Assessment of Liver Fibrosis in Non-Alcoholic Fatty Liver Disease Using Real-Time Elastography

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Abstract:

Background: Non-alcoholic fatty liver disease (NAFLD) become a challenge as it`s prevalence, difficulty to diagnose and complex pathogenesis representing about 20% of common liver disorders Patients with NAFLD are at higher risk for adverse outcomes such as cirrhosis, HCC and liver-related mortality. **Aim and objectives :** We aim to assess the applicability and performance of real-time elastography for the diagnosis of liver fibrosis.**Methods:** One hundred and thirty subjects attended damanhour medical national institute hepatology and gastroenterology outpatient clinic were divided into two groups; Group (I): Hundred cases having NAFLD by ultrasound, Group (II): thirty healthy subjects as control. **Results**: Fibrosis stages in NAFLD patients significantly higher than in the control group diagnosed by real time elastrography (P = 0.001). There was an agreement between Realtime Elastrography and FIB-4 index and NAFLD fibrosis score 93% and 86% respectively and diagnostic performance of real time elastrography in advanced liver fibrosis ≥F3 was assessed in comparing with FIB-4 index show sensitivity 90%, specifity 93.3%, PPV 60%, NPV 98.8%, accuracy 93%, AUC0.917, 95%CI 0.81-1.0 and (p = 0.001). **Conclusion :** Real time elastography may be useful in diagnosis of fibrosis in non-alcoholic fatty liver disease.

Key words : Non-alcoholic fatty liver disease (NAFLD), Hepatocellular carcinoma (HCC), Non-alcholic steato hepatitis (NASH), Real time Elastrography(RTE).

Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasing with the increase in the prevalence of obesity and other components of the metabolic syndrome (1).

(NAFLD) diagnosed when there is an evidence of hepatic steatosis by imaging or histology with lack of secondary causes of hepatic steatosis as high alcohol intake or long-term use of a steatogenic medication (2).

Most of patients with NAFLD are commonly associated with metabolic comorbidities such as obesity, DM and hyperlipidemia (2).

The overall global prevalence of NAFLD diagnosed by imaging is around 25.24% (95% CI, 22.10-28.65) the highest prevalence of NAFLD is reported from the Middle East (31.79% [95% CI, 13.48-58.23]) and South America (30.45% [95% CI, 22.74-39.440]) and the lowest prevalence rate is reported from Africa (13.48% [5.69-28.69]) (3).

Patients with NAFLD are at higher risk for adverse outcomes such as cirrhosis and liver-related mortality. (4) It has emerged as a major challenge because of its prevalence, difficulties in diagnosis, complex pathogenesis, and lack of approved therapies (1). As the burden of hepatitis C decreases over the next decade, NAFLD will become the major form of chronic liver disease in adults and children and could become the leading indication for liver transplantation (5).

Liver biopsy is currently the gold standard to determine the degree of fibrosis (6). But it has many drawbacks, approximately 1–3% of patients require hospitalization for complications, and 25% report post-procedural pain in addition, its diagnostic accuracy is strongly influenced by the quality of the specimen and by inter- or intra-observer variation (7).

Nowadays non-invasive methods are commonly used in the diagnosis of liver fibrosis in NAFLD patients include imaging (Elastrography) and biochemical tests such as (NAFLD fibrosis score and FIB-4 Index) instead of liver biopsy to overcome its draw backs as it is invasive and high coast (8),(9).

Real-Time ultrasonic elastography is a helpful noninvasive method for

identifying varying degrees of fibrosis in patients with NAFLD (10).

Real-time elastography (RTE) is technically different from FibroScan as (RTE) capture 2-dimensional (2D) strain images induced by internal heartbeats, and the strain images show progressively increasing patchiness with increasing severity of hepatic fibrosis,therefore, it can be used in obese patients and those with ascites (11)

Study subjects:

This study was conducted as a cross – sectional and case control study on 130 subjects attended to Hepatology and Gastroenterology outpatient clinic in Damanhou Medical National Institute from March 20, 2018, to September 1, 2019 divided in two groups **Group (I):** 100 patients diagnosed with fatty liver by ultrasound were admitted to this study after appropriate informed consent. Patients below the age of 18, patients with significant alcohol consumption, hepatitis C, hepatitis B, patients with symptoms or signs suggestive of haemochromatosis, Wilson's disease, alpha-one anti-trypsin deficiency, autoimmune hepatitis, history of intake of steatogenic medications, patients with decompensated cirrhosis, severe heart failure, severe renal failure or pregnancy were all excluded.

Group (II): 30 healthy individuals served as a control group with normal liver by ultrasound.

The study protocol was approved by the Ethical Committee of Faculty of Medicine, Benha University. An informed written concent was obtained from all patients participating in this study after explaining the study measures in details.

Methods:

For all patients and controls the FIB4-INDEX was calculated according to the formula: (Age x AST) / {Platelets x [sqr (ALT)]}. The result was interpreted as follows: FIB-4 Index <1.45 = F0-F1, FIB-4 Index 1.45-3.25 =F2-F3, FIB-4 Index >3.25 =F4 (12). Also, the NAFLD-FIBROSIS SCORE was calculated for all patients and controls according to the formula: -1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl) and the results were interpreted as follows: NAFLD Score < -1.455 = F0-F2, NAFLD Score -1.455 – 0.675 = Indeterminate score, NAFLD Score > 0.675 = F3-F4 (8). Patients and control subjects underwent real-time elastography (RTE) **(Siemens, Acuson S3000)**. The patients were examined in a supine position with the right arm elevated

above the head. Patients were instructed to continue breathing as usual. Because each elastography image is obtained in a few milliseconds, breathing did not cause any motion artifacts. The examination was performed on the right lobe of the liver through the intercostal space in all patients. An area was chosen where the liver tissue was at least 6 cm thick and was free of large blood vessels. The examination was performed with a 6-MHz transducer because, similar to B-mode imaging, higher frequencies allow better analysis of areas close to the transducer, and assessment of real-time elastography is optimized by the manufacturer on superficial tissues. The measurement depth was between 20 and 50 mm (mean, 35 mm) with a 350–500 mm2 area of measurement (mean, 420 mm2).

Images were evaluated according to the following standards:

One score: all green region of elasticity image with only a few (less than five) blue dot spots.

Two scores: liver elasticity image primarily green colored with blue spots but no confluent blue areas.

Three scores: obvious blue regions (confluent blue areas) collectively less than 50% of the total elastography window.

Four scores: more obvious blue regions collectively more than 50% of the total elastography window.

Score 1 corresponds to the normal liver (F0). Score 2 corresponde to mild insignificant fibrosis (F1 and F2). Scores 3 and 4 correspond to significant fibrosis (F3 and F4) (13), (14).

Statistical analysis :

Data were precoded and entered in Microsoft Excel and and analyzed using Statistical package for Social Science **(IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).** Categorical data were presented as number and percentages while quantitative data were expressed as mean±standard deviation (S.D), median, IQR and range. Chi square test (X2), or Fisher's exact test (FET) were used to analyze categorical variables.

Coordinate of correlation was assessed by Cohen Kappa test was used to assess the degree of agreement between 2 raters.Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P >0.05. Student "t" test was used to analyze normally distributed variables among 2 independent groups. Non-parametric variables were analyzed using Mann-Whitney U test.

Difference among 3 independent means was analyzed using Kruskal Wallis test (KW) for non parametric variables. Significant KW tests was followed by post hoc multiple comparisons using Bonferroni test to detect the significant pairs. Spearman's correlation coefficient (rho) was used to assess correlations. ROC curve analysis was constructed to assess the performance of real time in prediction of fibrosis among patients group.

Results

This prospective cross sectional study conducted on 130 subjects attending Damanhour Medical National Institute Hepatology and Gastroentrology out patient clinic in the period from March 20, 2018, to September 1, 2019. All subjects divided into 2 goups including 100 patients with non-alcholic fatty liver disease (group I) and 30 apparently healthy subjects as control (group II) .

An informed written concent was obtained from all patients participating in this study after explaining the study measures in details.

Table (1) Shows the demographic and laboratory data in the studied groups. The main demographic and laboratory variables of the included patients and control are presented in table 1 which shows statistically significant differences among means of: DM (P<.001), weight P<.001), BMI (P<.001), cholesterol(P<.001), HDL(P<.001), LDL(P<.001), TG(P<.001), AST(P<.001), ALT (P<.001), T.bilirubin (P<.001), S.albumin(P<.001), WBCs(P<.001), FIB-4 score(P<.001) and NAFLD –fibrosis score (P<.001) while other variables showed statistically insignificant differences.

Table (2) shows real time elastrography (RTE) in the studied groups .

Table (3) shows frequency of significant fibrosis among patients with NAFLD according to FIB-4 index, Nafld fibrosis score and RTE as 10%, 16% and 15% of NAFLD patients were categorized as having "significant fibrosis " (F3 & F4) by FIB4-INDEX, NAFLD-FIBROSIS SCORE and RTE respectively ($p > 0.05$).

Table (4) shows the degree of agreement between FIB4-INDEX and NAFLD-FIBROSIS SCORE, on the one hand, and real time elastography (RTE) on the other hand, were 93% & 86% respectively (Kappa test = 0.682 & 0.505; $P < 0.001$).

Table (5) show the degree of agreement between real time elastography and FIB-4 score results in diagnosis degree of liver fibrosis in NAFLD.

Table (6) show the degree of agreement between real time elastrograpgy and NAFLD fibrosis score results in diagnosis of liver fibrosis degree in NAFLD

Table (1): Demographic and laboratory data in the studied groups:

		Group I N=100	Group II N=30	*p*
Age (years)	**mean±SD**	47.1±11.6	43.1±9.9	0.086
Gender	**Males(N,%)**	55 55%	15 50%	0.63
	Females(N,%)	45 45%	15 50%	
Weight(kg)(mean±SD)		94.09 ±17.0	75.0±13.22	<0.001(HS)
Height(cm)(mean±SD)		167.6±6.16	169.1±6.72	0.25(NS)
BMI(mean ±SD), kg/m2		33.4±5.34	26.1±3.62	<0.001(HS)
Diabetes mellitus(N,%)		71(71%)	0(0%)	0.001(HS)
Cholesterol(mean±SD)		236.0±17.7	123.1±16.6	<0.001(HS)
HDL(mean±SD)		58.5±8.33	86.2±9.44	<0.001(HS)
LDL(mean±SD)		139.5±9.71	82.1±11.72	<0.001(HS)
AST(mean±SD,U/L)		35.8±29.4	20.0±5.8	<0.001(HS)
ALT(mean±SD,U/L)		39.7±29.8	19.1±7.3	<0.001(HS)
T.bilirubin(mean±SD,mg/dl)		0.72±0.17	0.86±0.09	<0.001(HS)
S.albumin(mean±SD, g/l)		4.15±0.36	4.55±0.43	<0.001(HS)
INR		1.00±0.01	1.00±0.00	1.0(NS)
S.creatinine(mg/dl)		0.90±0.15	0.91±0.07	0.63(NS)
HB(gm/dl)		13.1±1.85	12.9±1.52	0.48(NS)
WBCs(cm)		7171.4±2043.91	6302.5±1059	0.38(S)
PLTs(cm)		241.1±74.77	241.9±50.90	0.76(NS)
FIB-4 score points(mean±SD)		1.39±1.02	-0.75±	<0.001(HS)
NAFLD score points(mean±SD)		-.1.74±1.17	-2.75±0.91	<0.001(HS)

SD, standard deviation

Table (2): Real time elastography (RTE) in the studied groups.

Real time elastography	Group I (n = 100)		Group II (n = 30)		Total	
	No	%	No	%	No	%
F0	0	0.0%	19	63.4%	19	14.6%
F1	55	55%	7	23.3%	62	47.7%
F2	30	30%	4	13.3%	34	26.2%
F3	6	6%	0	0.0%	6	4.6%
F4	9	9%	0	0.0%	9	6.9%
Total	100	100%	30	100%	130	100%

Table (3): Frequency of significant fibrosis (F3 & F4) among patients with NAFLD according to FIB4-INDEX, NAFLD-FIBROSIS SCORE and RTE.

	FIB4-INDEX	**NAFLD-FIBROSIS SCORE**	**RTE**
Number of patients with No significant fibrosis	90	84	85
Number of patients with Significant fibrosis	10	16	15

The chi-square statistic is 1.7516. The p -value is .416533. The result is not significant at p < .05

Table (4): Degree of agreement between FIB-4 and NAFLD fibrosis score results in diagnosis the degree of liver fibrosis.

			NAFLD fibrosis score		**Total**
			< F3	**>/= F3**	
FIB-4	**< F3**	Count	80	10	90
		% within NAFLD	98.8%	52.6%	90.0%
	>/= F3	Count	1	9	10
		% within NAFLD	1.2%	47.4%	10.0%
Total		Count	81	19	100
		% within NAFLD	100.0%	100.0%	100.0%

Table (5): Degree of agreement between real time elastography and FIB-4 score results in diagnosis degree of liver fibrosis in NAFLD.

	Real time Elastography	FIB-4 Score		Total
		< F3	≥ F3	
<F3	Count	84	1	85
	% within Fib4	93.3%	85%	85%
≥F3	Count	6	9	15
	% within Fib4	6.7%	15%	15%
Total	Count	90	10	100
	% within Fib4	100%	100%	100%

Kappa test = 0.682; P<0.001 (HS); Degree of agreement=93%

Table (6): Degree of agreement between real time elastography and NAFLD fibrosis score results in diagnosis of liver fibrosis degree in NAFLD.

	Real time Elastography	NAFLD Fibrosis score		Total
		< F3	≥ F3	
<F3	Count	76	9	85
	% within NAFLD Score	93.8%	47.4%	85%
≥F3	Count	5	10	15
	% within NAFLD Score	6.2%	10%	15%
Total	Count	81	19	100
	% within NAFLD Score	100%	100%	100%

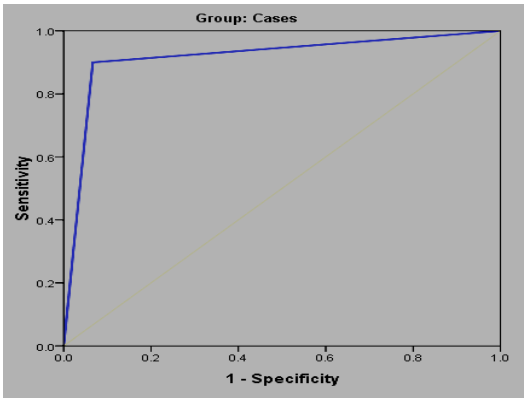

Fig. 1: ROC curve for the performance of real time elastography in prediction of F3 or more (taking FIB-4 as a standard)

The aim of this study was to assess the applicability and performance of real-time elastography for diagnosis of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD).

Patients and controls are age and sex matched. This eliminates confounding factors and validating further comparison.

Diabetes, dyslipidemia and obesity are more frequent among patients with NAFLD this expected because NAFLD is a manifestation of the metabolic syndrome.

Significant fibrosis (≥F3) was detected in 10% of patients by FIB-4 and in 16% by NAFLD Fibrosis Score (Table 3) . This discrepancy indicated that NAFLD Fibrosis Score is probably more sensitive in the detection of significant fibrosis, but it was not statistically significant ($p > 0.05$). However, it also indicates the need for other more reliable non-invasive methods for the detection of significant fibrosis. Significant

In the current study as much as 36.6% of healthy controls had F1 or F2 fibrosis by RTE (Table 2). This finding indicates that clinical methods are not sufficient by themselves in excluding subtle liver pathology. FIB-4 INDEX and NAFLD-FIBROSIS SCORE are two time-honored non-invasive methods for estimating and monitoring of significant liver fibrosis in patients with NAFLD (8),(12). The degree of agreement between FIB-4 and NAFLD-FIBROSIS SCORE, in the current study, was 89% (Table 4). When correlating RTE, a recently introduced technique, with FIB4 INDEX and NAFLD-FIBROSIS SCORE, the degree of agreement reached 93% and 86% respectively ($p < 0.001$) (Tables 5 & 6).

As a diagnostic method for significant fibrosis (F3 & F4), RTE had a sensitivity of 90% when FIB4 INDEX was taken as a gold standard (Figure1), and 52.6% when NAFLD-FIBROSIS SCORE was taken as a

gold standard (Figure 2). In both cases we are still far from finding the optimally utmost diagnostic tool. However, it is definitely clear that the sensitivity of RTE is more closely correlated to FIB4 INDEX than NAFLD-FIBROSIS SCORE.

The specificity of RTE in diagnosis of significant fibrosis was 93.3% and 93.8% when FIB4 INDEX and NAFLD-FIBROSIS SCORE were taken as gold standards (Figures 1 & 2). The accuracy and the AUC values of RTE were 93% and 0.917 when FIB4-INDEX was taken as a reference standard (Figures 1). When NAFLD-FIBROSIS SCORE was taken as reference, the accuracy and AUC value of RTE were 86% and 0.732 respectively (Figure 2). Overall, RTE proved to as equally reliable as FIB4 INDEX and NAFLD-FIBROSIS SCORE. However, it still has the added advantage of being immediately available for the patient and the physician during an office visit. This is important in the management of NAFLD patients which require repeated and frequent clinical interviews to monitor progress of a condition in which life style modifications are the most important part of treatment.

Our study has some limitations as the small number of patients in some of the univariate analyses might lead to insignificant findings in the statistical analyses, so larger studies should be considered to clarify diagnostic value of (RTE) in diagnosis liver fibrosis.

Finally we can conclude that (RTE) has a good diagnostic value in diagnosis advanced liver fibrosis .

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