

Study of Transient Elastography (FibroScan) findings: liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) in comparison with Ultrasonographic findings in evaluating Patients with Non-Alcoholic Fatty Liver Disease

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is considered the most common chronic liver pathology. There are many non-invasive ways for evaluating hepatic steatosis and fibrosis either laboratory or radiologically.

Objectives: To evaluate the correlation between laboratory biomarkers, ultrasonographic findings, transient elastography and controlled attenuation parameters in patients with (NAFLD).

Patients and methods: This cross-sectional study was carried out on 100 persons with NAFLD as determined by transabdominal ultrasound. A complete history, physical examination, and laboratory tests were done, besides pelviabdominal ultrasound scan. To evaluate the hepatic stiffness, transient elastography (TE) were utilized. Control Attenuation parameters (CAP), and liver stiffness measurements (LSM) and were evaluated. Grades concerning fatty liver and stiffness were determined for every patient by the following specific related equations: Fatty Liver Index [FLI], Hepatic Steatosis Index [HSI], and finally the Nonalcoholic fatty liver disease fibrosis score [NFS].

Results: We found that LSM and CAP were significantly increased in patients with grade III fatty liver disease (7.5 ± 2.1) (296.8 ± 43.8) when compared with those with grade II fatty liver disease (5.8 ± 1.8) (236 ± 1.8), and patients with grade I fatty liver disease (5.5 ± 1.1) (169.8 ± 35.8) respectively (p value < 0.001). Patients with grade III fatty liver disease had a statistically significant increase in NAFLD fibrosis score (p value = 0.027) and fatty liver index (p value < 0.001) compared with patients with grade II and grade I fatty liver disease.

Conclusions: The findings of LSM and CAP parameters of TE were significantly and positively correlated to ultrasonographic NAFLD stage. There was a significant positive correlation between liver fibrosis outcomes (FLI and NFS) and US NAFLD stage.

Keywords: NAFLD; Fibrosis; Elastography.

DOI: 10.21608/SVUIJM.2024.294942.1886

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Received: 24 June, 2024.

Revised: 12 July, 2024.

Accepted: 14 July, 2024.

Published: 20 December, 2024

Cite this article as: Mohammed AK, Abdallah E.M. Ali, Ghada M. Abdelrazek, Abd El Rahman Hamdy Ahmed, Mohammed Tag-Adeen Said Hussien.(2024). Study of Transient Elastography (FibroScan) findings: liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) in comparison with Ultrasonographic findings in evaluating Patients with Non-Alcoholic Fatty Liver Disease. *SVU-International Journal of Medical Sciences*. Vol.7, Issue 2, pp: 936-9.

Introduction

Non-alcoholic associated fatty liver disease (NAFLD), a disorder linked to obesity and reaching epidemic proportions, is characterized by the buildup of excess fat in liver cells. This condition can potentially result in liver cancer, cirrhosis, liver fibrosis, and inflammation (Loomba et al., 2013).

A recent analysis has revealed that NAFLD, with a global incidence of 25%, is the leading cause of chronic liver disease and places a substantial burden on both clinical and financial aspects (Younossi et al., 2016).

Non-alcoholic fatty liver disease (NAFLD) is influenced by various factors, including hepatitis C virus (HCV), hepatitis B virus (HBV), obesity, hyperglycemia, and insulin resistance (Adinolfi et al., 2016). NAFLD not only affects the liver itself but also has extrahepatic consequences, such as cardiovascular disease, which is closely linked to NAFLD and the condition known as metabolic syndrome. (Targher et al., 2016).

NAFLD is another symptom of metabolic syndrome, and pathophysiological considerations, laboratory studies, and clinical context all support the view that insulin resistance, a hallmark of metabolic syndrome, contributes to the development of NAFLD (Kim et al., 2016). Liver biopsy is the only accurate method to differentiate simple steatosis from NASH. However, liver biopsy is an invasive procedure with many potential consequences, so it is necessary to develop accurate non-invasive methods to determine the histological severity of NASH. Non-invasive tests for liver fibrosis aim to predict the stage of disease observed histologically. Tests are generally used to differentiate between patients with mild or no fibrosis (F0 to F1) and those with severe fibrosis (F2 to F4). Serological panels and radiological examinations are the two main

categories of non-invasive fibrosis tests (Chou et al., 2013).

Fibrosis index 4 (FIB-4), and the aspartate aminotransferase to platelet ratio (APRI score), are examples of serological tests used in evaluating hepatic fibrosis. One drawback of all blood tests is that they can be affected by renal clearance. Besides, they may not show specificity for hepatic affection as they may increase in other pathological conditions in tissues other than the liver. Real-time elastography, also known as FibroScan, measures liver stiffness using elastic shear waves generated by a vibrator attached to an ultrasound transducer probe. This is the non-invasive technique of choice today (Kaswala et al., 2016).

The controlled attenuation parameter (CAP) of the FibroScan system is a tool for hepatic steatosis assessment. It measures the attenuation of the ultrasound (US) beam, which is inversely proportional to the volume of liver fat. A liver stiffness value that varies from 100 to 400 decibels per meter (dB/m) can be reported (Ferraioli et al., 2021). The research's objective was to figure out if there is correlation in between transient elastography findings, ultrasonographic results, and levels of laboratory biomarkers in patients with fatty liver which are not known to be alcoholics.

Patients and methods

One Hundred Egyptian patients with non-alcoholic fatty liver disease (assessed by abdominal ultrasound) were involved in this study. A written release was signed by each participant. The study was approved by the regional ethics committees of South Valley University and the Qena Faculty of Medicine. Ethical approval code is (SVU-MED-MED018.1.3-21.140).

Setting: Hospitals of Qena University Outpatient Clinics of Internal Medicine Department.

I. Inclusion criteria: Patients proved to have Non-Alcoholic Fatty Liver Disease and aged more than 18 years.

II. Exclusion criteria: Patients with cirrhosis of the liver, chronic viral hepatitis (B and C) in the past, daily alcohol use of more than 20 grams, Congenital defects of the metabolism, patients with major comorbidities or concomitant malignancies were excluded.

All included diseased individuals were exposed to the subsequent:

I. Medical history taking and clinical assessment:

1) A comprehensive medical history was obtained, with details about any concomitant conditions

as DM, HTN, or cardiac disease. A history of drug use was collected.

2) A detailed clinical assessment: to look for signs of chronic liver disorders.

3) Measuring body weight, height to calculate the body mass index (BMI), also waist circumference (WC), was taken for every participant.

II. Biochemical Lab Studies:

Each participant had eight milliliters of blood from their veins drawn, which were split into three specimens:

a) 4 mls were placed in a plain tube not containing anticoagulant for lipid profile, and serum creatinine assessment, AST, ALT

b) the second and third (2 ml for each) were placed in 2 EDTA tubes used for CBC and HbA1c assays.

The following investigations were performed:

1. Fasting blood sugar (FBG), two hours post prandial blood sugar (2HPPG), Lipid profile and serum creatinine, ALT, AST levels were assessed by the Pantra C400 (Horiba, French) Automated chemistry analyzer.

2. CBC assessment by Cell dyne-Ruby (Abbott diagnostic-Santa-Clara-California, USA) automated cell counter.

3. HbA1c assessment by BioRad-D10 (Hercules, CA, USA) automated HbA1c analyzer.

III. Assessment of hepatic fibrosis:

a. Fatty Liver Index (FLI) is calculated as $ey / (1 + ey) 100$, where $y = 0.053 \ln(\text{waist circumference, cm} - 15.745) + 0.139 \ln(\text{GGT, U/L}) + 0.718 \ln(\text{GGT, U/L}) + 0.953 \ln(\text{triglycerides, mg/dL})$

b. Hepatic Steatosis Index [HSI] was determined using the formula: $8 \times \text{ALT/AST} + \text{BMI}$ (plus 2 if type 2 diabetes is present, plus 2 if female).

• The NAFLD-LFS, or NAFLD liver fat score, was computed using the following formula: $1.675 + 0.037 \text{ age (years)} + 0.094 \text{ BMI (kg/m}^2) + 0.013 \text{ platelet (109/L)} + 0.99 \text{ AST/ALT ratio} + 0.66 \text{ albumin (g/dL)}$ 1.13 impaired fasting glucose/diabetes (yes = 1, no = 0) Low NFS (1.445), ambiguous NFS (1.445 to 0.676), and high NFS (> 0.676) were the three possible interpretations of the outcome. (2015, Rinella).

IV. Imaging:

1. Abdominal Ultrasonography: assesses the hepatic surface, median and midclavicular diameters, and echogenicity.

2. NAFLD was identified employing a B-mode ultrasound device with great resolution performed by qualified ultrasonographers. Individuals who had two of the following three characteristics could be diagnosed with fatty liver, as defined by the international recommendation for the detection and treatment of nonalcoholic fatty liver disease; update 2010: (i) the liver's near-field echo is diffusely increased, more than the kidney; (ii) the structure of the intrahepatic duct is unclear; and (iii) the liver's far-

field echo is gradually decreasing (Fan et al., 2011).

The USG findings were then used to classify the degree of severity of the fatty liver: grade 1 (mild) showed a slightly increased echogenicity with normal diaphragm and intrahepatic vessel borders visualization; grade 2 (moderate) had a moderately increased echogenicity with slightly impaired diaphragm or intrahepatic vessel visualization; and grade 3 (severe) was indicated by a markedly increased echogenicity with poor visualization of the diaphragm, the intrahepatic vessels, and the posterior portion of the right lobe (Ferraioli G., 2021).

All ultrasonographic examinations were done by the same radiologist who was blinded to the clinical and laboratory details of the patients.

3. Transient elastography (FibroScan): We used FibroScan® (Touch 502, Echosens, Paris, France), to evaluate liver stiffness. It calculates the speed at which an electromagnetic wave travels throughout skin. The standard probe (M probe), was used by skilled operators to evaluate the liver's stiffness. Ultrasonography time-motion and A-mode imaging on the right lobe of the liver through the intercostal gap were used to facilitate the procedure while the patient was fasting and lying on his back with his right arm stretched to its maximum abduction. A 100% success rate was achieved with 10 successful shots. FibroScan's better-quality criteria (10 valid measurements, 30% IQR/M) were used to calculate the liver stiffness interquartile range (IQR) to the median (IQR/M). Liver stiffness was measured with transient

elastography at a range of values from 2.5 to 75.0 kPa; the average measurement for those in good health is 5.5 kPa. Data had been entered manually and the results were computed automatically by the FibroScan machine. The degrees of hepatic fibrosis are classified based on FibroScan information as following: F0-F1(Kpa 2-7): no or mild hepatic scarring. F2(7.5-10 Kpa): mild scarring of the liver. F3(10-14 Kpa): significant liver scarring. And finally, F4 (14 Kpa or more): extensive liver scarring (cirrhosis). (Afdhal, 2012).

CAP score has been used to find out steatosis grades. CAP score is measured in decibels per meter (dB/m). This score ranges from 100 dB/m to 400 dB/m. If CAP score is less than 237 dB/m (S0= no steatosis). CAP range 237.0-259.0 dB/m (S1= mild steatosis). While CAP range 259.0-291.0 dB/m (S2= moderate steatosis). Finally, 291.0-400.0 dB/m (S3=severe steatosis) (Kamali et al. 2019).

Ethical approval: The local ethical board of South Valley University's Qena Faculty of Medicine accepted the study with the ethical approval code (MED018) 1-3-21. Prior to their enrollment in the trial, the patients gave their informed consent.

Statistical analysis

It was performed on the data using SPSS version 24.0, statistical package for the social sciences. The frequency and percentage of the quantitative and qualitative data were compared using the Student's t-test. The mean standard deviation (MSD) and numbers (%) are presented. The chi-square test was used to compare the non-parametric data. At P 0.05, it was considered significant.

Results

Demographic data: there were 62 males (62%) and 38 females (38%) in the studied patients. As regard age, the mean age of all studied patients was 42.8 ± 4.9 years with minimum age of 32 years and maximum age of 52 years. As regard BMI, the mean BMI of all studied patients was

$31.3 \pm 4.8 \text{ kg/m}^2$ with minimum BMI of 25.3 kg/m^2 and maximum BMI of 46.7 kg/m^2 . As regard WC, the mean WC of all studied patients was $87.2 \pm 12.8 \text{ cm}$ with minimum WC of 70 cm and maximum WC of 114 cm. As regard smoking, there were 34 smokers (34%) in the studied patients. (Table.1).

Table 1. Description of demographic data in all studied patients.

Variables		Studied patients (N = 100)	
Sex	Male	62	62%
	Female	38	38%
Age (years)	Mean \pm SD	42.8 ± 4.9	
	Min – Max	32 – 52	
BMI (kg/m ²)	Mean \pm SD	31.3 ± 4.8	
	Min – Max	25.3 – 46.7	
WC (cm)	Mean \pm SD	87.2 ± 12.8	
	Min – Max	70 – 114	
Smoking	No	66	66%
	Yes	34	34%

Fatty lever grading by US: there was fatty liver grade I in 35 patients (35%), fatty

liver grade II in 35 patients (35%) and fatty liver grade III in 30 patients (30%). (Fig.1).

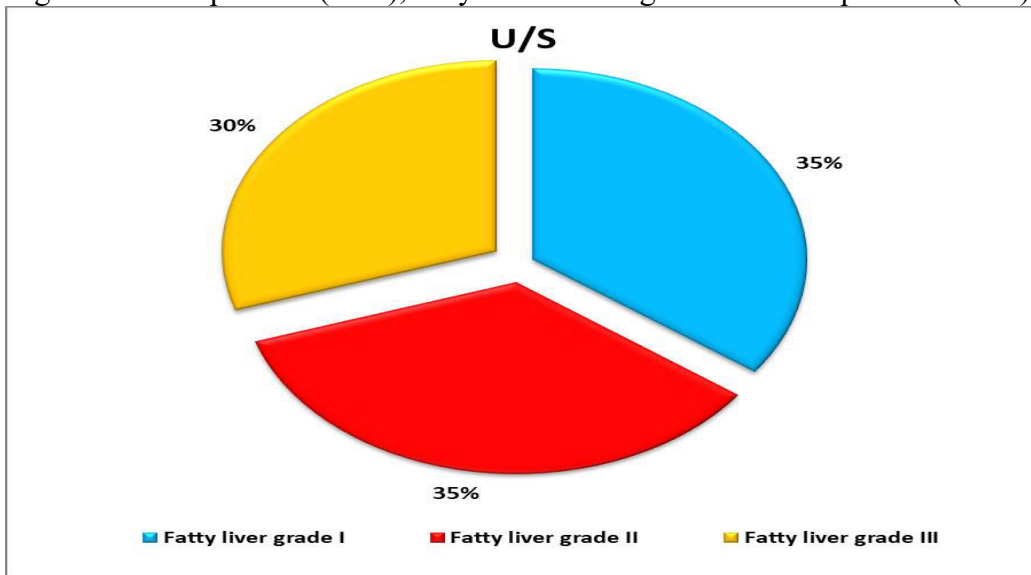


Fig.1. Description of U/S in all studied patients.

Fibro scan Findings: the mean LSM of all studied patients was 6.2 ± 1.89 with minimum LSM of 3.3 and maximum LSM

of 13. The mean CAP of all studied patients was 231.1 ± 73.5 with a minimum CAP of 130 and maximum CAP of 381. (Table .2).

Table 2. Description of Fibro scan in all studied patients.

(n = 100)	Minimum	Maximum	Mean	±SD
LSM	3.3	13	6.2	1.89
CAP	130	381	231.1	73.5

The mean NAFLD Fibrosis score of all studied patients was 0.5 ± 1.1 with minimum NAFLD Fibrosis score of -2.5 and maximum NAFLD Fibrosis score of 2.4 . The mean Fatty Liver Index of all studied patients was 58 ± 21.5 with minimum Fatty

Liver Index of 12.8 and maximum Fatty Liver Index of 97.1 . The mean Hepatic Steatosis Index of all studied patients was 49.3 ± 9.4 with minimum Hepatic Steatosis Index of 34.5 and maximum Hepatic Steatosis Index of 76.1 . (Table.3).

Table 3. Description of Hepatic Fibrosis Biomarkers in all studied patients.

(n = 100)	Minimum	Maximum	Mean	±SD
NAFLD Fibrosis score	-2.5	2.4	0.5	1.1
Fatty Liver Index	12.8	97.1	58.0	21.5
Hepatic Steatosis Index	34.5	76.1	49.3	9.4

There was a highly statistically significant (p -value < 0.001) increased LSM in grade III fatty liver patients (7.5 ± 2.1) when compared with grade II fatty liver patients (5.8 ± 1.8) and grade I fatty liver patients (5.5 ± 1.1). Also, a highly

statistically significant (p -value < 0.001) increased CAP in grade III fatty liver patients (296.8 ± 43.8) when compared with grade II fatty liver patients (236 ± 71.5) and grade I fatty liver patients (169.8 ± 35.8) was found. (Table .4).

Table 4. Relation between U/S and Fibro scan in studied patients.

Variables		U/S (fatty liver)			KW	P-value
		Grade I (n = 35)	Grade II (n = 35)	Grade III (n = 30)		
LSM	Mean	5.5	5.8	7.5	18.7	< 0.001 HS
	±SD	1.1	1.8	2.1		
CAP	Mean	169.8	236.0	296.8	45.7	< 0.001 HS
	±SD	35.8	71.5	43.8		

KW: Kruskal Willis test. HS: p -value < 0.001 is considered highly significant.

There was a statistically significant (p -value = 0.027) increased NAFLD fibrosis score in grade III fatty liver patients (0.9 ± 0.5) when compared with grade II fatty liver patients (0.5 ± 1.1) and grade I fatty liver patients (0.2 ± 1.3). Also, there was a highly statistically significant (p -value < 0.001) increased fatty liver index in grade III fatty

liver patients (74.7 ± 13.7) when compared with grade II fatty liver patients (54.2 ± 21.2) and grade I fatty liver patients (47.7 ± 19). No statistically significant difference (p -value = 0.716) between studied fatty liver grades (I, II & III) was found, as regards hepatic Steatosis index. (Table .5).

Table 5 . Relation between U/S and Hepatic Fibrosis Biomarkers in studied patients.

Variables		U/S (fatty liver)			KW	P-value
		Grade I (n = 35)	Grade II (n = 35)	Grade III (n = 30)		
NAFLD Fibrosis score	Mean	0.2	0.5	0.9	7.2	0.027 S
	±SD	1.3	1.1	0.5		
Fatty Liver	Mean	47.7	54.2	74.7	28.7	< 0.001

Index	±SD	19.0	21.2	13.7		HS
Hepatic Steatosis Index	Mean	49.5	48.4	50.3	0.66	0.716 NS
	±SD	9.3	9.2	10.1		

Discussion

The current study's findings indicated a connection between rising BMI and NAFLD. These findings are consistent with those of **Hegazy et al. (2019)**, who found that hepatic steatosis significantly impacted both the weight-to-size ratio and serum triglycerides (P 0.001 and P 0.001, respectively). Even in the absence of obesity, Alo, Younossi et al. discovered that the incidence of NAFLD increased linearly with BMI, triglyceride levels, and low-density lipoprotein cholesterol levels (**Younossi et al., 2018**).

Consistent with our findings, **Mansour and his team** reported that the average BMI ± standard deviation of NAFLD patients was 35.59 ± 5.77 Kg/m² and the waist circumference (cm) was 109.44 ± 11.54. Furthermore, MAFLD is closely associated with weight and metabolic syndrome; additionally, hepatic steatosis and fibrosis showed a statistically significant positive correlation with BMI, WC (**Mansour et al., 2020**). Furthermore, **Hu and his colleagues**. reported that the BMI was higher in the NAFLD group than in the non-NAFLD group (**Hu et al., 2018**).

According to our findings, patients with grade III fatty liver had a statistically significant higher (LSM) compared to patients with grade II fatty liver and grade I fatty liver. Additionally, patients with grade III fatty liver had a statistically significantly higher Controlled Attenuation Parameter (CAP) compared to patients with grade II fatty liver and grade I fatty liver.

Also, we found that patients with grade III fatty liver had a statistically significant increase in NAFLD fibrosis score compared to patients with grade II fatty liver and grade I fatty liver. Additionally, patients with grade

III fatty liver had a statistically significant increase in fatty liver index compared to patients with grade II fatty liver and patients with grade I fatty liver.

The current study's findings concurred with a cross-sectional analysis of 90 adult NAFLD patients identified by abdominal ultrasonography, which was carried out by **Mansour et al. (2020)**.

We found a statistically significant correlation between liver fibrosis and NFS value. In concordance with our results, there was a study done by **Mansour and his colleagues**, who looked at 60 persons with fatty liver and who were not alcoholics utilizing TE to evaluate the LSM, FIB-4 score, NFS, and fibrotest in the diagnosis of liver stiffness based on hepatic sonar guided biopsy. They concluded that the NFS score and the LSM via TE were shown to be statistically correlated.

In agreement with our findings, also a study made by **Motamed and his colleagues** on 5052 people, discovered a significant positive correlation between serum Fatty Liver Index (FLI) and NAFLD. They illustrated that the probability of getting NAFLD increased by 5.8% for every unit higher FLI. This correlation was verified using binary regression. Moreover, their study showed outstanding prediction in the diagnosis of NAFLD (**Motamed et al. 2016**).

Many other studies have shown that individuals with NAFLD had significantly higher mean FLI, BMI, WC, TG, and GGT readings than those without the illness. These findings are supported by the **Dehnavi et al.** study, which included 212 patients with NAFLD. Additionally, they discovered a substantial link between FLI and NAFLD. Additionally, they found that

for every unit increase in FLI, the probability of developing NAFLD increased by 6.2% (Dehnavi et al. 2018).

Conclusion

Transient elastography (TE) (FibroScan) findings including LSM and CAP has significant positive correlation with the US's grading of NAFLD. There was significant positive correlation between scores of liver fibrosis (FLI and NFS) and grading of NAFLD using US

Conflict of Interest: There are no competing interests between the authors and this publication.

List of Abbreviations

Abbreviation	Full Term
BMI	Body Mass Index
CAP	Controlled attenuation parameter
CBC	Complete Blood Count
DM	Diabetes Mellitus
FLI	Fatty Liver Index
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HSI	Hepatic Steatosis Index
IU/ml	International Unit/Milliliter
LSM	liver stiffness measurement
NAFLD	Non-Alcoholic Fatty Liver
NASH	non-alcoholic steatohepatitis
NFS	Nonalcoholic fatty liver disease fibrosis score
SD	Standard Deviation
SPSS	Statistical Program for Social Science
TG	Triglycerides
TE	Transient elastography

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