

Correlation between ultrasonography and MR proton density fat fraction techniques in evaluating the severity of liver steatosis

 Betül Kizildag¹,  Murat Baykara²,  Nursel Yurttutan¹,  Halit Vicdan¹

¹Department of Radiology, Sutcu Imam University School of Medicine, Kahramanmaraş, Türkiye; ²Department of Radiology, Firat University School of Medicine, Elazığ, Türkiye

Abstract

Background and Aim: To investigate the relationship between ultrasonography (US) and magnetic resonance (MR) proton density fat fraction (PDFF) techniques, using the modified DIXON method, in determining the severity of liver steatosis.

Materials and Methods: This study included seventy consecutive patients who underwent upper abdominal MRI for various reasons between June 2016 and January 2017. Fatty liver staging was performed using US as indicated. The liver fat percentage was measured and staged according to PDFF values.

Results: In the study, of the 70 cases, 36 were male and 34 were female. On US, 18.5% of the cases had stage 0, 32.8% had stage 1, 42.8% had stage 2, and 5.7% had stage 3 liver steatosis. A significant correlation was found between ultrasonographic evaluation and PDFF in determining the percentage of liver fat ($r=0.775$, $p<0.001$). When comparing the percentages, MR-evaluated PDFF and ultrasonographic staging were most compatible at grade 3 and least compatible at grade 2. When the PDFF threshold value was set at 8.1%, the sensitivity of US in distinguishing between obvious and indistinct steatosis was 97.1%, and the specificity was 88.9%.

Conclusion: Ultrasound continues to be a useful tool for detecting fatty liver disease. However, magnetic resonance (MR) proton density fat fraction (PDFF) imaging is essential for accurately determining the severity and prevalence of steatosis. Our study revealed inconsistencies between US and MR PDFF in grading liver steatosis, showing higher agreement in severe cases and lower agreement in moderate cases. Therefore, we recommend classifying steatosis as either uncertain or apparent rather than using a grading system in US.

Keywords: Fat quantification; liver steatosis; magnetic resonance imaging; proton density fat fraction; ultrasonography.

Introduction

Hepatic steatosis is a common abnormal finding observed in cross-sectional imaging of the abdomen, characterized by the accumulation of triglycerides as droplets in the cytoplasm of hepatocytes. Non-alcoholic fatty liver disease (NAFLD), formerly described as a clinical condition characterized by non-alcohol-related liver fat accumulation, has seen its prevalence range from 10% to 33% in recent years. It can progress to end-stage liver disease.^[1] With recent advancements, the terminology has shifted from NAFLD to MAFLD/MASLD, Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), as highlighted in a recent consensus paper.^[2] It is essential to mention the ongoing debate surrounding this transition.^[3] NAFLD is a complex condition that affects multiple systems in the body and is closely linked to obesity, insulin resistance, type 2 diabetes, metabolic syndrome, and heart disease. In Western nations, NAFLD has risen to be the leading chronic liver condition, with a growing number of patients with NAFLD-related cirrhosis awaiting liver transplants.^[4] NAFLD can manifest as simple steatosis (fatty liver) or progress to more severe conditions such as non-alcoholic steatohepatitis (NASH), characterized by necrosis and inflammation due to fat accumulation in liver cells, parenchymal fibrosis, cirrhosis, and even hepatocellular carcinoma (liver cancer).^[5,6] Liver biopsy and histological analysis are the diagnostic reference standards for NAFLD.

Patients with NAFLD are mostly asymptomatic. Abnormal liver enzyme elevation, hepatomegaly, or suspicion of liver fat accumulation through imaging methods are often identified as part of routine physical examinations. Although the definitive diagnosis is made through liver biopsy, it is an invasive procedure with approximately 1–3% morbidity and a 1 in 10,000 mortality risk, which restricts its use.^[7] For this reason, biopsy is not preferred in the diagnosis and monitoring of NAFLD. Additionally, since a liver biopsy samples only a small portion of the liver, it may result in high error rates in patients with heterogeneous fat distribution.^[7]

Non-invasive methods such as ultrasonography (US), computed tomography (CT), and conventional magnetic resonance imaging (MRI) are used in diagnosing NAFLD.^[8] However, these techniques' main disadvantages include the potential for confusing hepatosteatosis with other conditions and the inability to quantify the degree of fat accumulation as numerical data.

In ultrasonography, fatty liver cases involve fat-filled vacuoles leading to increased scattering and attenuation of sound waves.^[9] Diffuse liver steatosis appears as a general increase in echogenicity in ultrasound images. The sensitivity of US in detecting steatosis has been found to be between 60–94%, and its specificity has been found to be between 66–95%.^[10,11]

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Corresponding author: Betül Kizildag; Sutcu Imam Universitesi Tıp Fakültesi, Radyoloji Anabilim Dalı, Kahramanmaraş, Türkiye
Phone: +90 505 255 64 61; **e-mail:** dr.betulkizildag@hotmail.com



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Magnetic Resonance Spectroscopy (MRS) is an MR method that enables quantitative evaluation based on separate measurement of signals from water and fat, providing a fat-signal ratio for hepatosteatois. However, MRS has significant disadvantages, such as measuring signals only from the examined voxel rather than the entire liver, longer examination times, the need for experienced evaluation, and limited availability in small centers.^[12]

Another method for the quantitative measurement of hepatosteatois using MRI is chemical shift-based water-fat separation techniques.^[13,14] Among these, the traditionally used dual-echo chemical shift MRI is based on the different resonances of fat and water molecules at distinct frequencies and utilizes signal loss for detecting intravoxel fat.

In recent years, modified complex-based methods calculating liver fat percentage have been introduced using the DIXON technique. They are being used with different commercial names on various devices.^[15] When compared to histopathological results, these complex-based fat percentage methods have been reported to correlate with histopathological data, demonstrating high sensitivity and specificity in detecting and quantifying liver steatosis.^[16] In this study, liver fat percentage was calculated using the modified DIXON (mDIXON) method, accepted as the liver fat imaging standard by the MR research community, approved by the United States Food and Drug Administration (FDA), and ready for clinical use.^[17]

This study aims to investigate the correlation between the semi-quantitative staging of liver steatosis on ultrasonography (US) in patients with non-alcoholic fatty liver disease (NAFLD) and the quantitatively calculated liver fat percentage using the mDixon method on magnetic resonance imaging (MRI).

Materials and Methods

This prospective study was approved by the Ethics Committee (Approval date: June 1, 2016, Protocol No: 2016/10-12). Informed written and verbal consent forms were obtained from all patients included in the study before the radiological evaluation.

Selection of Patients

Between June 1, 2016, and January 1, 2017, a total of 100 consecutive patients who underwent upper abdominal MRI for various reasons (non-specific abdominal pain or discomfort, adrenal mass, pancreatic cysts, etc.), from outpatient clinics, were screened for inclusion criteria. Among them, 70 patients who met the study criteria were included in our study. After the upper abdominal MRI examination, liver steatosis was staged using B-Mode Ultrasonography in our unit according to the method described in reference.^[9] Anthropometric measurements, including weight (kg) and height (m), were recorded for all participants, and body mass index (BMI) was calculated using the formula (kg/m^2). Serum AST, ALT, total bilirubin, GGT, cholesterol, triglycerides, VLDL, HDL, and LDL values were obtained from the patient records.

The inclusion criteria for the study involved patients between the ages of 18 to 65 years, who consumed alcohol within the limits of 140 grams per week, and had no diagnosis of acute or chronic hepatitis, malignancy, or metabolic liver diseases (such as Wilson's disease, hemochromatosis, etc.). Patients with movement artifacts on MRI, occupying lesions in the liver, parenchymal coarseness, and lobulation in liver contours were excluded from the study.

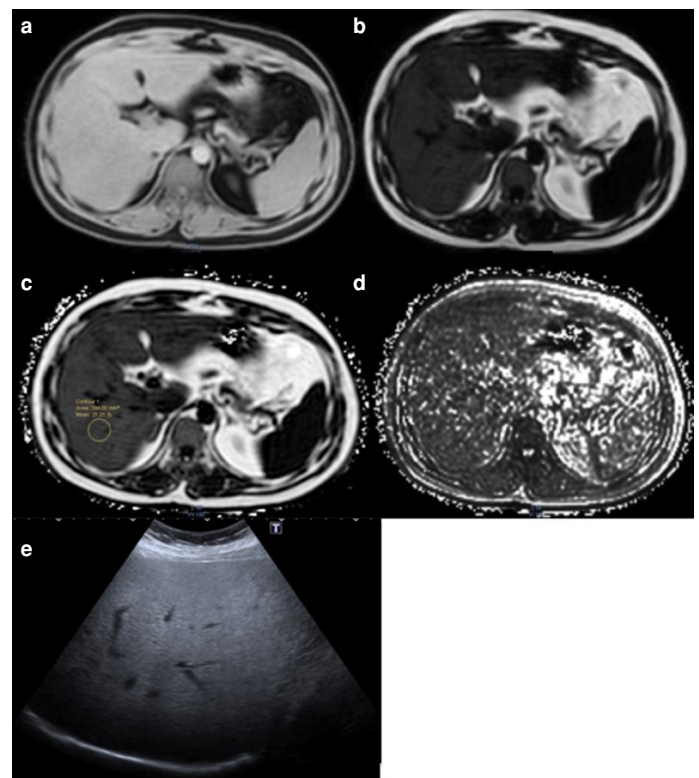


Figure 1. Axial section of MR mDixon-Quant and ultrasound scan of a 47-year-old male patient. (a) Water Value: 1019.04, (b) Fat Value: 273.84, (c) Fat Percentage: 21.16%, (d) T2* Value: 24.94 ms, (e) Ultrasonographic findings reveal Grade 2 hepatic steatosis.

Sonographic Examination

The study participants underwent ultrasound examinations performed by a radiology resident with four years of experience, following at least an 8-hour fasting period. The examinations were conducted in the supine and left lateral decubitus positions, with approximately 5-second breath-holding intervals, using a subcostal approach. All examinations were carried out using a Color Doppler Ultrasonography device (Toshiba Aplio 400, Tokyo, Japan) with a 3.5 MHz convex probe.

The liver parenchyma echogenicity was categorized into different stages of fatty liver based on the previously mentioned criteria^[9] in segments 6–7. The stages are as follows: absence (grade 0) indicates normal echotexture of the liver; mild (grade 1) is characterized by a slight and diffuse increase in liver echogenicity with normal visualization of the diaphragm and portal vein wall; moderate (grade 2) indicates a moderate increase, and severe (grade 3) indicates a severe increase in liver echogenicity. In cases where the grade could not be determined, a consensus decision was reached with an experienced radiologist (with 20 years of experience) as shown in Figure 1.

Magnetic Resonance Imaging

All participants underwent upper abdominal MRI examinations using the MR mDixon-Quant sequence on a Philips 1.5 T Ingenia MRI device with an 8-channel phased-array body coil (Philips Healthcare Nederland B.V., Best, Netherlands). The MR mDixon-Quant sequence parameters in our unit were as follows: TR (Repetition Time) shortest: 5.3 ms, FOV (Field of View): 400352231 mm, matrix size:

Table 1. Characteristics of the 70 NAFLD patients included in the study

	Minimum	Maximum	Average	SD
Age	23	77	51.46	14.70
Height	147	195	164.66	10.33
Weight	44	129	82.84	16.11
BMI	18.50	53.30	30.60	6.15
Water value	523.23	1506.26	1056.55	210.47
Fat value	26.53	509.59	145.78	111.21
Fat percentage	2.50	40.09	12.12	9.38
T2*	7.96	84.17	30.45	10.70
AST	11	496	38.77	65.93
ALT	8	932	46.41	115.68
GGT	7	770	57.93	117.14
Total bilirubin	0.29	5.60	0.76	0.81
LDL	58	264.80	122.26	42.77
HDL	38	53	46.98	7.21
VLDL	10	57	29.09	11.41
TGL	50	286	145.06	57.21

SD: Standard deviation; NAFLD: Non-alcoholic fatty liver disease; BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotran ferase; GGT Gama glutamyl transpherase; LDL Low density lipoprotein; HDL: High density lipoprotein; VLDL: VLDL Very low density lipoprotein; TGL: Trigliseride.

132*116, reconstruction matrix size: 192, bandwidth: 2869.4 Hz, flip angle: 5°, slice thickness: 3 mm, and approximately 77 slices were used. The sequence duration was around 17 seconds with a single breath-holding period. Six different TE (Echo Time) values were employed during the acquisition.

All measurements of the patients were performed on the workstation (IntelliSpace Portal, Philips v6.03.13200; Philips Healthcare Nederland B.V., Best, Netherlands) using quadruple images consisting of fat value, water value, fat percentage, and T2* (Fig. 1). A region of interest (ROI) measuring 4 cm² was placed on the liver’s right lobe segment 5–6, avoiding large vessels (Fig. 1). The measurements were conducted blindly by a senior radiology specialist based on the ultrasonographic stages. Previously, following the values specified for each histological steatosis grade according to liver fat percentages in Tang et al.’s study, the patients were staged.^[18,19]

Statistical Analysis

The statistical analysis of all patient data was conducted using the SPSS 22.0 statistical software package. The measurements were expressed as mean±standard deviation or median (minimum–maximum). The Pearson correlation coefficient was used to assess the relationship between the obtained values, and the Student’s T-test was used for comparisons. A p-value less than 0.05 was considered statistically significant.

Results

The study included 70 patients, with 36 males and 34 females. The average age of the patients was 51.46±14.7 years. The average BMI of the patients was calculated to be 30.60±6.15. Among the patients, 22.8% (16/70) had Type 2 Diabetes Mellitus (DM), 31.4% (22/70) had

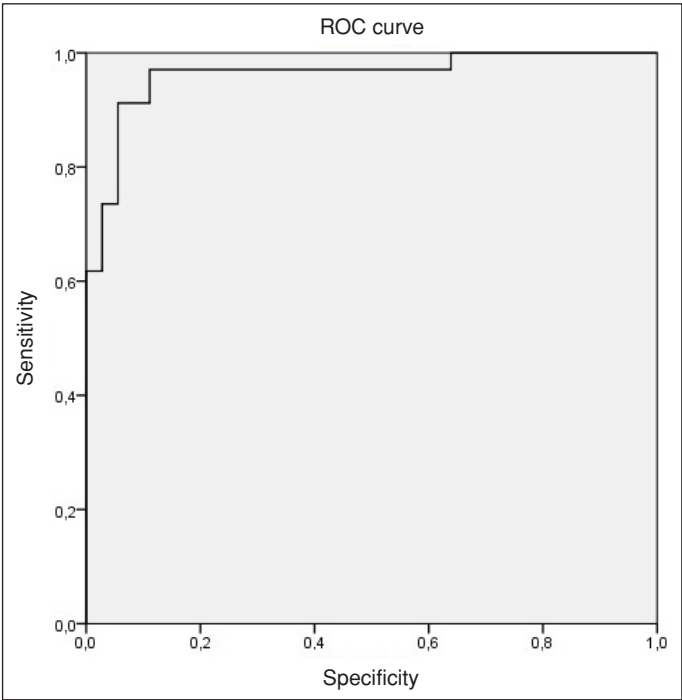


Figure 2. Receiver operating characteristic (ROC) curve depicting the diagnostic performance of ultrasound in distinguishing between indistinct and overt hepatic steatosis when MR mDixon-Quant technique is considered as the gold standard.

hypertension, and 5.7% (4/70) had hyperlipidemia. The demographic characteristics and laboratory data of the patients included in the study are presented in Table 1.

Ultrasonographic Evaluation

According to the ultrasound findings, 13 patients (18.5%) had grade 0 steatosis (absent), 23 patients (32.8%) had grade 1 steatosis (mild), 30 patients (42.8%) had grade 2 steatosis (moderate), and 4 patients (5.7%) had grade 3 steatosis (severe). Additionally, when the patients were categorized into two separate groups, with grade 0–1 steatosis as “indeterminate steatosis” and grade 2–3 steatosis as “definite steatosis,” 36 patients (51.43%) had indeterminate steatosis, and 34 patients (48.57%) had definite steatosis.

MR mDixon-Quant Measurements

The average fat percentage measured using MR mDixon-Quant was found to be 12.12%±9.38. The mean fat value was 145.78±111.2, the mean water value was 1056.55±210.47, and the mean T2* value was 30.45±10.70 ms. Table 2 shows the mDixon fat percentage values corresponding to the steatosis stages observed in the ultrasound (US) examination for all patients in the study. In patients with indeterminate steatosis on ultrasound, the average MR mDixon-Quant fat percentage was measured as 5.76%±2.68. In patients with definite steatosis, the average MR mDixon-Quant fat percentage was measured as 18.86%±9.25.

When ROC analysis was performed, the MR mDixon-Quant fat percentage threshold of 8.1% was chosen, resulting in a sensitivity of 97.1% and specificity of 88.9% in distinguishing definite from indeterminate steatosis on ultrasonography (AUC: 0.962) (Fig. 2).

Table 2. Corresponding MR mDixon fat percentages for liver steatosis grades on ultrasonography

USG steatosis grade	mDixon-quant fat percentage range	Average fat percentage	SD
0	%2.96–6.7	4.30	1.24
1	%2.5–12.81	6.11	2.30
2	%8.12–38.87	17.25	7.32
3	%26.1–40.09	33.62	6.36

USG: Ultrasonography; MR: Magnetic resonance; SD: Standard deviation.

The comparison between the degree of steatosis on MR mDixon-Quant and ultrasound (US) is summarized in Table 3. According to the results: among the 13 patients classified as grade 0 on ultrasound, 11 patients (85.6%) showed grade 0 on MR mDixon-Quant, and 2 patients (15.3%) showed grade 1. Among the 23 patients classified as grade 1 on ultrasound, 11 patients (47.8%) showed grade 0 on MR mDixon-Quant, and 12 patients (52.1%) showed grade 1. Among the 30 patients classified as grade 2 on ultrasound, 16 patients (53.3%) showed grade 1 on MR mDixon-Quant, 10 patients (33.3%) showed grade 2, and 4 patients (13.3%) showed grade 3. Lastly, among the 4 patients classified as grade 3 on ultrasound, all 4 patients (100%) showed grade 3 on MR mDixon-Quant.

Pearson correlation analysis revealed a significant correlation between the degree of steatosis on ultrasound and the calculated fat percentage using the MR mDixon-Quant technique ($r=0.775$, $p<0.001$) (Fig. 3). There was also a significant correlation between the ultrasound steatosis grade and the MR mDixon-Quant steatosis grade ($r=0.770$, $p<0.001$).

Among the patients categorized into two separate groups as “indeterminate steatosis” and “definite steatosis” based on the ultrasound findings, a significant correlation was found between the MR mDixon-Quant fat percentages ($r=0.703$, $p<0.001$) (Fig. 4). This indicates a strong relationship between the fat percentages measured by MR mDixon-Quant in patients with indeterminate and definite steatosis on ultrasound.

According to the results, there was a negative correlation between the ultrasound steatosis grade and the water value measured by MR mDixon-Quant. This means that as the ultrasound-detected steatosis grade increased, the water value significantly decreased ($r=-0.614$, $p<0.001$). In other words, as the degree of liver steatosis increased, the water content in the liver decreased, indicating higher fat accumulation.

The results indicated a significant correlation between the MR mDixon-Quant T2* value and Type 2 Diabetes Mellitus (T2DM). The correlation coefficient ($r=0.366$) and the p-value ($p<0.05$) suggest a positive correlation between the T2* value and T2DM, meaning that as the T2* value increases, the liver iron content decreases, indicative of reduced liver iron accumulation in patients with Type 2 Diabetes Mellitus.

The results show significant correlations between Type 2 Diabetes Mellitus (T2DM) and the following factors: increase in BMI ($r=0.303$, $p<0.05$), MR mDixon-Quant T2* value ($r=0.366$, $p<0.05$), and the presence of definite steatosis ($r=0.288$, $p<0.05$). These findings suggest that in patients with Type 2 Diabetes Mellitus, there is a positive correlation between BMI, T2* value (indicative of liver iron content), and the presence of definite steatosis (severe fatty liver).

Regarding gender, the average MR mDixon-Quant fat percentage in females was $10.79\% \pm 7.80$, while in males, it was $13.39\% \pm 10.63$. There was no significant difference in MR mDixon-Quant fat percentage between females and males, indicating that the average fat

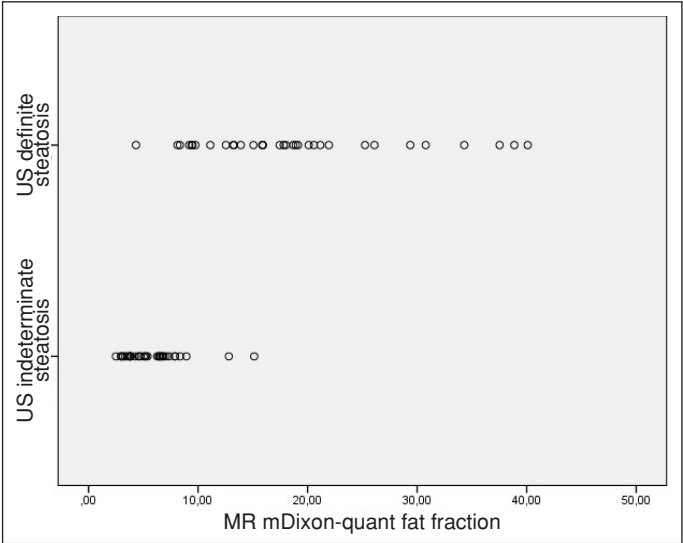


Figure 3. Correlation between ultrasound steatosis grade and MR mDixon-quant fat percentage.

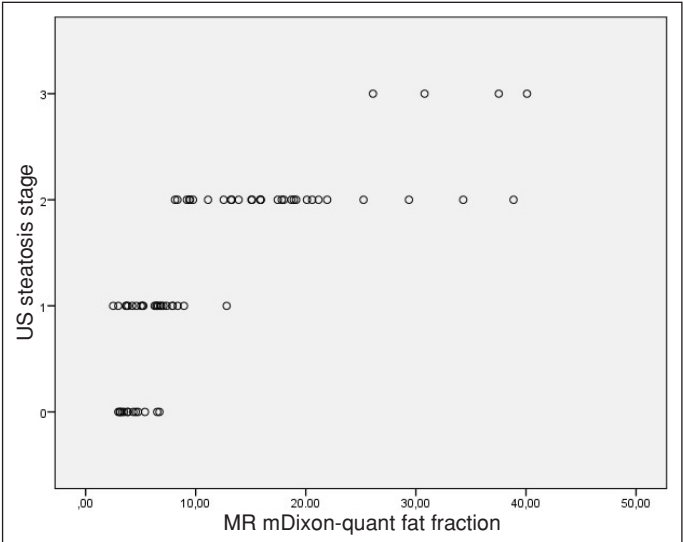


Figure 4. Correlation between ultrasound indeterminate and definite steatosis and MR mDixon-quant fat percentage.

percentage measured by MR mDixon was similar in both genders, with no statistically significant difference between them.

Discussion

In this study, conventional ultrasonography and a new magnetic resonance (MR) fat measurement method called Proton Density Fat Fraction (PDFF), calculated by the MR mDixon-Quant technique, were compared in terms of liver fat quantification. A significant correlation was found between the ultrasonographic grading of liver steatosis and the fat percentage calculated by the MR mDixon-Quant technique. The MR mDixon-Quant fat percentages corresponding to the sonographically graded hepatosteatoses were measured, and a significant correlation was observed between liver fat percentages measured by ultrasonography and MR mDixon-Quant ($r=0.775$, $p<0.001$). This indicates their agreement in determining the degree of liver fat accumulation.

Table 3. Comparison of liver steatosis grading between MR mDixon-quant and ultrasonographic evaluation

MRI steatosis stage	USG stage				Total
	0	1	2	3	
0					
Number	11	11	0	0	22
(%)	50.0%	50.0%	0.0%	0.0%	100.0%
1					
Number	2	12	16	0	30
(%)	6.7%	40.0%	53.3%	0.0%	100.0%
2					
Number	0	0	10	0	10
(%)	0.0%	0.0%	100.0%	0.0%	100.0%
3					
Number	0	0	4	4	8
(%)	0.0%	0.0%	50.0%	50.0%	100.0%
Total					
Number	13	23	30	4	70
(%)	18.6%	32.9%	42.9%	5.7%	100.0%

USG: Ultrasonography; MR: Magnetic resonance; MRI: Magnetic resonance imaging.

The correlation between MR mDixon-Quant values and PDFF values in grading liver steatosis was evaluated. The highest concordance was observed for Grade 3 steatosis, while the highest discordance was seen in Grade 2 steatosis. Setting the MR mDixon-Quant fat percentage threshold at 8.1%, ultrasonography demonstrated a sensitivity of 97.1% and a specificity of 88.9% in distinguishing definite and indeterminate steatosis, with an Area Under the Curve (AUC) value of 0.962.

The MR mDixon method is a state-of-the-art quantitative technique that calculates liver fat percentage through complex-based fat measurement. Techniques such as low flip angle, T2* correction, spectral modeling, and eddy current correction are used to calculate PDFF. Factors like T1 bias, T2* decay, noise effect, eddy currents, and field strength are minimized in the PDFF technique. The MR mDixon-Quant method captures images at six different echo times (TE) simultaneously, generating images of water, fat, fat percentage, and T2*.^[20,21]

In a study involving 506 adult patients, the PDFF technique demonstrated high specificity for assessing steatosis, unaffected by various histological and clinical factors such as BMI, inflammation, and fibrosis.^[22] A study by İdilman and colleagues with 70 patients showed a good correlation between PDFF and histological liver steatosis.^[23] Another study indicated that PDFF could detect changes in liver steatosis over time with a sensitivity of 93% and specificity of 85%.^[24] In our study, liver steatosis stages determined by ultrasonography were quantified by assessing liver fat percentage using MR mDixon measurements. Comparing the semiquantitative assessment by ultrasonography with the fat percentages measured by MR, our study could not directly correlate the results of both modalities with histopathological data. Consequently, the diagnostic sensitivity and specificity of these modalities for liver steatosis could not be calculated. Previous studies reported sensitivity and specificity ranges of 60–96% and 84–100% for ultrasonography, respectively.^[10,11] For PDFF, sensitivity and specificity rates of 93% and 85% were reported.^[23] In our study, when PDFF was considered the gold standard for assessing steatosis, ultrasonography demonstrated a sensitivity of 97.1% and specificity of

88.9% in distinguishing between indistinct and distinct steatosis (AUC: 0.962). These results were consistent with findings in the literature.^[23]

Considering the high sensitivity and specificity rates compared to the gold standard of liver biopsy, the MR mDixon technique for liver fat quantification can be considered the reference method. The sensitivity of ultrasound in detecting steatosis ranges from 60% to 94%, and its specificity ranges from 66% to 95%.^[10,11] The main advantage of ultrasound is its cost-effectiveness and applicability to all types of patients, regardless of age or other underlying conditions, including pregnancy. However, the quality and sensitivity of ultrasound can vary depending on the operator, equipment, and patient factors (such as intestinal gas and body habitus).^[25] Consequently, it can be suggested that ultrasonography (USG) may be more diagnostically effective in distinguishing between definite and indeterminate hepatic steatosis. Therefore, instead of staging liver steatosis using USG, it may be more appropriate to use terms such as definite steatosis or indeterminate steatosis in the reports. This approach would provide clearer and more clinically relevant information for the management of patients with fatty liver disease.

In previous studies, Tang and colleagues^[18,19] proposed PDFF ranges for each histological steatosis grade as follows: grade 0 (0–6.4%), grade 1 (6.5–17.4%), grade 2 (17.5–22.1%), and grade 3 (above 22.2%). This study similarly categorized mDixon measurements based on the calculated fat percentages. The MR mDixon-Quant-based grading was compared with ultrasonographic grading. Among the 22 patients graded as MR mDixon-Quant grade 0, 50% were classified as ultrasonographic grade 0, and the remaining 50% as ultrasonographic grade 1. Among the 30 patients graded as MR mDixon-Quant grade 1, 6.7% were classified as ultrasonographic grade 0, 40% as ultrasonographic grade 1, and 53.3% as ultrasonographic grade 2. All 10 patients graded as MR mDixon-Quant grade 2 were classified as ultrasonographic grade 2. Among the eight patients graded as MR mDixon-Quant grade 3, 50% were classified as ultrasonographic grade 2, and the remaining 50% as ultrasonographic grade 3.

The comparison of ultrasonography and MR mDixon-Quant revealed the highest agreement between grades 3 and 0, with less agreement observed in grade 1, and the lowest in grade 2. The study identified differences between ultrasonography and MR mDixon-Quant in assessing steatosis grade. Notably, all patients categorized as MR mDixon-Quant grade 3 were also classified as ultrasonographic grade 3, suggesting that the reliability of both MR mDixon-Quant and ultrasonography increases with the degree of steatosis. However, a one-to-one correspondence between the MR mDixon-Quant grade and the ultrasonographic grade is not always expected. In a previous study, the detection of moderate to severe fatty liver (>20–30% steatosis) showed that ultrasound (US) has similar performance to computed tomography (CT) or magnetic resonance imaging (MRI),^[26] while MRI and MRS were better at detecting mild steatosis than CT or US.^[26] These results are consistent with our study and may be attributed to high US inter/intraobserver variability. Another study by Sahannun and colleagues on 208 children demonstrated a good correlation between ultrasonographic and histological steatosis; however, 83% of children with normal ultrasonographic findings had steatosis on liver biopsy.^[27] Additionally, ultrasonography had a sensitivity of 55% for detecting mild steatosis.^[27]

Overall, the findings suggest that MR mDixon-Quant and ultrasonography may differ in assessing steatosis grade, but agreement between the two methods improves as the degree of steatosis increases. It is important to note that complete concordance between the two methods might not always be achievable due to their inherent differences in sensitivity and ability to detect different grades of steatosis.

The main limitation of our study was the inability to compare the fatty liver grades obtained from MRI and USG with histopathological results. Liver biopsy is an invasive procedure, and none of our patients required this method. However, liver biopsy samples only a small portion of the liver, leading to potentially high error rates in patients with heterogeneous fat distribution. Another limitation is the relatively small number of patients in our study, which can lead to less powerful and less generalizable results. Future studies with larger patient samples and histopathological confirmation may yield more reliable and meaningful results.

Conclusion

Ultrasound remains a useful tool for detecting fatty liver disease. However, for accurate determination of steatosis severity and prevalence, MR proton density fat fraction (PDFF) imaging is essential. Our study revealed inconsistencies between ultrasonography and MR PDFF in grading liver steatosis, with higher agreement in severe cases and lower agreement in moderate cases. Therefore, it is recommended to classify steatosis as either uncertain or apparent instead of using a grading system in ultrasonography.

Ethics Committee Approval: The Sutcu Imam University University Clinical Research Ethics Committee granted approval for this study (date: 01.06.2016, number: 2016/10-12).

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Author Contributions: Concept – BK; Design – BK, NY; Supervision – BK, HV; Fundings – HV; Materials – HV, MB; Data Collection and/or Processing – HV, MB; Analysis and/or Interpretation – BK, NY; Literature Search – BK, HV; Writing – BK, HV; Critical Reviews – MB, NY.

Conflict of Interest: The authors have no conflict of interest to declare.

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