions

HN, AAC, and FT contributed equally to conducting this project and writing this article.

Ethics approval

Ethical approval for this research was obtained from Zanjan University of Medical Sciences with ID: A-11-918-5.

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