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Multi-parametric magnetic resonance imaging as a diagnostic tool of hepatic steatosis for non-alcoholic fatty liver disease in Egyptian patients.

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Abstract

Background and Aim: Non-invasive assessment of non-alcoholic fatty liver disease (NAFLD) is increasing in desirability due to the invasive nature and costs associated with the current form of assessment by liver biopsy. Quantitative multi-parametric magnetic resonance imaging (mpMRI) to measure liver fat (proton density fat fraction) is an emerging alternative which could be utilized as safe surrogate to liver biopsy. The aim of this study is to evaluate the diagnostic utility of multi-parametric MRI for hepatic steatosis in patients having NAFLD.

Materials and Methods: Sixty patients with NAFLD were recruited into this cross-sectional study and were screened using non-invasive imaging technique; mpMRI. Patients were biopsied, and samples were scored by one expert hepato-pathologist. The diagnostic performance of mpMRI was assessed using area under receiver operating characteristic curve (AUC) with the median of the histology scores as the gold standard diagnoses.

Results: AUROC for Magnetic resonance spectroscopy proton density fat fraction (MRS-PDFF) was 1.000 (p =0.0001; 95% CI = 1.000–1.000), a cut-off value of 32.5 had the best sensitivity (97.5%) and specificity (100%).The AUROC for fat fraction (FF) index was 0.995 (p =0.0001; 95% CI = 1.000–0.985), a cut-off value of 22.5 had the best sensitivity (97.2%) and optimal specificity (84%). The AUROC for water fraction index (WF) was 0.998 (p =0.0001; 95% CI = 1.000–0.991), a cut-off value of 22.5 had the best sensitivity (97.2%) and optimal specificity (92%). **Conclusions***:* Quantitative mpMRI is an effective alternative to liver biopsy for diagnosing non-alcoholic fatty liver, and thus may offer clinical utility in patient management.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting approximately 25% of the general population international [1].

Individuals with NAFLD often have metabolic comorbidities and may place a burden on health care costs because of the need for their management and treatment [2]. It is defined as fat accumulation (steatosis) in > 5% of hepatocytes in absence alcohol intake, viral hepatitis, hereditary liver diseases, or long-term use of steatogenic medications (corticosteroids, methotrexate, amiodarone, isoniazid, highly active anti-retroviral therapy, etc.) so, it is diagnosed by exclusion of any secondary causes [3]. The scope of NAFLD encompasses ,simple steatosis or NAFL (hepatic steatosis without inflammation), to non-alcoholic steatohepatitis (NASH) which differ in both clinical significance and prognosis [4]. NASH leads eventually to cirrhosis [5] and hepatocellular carcinoma (HCC) [6], so that, it is predicted to become the leading cause of liver transplant over the coming decade [1].

Percutaneous biopsy is the gold standard method to estimate liver fat and detect associated complications such as steatohepatitis and hepatic fibrosis [7]. liver biopsy is an invasive procedure with a various limitations (pain, bleeding), so, there is a persistent need for development of non-invasive procedures for diagnosis of NAFLD either chemical or radiological biomarkers [8].

Multi parametric MRI which is a relatively new method for the diagnosis of NAFLD with specific liver tissue quantification of fat, iron, and fibrosis [9] is a combination of two or more sequences [10]. MRI quantification of liver fat content can be performed with different techniques. The aim of this study is to evaluate the diagnostic utility of multi-parametric MRI for hepatic steatosis in patients having NAFLD.

Materials and methods

This study is a cross sectional study that included 60 patients, 18 years old or more having different grades of fatty liver by ultrasound. Patients were recruited from outpatient clinic of Tropical Medicine Department, Mansoura University Hospitals, Dakahleya, Egypt between October 2018 and June 2021. Patients were divided according to percentage of hepatic steatosis by liver biopsy into:

i. Grade I (S1): less than 33% steatosis
ii. Grade II (S2): from 33% to 66%.
iii. Grade III (S3): more than 66% steatosis.

All members of the study signed a written informed consent before inclusion in this study. All procedures were approved by the Institutional Research Board (IRB) of the Faculty of Medicine, Mansoura University (approval number 114/2013) as a single-center study.

Inclusion criteria:

Obesity (BMI >25), type 2 diabetes mellitus (T2DM), dyslipidemia, patients with metabolic syndrome (MetS)[11],

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patients with ultrasound criteria of NAFLD and chronically elevated liver enzymes for at least 6 months without identifiable cause [12].

Exclusion criteria:

Common causes of hepatic steatosis: Significant alcohol consumption, previous use of steatosis-inducing drugs, active intravenous drug addiction, parenteral nutrition, severe malnutrition and pregnancy. Hepatocellular carcinoma (HCC) and other etiologies for chronic liver disease (CLD) e.g.**;** hemochromatosis, autoimmune liver disease, chronic viral hepatitis, Alpha-1 antitrypsin deficiency and Wilson disease.

All participants of the study were subjected to detailed clinical assessment (history taking, physical examination). Laboratory workup was done including: Complete Blood Count (CBC), liver function tests, virology (HCV antibody, HBS antigen), lipid profile, random blood sugar, fasting, 2hour post prandial blood sugar and glycated hemoglobin (HB A1C) in diabetic patients.

Radiological Evaluation.

Abdominal ultrasound examination using Toshiba Xario XG ultrasound machine (Jaban), to assess presence and grading of fatty liver [13]. Multi parametric magnetic resonance imaging (mp MRI) using "Philips Ingenia" 1.5 T MRI machine (Netherlands) using a torso phased- array coil for signal reception. It included the following sequences: mDixon MR imaging, dual-gradient echo MR (DGE-MRI) and MR spectroscopy (MRS). Post processing of magnetic resonance data was conducted on a dedicated work station (Extended MR Work Space, ver. 2.6.3.5; Philips Medical Systems, The Netherlands). On mDixon MRI,DGE-MRI sequences, 3 regions of interest (ROIs) that avoided large vessels and organ boundaries were drawn in the liver parenchyma. Percentage of fat can be assessed by DGE-MRI as Fat Fraction index (FF index) which is calculated from the in-phase and opposed-phase signals (SII and SIO) using the following equation: SI index = (SII − SIO) / SII × 100 (%) [14] and by mDixon as water fraction index using signal intensities acquired from the fat image and water image (SIF and SIW) with the following equation : WF index = SIF / (SIW + SIF) × 100 (%) [15].

For data analysis, the average of 3 calculated HFF values was used. A single-voxel MR spectroscopy in a 10-20 mm3 voxel was used and placed avoiding intrahepatic vessels and at least 0 mm from the edge of the liver in all dimensions. For hepatic fat quantification, the ratio between the fat signal peaks and the sum of the fat and water peaks was calculated [fat signal peak / (fat signal peak area + water peak area)].

Liver Biopsy.

Liver biopsy was performed with pericussions requisites to liver biopsy before mp MRI or two weeks after it to avoid radiological artifacts. Liver biopsy (trans-costal or subcostal) was performed by expert hepatologist from the right liver lobe using a 16-gauge needle under ultrasound guidance at a minimum of 1.5-cm-length core which is suitable for interpretation. Samples were assessed by one expert pathologists who were blinded to the imaging findings. Biopsies were fixed in 4% buffered fromaldehyde and embedded in paraffin. Sections (4 µm thick) were cut and routinely stained with hematoxylin and eosin.

In formalin-fixed, paraffin embedded tissue, fat is represented by velar, empty, variable sized vacuoles. Hepatocytes were evaluated for the presence of micro-vesicular changes characterized by numerous uniform intra-cytoplasmic vacuoles, that are smaller than the centrally located nucleus, or for macro-vesicular changes with vacuoles the size of the nucleus or larger and with frequently displaced nucleus.

Statistical analysis

Statistical analysis of the data was done by using Statistical Package for Social Science (SPSS) version 25.0. The normality of the distribution was checked by Kolmogrov Smirnov test to determine parametric or nonparametric distribution. The data were presented in the form of range, median, mean, standard deviation and 95% confidence interval. Quantitative data were expressed as Mean± SD for parametric data and as median and range for non-parametric data while qualitative data were expressed as f and percent. Significance was considered when P value ≤ 0.05. ROC curve analysis to determine the best cutoff point for hepatic steatosis for each imaging modality was done.

Results

The demographic, anthropometric and biochemical parameters of the study population are summarized in **(Table 1)**.

The percentage of different steatosis grades of the patients according to liver biopsy were as follows: S1 was found in 40% of patients, S2 was found in 33.3% of patients and S3 was present in 26.7% of patients **(Table 2).**

The percent of hepatic steatosis by different assessment modalities was as follow: liver biopsy steatosis percentage of 36% (5%-75%), MRS-PDFF is 35 (8– 46), DGE-MRI fat fraction (FF index) is 0.69 (± 0.1) and mDixon-MRI fat fraction (WF) index of 5.5 (3.1- 18) **(Table 3)**. There was a statistically significant positive correlation between percentage of hepatic steatosis by liver biopsy and MRI parameters (MRS-PDFF, Fat Fraction index and Water Fraction index), ALT, ALT levels however, no significant correlation between with visceral adiposity index, age, body mass index, waist circumference, serum triglycerides, cholesterol level and glycated hemoglobin **(Table 4)**.

The AUROC for MRS-PDFF was 1.000 (p =0.0001; 95% CI = 1.000–1.000), a cut-off value of 32.5 had the best sensitivity (97.5%) and specificity (100%).AUROC for FF index was 0.995 (p =0.0001; 95% CI = 1.000–0.985), a cut-off value of 22.5had the best sensitivity (97.2%) and optimal specificity (84%).

The AUROC for WF index was 0.998 (p =0.0001; 95% CI = 1.000–0.991), a cut-off value of 22.5had the best ensitivity (97.2%) and optimal specificity (92%) **(Table 5and figure 1)**.

Table 1. Demographic, anthropometric and biochemical parameters of the studied patients.

Parameter		Mean ± SD / Median (range)
Age/year		46.8 ± 9.5
Sex	**M**	12 (20%)
	F	48 (80%)
Weight/ kilograms		93 ± 10
Height/ centimeters		164 ± 6.2
BMI (Kg/m^2)		34.8 ± 4.8
Waist circumference/ centimeters		111.3 ± 11.4
RBS (mg/dl)		125 (85 – 295)
Hba1c (%)		6.35 ± 1.3
Cholesterol (mg/dl)		208 ± 46.2
TG (mg/dl)		158 ± 45
LDL (mg/dl)		107 ± 44
HDL (mg/dl)		51 ± 10.4
AST (U/L)		72 ± 21.3
ALT(U/L)		79 ± 16.2
Albumin (g/dL)		4 ± 0.3
Bilirubin (mg/dl)		0.9 ± 0.15
INR		1.08 ± 0.08
Creatinine (×10^3/µL)		0.8 ± 0.14
WBCs (×10^3/µL)		6.3 ± 1.8
PLT (×10^3/µL)		255 ± 75

BMI:*Body mass index*, SD: *Standard deviation*, ALT: *Alanine Transaminase*, AST: *Aspartate Transaminase*, FBS: *Fasting Blood Sugar*, Hba1c: *glycated hemoglobin*, HDL*: High Density Lipoprotein*, INR: *International Normalized Ratio*, LDL: *Low Density Lipoprotein*, PLTs: *Platelets*, TG: *Triglycerides*, WBCs: *White Blood Cells*.

Table 2. Descriptive data of patients according to steatosis grade (by liver biopsy).

Parameter		Frequency (percentage)
Steatosis grade	S1 (5%-33%)	24 (40%)
(60 patients)	S2 (>33%-66%)	20 (33.3%)
	S3 (>66%)	16 (26.7%)

Table 4. Descriptive data of steatosis parameters

Parameter	Mean ± SD / Median (range)
Biopsy steatosis	36% (5%-75%)
MRS steatosis	35 (8 – 46)
Fat Fraction ratio	0.69 ± 0.1
Water Fraction index	5.5 (3.1- 18)

Table 4. Correlations of biopsy percentage of steatosis with mp MRI and other biochemical and anthropometric parameters

Parameter	R	P-value
MRS (%)	0.94	< 0.0001
FF ratio	0.95	< 0.0001
WF index	0.94	< 0.0001
AST (U/L)	0.4	0.002
ALT (U/L)	0.3	0.01
Age (year)	-0.01	0.8
BMI(Kg/m^2)	-0.09	0.5
WC (centimeters)	-0.04	0.7
TG (mg/dl)	-0.15	0.2
Cholesterol (mg/dl)	-0.17	0.2
Hba1c (%)	0.08	0.5

P: *significance (probability)*. **r**: *Spearman correlation co-efficient*, **MRS**: *Magnetic resonance spectroscopy*, **FF ratio**: *Fat fraction ratio*, **WF index**: *Water fraction index*, **BMI**: *Body mass index*, **ALT**: *Alanine Transaminase*, **AST**: Aspartate Transaminase, **Hba1c:** *glycated hemoglobin*, **TG:** *Triglycerides*, **WC:** *Waste circumference*.

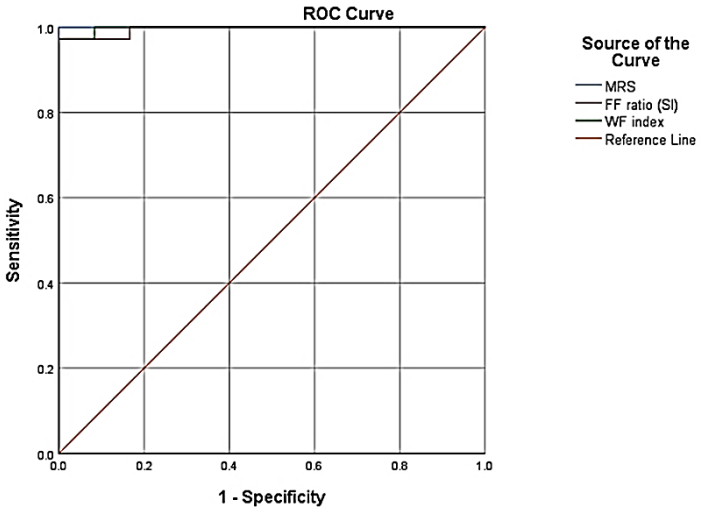

Figure 1. Diagnostic accuracy of MRS-PDFF, FF index and WF index for diagnosis of hepatic steatosis in NAFLD.

Table 5. Accuracy of MRS-PDFF, FF index and WF index for diagnosis of hepatic steatosis in NAFLD.

	AUC	P-value	95% CI	cutoff	Sensitivity	specificity
MRS-PDFF	1.000	0.0001	1.000-1.000	32.5	97.2%	100%
FF index	0.995	0.0001	1.000-0.985	22.5	97.2%	84%
WF index	0.998	0.0001	1.000-0.991	25.5	97.2%	92%

AUC: *Area under the curve*, **p:** *Probability*, **CI:** *Confidence interval*, **MRS:** *Magnetic resonance spectroscopy*, **PDFF:** *Proton density fat fraction*, **FF index:** *Fat fraction index*, **WF index:** *Water fraction index.*

Discussion

The present study evaluated three imaging techniques (MRS, DGE-MRI and mDixon) to quantify hepatic fat fraction (HFF) as potential alternative methods to liver biopsy for the diagnosis and grading of hepatic steatosis. Our study demonstrated a statistically significant strong positive correlation between percentage of hepatic steatosis by liver biopsy and MRS-PDFF (r 0.94, p < 0.0001) which is consistent with previous studies by Idilman et al, where was (r = 0.712, *P*< 0.001) [16] and Van Werven et al, where was (r = 0.86, *P*=.001) [17]. Nakamura et al, also reported a significant correlation between fat spectroscopy and lipid content by liver biopsy (r=0.876, p< 0.001) [15].

This result also agreed with the study of Van Werven et al, which observed that spectroscopic measurements of hepatic fat had stronger positive correlation with histopathologic steatosis assessment (r = 0.86, P<0.001) than ultrasound and CT also, there was a strong correlation found between T1-weighted dual-echo MR imaging (in phase – out of phase) and histopathologic examination (r = 0.85, P< .001) [17].

The current study also detected a statistically significant strong positive correlation between percentage of hepatic steatosis by liver biopsy and mDixon-MRI (r= 0.94, p< 0.0001) which approved with the results of Bhat et al., which noted that there was significant correlation between estimated fat by mDixon-MRI and histology for both left liver lobe (LLL) and right liver lobe (RLL) (LLL p=0.4, p=0.03, RLL p=0.51 p=0.004) [18].

There was also a significant correlation between percentage of hepatic steatosis by liver biopsy with ALT and ALT levels which was consistent with the study of Cuenza et al, where there was a significant correlation of increased levels of liver enzymes with the grade of steatosis [19]. Also there is agreement with Cuenza et al study in that age, serum cholesterol and HDL levels have no correlation with hepatic steatosis, in contrast, in our study, BMI and triglyceride level showed no significant correlation with hepatic steatosis the findings that not found in Cuenza et al study. This controversy could be explained by that Cuenza et al., in its study depended on ultrasound finding without doing liver biopsy.

This study showed that the AUROC for MRS-PDFF was 1.000 (p =0.0001; 95% CI = 1.000–1.000), a cut-off value of 32.5had the best sensitivity (97.5%) and specificity (100%) for diagnosis of hepatic steatosis in NAFLD patients. This results agreed with Assingnies et al., who demonstrated that the ROC curves obtained with MRS results for the combined data of steatosis patients showed nearly perfect curves, with areas under the curve (AUCs) of 1.000, sensitivity was 0.95 (IC 95%: 0.73-0.99) and the specificity 1.0 (IC 95%: 0.83-1.0) [20]. The study of Georgoff et al., nearly reached the same results where the diagnostic accuracy of H-MRS was very good with an AUC of 0.94 (95% CI0.88–1.0) [21].

It was also demonstrated by the authors of the current study that the AUROC for FF index was 0.995 (p =0.0001; 95% CI = 1.000–0.985), a cut-off value of 22.5had the best sensitivity (97.2%) and optimal specificity (84%), Partially agreeing with these results, the study of Van Werven et al., demonstrated thatT1-weighted dual-echo MR imaging (In phase – Out of phase) had a sensitivity of 90% and specificity of 91% which was higher than that of CT and US but slightly the same as MRS (sens. 91%, spec.87%) [17].

The AUROC for WF index was 0.998 (p =0.0001; 95% CI = 1.000–0.991), a cut-off value of 22.5had the best sensitivity (97.2%) and optimal specificity (92%).This was matched with the results of Eddowes et al., which stated that AUROC (95% CI) for the identification of steatosis for PDFF-mDixon was 1.00(1.00-1.00) [22].

Conclusion

Multi-parametric MRI can be used as a non-invasive substitute to liver biopsy as regard diagnosis of hepatic steatosis in Egyptian patients having NAFLD.

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