Al-Kindy College Medical Journal (KCMJ)

Research Article

The Combination of Non-Invasive Tests and Fibroscan for the Assessment of Liver Fibrosis among Patients with Non-Alcoholic Fatty Liver Disease

Muntadher Abdulkareem Abdullah[1*], Raghad Jawad Al-Akayshee[2], Kamal Breesam Lafta[3]

[1] Department of Medicine, Basrah College of Medicine, Basrah Gastroenterology and Hepatology Hospital, Basrah, Iraq
[2] Baghdad Medical City, Baghdad Gastroenterology and Hepatology Teaching Hospital, Baghdad, Iraq
[3] Basrah College of Medicine, Basrah Gastroenterology and Hepatology Hospital, Basrah, Iraq
* Corresponding author's email: muntaderaltememy984@gmail.com

Article history:
Received 9 June 2023
Accepted 21 August 2023
Available online 30 December 2023

https://doi.org/10.47723/kcmj.v19i3.1058

Keywords: Fibroscan, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver stiffness measurement, transient elastography, controlled attenuation parameter

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license http://creativecommons.org/licenses/by/4.0/

ABSTRACT

Background: Non-alcoholic fatty liver disease is the most prevalent chronic liver disease in developed and developing countries. It encompasses a broad spectrum of diseases ranging from fatty liver, which often has a benign, non-progressive course, to non-alcoholic steatohepatitis, a more serious variant that can lead to cirrhosis and end-stage liver disease. The gold standard for defining the extent of hepatic fibrosis is liver biopsy; however, a number of non-invasive tests have been suggested for setting the diagnosis and assessing treatment.
Objective: to assess the effectiveness of combined Fibroscan and noninvasive biochemical tests and scoring systems in assessing liver fibrosis among patients with non-alcoholic fatty liver disease.
Subjects and Methods: We conducted a cross-sectional prospective study at the outpatient clinic of Baghdad Gastroenterology and Hepatology Teaching Hospital from October 2018-March 2020. One hundred patients with fatty liver were recruited and data were collected through a well-designed questionnaire based on Fibroscan finding and certain serum markers.
Results: There were fifty-five (55%) males and forty-five (45%) females. The mean age was (45 ±12.24) years and females were significantly older. The mean stiffness score was 11.7(SD:5.29) KPa. Forty-six (46%) patients had advanced fibrosis. The positive correlation between Fibroscan examination results and the noninvasive scores results was statistically significant. There was a positive statistically significant correlation between age and stiffness score while a statistically significant negative correlation was found between platelet count and stiffness score.
Conclusions: Approximately half of the patients had advanced fibrosis, highlighting the need for early detection and management. Implementation of Fibroscan with noninvasive fibrosis scoring tools could play a role in the diagnosis and management and decrease the need for liver biopsy.

Introduction

The definition of non-alcoholic fatty liver disease (NAFLD) is the presence of hepatic steatosis in the absence of other causes of fat accumulation (1). It encompasses cirrhosis, non-alcoholic steatohepatitis (NASH), simple steatosis, and steatosis associated

with different degrees of inflammation and fibrosis (2). The most prevalent chronic liver disease (CLD) is NAFLD, and simple steatosis and NASH are present in 20%–30% and 5%–12% of the general population, respectively (3, 4, 5). A relatively low risk of developing cirrhosis was linked to simple steatosis (6–8). In contrast, cirrhosis develops in about 7% of NASH patients within 3 years (6–8). Additionally, a number of prospective studies revealed that NASH was independently linked to an increase in mortality from both cardiovascular and liver disease-related causes (9, 10). Therefore, it is imperative to distinguish between patients with simple steatosis and those with NASH. The gold standard for determining fatty liver and distinguishing simple steatosis from NASH is still liver biopsy. But a biopsy is an intrusive procedure that has a small but real risk of consequences (11, 12). Given the limitations of liver biopsy, a number of noninvasive markers, such as imaging techniques and serological indices, have been investigated for the diagnosis of simple steatosis and NASH. As a result, researchers have looked into other non-invasive techniques for evaluating liver fibrosis in these patients (13).

The serological tests and the noninvasive scorings include the Aspartate aminotransferase /Alanine aminotransferase ratio (AST/ALT ratio), the aspartate aminotransferase/platelet ratio index (APRI), the Fibrosis 4 (FIB-4) score and the nonalcoholic fatty liver disease fibrosis score (NAFLD fibrosis score). The other popular and thoroughly tested noninvasive techniques for determining liver fibrosis are transient elastography (TE) (Fibroscan), an ultrasound-based procedure (14). This technique has been used to diagnose liver stiffness and fibrosis in the past (15, 16). Thus, monitoring the stiffness of the liver over time can show how some liver disorders, including NASH, progress (17). The fundamental idea behind TE is that a homogenous tissue's elasticity, which is connected with the degree of fibrosis in the liver, determines how quickly a wave propagates through it.

Fibroscan is an ultrasound-based technique and one of the most extensively used and well-validated non-invasive methods for the assessment of liver fibrosis. Diagnosis of liver stiffness and fibrosis by this method was reported previously (15,16). Thus, serial evaluation of liver stiffness can provide evidence about the progression of liver diseases like NASH. Transient elastography (TE or FibroScan ® - FS) was first described in France in 2001, then in other parts of the world (18).

Transient elastography (TE) involves the basic principle that the propagation velocity of a wave through a homogenous tissue is proportional to its elasticity, which is correlated with the amount of fibrosis in the liver. Briefly, TE consists of an ultrasound transducer mounted on the axis of the vibrator, which produces vibration of a mild amplitude and low frequency (50 Hz), consequently inducing elastic shear wave that propagates through the liver. Pulse-echo ultrasound follows the propagation of the shear wave and measures its velocity, which is related to liver tissue stiffness. It is reported that the velocity of elastic waves is faster in fibrotic liver than normal livers in previous study.

Fibroscan is equipped with a probe including an ultrasonic transducer and a vibrator. A vibration of mild amplitude and low frequency is transmitted from the vibrator placed on the body surface toward the liver through the intercostal space. The vibration induces an elastic shear wave that propagates through the liver tissue. The pulse-echo ultrasound acquisitions follow the propagation of the shear wave and determine its velocity. The velocity is directly related to tissue stiffness; the harder the tissue, the faster the shear wave propagates. Liver stiffness measurements (LSM) is calculated from velocity and expressed in kilo Pascale (kPa). LSM was performed after an overnight fast. Ten successful acquisitions were performed on each measurement and the median value was adopted as representative of LSM. To improve test reliability, a minimum of 10 valid readings, with at least a 60% success rate and an interquartile range of ≤30% of the median value were taken. FibroScan® is principally used to estimate the degree of liver scarring present (ie. stage of liver disease). This is especially useful in the assessment of patients with chronic liver disease, including chronic hepatitis C, chronic hepatitis B, chronic alcohol abuse, and fatty liver.

The diagnostic performance of TE using CAP in detecting liver steatosis is very high with a sensitivity and specificity of nearly 90% (19).

This reading may used to:

- Estimate the existing degree of liver damage.
- Monitor disease progression or regression via serial measurements.
- Guide prognosis and further management, including treatment.

The ultrasound-based technique requires an adequate visualization of the liver to obtain readings. In our experience, accurate readings are obtained in <50% of patients who have a body mass index (BMI) >35 kg/m2 using the standard M probe. This problem is partially overcome through the development of different probes (such as the XL probe), that allows deeper penetration of vibration wave. There are no absolute contraindications for the test, although the presence of ascites prevents propagation of the vibration wave and therefore frequently results in failure to obtain readings. The manufacturer also advises against the use of this device in pregnancy and in patients with a pacemaker.

Prevalence and Distribution of NAFLD

The worldwide increase in the prevalence of well-established risk factors for NAFLD, such as diabetes, obesity, and age, has had an accompanying increase in the prevalence of NAFLD. The estimated global incidence of NAFLD is 47 cases per 1,000 population and is higher among males than females. The estimated global prevalence of NAFLD among adults is 32% and is higher among males (40%) compared to females (26%). The global prevalence of NAFLD has increased over time, from 26% in studies from 2005 or earlier to 38% in studies from 2016 or beyond. The prevalence of NAFLD varies substantially by world region, contributed by different rates of obesity, and genetic and socio-economic factors (20). A recently published meta-analysis reported NAFLD prevalence in Latin America (44.37%; 30.66%-59.00%), Middle East and North Africa (MENA) (36.53%; 28.63%-45.22%), South Asia (33.83%; 22.91%-46.79%), South-East Asia (33.07%; 18.99%-51.03%), North America (31.20%; 25.86%-37.08%), East Asia (29.71%; 25.96%-33.76%), Asia Pacific (28.02%; 24.69%-31.60%), and Western Europe (25.10% ; 20.55%-30.28%) (21).

In the pooled analysis comprising 101028 individuals in 2022 found that the prevalence of NAFLD in the overweight population was

69·99% (95% CI 65·40–74·21 I2=99·10%), the prevalence of NAFL was 42·49% (32·55–53·08, I2=96·40%), and the prevalence of NASH was 33·50% (28·38–39·04, I2=95·60%). Similar prevalence estimates were reported in the obese population for NAFLD [(75·27%) (95% CI 70·90–79·18); I2=98·50%], NAFL [(43·05%) (32·78–53·97); I2=96·30%)] and NASH [(33·67%) (28·45–39·31); I2=95·60%). The prevalence of NAFLD in the overweight population was the highest in the region of the Americas [(75·34%) (95% CI: 67·31–81·93); I2=99·00%)]. Clinically significant fibrosis (stages F2–4) was present in 20·27% (95% CI 11·32–33·62; I2= 93·00%) of overweight individuals with NAFLD and in 21·60% (11·47–36·92; I2=95·00%) of obese patients with NAFLD while 6·65% (4·35–10·01; I2=58·00%) of overweight individuals with NAFLD and 6·85% (3·85–11·90; I2=90·00%) of obese individuals with NAFLD had advanced fibrosis (stages F3–4) (22). The increasing prevalence of NAFLD in the US parallels the increase in prevalence of NAFLD-related risk factors, which include insulin resistance, obesity, hypertension, and dyslipidemia.

Pathophysiology of NAFLD

The pathogenesis of NAFLD is multi-factorial. Genetic factors cooperate with metabolic and environmental factors to promote the accumulation of fat in hepatocytes. In the last decade of the 20th century, the most corroborated theory was the “two hit pathogenesis”. It stated that insulin resistance leads to triglyceride deposition in the liver, thus steatosis, rendering it more susceptible to the action of second hits, such as oxidative stress, ATP depletion and endotoxins, finally leading to inflammation, fibrosis and cancer. Cell death and inflammation are the key drivers of fibrosis in NASH and other forms of chronic liver disease. The primary pathophysiological mechanisms of insulin resistance induced by inflammatory mediators are probably the result of interference with insulin signaling (23). Insulin acts in all cells by binding to its specific receptor and activating a cascade of intracellular signaling. Upon insulin binding, the insulin receptor phosphorylates itself and several members of the insulin receptor substrate (IRS) family. IRS1 and IRS2 are the main mediators of insulin signaling in the liver, where they control insulin sensitivity (24). Nowadays, this theory has been replaced by the “multiple hit pathogenesis”. This states that multiple etio-pathogenic factors act in a parallel or sequential and somehow synergic way on a genetically predisposed subject, to cause NAFLD and thus defining the spectrum of the disease phenotype. Particularly, some patients will develop NAFLD and consequently NASH, but others will directly present inflammation and fibrosis, probably because of the influence of genetic and epigenetic factors.

The present study aims to assess the effectiveness of the combination of noninvasive testing and scoring systems with fibroscan for the assessment of fibrosis among Iraqi NAFLD patients attending the outpatient medical clinic of the Gastroenterology and Hepatology Teaching Hospital in Baghdad.

Subjects and Methods

A cross-sectional observational prospective study was carried out to assess the effectiveness of non-invasive testing and scoring systems with Fibroscan for the assessment of fibrosis among Iraqi NAFLD patients. One hundred patients who were diagnosed with NAFLD or NASH between October 2018 and March 2020 at the Gastroenterology and Hepatology Teaching facility (GI and Hep Hospital) in Baghdad, Central Iraq, based on ultrasound examination of abdominal and testing for serum liver enzyme that were done in our facility were included in this study. All patients, and occasionally their family, completed pre-designed questionnaires for this study. The study's inclusion and exclusion criteria were used to choose the participants. Participants had to be at least 18 years old, had undergone a Fibroscan examination within the study period, had an abdominal ultrasound that revealed evidence of a fatty liver, and had blood tests that revealed elevated liver enzyme levels. Patients whose data were insufficient were excluded. Also, patients were disqualified if they had a history of alcohol-related liver illness, autoimmune hepatitis (AIH), hepatitis B or C , as demonstrated by an autoimmune positive antibody test or a positive response to corticosteroids. Patients with advanced liver illness, heart failure, and hepatic congestion were also disqualified, as were those on hepatotoxic drugs. Participants were also excluded if they had clinical or ultrasound indications of decompensated cirrhosis or were unable to perform Fibroscan exams due to very high BMI or other extenuating circumstances. Each patient's demographic information, including age, sex, and country of origin, was gathered. Within two weeks of the Fibroscan examination, all findings from the laboratory tests that were assessed were acquired. These tests were conducted in our hospital. The normal range laboratory reference for serum alanine aminotransferase (ALT) in the GI and Hep.hospital is 12-78 U/L. Since it is difficult to determine a cutoff value for serum ALT and most studies still use the 30 U/L as upper cutoff value, we used this cutoff value for the upper limit of serum ALT in this study (25). The typical reference for platelet counts range is 150000-400000/microliter, while the usual reference range for serum aspartate aminotransferase (AST) was 15-37 U/L.

A SIEMENS Dimension X Pand Plus clinical chemistry system (Flex Reagent Cartridge) was used to detect liver enzymes. The alanine aminotransferase/ aspartate aminotransferase ratio (AST/ALT ratio), the aspartate aminotransferase/ platelet ratio (APRI score), nonalcoholic fatty liver disease fibrosis score (NAFLD fibrosis score) and the fibrosis-4 score (FIB-4 score) for each patient were measured using a computerized software application provided by (MD calculator and calculated by (QxMD) using the following formulas:

$$APRI = \frac{AST\ Level\ IU/L\ /(AST(ULN)IU/L}{Platelates\ Counts(10^9/L)} \times 100$$

$$FIB-4 = \frac{Age(y) \times AST\ (U/L)}{Platelates\ Count\ 10^9/L \times \sqrt{AST(U/L)}}$$

NAFLD fibrosis score = −1.675 + 0.037 3 ages (years) + 0.094 3 BMI (kg/m2) + 1.13 3 IFG/diabetes (yes = 1, no=0) +0.99 3 AST/ALT ratio – 0.013 3 platelet (3109/L) – 0.66 3 albumin (g/dL)

The instrument utilized in the investigation was an Echosens Fibroscan530 COMPACT (2018) (30 Place d'Italie 75013 Paris, France) The patient prepared for the test should fast for 4-6 hours, lay on his /her back with the right arm under the head, the abdomen and the lower chest exposed and the fibroscan probe is positioned in an intercostal space near the right lobe of the liver usually in the ninth – eleventh intercostal space , anterior axillary line, For a test to be judged effective, ten valid Fibroscan readings are required (according to the manufacturer's recommendations and results from earlier research) (26,27). For every patient that was a part of the trial, a success rate of > 90% was attained.

Based on numerous prior research and references offered by the manufacturer, the following fibrosis stages were identified: F0 = 1-6

KPa, F1 = 6.1-7 KPa , F2 = 7-9 KPa , F3 = 9.1-10.3 KPa and F4 > = 10.4 KPa (28, 29).

Statistical analyses were performed using IBM's Statistical Package for the Social Sciences (SPSS) version 26. A descriptive data and a student's t-test were used to compare the differences in the mean ages and serum ALT values between the males and the females. Age, platelet count, and serum ALT levels were compared with a correlation analysis to determine the degree of hepatic fibrosis. The association between the results of the Fibroscan and the NAFLD fibrosis score, AST/ALT ratios, APRI scores, and FIB-4 scores was examined using a straightforward dot graph. To compare the AST/ALT ratio, the APRI and FIB-4 scores, and the NAFLD fibrosis score between patients with advanced fibrosis (higher than F2) and patients with mild-moderate fibrosis of (F2 or less), a student's t-test were used.

Results

Of one hundred patients included in the study; fifty-five (55%) were males and forty-five (45%) were females. The mean age of the studied patients was 45 years (range 20-75 years). Female patients were significantly older than male patients (47 years (SD:11) versus 43 years (SD:13), respectively, P= 0.01). Forty-two (42%) patients had hypertension and 39(39%) had diabetes.

The study showed that the mean waist circumference for the studied patients was (99.5±3.5 cm). The waist circumferences were significantly higher in females in comparison with males (mean=102±4 for the females and 97±3for the males, P = 0.03). The mean BMI for the studied patients was 32.41±4.05 and it was significantly higher in females comparing to males (mean=33.64±4.33 and 31.19±3.78 respectively, P = 0.04). The mean serum cholesterol was 253±30 mg/dl and it was significantly higher in females compared to males [265 mg/dl (SD=32) versus 241 mg/dl(SD=28),respectively, P=0.02]. The mean serum triglyceride value was 232 ±21 mg/dl and these values were significantly higher in female compared with the males [247 mg/dl (SD=25) versus 217 mg/dl (SD=17), respectively, P=0.03]. The mean serum high density lipoprotein (S.HDL) was 42±9 mg/dl and the value was found to be lower in females compared to males patients [39 mg/dl SD=8) versus 45 mg/dl (SD=10), respectively, P=0.02] (Table-1).

Most of the patients showed either no fibrosis (36%) or a severe grade of fibrosis (30%), and the mean stiffness score was 11.7(SD:5.29) KPa. According to the stage of fibrosis, the distribution of the patients under study is shown in Table-2.

Male patients showed higher stiffness scores compared to females and the difference was statistically significant [12.93 (SD:6.02) versus 10.47 (SD:4.57) KPa, respectively, P=0.02] (Table-3). The study also showed that the mean ALT for male patients was significantly higher than that in female patients [78.81IU\ml (SD:52.31) versus 51.88(SD:33.06), respectively, P=0.03] (Table-3), (also see table 4 for mean serum ALT, AST and platelets count).

The statistical analysis revealed a significant positive correlation between patients age and stiffness score (r=0.49 for Pearson correlation, p=0.01) (Table-5). On the other hand, there was a strong negative correlation between platelets count and fibrosis score (r=-0.339 for Pearson correlation, P= 0.001) (Table-5). In addition, a significant positive correlation was found between the serum ALT level and stiffness score (r=0.45 for Pearson correlation, P=0.01) (Table-6). A negative correlation was found between serum ALT and the patients' age (r= -0.38 for Pearson correlation, P=0.03) (Figure-1).

Table-1: The demographic characteristics, fibroscan findings, and serum markers in the studied patients.

Characteristic	Overall	Male	Female	P-Value
Number of Patients**	100	55 (55%)	45 (45%)	-
Age (years)*	45 ± 12	43 ± 13	47 ± 11	0.01
BMI (Kg/m²)*	32.41 ± 4.05	31.19 ± 3.78	33.64 ± 4.33	0.04
Waist Circumference (cm)*	99.5 ± 3.5	97 ± 3	102 ± 4	0.03
Hypertension**	42%	-	-	-
Diabetes Mellitus**	39%	-	-	-
Fibrosis Score (KPa)*	11.7 ± 5.29	-	-	-
Steatosis Score (dB/m)*	285.64 ± 62.87	-	-	-
S. Cholesterol (mg/dl)*	253 ± 30	241 ± 28	265 ± 32	0.02
S. Triglyceride (mg/dl)*	232 ± 21	217 ± 17	247 ± 25	0.03
S. HDL (mg/dl)*	42 ± 9	45 ± 10	39 ± 8	0.02

* Data presented as mean ± standard deviation.
** Data presented as number and percentage.

Table-2: Distribution of patients according to the stage of liver fibrosis

Stage of fibrosis (Kpa) %	NO
F0 (0-5.9)	36(36%)
F1 (6-6.9)	14(14%)
F2 (7-9)	4(4%)
F3 (9.1-10.3)	16(16%)
F4 >=10.4	30(30%)
Total	100(100%)

There were significant differences in the stiffness scores for NAFLD fibrosis score, FIB4 score, APRI score, and AST/ALT ratio score calculations; the most significant were for NAFLD fibrosis score and FIB4 calculations between patients with advanced fibrosis (more than F2 score on FibroScan) [46(46%] and those with mild to moderate fibrosis (F2 score and less on FibroScan) [54(54%] (Table-7).

The stiffness Scores on the Fibroscan examination were correlated significantly with the results obtained from NAFLD fibrosis score, FIB4 score, AST/ALT score and APRI score; the most significant positive correlation was found for the fibrosis results obtained by NAFLD fibrosis score and FIB4 score(figures2,3,4-5).

Table-3: Stiffness score and serum ALT level in males and females

	Sex	Mean±SD	P. Value
Fibrosis Score(KPa)	Male	12.93±6.02	0.02
	Female	10.47±4.57	
Serum ALT level(U/L)	Male	78.81±52.31	0.003
	Female	51.88±33.06	

Table-4: Mean platelet counts, serum Alanine Transaminase levels (ALT), and Aspartate Amino Transferase levels (AST)

Lab Test	Mean ±SD	Normal reference range
Platelets k/µL	235.20± 79.92	150-400
Serum ALT U/L	65.34± 42.68	12 - 40
Serum AST U/L	45.13± 25.53	15-37

Table-5: The distribution of the patients according to the platelet level

Platelets level(k/µL)	No. (%)
50000-100000	17(17%)
100000-150000	33(33%)
>= 150000	50(50%)

Table-6: The statistical correlation of patients' age, serum ALT level and platelets count with stiffness score

Characteristic	r value	P value
Age(years)	0.49	0.01
Serum ALT(U/L)	0.45	0.01
Platelets Count	- 0.339	0.001

Table-7: Differences in stiffness scores, NAFLD fibrosis score, AST/ALT ratio, APRI score and FIB-4 score between patients with mild to moderate fibrosis and those with advanced fibrosis

	> F2 (N.=46)	F2 or less (N.=54)	P value
Stiffness Score	18.7±2.1	4.8±1.5	0.001
NAFLD fibrosis score	0.89±0.17	- 1.66± - 0.12	0.001
AST/ALT ratio score	2.3±0.2	0.61±0.11	0.04
APRI score	1.36±0.26	0.31±0.21	0.02
FIB-4	2.96±0.35	0.87±0.63	0.01

Values are expressed as Mean±SD

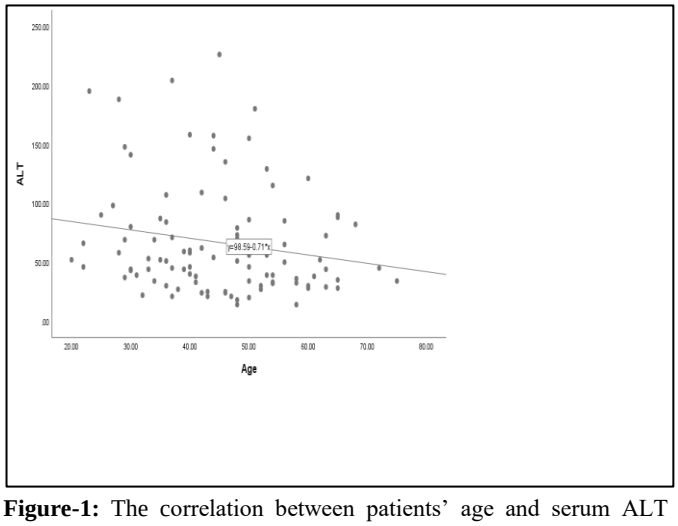


Figure-1: The correlation between patients' age and serum ALT levels (r= - 0.38, P=0.03)

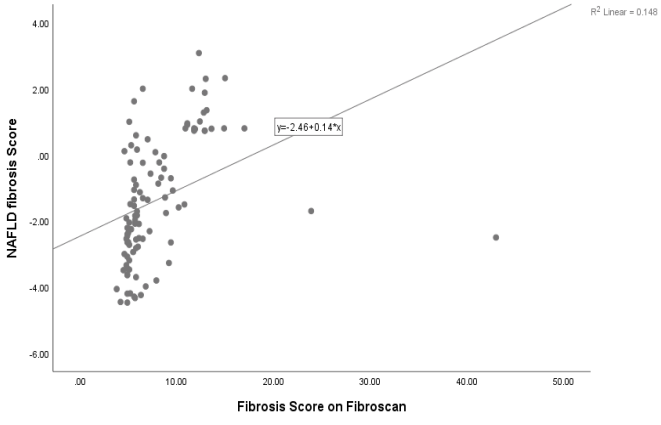


Figure-2. The correlation between fibrosis scores on Fibroscan and NAFLD fibrosis score (r = 0.38, P=0.001)

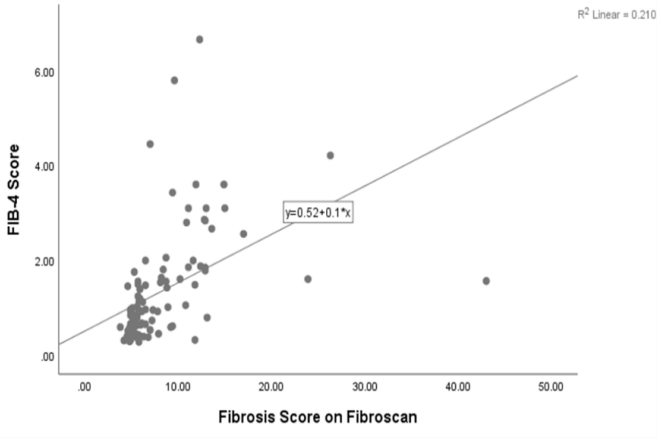


Figure-3. The correlation between Fibrosis Scores on Fibroscan and FIB-4 scores (r=0.46, P=0.001)

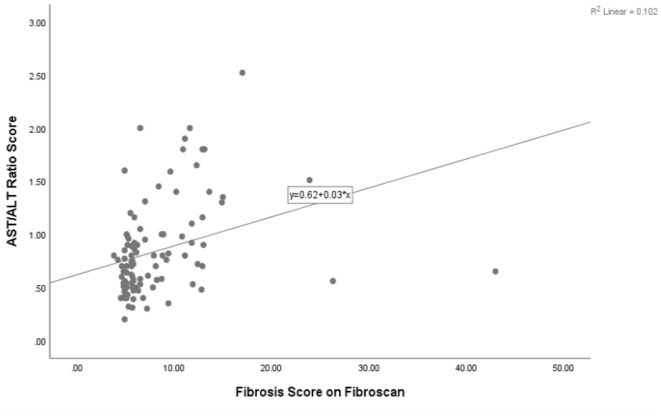
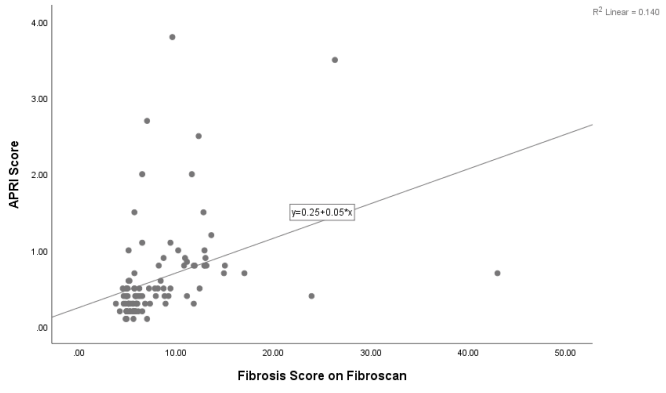


Figure-4. The correlation between fibrosis scores on Fibroscan and AST/ALT ratio scores (r=0.32, P=0.01)



Figur-5. The correlation between fibrosis scores on Fibroscan and APRI scores (r=0.37, P=0.001)

Discussion

Across the world, NAFLD is becoming more common. With catastrophic morbidities, it is a significant contributor to liver cirrhosis and hepatocellular cancer. The percentage of chronic liver disease is as the following: NAFLD (59%), followed by HBV (29%), HCV (9%), and autoimmune hepatitis (2%), while other liver diseases such as primary biliary cholangitis, primary sclerosing cholangitis, alpha-1-antitrypsin deficiency, Wilson's disease, and autoimmune hepatitis, account for 1% of cases (30).

According to the findings of the Fibroscan tests, the study revealed that a significant proportion of the NAFLD patients had advanced fibrosis stages. The Fibroscan results and the outcomes of the non-invasive fibrosis scoring methods (NAFLD, FIB-4, AST/ALT, and APRI scores) showed a significant association, which validated these findings.

In the study, advanced fibrosis was seen at a younger age in males compared to females, which was consistent with findings by Ezhilarasan et al, which showed that women developing chronic liver disease at older age (mean age 60 years) than men (mean age 45 years) and this could be explained by the protective effect of female sex hormones on the progression of hepatic fibrosis , and this might explain why male patients typically have more severe liver disease compared to female patients for the majority of liver-related etiologies (31).

In Iraqi patients with metabolic syndrome and type 2 diabetes mellitus, the high rate of advanced NAFLD is alarming. Both conditions are prevailing among Iraqi populations since Asmaa et al reported that 14% had type 2 diabetes and 66.9% had overweight/obesity (32).

Taking into consideration the association between severe fibrosis and thrombocytopenia, the study demonstrated that there was a strong negative association between the stiffness score and platelet count, which is consistent with study by Desai and Subramanian who found that patients with stage 4 fibrosis have mean platelets count of 80000/microliter (33).

Additionally, the results of this study support previous researches by Chalasani et al , Liu et al and Fan et al who demonstrated that liver fibrosis severity increased with age (42% had F3-F4 fibrosis at age 65 year) and that age was related with a more advanced level of fibrosis. This can be explained by the fact that insulin resistance, which is known to be a primary cause of the NAFLD, is a major component of the metabolic syndrome, which is often observed in elderly people. Aging, which is accompanied by abdominal obesity and excessive visceral fat, causes insulin resistance and an increased secretion of proinflammatory cytokines and, subsequently, results in the metabolic syndromes and type 2 diabetes, activation of p300-C/EBP-dependent neutral fat synthesis, telomere shortening, a decreased autophagy, increased M1 macrophage inflammatory responses , and activation of nuclear factor-κB pathways (2, 34, 35).

The study showed that ultrasound examination of abdomen alone is insufficient in the evaluation of advanced fibrosis, but that the implementation of the serum markers to ultrasonic abdominal examination can further categorize patients into those with mild and those with advanced NAFLD, which was consistent with the findings of Razavizade et al who found that combining serum markers with ultrasonic finding increase the detection rate of advance fibrosis to 85-90 % (36).

In this study, all patients had baseline abdominal ultrasound examinations that showed evidence of fatty liver. This finding may further support the use of straightforward, non-invasive fibrosis scoring in conjunction with abdominal ultrasound in the diagnosis of NAFLD when more sophisticated techniques like MRI elastography or transient elastography (Fibroscan) are unavailable or when performing liver biopsy is not necessary.

In contrast to numerous general population studies that revealed that level of serum ALT increases with advancing age (16), the findings from our study demonstrated an inverse connection between serum ALT level and patient's age. This can be explained by disease activity and the onset of NASH in younger people, which can cause cirrhosis with advancing age and return of ALT to normal levels in older people. Chalasani et al, Reddy et al, Dai et al and Lee et al, reported comparable results of decreasing serum ALT (mean=10 U/ml) with advancing age (mean age of 60 year) which further support the findings in our study (2, 37, 38, 39).

This study also demonstrated that the FibroScan examination and non-invasive scoring tools (FIB-4, NAFLD fibrosis score, APRI, and AST/ALT score) could distinguish between mild to moderate and advanced fibrosis; the best outcomes were obtained from the FibroScan, FIB-4, and NAFLD fibrosis scores, while the least favorable outcomes were with the AST/ALT ratio score. These outcomes were comparable to that in the United Kingdom by Masuzaki R. et al (40).

The study revealed that the majority of the patients had advanced levels of steatosis, which was previously verified in other locally-conducted studies by Asmaa et al on the high incidence of metabolic syndrome and type II diabetes mellitus in the Iraqi community, where 33.9% are obese with BMI > 30 Kg\m2 and the prevalence of overweight/obesity was 66.9% (32) .

Limitations

The number of included patients is small in view of the national growing prevalence of the NAFLD risk factors among Iraqi population, but this could be compensated for by the strict inclusion criteria.

Liver biopsy which is the gold standard for the diagnosis of NAFLD/NASH was not used in this study, due to the complication of this procedure and the absence of clear indications among the studied patients. Itt should not be used for every patient with NAFLD.

The study was conducted in a single center study and the limitation of the availability of the Fibroscan machines**.**

The study was approved by the Ethics Committee of the College of Medicine, University of Basrah (N0. 030408-030-2022 in 2020). Verbal and written informed consent were taken from the patients enrolled in the study and to support privacy and confidentiality, we concealed the unique identifying information of people in the data gathering.

Conclusion

In evaluating liver fibrosis and planning therapy in NAFLD patients, FibroScan in conjunction with non-invasive scoring tools (NAFLD fibrosis score, AST/ALT ratio score, APRI score, and FIB-4 score) is an effective method and it could lessen the need for an unnecessary liver biopsy.

Consent to publication

Written informed consent had been taken from participants.

Competing Interests

Authors have declared that no competing interests exist.

Acknowledgments

The authors are grateful to all patients who agreed to participate in this study.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors' contributions

Concept and design: Muntadher Abdulkareem Abdullah. Acquisition, analysis or interpretation of data: all authors. Drafting of the manuscript: Muntadher Abdulkareem Abdullah. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Muntadher Abdulkareem Abdullah and Kamal Breesam Lafta. Supervision: Raghad Jawad.

Data availability

Data are available upon reasonable request.

ORCID

Muntadher Abdullah 0000-0002-8893-1920
Raghad Al-Akayshee 0000-0002-3436-7105
Kamal Lafta 0000-0002-7890-2405

References

[1] Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. Journal of Gastroenterology. 2021;56(11):951-63. https://doi.org/10.1111/hepr.13688

[2] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328-57. https://doi.org/10.1002/hep.29367

[3] Henry L, Paik J, Younossi ZM. the epidemiologic burden of non-alcoholic fatty liver disease across the world. Alimentary pharmacology & therapeutics. 2022;56(6):942-56. https://doi.org/10.1111/apt.17158

[4] Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. Clinical liver disease. 2021;17(5):365. https://doi.org/10.1002/cld.1061

[5] Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. Gastroenterology. 2019;156(5):1264-81. e4. https://doi.org/10.1053/j.gastro.2018.12.036

[6] Loomba R, Wong R, Fraysse J, Shreay S, Li S, Harrison S, et al. Nonalcoholic fatty liver disease progression rates to cirrhosis and progression of cirrhosis to decompensation and mortality: a real world analysis of Medicare data. Alimentary pharmacology & therapeutics. 2020;51(11):1149-59. https://doi.org/10.1111/apt.15679

[7] Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. Metabolism. 2020;111S:154170. https://doi.org/10.1016/j.metabol.2020.154170

[8] Seval GC, Kabacam G, Yakut M, Seven G, Savas B, Elhan A, et al. The natural course of non-alcoholic fatty liver disease. Hepatology Forum.2020;1(1):20-24. https://doi.org/10.14744/hf.2020.0008

[9] Fujii H, Ko T, Fukuma T, Kadono T, Asaeda K, Kobayashi R, et al. Frequently abnormal serum gamma-glutamyl transferase activity is associated with future development of fatty liver: a retrospective cohort study. BMC gastroenterology. 2020;20(1):1-9. https://doi.org/10.1186/s12876-020-01369-x

[10] Song Q-R, Liu S-L, Bi Y-G, Chen S-H, Wu S-L, Cai J. Non-alcoholic fatty liver disease is associated with cardiovascular outcomes in subjects with prediabetes and diabetes: a prospective community-based cohort study. Frontiers in Cardiovascular Medicine. 2022;9:889597. https://doi.org/10.3389/fcvm.2022.889597

[11] Thomaides-Brears HB, Alkhouri N, Allende D, Harisinghani M, Noureddin M, Reau NS, et al. Incidence of complications from percutaneous biopsy in chronic liver disease: a systematic review and meta-analysis. Digestive Diseases and Sciences. 2022;67(7):3366-94. https://doi.org/10.1007/s10620-021-07089-w

[12] Alboraie M, Khairy M, Elsharkawy A, Asem N, El Kassas M, Elgendy AA, et al. Role of liver biopsy versus non-invasive biomarkers for diagnosis of significant fibrosis and cirrhosis: a web-based survey. Egyptian Liver Journal. 2021;11(1):1-8. (1-27) https://doi.org/10.1186/s43066-021-00166-9

[13] Nascimbeni F, Ballestri S, Machado MV, Mantovani A, Cortez-Pinto H, Targher G, et al. Clinical relevance of liver histopathology and different histological classifications of NASH in adults. Expert review of gastroenterology & hepatology. 2018;12(4):351-67. https://doi.org/10.1080/17474124.2018.1415756

[14] Masuzaki R, Kanda T, Sasaki R, Matsumoto N, Ogawa M, Matsuoka S, et al. Noninvasive assessment of liver fibrosis: current and future clinical and molecular perspectives. International Journal of Molecular Sciences. 2020;21(14):4906. https://doi.org/10.3390/ijms21144906

[15] Gheorghe G, Bungău S, Ceobanu G, Ilie M, Bacalbaşa N, Bratu OG, et al. The non-invasive assessment of hepatic fibrosis. Journal of the Formosan Medical Association. 2021;120(2):794-803. https://doi.org/10.1016/j.jfma.2020.08.019

[16] Aleknavičiūtė-Valienė G, Banys V. Clinical importance of laboratory biomarkers in liver fibrosis. Biochemia Medica. 2022;32(3):346-56. https://doi.org/10.11613/bm.2022.030501

[17] ElShahawy A, El-Raziky M, Sharaf S, Elsharkawy A, Enayet A, Taher H. Accuracy of noninvasive methods for the diagnosis of liver fibrosis in children with chronic viral hepatitis. BMC gastroenterology. 2022;22(1):1-6. https://doi.org/10.1186/s12876-022-02570-w

[18] Ajmera V, Loomba R. Imaging biomarkers of NAFLD, NASH, and fibrosis. Molecular metabolism. 2021;50:101167. https://doi.org/10.1016/j.molmet.2021.101167

[19] Kamali L, Adibi A, Ebrahimian S, Jafari F, Sharifi M. Diagnostic performance of ultrasonography in detecting fatty liver disease in comparison with fibroscan in people suspected of fatty liver. Advanced biomedical research 2019;8:69. https://doi.org/10.4103/abr.abr_114_19

[20] Teng ML, Ng CH, Huang DQ, Chan KE, Tan DJ, Lim WH, et al. Global incidence and prevalence of nonalcoholic fatty liver disease. Clinical and Molecular Hepatology. 2023;29(Suppl):S32. https://doi.org/10.3350/cmh.2022.0365

[21] Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology (Baltimore, Md). 2023;77(4):1335. https://doi.org/10.1097/hep.0000000000000004

[22] Quek J, Chan KE, Wong ZY, Tan C, Tan B, Lim WH, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. The Lancet Gastroenterology & Hepatology. 2023;8(1):20-30. https://doi.org/10.1016/s2468-1253(22)00317-x

[23] Pouwels S, Sakran N, Graham Y, Leal A, Pintar T, Yang W, et al. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC endocrine disorders. 2022;22(1):1-9. https://doi.org/10.1186/s12902-022-00980-1

[24] Spremović Rađenović S, Pupovac M, Andjić M, Bila J, Srećković S, Gudović A, et al. Prevalence, risk factors, and pathophysiology of nonalcoholic fatty liver disease (NAFLD) in women with Polycystic Ovary Syndrome (PCOS). Biomedicines. 2022;10(1):131. https://doi.org/10.3390/biomedicines10010131

[25] Valenti L, Pelusi S, Bianco C, Ceriotti F, Berzuini A, Prat LI, et al. Definition of healthy ranges for alanine aminotransferase levels: a 2021 update. Hepatology Communications. 2021;5(11):1824-32. https://doi.org/10.1002/hep4.1794

[26] Xia S, Ren X, Ni Z, Zhan W. A noninvasive method—Shear-wave elastography compared with transient elastography in evaluation of liver fibrosis in patients with chronic hepatitis B. Ultrasound quarterly. 2019;35(2):147-52. https://doi.org/10.1097/ruq.0000000000000399

[27] Chuah KH, Lai LL, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Liver stiffness measurement in non-alcoholic fatty liver disease: Two is better than one. Journal of gastroenterology and hepatology. 2020;35(8):1404-11. https://doi.org/10.1111/jgh.14978

[28] Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 2023;77(5):1797-1835. https://doi.org/10.1097/hep.0000000000000323

[29] Söderberg C, Stål P, Askling J, Glaumann H, Lindberg G, Marmur J, et al. Decreased survival of subjects with elevated liver function tests during a 28-year follow-up. Hepatology. 2010;51(2):595-602. https://doi.org/10.1002/hep.23314

[30] Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. Clinical liver disease. 2021;17(5):365. https://doi.org/10.1002/cld.1061

[31] Ezhilarasan D. Critical role of estrogen in the progression of chronic liver diseases. Hepatobiliary & Pancreatic Diseases International. 2020;19(5):429-34. https://doi.org/10.1016/j.hbpd.2020.03.011

[32] Asmaa A, Ali H, Abdilkarim Y. Metabolic syndrome among obese adults in Baghdad, Iraq. Saudi Journal of Obesity. 2017; 5(1):8-14. https://doi.org/10.4103/sjo.sjo_3_17

[33] Desai S, Subramanian A. Thrombocytopenia in chronic liver disease: challenges and treatment strategies. Cureus. 2021;13(7): e16342. https://doi.org/10.7759/cureus.16342

[34] Liu Y, Zhong G-C, Tan H-Y, Hao F-B, Hu J-J. Nonalcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: a meta-analysis. Scientific reports. 2019;9(1):11124. https://doi.org/10.1038/s41598-019-47687-3

[35] Fan J, Luo S, Ye Y, Ju J, Zhang Z, Liu L, et al. Prevalence and risk factors of metabolic associated fatty liver disease in the contemporary South China population. Nutrition & metabolism. 2021;18:1-10. https://doi.org/10.1186/s12986-021-00611-x

[36] Razavizade M, Jamali R, Arj A, Talari H. Serum parameters predict the severity of ultrasonographic findings in non-alcoholic fatty liver disease. Hepatobiliary & pancreatic diseases international. 2012;11(5):513-20. https://doi.org/10.1016/s1499-3872(12)60216-1

[37] Reddy YK, Marella HK, Jiang Y, Ganguli S, Snell P, Podila PS, et al. Natural history of non-alcoholic fatty liver disease: a study with paired liver biopsies. Journal of Clinical and Experimental Hepatology. 2020;10(3):245-54. https://doi.org/10.1016/j.jceh.2019.07.002

[38] Dai W, Ye L, Liu A, Wen SW, Deng J, Wu X, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. Medicine. 2017;96(39). https://doi.org/10.1097/md.0000000000008179

[39] Lee CH, Lui DT, Lam KS. Non-alcoholic fatty liver disease and type 2 diabetes: An update. Journal of Diabetes Investigation. 2022;13(6):930-40. https://doi.org/10.1111/jdi.13756

[40] Masuzaki R, Kanda T, Sasaki R, Matsumoto N, Ogawa M, Matsuoka S, et al. Noninvasive assessment of liver fibrosis: current and future clinical and molecular perspectives. International Journal of Molecular Sciences. 2020;21(14):4906. https://doi.org/10.3390/ijms21144906

To cite this article: Abdullah M, Al-Akayshee RJ, Lafta KB. The Combination of Non-Invasive Tests and Fibroscan for the Assessment of Liver Fibrosis among Iraqis' Nonalcoholic Fatty Liver Disease Patients. AL-Kindy College Medical Journal. 2023;19(3):12–20. https://doi.org/10.47723/kcmj.v19i3.1058