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Diabetic and Non-Diabetic Non-Alcoholic Fatty Liver Disease Patients: Assessment Using Non-Invasive Diagnostic Tools

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) incidence is rapidly increasing.

Objective: Our study aimed to assess diabetic and non-diabetic NAFLD using non-invasive imaging tools; conventional US, Chemical shift MRI, and MRE to evaluate the degree of liver steatosis and fibrosis.

Patients and methods: In this case-control study, a total of 130 subjects including 90 NAFLD patients {50 diabetic (Group I) & 40 non-diabetic (Group II), as well as 40 controls (Group III) }, were enrolled. The mean age in the present study was 51.46 ± 7.54 years in group I, 48.40 ± 7.47 years in group II, and 49.55 ± 7.95 years in group III (controls). Most of our subjects were females (78%, 77.5%, and 65% in groups I, II, and III respectively). Assessment of liver steatosis was done using conventional US and chemical shift (in-phase and out-of-phase) MR imaging. Assessment of liver stiffness to detect the degree of fibrosis/cirrhosis was done using magnetic resonance enterography (MRE).

Results: Diabetic NAFLD patients (group I) had a higher degree of liver steatosis (scores 2 and 3), measured by conventional US, compared to group II (non-diabetic NAFLD). Group I patients had a significantly higher degree of liver steatosis (p = 0.007), measured by chemical shift (in-phase and out-phase) MRI, compared to group II. Liver stiffness measurement by MRE was significantly higher (p = 0.038) in group I (Mean 2.88 ± 0.81 kPa) than in group II (mean 2.46 ± 0.71kPa). Liver fibrosis (≥ F2) was significantly higher (p = 0.021) in group 1 (20 of 50 patients (40%)) than in group II (7 of 40 patients (17.5%)).

Conclusions: Current non-invasive imaging methods demonstrated their value as non-invasive imaging biomarkers to evaluate the degree of liver steatosis in NAFLD and its progression into fibrosis/cirrhosis.

Keywords: NAFLD, Diabetic, Non-diabetic, Non-invasive, US, MRI, Chemical-shift, MRE.

INTRODUCTION

The incidence of non-alcoholic fatty liver disease (NAFLD) is on the rise, particularly in Western nations. Increasing rates of obesity, unhealthy fast food consumption, sedentary lifestyles, an increase in childhood obesity, and an extended life expectancy are all potential explanations [(1)].

Egypt is one of the top ten countries with the highest obesity rates worldwide. Although there is little evidence on the scale of metabolic-associated fatty liver disease (NAFLD) in Egypt, existing statistics indicate that the country has one of the highest MAFLD prevalence rates [(2)].

In cases where NAFLD is suspected to be the underlying disease or a coexisting condition, steatosis should be diagnosed using US, as it is a more accessible and cost-effective method compared to MRI [(3)]. Ultrasound has a sensitivity of 81.8-100% and a specificity of 98% when diagnosing NAFLD with mild to severe steatosis. When the hepatic fat concentration is less than 20%, the sensitivity reduces to 55%. Sensitivity and specificity for computed tomography (CT) in detecting NAFLD are 50-85% and 75-87%, respectively. CT, like US, is more reliable in detecting mild to severe steatosis. MRI offers the greatest diagnostic accuracy for fatty liver disease, detecting as little as 3% fatty infiltration [(4)].

The usual approach for diagnosing nonalcoholic steatohepatitis (NASH) is liver biopsy, which is the only test capable of distinguishing among NAFL and NASH, regardless of sample variability [(5)].

The stage of fibrosis is a critical indicator for the development of other co-morbidities, including cardiovascular disease and type 2 diabetes (T2DM), and the most significant determinant of liver-related progression and long-term outcomes, including mortality, in patients with NAFLD. This emphasizes the significance of accurately diagnosing fibrosis [(6)]. Nowadays, functional MRI is the most effective radiological noninvasive method for detecting hepatic fibrosis and steatosis in NAFLD patients [(7)].

Our study's goal was to analyze diabetic and non-diabetic NAFLD utilizing non-invasive conventional diagnostic imaging methods (Conventional US, Chemical shift (in-phase and out-of-phase) MRI, and MRE to evaluate the degree of liver steatosis and fibrosis.

PATIENTS AND METHODS

From August 2020 to January 2022, this case-control research was carried out at the National Liver Institute (NLI), Menoufia University, Egypt, in the departments of Hepatology and Gastroenterology and Diagnostic Medical Imaging and Intervention Radiology.

The study included 130 subjects who were divided into three groups:

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- **Group I:** Involved (50) NAFLD subjects with type 2 DM
- **Group II:** Involved (40) non-diabetic NAFLD subjects.
- **Group III:** Involved (40) healthy individuals as controls (both age- and gender-matched)

Inclusion criteria: Age (18-65) years old. Bright fatty liver by ultrasonography.

Exclusion criteria: Age less than 18. Other causes of chronic liver disease (autoimmune, viral, and metabolic). Individuals who exceed the weekly alcohol consumption limit of 14 drinks for women and 21 drinks for men. ≥10% weight loss within 6 months. Patients utilizing medicines that induce fatty liver include amiodarone and steroids. Patients with hepatic encephalopathy, hepatic decompensation, ascites, variceal bleeding. Patients with chronic kidney diseases, autoimmune diseases, thyroid abnormalities, malignancy, or patients with sepsis. Patients have claustrophobia or metallic objects (For MRI examination).

All subjects underwent:

1. **Complete history taking:** Particularly: Age, gender, history of comorbidities: DM, HTN, or thyroid abnormalities, and drug history.
2. **Physical examination including anthropometric measurements:**
   Systolic and diastolic blood pressure measurement. Circumference of the waist in centimeters. Circumference of the hip in centimeters. Wight (kg). Height (m). [BMI] is measured in kg/m^2, and waist/hip circumference ratio.
3. **Laboratory investigations:**
   Liver function tests (AST, ALP, ALT, GGT, INR & S. albumin), CBC, RFT. Lipid profile (total cholesterol, HDL, LDL & s. triglycerides). HBsAg and HCV Ab. Fasting blood sugar. HbA1c. Fasting insulin, and HOMA-IR.

4-Radiological Investigations:

I. Conventional ultrasonography (US):

Conventional ultrasonography examination was done for all subjects using the US system (iU22, Philips Medical Systems, Bothell, WA, USA). Steatosis presents as increased echogenicity in liver tissue relative to kidney tissue. Steatosis is graded ultrasonographically as non-existent (Score 0), mild (Score 1), moderate (Score 2), or severe (Score 3) **[8].** Conventional US can also reveal signs of cirrhosis (Coarse parenchyma, surface irregularity, caudate lobe prominence, and hepatic vessel attenuation).

II. Magnetic resonance imaging (MRI):

A:Chemical shift (In-Phase and Out-Phase) MR imaging (for assessment of hepatic steatosis): It provides two high-quality anatomic images of the liver, "weighted to T1" and acquired in two distinct phases IP and "out-phase" (OP) which facilitate the qualitative and visual identification of adipose tissue. The liver parenchyma exhibits an identical signal in both the IP and out-phase images in a healthy individual. Steatosis patients exhibit signal decay in "out-phase" images, which results in a darkening of the liver image.

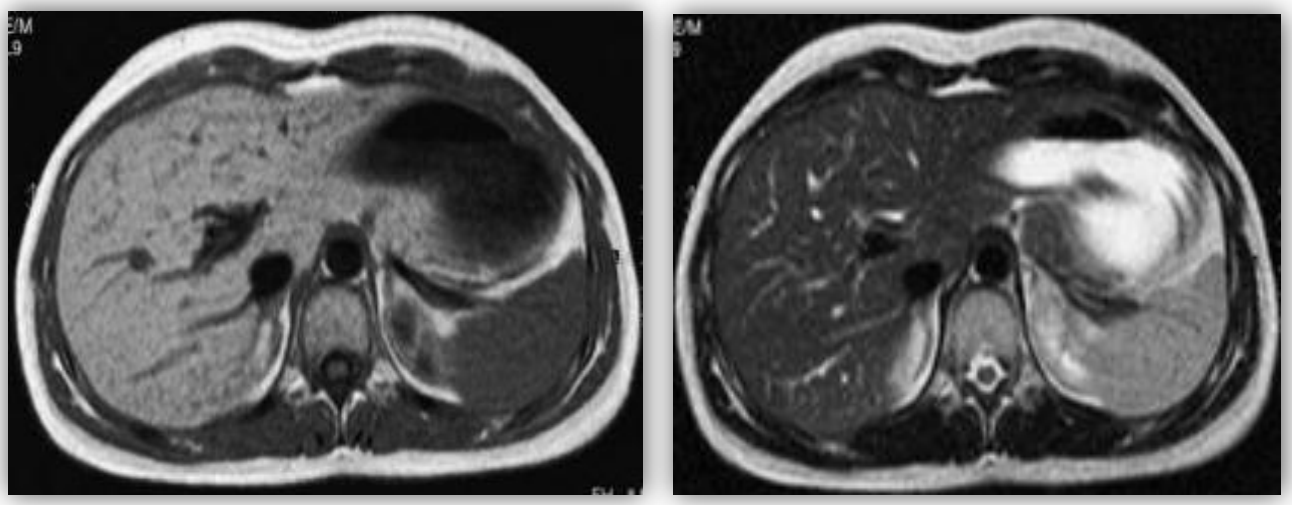
MR (in-phase & out-phase) (IPOP) imaging was performed for all patients utilizing a 1.5-T MR imaging system (GE, USA; 1.5 T Optima 450W GEM suite). Six hours of fasting were prescribed for the patients prior to the MR examination. An integrated body coil and a linear general-purpose flexible surface coil were used. A coronal reconnaissance image of the abdomen was acquired. Following that, transverse T1-weighted dual-echo fast spoiled gradient-recalled images of the whole liver during intake and exhale. The imaging parameters consisted of the following: 75-100 ms for repetition time, 4.6 (IP) or 2.3 (out-phase) msec for echo time, 7-8 mm for section thickness, and a 23-second acquisition time.

Image Interpretation:

In the absence of the patient's clinical examination, medical history, and pathological findings, the radiologist utilized qualitative and quantitative methods to interpret the MR examinations on a picture archive and communication system (PACS) workstation in order to determine the presence and extent of hepatic steatosis.

1-Qualitative Assessment:

An assessment of the overall image quality was conducted subjectively. The identification of fatty infiltration was conducted by analyzing distinctive signal characteristics, which were identified as areas of the liver exhibiting a reduced signal intensity (SI) on out-phase (OP) images relative to IP images. SI alterations were observed in skeletal muscle and the spleen, which were utilized as reference tissues due to their low intracellular lipid content **(Figure 1).**



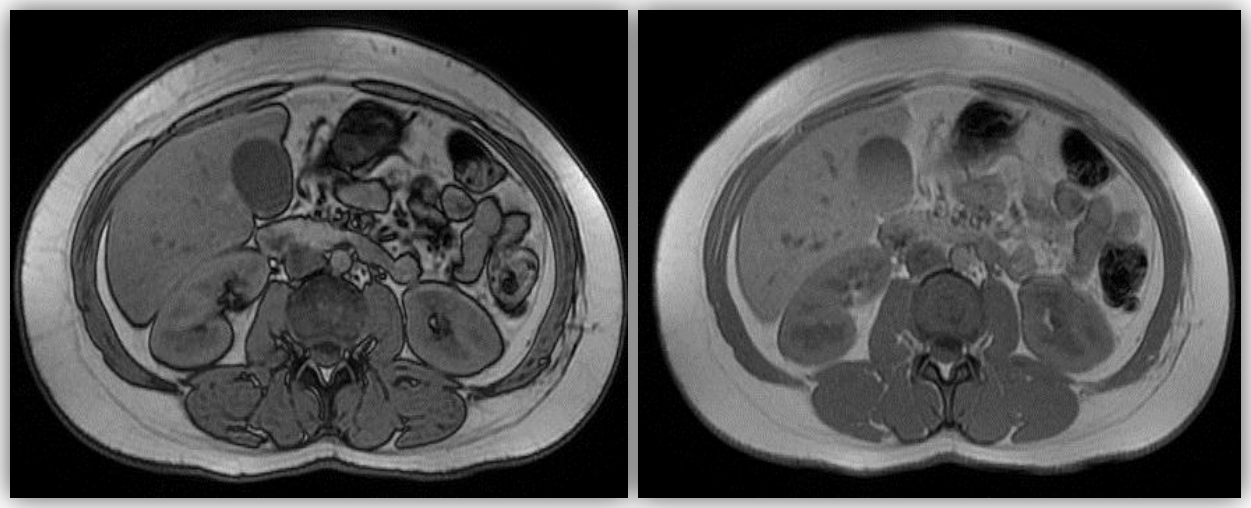
(a) **(b)**

Figure (1): A case of NAFLD in our study; (a) T1-weighted non-contrast axial MRI image, the liver appears brighter than the spleen, (b) T2-weighted non-contrast axial MRI image, the liver appears darker than the spleen.

2-Region-of-Interest (ROI) Analysis:

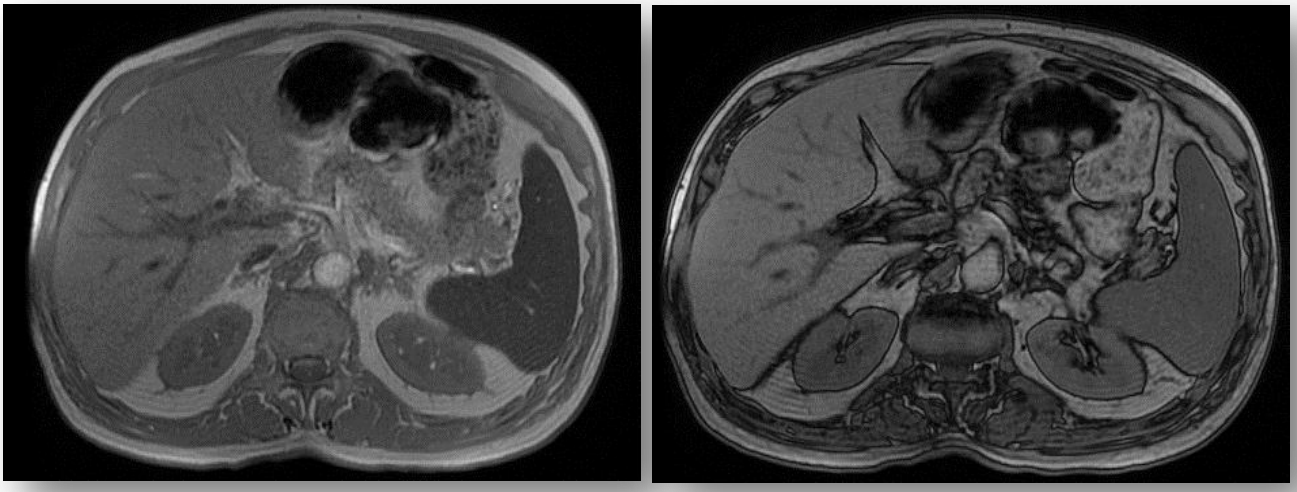
T1-weighted IP and OP sequence signal intensities from regions of interest (ROI) in the liver were documented. A 2 cm^2 region was designated as the area of interest in order to encompass a representative portion of the parenchyma devoid of any artifacts or blood vessels. In order to avoid motion artifacts and areas with vasculature, for each sequence, the region of interest in the liver parenchyma was matched at the same location. Using an equation derived from the **SI** differences between IP and out-phase images, fat indices **(FI)** were calculated: **FI = (SI in – SI out) ÷ (SI in) X100**

The steatosis grades were classified using the FI equation as follows: grade 0 (minimal steatosis) ≤ 5%, grade 1 (mild) > 5–≤ 33%, grade 2 (moderate) > 33–≤ 66%, and grade 3 (severe) > 66% **(9)** (Figures 2, 3 and 4**).**



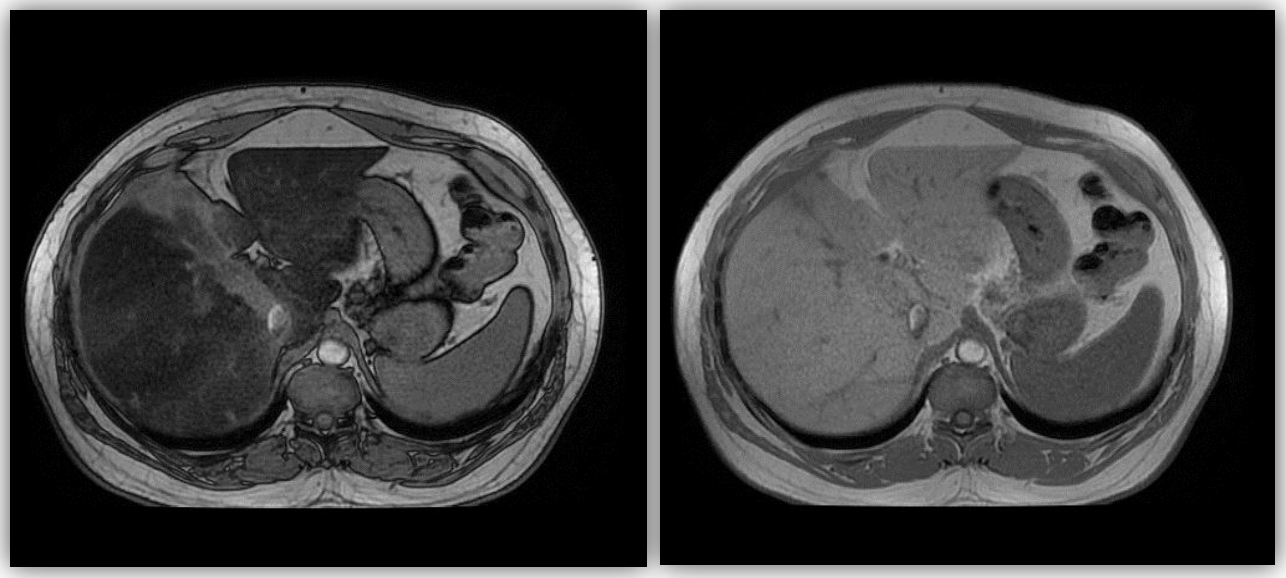
(a) Out-of-phase. **(b) In-phase.**

Figure (2): A 40-year-old non-diabetic male with diffuse hepatic hyperechogenicity by US (Score1). BMI was 31. Out-of-phase MR image of the liver at the level of segments V, and VIII show high signal intensity with its value of (980) (a). In-phase MR image of the liver at the same level of segments V, and VIII showed a drop of the signal intensity with the mean value of 867 (b). MR IPOP result by the FI equation was 11.5% , (grade 1) mild steatosis.



(a) Out-Phase MRI. **(b) In-phase MRI.**

Figure (3): A 58-year-old diabetic female with diffuse hepatic hyperechogenicity by US (Score 2). BMI was 40.8. Out-phase MR image of the liver at the level of segment VI showed high signal intensity with a mean value of 400 (a). In-phase MR image of the liver at the same level of segment VI showed a drop of the signal intensity with a mean value of 260 (b). MR IPOP result by the FI equation was 35.5%, (grade II) moderate steatosis.



(a) Out-of-phase MRI **(b) In-phase MRI**

Figure (4): A 48-year-old diabetic male patient with diffuse hepatic hyperechogenicity by US (Score 3). BMI was 34.3. Out-phase MR image of the liver at the level of segments VII and VIII showed high signal intensity with a mean value of (503) (a) . In-phase MR image of the liver at the same level of segments VII and VIII showed a drop of the signal intensity with a mean value of (160) (b). MR IPOP result by the FI equation was 68.2%, (grade III) marked steatosis.

B-Magnetic resonance elastography (MRE): (for evaluation of liver stiffness). Using a 1.5-T MR imaging system (1.5 T Optima 450W GEM suite, GE, USA)**.(Figure 5)**

Hepatic MRE protocol:

Mechanical shear waves can be created by transmitting a continuous longitudinal vibration at 60 Hertz from the active driver to the passive driver. The acquisition of these waves was achieved by employing a specific MRI pulse sequence that incorporated synchronized motion sensitizing gradients (MSGs). These gradients were designed to produce a shear wave image, which depicted the micron-level cyclic displacements induced by the propagating waves, in which the amount of wave displacement was measured in micrometers (μm); stiff tissue showed longer wavelength, while the softer tissue showed shorter wavelength.

Automated processing of the obtained data in the wave images with an inversion algorithm to create (elastogram images), which are quantitative maps with mechanical properties for estimation of tissue stiffness in Kilopascal (kPa). In order to conduct LSM, ROI was delineated on the elastograms.

ROIs encompassed areas of the liver that exhibited adequate wave amplitude while preventing edge effects (at least one-half wavelength spacing from the liver margin). This included large vessels, the gallbladder fossa, and any regions susceptible to cardiac and vascular anomalies. Ample-amplitude planar shear waves "illuminated" the entire liver parenchyma in the cross-section in an even fashion. The mean LSM of the liver was calculated by averaging the mean ROIs of the four obtained sections.

The thresholds utilized to differentiate fibrosis into stages 1, 2, 3, and 4 were 2.88, 3.54, 3.77, and 4.09 kPa, respectively [18] .

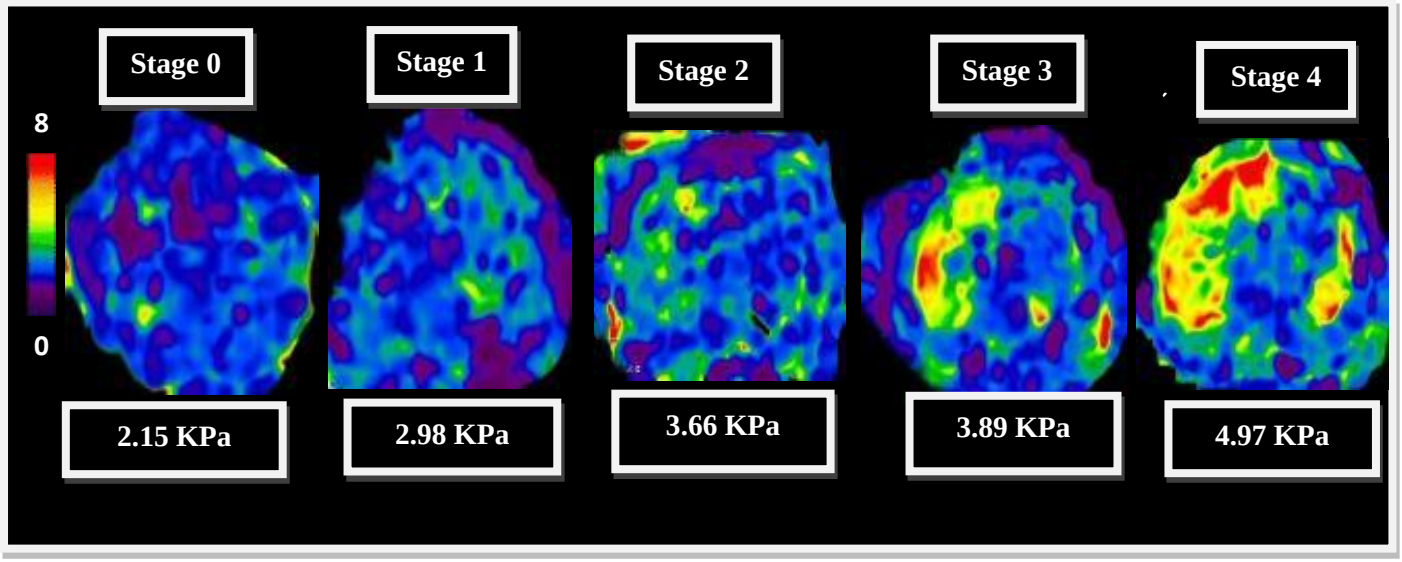


Figure (5): MRE stiffness maps of 5 patients in with NAFLD and different stages (stage 0, 1, 2, 3 and 4) of liver fibrosis. As shown in the color lookup table at the right, the stiffness values rangeed from near zero (dark purple) to 8 kPa (red). The MRE-determined mean liver stiffness was shown at the bottom of each image. Notice that the stiffness values were greater in patients with more advanced fibrosis.

Ethical approval: All study protocols were approved by the NLI Institutional Review Board at Menoufia University, and all participants provided informed consent. The Helsinki Declaration was observed throughout the study's conduction.

Statistical analysis

SPSS version 22.0 for Windows® was used to code, process, and analyse the gathered data. Frequencies and relative percentages were used to display the qualitative data. The mean ± SD was used to convey quantitative data. Pearson coefficient, X^2-test, Fisher's exact or Monte Carlo correction, F-test (ANOVA), Spearman coefficient, and Mann-Whitney test were the tests used to analyze data. P values with two tails ≤ 0.05 were regarded as significant.

RESULTS

In total 130 subjects including 90 NAFLD patients {50 diabetic (Group I) & 40 non-diabetic (Group II), as well as 40 controls (Group III)}, were enrolled. The mean age in the present trial was 51.46 ± 7.54 years in group I (diabetic NAFLD), 48.40 ± 7.47 years in group II (nondiabetic NAFLD) and 49.55 ± 7.95 years in group III (control). Most of our subjects were females (78%, 77.5% and 65% in groups I, II and III respectively). The studied groups were sex- and age-matched **(Table 1)**.

Table (1): Demographic data among the studied patients.

	Group I (n = 50)		Group II (n = 40)		Group III (n = 40)		p
	No.	%	No.	%	No.	%	
Sex:							
• **Male**	11	22	9	22.5	14	35	0.310
• **Female**	39	78	31	77.5	26	65	
Age (years) :							
• **Min. – Max.**	39– 60		39 – 60		38– 60		0.161
• **Mean ± SD.**	51.46 ± 7.54		48.40 ± 7.47		49.55 ± 7.95		

Mean ± SD: (mean± standard deviation), No: number, Min. – Max: minimum-maximum,p: p-value for comparing between the studied groups.

In comparison with the control group, all anthropometric measurements (weight, BMI, waist circumference (females), hip circumference (females), waist/hip ratio (females), and weight) differed significantly among NAFLD subgroups ($p < 0.001$). In a similar fashion, male diabetic NAFLD patients (group I) exhibited significant differences in waist circumference, hip circumference, and waist-to-hip ratio compared to the control group (group III). No significant anthropometric differences were observed among the subgroups of NAFLD **(Table 2)**.

Table (2): Comparison among the three studied groups according to anthropometric measurements**.**

	Group I (n = 50)	Group II (n = 40)	Group III (n = 40)	p	Sig. bet. Groups		
					I vs II	I vs III	II vs III
Waist circumference (cm):							
• **Male:**							
Min. – Max.	77 – 132	77 – 119	79– 99	**0.001***	0.317	**0.001***	0.067
Mean ± SD.	112.09 ± 15.57	104.1 ± 13.91	92.14 ± 6.35				
• **Female:**							
Min. – Max.	92 – 140	90 – 140	77 – 94	**<0.001***	0.666	**<0.001***	**<0.001***
Mean ± SD.	117.82 ± 11.54	115.6 ± 12.12	86.85 ± 4.42				
Hip circumference (cm):							
• **Male:**							
Min. – Max.	88 – 133	97 – 131	97 – 112	**0.029***	0.359	**0.022***	0.460
Mean ± SD.	116.64 ± 11.74	111 ± 10.71	106.36 ± 4.2				
• **Female:**							
Min. – Max.	103 – 148	106 – 160	97 – 111	**<0.001***	0.853	**<0.001***	**<0.001***
Mean ± SD.	125.9 ± 11.7	124.61± 11.25	105.65 ± 4.4				
Waist/hip ratio:							
• **Male:**							
Min. – Max.	0.78 – 1.15	0.79 – 1.05	0.79 – 0.94	**0.020***	0.888	**0.024***	0.099
Mean ± SD.	0.95 ± 0.1	0.94 ± 0.07	0.87 ± 0.05				
• **Female:**							
Min. – Max.	0.84 – 1.04	0.77 – 1.06	0.79 – 0.91	**<0.001***	0.884	**<0.001***	**<0.001***
Mean ± SD.	0.94 ± 0.05	0.93 ± 0.07	0.82 ± 0.02				
Weight (Kg):							
Min. – Max.	56.1 – 121.3	65.5 – 116	52.2 – 75.6	**<0.001***	0.918	**<0.001***	**<0.001***
Mean ± SD.	93.50 ± 15.67	94.53 ± 11.36	64.55 ± 7.23				
BMI(Kg/m^2):							
Min. – Max.	18.3 – 50.1	20.2 – 44.9	19 – 24.9	**<0.001***	0.159	**<0.001***	**<0.001***
Mean ± SD.	37.22 ± 6.84	35.13 ± 5.60	23.38 ± 1.77				

No: number, M ± SD: mean± standard deviation, Min. – Max: minimum -maximum, p: p-value for comparing between the studied groups, BMI: body mass index.

Regarding laboratory characteristics, NAFLD subgroups (I, II) had significantly higher values as regards AST and ALT compared to control group (III). Patients with diabetic NAFLD (group I) had a significantly lower serum albumin level (p=0.011) than controls (group III). The tabulated lipid profile parameters, showed a significant difference among the studied groups as regards total cholesterol and LDL, while triglycerides showed a significant difference among NAFLD subgroups and control group at the verge of significance. Moreover, HDL showed a significant difference among diabetic NAFLD and the control group (p=0.022). Diabetic subjects with non-insulin-dependent NAFLD (group II) had significantly lower levels of FBS and HBA1c than non-diabetic subjects with NAFLD (group I) (p=0.001). Serum insulin levels were also higher in NAFLD subgroups than in the control group, but there was no distinction between diabetic and non-diabetic NAFLD. Additionally, HOMA IR revealed a statistically significant difference among all groups examined, with the greatest value observed in diabetic NAFLD (group I).

Regarding liver steatosis measurement in NAFLD subgroups, diabetic NAFLD patients (group I) had a significantly higher degree of liver steatosis (scores 2 and 3), measured by conventional US compared to group II (non-diabetic NAFLD) **(Table 3).**

Table (3): Comparison between group I (diabetic NAFLD) and group II (nondiabetic NAFLD) according to the grades of liver steatosis, measured by ultrasonography according to **Mottin *et al.*** [8]

Grade of steatosis	Group I (n = 50)		Group II (n = 40)		Group III (n = 40)	
	No.	%	No.	%	No.	%
• **Absent (score 0)**	0	0	0	0	40	100
• **Mild (score 1)**	25	50	28	70	0	0
• **Moderate (score 2)**	17	34	10	25	0	0
• **Sever (score 3)**	8	16	2	5	0	0

\The extent of liver steatosis in patients with diabetic NAFLD (group I) was significantly greater than in group II (non-diabetic NAFLD), as determined by chemical shift MRI (p-value 0.007) **(Table 4)**.

Table (4): Comparison between group I (diabetic NAFLD) and group II (nondiabetic NAFLD) according to the degree of liver steatosis, measured by chemical shift (in-phase and out-of-phase) MRI.

Steatosis MRI (%)	Group I (n = 50)	Group II (n = 40)	p
• **Min. – Max.**	6– 89	5.3– 78	**0.007***
• **Mean ± SD.**	44.78 ± 23.41	32.15 ± 20.9	

Measuring liver steatosis by equation (**FI= (S_{in} – SI_{out}) ÷ (SI_{in}) X100**, **SI_{in}** (signal intensity in phase), SI_{out} (signal intensity out phase). The following grading system was used for reporting steatosis grades (according to FI equation): grade 0 (minimal steatosis) ≤ 5%, grade 1 (mild) > 5–≤ 33%, grade 2 (moderate) > 33– ≤ 66% and grade 3 (severe) > 66% [9].

There was a significantly positive correlation among the degree of liver steatosis by MRI and anthropometric measurements (waist circumference and BMI) (p-value <0.001), and triglyceride level (p-value 0.001) in NAFLD group **(Table 5).**

Table (5): Correlation between the degree of liver steatosis by chemical-shift MRI and different parameters

	Liver Steatosis MRI(%)					
	Total patients		Group 1		Group 2	
	r_s	p	r_s	p	r_s	P
• **Waist circumference(cm)**	0.737	**<0.001***	0.668	**<0.001***	0.668	**<0.001***
• **BMI(Kg/m^2)**	0.637	**<0.001***	0.738	**<0.001***	0.738	**<0.001***
• **T. Cholesterol (mg/dl)**	0.258	0.070	0.118	0.468	0.118	0.468
• **LDL (mg/dl)**	0.208	0.147	0.088	0.590	0.112	0.491
• **HDL (mg/dl)**	-0.082	0.570	0.112	0.491	0.088	0.590
• **Triglycerides (mg/dl)**	0.468	**0.001***	0.169	0.296	0.169	0.296

rs: Spearman coefficient

The measurement of liver stiffness (LSM) using MRE was significantly greater in the diabetic NAFLD group (mean 2.88 ± 0.81 kPa) (p-value 0.038) compared to the non-diabetic NAFLD group (mean 2.46 ± 0.71 kPa) **(Table 6).**

Table (6): Comparison between NAFLD subgroups according to liver stiffness measurement (LSM) by MRE

LSM (MRE) (kPa)	Group I (n = 50)	Group II (n = 40)	p
Min. – Max.	1.6 – 4.72	1.4 – 4	**0.038***
Mean ± SD.	2.88 ± 0.81	2.46 ± 0.71	

LSM: liver stiffness measurement, MRE: magnetic resonance elastography, kPa: kilopascal, Min. – Max: minimum-maximum, M ± SD.: mean± Standard deviation: p-value for comparing between the studied groups, cut-offs for distinguishing stage1, stage 2, stage 3 and stage 4 fibrosis were 2.88, 3.54, 3.77, and 4.09 kPa**.**

Diabetic NAFLD patients (20 of 50 patients, or 40%) had significantly more significant liver fibrosis (≥ F2) than nondiabetic NAFLD patients (7 of 40 patients, or 17.5%) (group II), with a p-value of 0.021 **(Table 7).**

Table (7): Comparison between NAFLD subgroups according to fibrosis stages by MRE.

Liver Fibrosis	Group I (n = 50)		Group II (n = 40)		p
	No.	%	No.	%	
F0	23	46	25	62.5	0.119
F1	7	14	8	20	0.448
F2	11	22	6	15	0.399
F3	6	12	1	2.5	0.094
F4	3	6	0	0	0.251
<F2	30	60	33	82.5	**0.021***
≥ *F2*	20	40	7	17.5	

No: number, p: p-value for comparing between the studied groups, cut-offs for distinguishing stage 1, stage 2, stage 3, and stage 4 fibrosis were 2.88, 3.54, 3.77, and 4.09 kPa.

DISCUSSION

NAFLD is significantly more prevalent globally than was previously estimated, and its alarming rate of increase continues. According to a study by **Riazi *et al.*** [(11)] that was recently published, the global prevalence of NAFLD is estimated to be 32.4%. From 2005 to 2016, the prevalence increased substantially, from 25.5% prior to that year to 37.8%**.** A multitude of guidelines advise the implementation of primary care surveillance for NAFLD patients with T2DM and prediabetes, owing to the elevated risk of advanced fibrosis that these individuals encounter [(12)]**.**

This trail aimed to assess the degree of liver steatosis and fibrosis in both diabetic and nondiabetic NAFLD using noninvasive diagnostic tools, conventional US, chemical shift (in-phase and out-phase) MRI, and MRE and its relation to laboratory and clinical data. In this study, we enrolled 130 subjects including 90 NAFLD patients (50 diabetic & 40 non-diabetic) as well as 40 controls to assess the degree of liver steatosis and fibrosis in both diabetic and non-diabetic NAFLD using functional MRI. The mean age in the present trial of NAFLD subjects with T2DM was 51.46 ± 7.54 years, while it was 48.40 ± 7.47 years in the non-diabetic NAFLD group, which is comparable to several other studies in which NAFLD subjects with T2DM were more likely to be older [(13,14)]**.**

Most NAFLD patients in our study were females (78%) of diabetic NAFLD and 77.5% of non-diabetic NAFLD. NAFLD is more prevalent among men than among women, according to a number of prior trials [(15)]**.** In a recent investigation, **Succurro *et al.*** [(16)] examined whether there are any disparities in the prevalence of NAFLD, prediabetes, and T2DM based on gender. Comparatively, the incidence of NAFLD was higher among diabetic women than men. This phenomenon could be attributed to the correlation between impaired glucose homeostasis and a more severe exacerbation of metabolic risk factors in females, thereby explaining the greater impact of T2DM on NAFLD in this demographic. The inconsistency in question may be ascribed to the neglect of glucose tolerance status in a multitude of inquiries [(17)]**.**

In the present trial, subjects underwent anthropometric measurements. NAFLD subgroups exhibited significantly greater waist circumference (WC), waist/hip circumference ratio, and BMI values than the control group (p<0.001). These results are previously validated by numerous studies examining the anthropometric measurements of individuals with NAFLD [(18, 19)]**.**

In this trial, there was a significant difference between diabetic and non-diabetic NAFLD (p<0.001) as regards the presence of metabolic syndrome features

(88% Vs 47.5%) and this agrees with **Tanase *et al.*** [20] who found that T2DM NAFLD had more IR, visceral obesity, more triglycerides, and lower HDL**.**

Regarding laboratory data, significantly higher AST & ALT levels (p <0.001) were found in NAFLD subgroups than in the control group. Elevated transaminases are the most conspicuous, as previously documented [21]**.** Compared to controls (group III), diabetic NAFLD (group I) had significantly elevated gamma-glutamyltransferase (GGT) (p0.001) and uric acid (p0.012). According to a study by **Tekeli *et al.*** [22]**,** it confirmed the relationship between high serum GGT levels and hyperuricemia in T2DM and metabolic syndrome. Patients with diabetic NAFLD (group I) had a significantly lower serum albumin level (p=0.011) than controls (group III). This finding may underscore the renal impact of T2DM [23].

As regards lipid profile in our study, NAFLD subgroups showed significantly higher values of total cholesterol, LDL, and triglyceride than the control group**. Xepapadaki *et al.*** [24] mentioned that the hyperinsulinemia and hyperglycemia that develop in T2DM lead to reduced HDL levels and deterioration of its function via various alterations in its characters**.** Moreover, diabetic NAFLD patients in the current study had significantly higher total cholesterol and LDL (p=0.003) than the non-diabetic NAFLD group. In previous research, a significant difference was identified in terms of total cholesterol and LDL levels between diabetic and non-diabetic NAFLD [25]**.**

In our study, a Conventional ultrasonography examination was done for all subjects. Steatosis is characterized by elevated echogenicity in liver tissue relative to kidney tissue. Steatosis is further categorized into four degrees: absent, mild, moderate, or severe. In contrast, in 2019 a study by **Castera *et al.*** [3] stated that conventional ultrasound is the most frequently utilized non-invasive imaging technique for detecting hepatic steatosis due to its affordability, accessibility, tolerability, and low cost. In patients with a BMI exceeding 40 kg/m^2, however the functionality of US is restricted, and its ability to detect mild steatosis with sensitivity and specificity is inadequate. Furthermore, the quantification of hepatic steatosis is unattainable due to the observer effect of conventional US. In our study, diabetic NAFLD patients (group I) had a significantly higher degree of liver steatosis (scores 2 and 3), measured by conventional US, compared to group II (non-diabetic NAFLD).

In the present trial, MRI was used to assess hepatic steatosis in NAFLD patients by using the chemical shift (In-phase and Out-phase) MRI technique. The technique yields two high-quality anatomical images of the liver, "weighted to T1" and acquired in two separate phases: IP and "out-phase" (OP). These images facilitate the qualitative and visual identification of adipose tissue. The liver parenchyma exhibits an identical signal in both the IP and out-phase images in a healthy individual. Steatosis patients exhibit signal decay in "out-phase" images, which results in a darker appearance of the liver. Diabetes is widely acknowledged as the principal risk factor linked to the development and progression of hepatic steatosis [26]**.**

In our study, the mean liver steatosis measured by chemical-shift MRI in diabetic NAFLD (group I) was 44.78 ± 23.41, which was higher than the degree of steatosis in group II non-diabetic NAFLD (32.15 ± 20.90) with a significant difference (p=0.007). A study conducted by **Gamsiz and Köroğlu** [26] on 116 NAFLD subjects showed that the degree of hepatic steatosis measured by the Controlled Attenuation Parameter (CAP) was significantly higher in diabetic patients (p<0.001).

In the present study, liver steatosis in diabetic NAFLD subjects was positively correlated (p<0.001) with WC and BMI. A similar study described the relation between WC & BMI and NAFLD as classical anthropometric indicators of visceral obesity, which is the most important risk factor for hepatic steatosis [27]**.**

Steatosis does not impact liver-related outcomes in patients with NAFLD, rather it is the advanced stage of fibrosis that does [28]**.** Elastographic techniques can significantly enhance the value of NAFLD screening and diagnostic procedures in diabetic patients, thereby contributing to the management of NAFLD [29]**.**

The aim of liver elastography is to acquire in vivo and non-invasive data regarding the mechanical characteristics of the parenchyma subsequent to subjecting it to a deforming stressor. Elastography can be executed using either magnetic resonance (MRE) or ultrasound (USE). MRE is predicated on the transmission of low-frequency longitudinal wavelengths of approximately 65Hz through an instrument positioned near the liver in the rib cage. Transversal wavelengths, referred to as cuts or shears, are generated from longitudinal wavelengths that traverse the organ. These shears are computed using the shear modulus and are propagated by the liver parenchyma. The units of measurement utilized for quantification are kilopascals (kPa) [30]**.**

In the present investigation, liver stiffness in patients with NAFLD was assessed via MRE. Significant different liver stiffness measurements (LSM) were obtained by MRE in diabetic NAFLD (2.46 ± 0.71 kPa) and non-diabetic NAFLD (2.88 ± 0.81 kPa; p=0.038).

The fibrosis grades were distinguished among the two subgroups of NAFLD. A significant difference in the prevalence of significant liver fibrosis (≥ F2) was observed among patients with diabetic NAFLD (40% vs 17.5%) and those without diabetes. **Park *et al.*** [31] discovered that the diabetic NAFLD group had a greater risk of significant fibrosis than the control group without glucose intolerance NAFLD.

LIMITATIONS

In our work, there was a relatively small number of the studied subjects and the absence of liver biopsy, which is considered the gold standard for detection of liver fibrosis and disease activity degree, were the two important limitations.

CONCLUSION

Conventional US, chemical shift (in-phase and out-phase) MRI, and MRE are non-invasive diagnostic tools that are used to evaluate the liver steatosis degree and the liver stiffness respectively in NAFLD patients either diabetic or nondiabetic, being more accurate and able to overcome the limitations of other non-invasive methods.

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