Hong Kong J Radiol. 2015;18:4-10 | DOI: 10.12809/hkjr1414261

ORIGINAL ARTICLE **CME**

Semi-quantification of Hepatic Steatosis in Patients with Chronic Liver Disease Using the Multiecho Two-Point Dixon Technique with Histopathology as the Reference Standard

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ABSTRACT

Objectives: *To determine the utility of the multiecho two-point Dixon technique in the semi-quantification of hepatic fat in patients with chronic liver disease and compare these results with the histopathology outcomes.*

Methods: *In a retrospective study, patients with chronic liver disease who underwent both magnetic resonance imaging and liver biopsy on the same day between June 2012 and May 2013 were included. The hepatic fat fraction was calculated from water and fat images and reported by their mean. Liver specimens were reviewed by a hepatopathologist blinded to the radiological data.*

Results: *Among 21 patients with histological evaluation, steatosis was present in 15 patients and was of grade 1 in nine patients, grade 2 in four patients, and grade 3 in two patients. No hepatic steatosis (grade 0) was found in six patients. The median (range) of the hepatic fat fraction of patients with steatosis grades 0, 1, 2, and 3 were 2.4 (1.9-3.3), 6.4 (2.7-10.6), 19 (14.9-26.4), and 45.7 (20.1-71.3), respectively ($p < 0.001$).*

Conclusion: *Semi-quantification of hepatic steatosis using the multiecho two-point Dixon technique is a non-invasive method that provides reasonably accurate data about the severity of fat deposition in a range of liver diseases. The implementation of this sequence into the routine hepatic steatosis magnetic resonance imaging protocol could be of great benefit.*

Key Words: *Fatty liver; Image enhancement; Information storage and retrieval; Magnetic resonance imaging; Non-alcoholic fatty liver disease*

中文摘要

多迴波兩點Dixon法聯合組織病理學作為慢性肝病患者中肝脂肪變性半定量檢測的參考標準

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目的：評估多迴波兩點Dixon法為慢性肝病患者作肝脂肪變性半定量檢測的效用，並與組織病理學的結果作比較。

方法：把2012年6月至2013年5月期間於同一天內接受磁共振成像和肝活檢的慢性肝病患者納入本回顧研究。從水和脂肪圖像計算出肝臟脂肪分數，並報告其平均值。肝臟樣本則由不知道影像數據的

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Submitted: 18 Aug 2014; Accepted: 17 Nov 2014.

肝臟病理學醫生審查。

結果：21名已進行病理學評估的病人中，脂肪肝有15例，其中9例屬一級，4例屬二級，2例屬三級。其餘6例並無脂肪肝（零級）。患者的肝臟脂肪分數的中位數（範圍）為：零級2.4（1.9-3.3），一級6.4（2.7-10.6），二級19（14.9-26.4），三級45.7（20.1- 71.3）；$p < 0.001$。

結論：使用多迴波兩點Dixon法為肝脂肪變性作半定量檢測是一種非侵入性的方法，能為提供一系列肝病中脂肪沉積嚴重度的合理而準確的數據。把該序列納入常規脂肪肝磁共振成像方案大有裨益。

INTRODUCTION

Hepatic steatosis refers to large vacuoles of triglyceride fat that accumulate in liver cells, including in alcoholic and non-alcoholic fatty liver disease.[1] Other causes of steatosis include hepatitis B and C infection. Non-alcoholic fatty liver disease can range from simple steatosis to steatohepatitis, and can subsequently progress to cirrhosis or hepatocellular carcinoma.[1] Thus, evaluation methods for hepatic steatosis are of paramount importance to assess the suitability of donor livers before transplantation to reduce the high incidence of primary non-function. Currently, liver biopsy is the standard reference for steatosis assessment, but there are limitations such as procedure-related complications and high sampling variability.[1-3]

In terms of non-invasive and accurate radiological diagnostic value, magnetic resonance imaging (MRI) and MR spectroscopy (MRS) have become acceptable and promising methods for assessment of steatosis.[2,3] However, MRS is not suitable for routine clinical practice as it is time-consuming and requires specialised software and clinical expertise.[4] Currently, the chemical shift-based technique is one of the non-invasive MRI techniques for quantitative assessment of hepatic steatosis. The major limitation of the conventional chemical shift-based technique called 'dual phase' is that it is unable to evaluate the complete dynamic range of 0% to 100% fat fraction, even in patients with hepatic fat fraction (HFF) approaching 50%, and is limited to evaluating patients with severe steatosis.[2,4] Therefore, the modified chemical shift-based technique known as the Dixon method has been adjusted to evaluate hepatic steatosis. The technique is widely available and easy to perform. Nevertheless, the accuracy of the Dixon method for quantification of hepatic steatosis can be affected by acquisition parameters.[5,6]

Based on a number of acquired images of the Dixon technique, the multipoint method tends to be less vulnerable to reconstruction errors than the two-point method, although in clinical practice, with critical acquisition times, the two-point method is more desirable. The multiecho two-point Dixon (mDixon) technique with flexible echo times has been shown to improve fat-water separation, have better signal-to-noise ratio, and have the feasibility of breath-hold.[7,8] To our knowledge, there is limited documentation of semi-quantitative assessment of hepatic steatosis with the mDixon technique using histopathological results as a standard reference. This study aimed to determine the use of the mDixon technique in the semi-quantification of hepatic fat in patients with chronic liver disease and to compare these results with the histopathology results.

METHODS

Study Population

From June 2012 to May 2013, patients with chronic liver disease who had indications for liver biopsy were recruited from the Hepatology Outpatients Clinic at Siriraj Hospital, Bangkok, Thailand. Patients who underwent liver MRI for evaluation of the severity of hepatic fibrosis and hepatic fat before undergoing liver biopsy on the same day were included.

This retrospective study was approved by the Institutional Review Board of the Faculty of Medicine, Siriraj Hospital, Mahidol University, Thailand. The study was conducted according to the principles of the Declaration of Helsinki.

Magnetic Resonance Imaging

MRI examination of the liver was performed on a 3.0 T Achieva MRI scanner (Philips Medical Systems, Eindhoven, The Netherlands) using a Sense body coil (Philips Medical Systems) with the two-point Dixon without R2* (1/T2*) correction option. The sequence was a three-dimensional T1 fast-field multiecho MR sequence scanned within a breath-hold time (approximately 15 seconds) with the imaging parameters as follows: repetition time / echo time 1 / echo time 2 of 3.5 / 1.4 / 2.7 ms, respectively, matrix

of 292 x 292, slice thickness of 2.5 mm, and field of view of 350 x 290 mm. Four sets of images were reconstructed from the scan: in-phase, opposed phase, water-only, and fat-only images.

Three circular regions of interest (ROIs) of approximately 2.30 cm^2 were manually placed around the suspected liver biopsy area on the mid-axillary area of the liver parenchyma at the right lobe of the liver, extending from the liver capsule for about 1.0 to 1.5 inches, excluding the region of the vessels, signal artefacts, and focal fat sparing or infiltration, by a radiologist and co-localised into both water and fat images in the same liver parenchyma area. The HFF was calculated from the mean ROIs of water and fat signals on water-only and fat-only images using the following formula:

$$HFF = \frac{S_{Fat}}{S_{Fat} + S_{Water}} \times 100$$

where S_{Fat} and S_{Water} are fat and water signals, respectively

For each patient, three HFFs were analysed and the mean was reported.

Histopathological Grading

Percutaneous liver biopsies under sonographic guidance were performed at the right lobe of the liver, located on the mid-axillary line from the liver capsule for about 1.0 to 1.5 inches with at least two usable sampling tissues in fully inspirated patients on the same day as the hepatic MRI examination by an experienced gastroenterologist. After routine processing and staining, a pathologist who specialised in the hepatobiliary system and was unaware of the patients' clinical and radiological details performed interpretation of the biopsy specimens independently. All biopsies were of an appropriate size and included enough portal tracts for confident pathological grading and staging of the histological features.

The severity of fatty infiltration, necroinflammation, and fibrosis was recorded and scored according to the schema outlined by Kleiner et al.[9] Hepatic steatosis was classified from grade 0 to 3: steatosis grade 0 (<5% macrovesicular fat in hepatocytes), grade 1 (5%-33%), grade 2 (34%-66%), and grade 3 (>66%). A diagnosis of fatty liver was made when there was steatosis of grade 1 or greater. Lobular inflammation was assessed per foci of inflammation under x 200 magnification. Grading severity of lobular and portal inflammation is shown in Table 1. Fibrosis was classified into five stages as follows: stage 0, none; stage 1, periportal or perisinusoidal; stage 2, periportal and portal or periportal; stage 3, bridging fibrosis; and stage 4, cirrhosis.

Table 1. Grading severity of lobular and portal inflammation.

Grade	Lobular inflammation	Portal inflammation
0	No inflammation	None
1	<2 foci	Mild
2	2-4 foci	Moderate
3	>4 foci	Marked portal hypertension

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 19 (IBM Corp, Armonk [NY], USA). Data were summarised using descriptive statistics. Continuous variables were compared using the Kruskal-Wallis test and a non-parametric multiple comparison test (Dunn's test). P values of ≤0.05 were considered statistically significant.

RESULTS

There were 24 patients eligible for the study, of whom three who had no available images for analysis were excluded. The mean age of the 21 patients was 47 years (range, 23-77 years) and 57% were women. The mean body mass index (BMI) was 24.6 kg/m^2 (range, 14.0-33.9 kg/m^2). Obesity (BMI ≥28 kg/m^2) was present in four (19%) of the patients. The mean value of alanine aminotransferase was 124 IU/L (range, 23-414 IU/L). Indications for liver biopsy included assessment of severity of chronic hepatitis B or C infection in 13 patients, evaluation of drug-induced hepatotoxicity in one patient, and identification of the aetiologies of chronic hepatitis in seven patients. On the basis of the histological findings, six (29%) patients were diagnosed with non-alcoholic steatohepatitis (NASH).

Figure 1 shows an example of a representative ROI placement in in-phase, opposed phase, water-only, and fat-only images. The HFF of the patients included in the study are summarised in Table 2. The median and range of the HFFs of patients with steatosis grades 0, 1, 2, and 3 were 2.47 (1.96-3.37), 6.43 (2.74-10.66), 19.08 (14.91-26.45), and 45.77 (20.17-71.37), respectively ($p < 0.001$). A steady stepwise increase in HFF was observed with the increasing histological severity of hepatic steatosis (Figure 2). In addition, the HFFs were significantly different among the subgroups ($p < 0.05$).

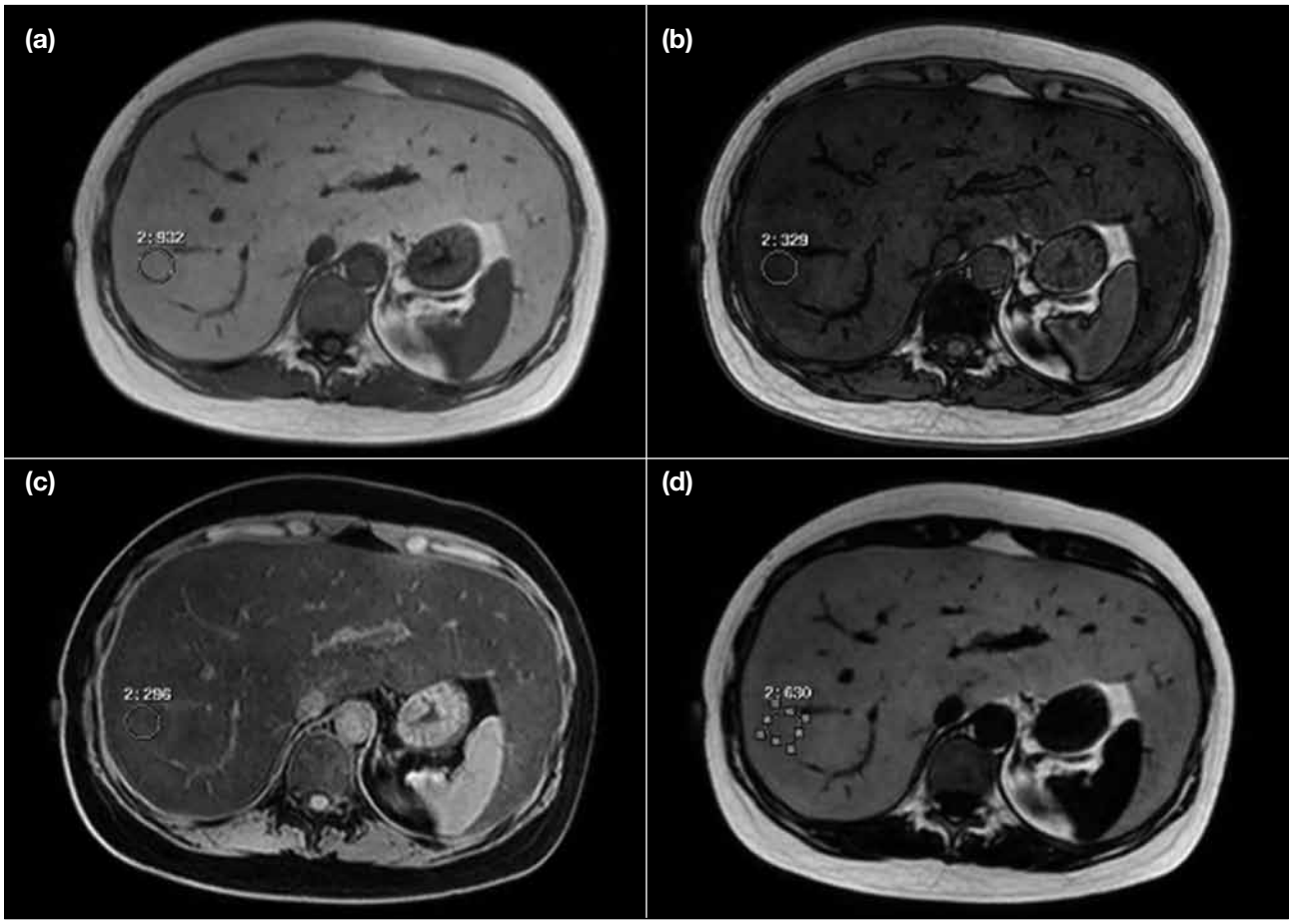

Figure 1. A 33-year-old woman with severe hepatic steatosis (grade 3).
Representative images obtained in multiecho two-point Dixon sequences: (a) in-phase, (b) opposed phase, (c) water-only, and (d) fat-only. By using water-only and fat-only images, the calculated hepatic fat fraction was 630/(296+630) = 68%.

Table 2. Descriptive statistics of hepatic fat fraction in subgroups divided according to histopathological grading.

Histopathological grading	Hepatic fat fraction (%)				p Value
	Mean	Standard deviation	Median	Range	
Grade 0	2.51	0.47	2.47	1.96-3.37	
Comparison with:					
Grade 1					0.003
Grade 2					0.002
Grade 3					0.009
Grade 1	6.70	3.42	6.43	2.74-10.66	
Comparison with:					
Grade 2					0.297
Grade 3					0.281
Grade 2	19.73	4.20	19.08	14.91-26.45	
Comparison with:					
Grade 3					1
Grade 3	45.77	36.20	45.77	20.17-71.37	
Total	12.33	15.50	7.51	1.96-71.37	
Comparison across groups	-	-	-	-	<0.001

From the histological features of the 21 patients, steatosis was present in 15 (71.4%) and was of grade 1 in nine (42.9%), grade 2 in four (19.0%), and grade 3 in two (9.5%). No hepatic steatosis (grade 0) was found in six patients. Lobular inflammation was found in 18 patients (85.7%; 11 with grade 1 and 7 with grade 2)

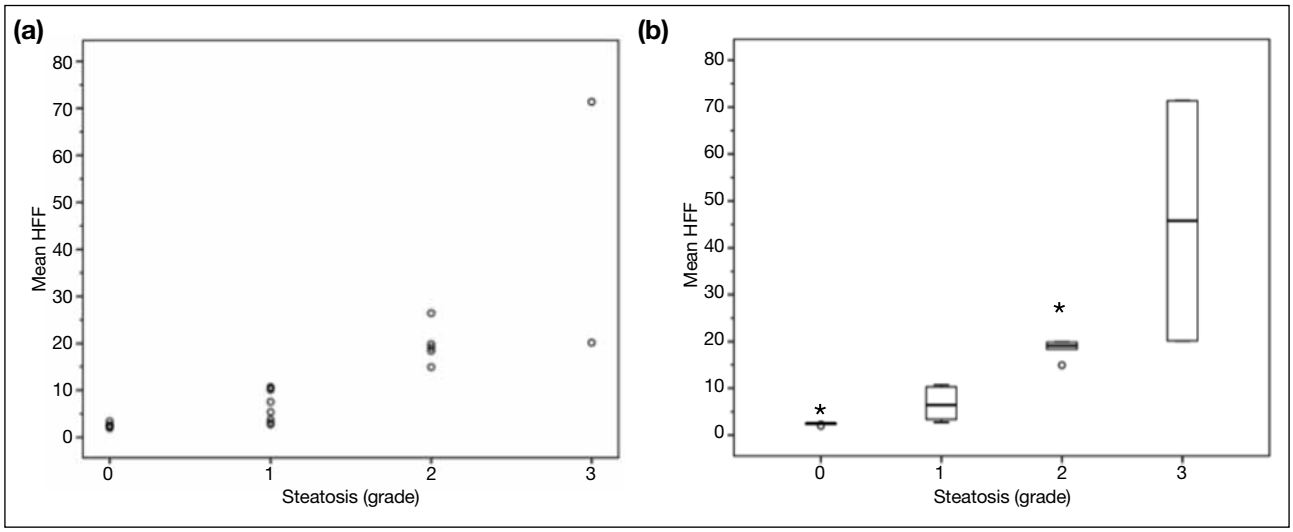

Figure 2. Hepatic fat fraction (HFF) plots for 21 patients.
(a) Scatter plot showing means (circles) of HFF among different grades of hepatic steatosis, and (b) box plot showing the ranges (boxes), means (thick lines), and outliers (circles and asterisks) of HFF among different grades of hepatic steatosis.

Table 3. Hepatic fat fraction across subgroups according to lobular inflammation, portal inflammation, fibrosis, and diagnosis of NASH.

	No.	Hepatic fat fraction (%)				p Value
		Mean	Standard deviation	Median	Range	
Lobular inflammation						0.122
Grade 0	3	2.73	0.92	2.48	1.96-3.74	
Grade 1	11	14.66	20.46	5.35	2.31-71.37	
Grade 2	7	12.78	6.83	10.66	2.46-20.17	
Portal inflammation						0.333
Grade 0	4	26.01	32.18	15.09	2.48-71.37	
Grade 1	10	11.55	7.81	12.78	1.96-20.17	
Grade 2	7	5.61	3.64	3.37	2.31-10.44	
Fibrosis						0.760
Stage 0	4	12.94	11.75	11.41	2.48-26.45	
Stage 1	9	16.73	21.94	10.65	1.96-71.31	
Stage 2	7	6.58	4.65	5.35	2.31-14.91	
Stage 3	1	10.44	-	-	-	
Stage 4	0	-	-	-	-	
Diagnosis of NASH						0.002
NASH	6	25.88	22.56	19.11	10.66-71.37	
Non-NASH	15	6.90	7.15	3.34	1.96-26.45	

Abbreviation: NASH = non-alcoholic steatohepatitis.

and the remaining 3 (14.3%) patients had no lobular inflammation. Portal inflammation was seen in 17 patients (81.0%; 10 with grade 1 and 7 with grade 2); four (19.0%) patients had no portal inflammation. Stage 1 fibrosis was seen in nine (42.8%) patients, stage 2 was seen in seven (33.3%), and stage 3 was seen in one (4.8%). Four (19%) patients had no fibrosis (Table 3).

To better understand the potential role of hepatic necroinflammation on the measurement of HFF in patients with fatty liver, we examined the relationship of the presence of steatohepatitis with the percentage of HFF (Table 3). The HFF of six patients with NASH was significantly higher than those of the remaining patients without NASH ($p = 0.002$). In addition, the HFF was higher in patients with lobular inflammation than in those without inflammation, although this did not reach statistical significance ($p = 0.122$). However, no significant difference of HFF was observed between patients with and without portal inflammation.

DISCUSSION

The chemical-shift MRI, which is commonly used in clinical practice for detecting fatty changes in the liver, is limited in its inability to quantify hepatic fat content beyond a 50% fat fraction due to water-fat signal ambiguity and degrading image quality caused by susceptibility artefacts from the air-tissue interface.[4,10] The modified chemical-shift MRI known as the mDixon sequence, which is a three-dimensional fast-field chemical-shift–based sequence using multiple echoes for water-fat separation, has a shorter scan time and provides better signal-to-noise ratio for semi-quantification of liver fat content.[7] However, the mDixon technique, based on the two-point Dixon method, might have uncorrected T2* effects, which are more often interfered with by several confounding factors such as hepatic inflammation, fibrosis, and iron deposition in the liver parenchyma.[2,4] In patients with iron deposition and high-grade fibrosis or cirrhosis, T2* decay is an important confounding factor of the chemical-shift MRI technique and is corrected for by using the T2* correction method or the proton density fat fraction technique.[11] Our results showed significant correlation of HFF and histological grading of steatosis, particularly for the diagnosis of moderate-to-severe steatosis, with lower effects of hepatic inflammation and fibrosis on the semi-quantitation of hepatic steatosis, as there were some results that recorded no effect of fibrosis on hepatic fat quantification.[12-14] These results may be explained by the small number of patients with severe liver fibrosis (grades 3 or 4) in this study. Additionally, the measured ROIs were placed in the middle of the liver parenchyma where the susceptibility effect is less prominent than the near-hepatic diaphragm region. Nevertheless, further studies to assess the effect of T2* decay on quantification of hepatic steatosis in patients with coexisting iron deposition and the calculation of proton density fat fraction using the mDixon sequence with T2* correction are warranted.

With regard to histopathology, hepatic steatosis is defined by the accumulation of 5% or more fat in hepatocytes. Unfortunately, no exact cutoff value for MRI-measured HFF has yet been defined to discriminate between normal and abnormal levels of hepatic fat. In a study using the two-point Dixon method with MRS as the reference standard, the investigators proposed a cutoff value for HFF of 3.6% for detection of fatty liver,[8] whereas in a study using the different chemical-shift–based techniques compared with liver biopsy as the reference standard, the HFF values were reported to range from –1.46% to 2.94%.[8] Even our semi-quantification of HFF using the mDixon technique is a reliable non-invasive measure, particularly for diagnosis of moderate-to-severe steatosis, we were still unable to define a cutoff point to detect the hepatic steatosis due to the small number of patients in each steatotic subgroup. The exact cutoff value of HFF in our study should be in the range of 3.37-14.91. However, the various cutoff values may be, in part, due to different MR sequences and acquisition techniques used at each centre. A larger study would more accurately determine the cutoff point between normal liver and fatty liver. The discrepancy between the MRI measurement and histopathological evaluation may be explained by the difference in targeted measurement between MRI and histopathology. MRI quantitative hepatic steatosis is calculated by the fraction of protons in lipid versus water, while the degree of steatosis in a liver biopsy specimen was interpreted based on the fraction of fat-containing hepatocytes.

A previous study comparing paired biopsy specimens of 51 patients has reported discordance between the first and second biopsy in 22% of patients.[15] However, liver biopsy is regarded clinically as the reference standard for diagnosis of hepatic steatosis, although it may be associated with potential sampling errors. To avoid the mismatch between ROI placement and biopsy site, we performed the liver biopsy and MRI on the same day in all patients, while keeping the location target of the MRI nearly the same as the liver biopsy area, thus permitting good correlation of hepatic fat assessment. However, many causes of mismatched ROI errors — such as respiratory, diaphragmatic, and cardiac motion as well as the selection of a safe biopsy site and size far from blood vessels — are inevitable and continue to be a concern.

Limitations of this study are the small number of patients and the unequal distribution of steatosis grading. Only two patients had steatosis grade 3 resulting in a non-statistical power difference for HFF between grade 3 steatosis and the other subgroups. A larger study is required for better assessment of the difference in HFF between each of the steatosis subgroups. Secondly, we graded histological steatosis on a discrete scale of 0 to 3, which limited the relevance of correlation with a continuous value of HFF. Histological quantification of the exact percentage of fat deposition in the hepatocytes or the semi-automatic vacuole segmentation method may be both more accurate and more valuable for

further studies. Thirdly, we did not evaluate the regional heterogeneity of fat deposition in liver tissue, which became a concern for inaccurate hepatic-fat calculation because the small sample size may have been a cause of unreliable data. Finally, our study did not enrol a healthy control population and included patients with a variety of liver diseases, which is important for determining the baseline HFF of normal liver and the different underlying parameters of liver diseases that may have effects on the different patterns of fatty changes on liver parenchyma.

CONCLUSION

Semi-quantification of fat deposition in liver parenchyma using the mDixon technique is a fast and reliable method for diagnosis of hepatic steatosis, particularly for diagnosis of moderate-to-severe disease, without any interference from coexisting hepatic fibrosis or inflammation. In clinical practice, the implementation of this sequence into the routine hepatic steatosis MRI protocol could be of great benefit.

DECLARATION

No conflicts of interest were declared by authors.

ACKNOWLEDGEMENT

The authors thank Suthipol Udompumturale of the Biostatistics Unit at the Siriraj Biomedical Research Institute, Bangkok, Thailand, for statistical assistance.

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