Fatty infiltration of the liver
Detection and grading using dual T1 gradient echo sequences on clinical MR system

Frank PILLEUL (1), Géraldine CHAVE (1), Jérome DUMORTIER (2), Jean-Yves SCOAZEC (3), Pierre-Jean VALETTE (1)
(1) Département d’Imagerie Digestive, (2) Département de Gastroentérologie, (3) Département d’Anatomopathologie, Hôpital Edouard Herriot, Lyon.

SUMMARY

Aim — To evaluate the relationship between histopathology results and magnetic resonance imaging (MRI) on in and opposed-phase sequences grading of fat deposition within human liver.

Materials and methods — In and opposed-phase T1-weighted gradient-echo sequences (double echo time 2.3 ms and 4.6 ms) were performed in 25 patients, using a 1.5-T clinical MR imaging system. Fat/water ratio on in- and opposed-phase images of the liver was compared with pathologically defined degree of steatosis. The signal intensity in the images was acquired with operator-defined regions of interest at the same location in both fat and water images and the ratio was calculated by dividing signal intensity of liver in opposed phased sequence on signal intensity of liver in phased sequence. Fat/water ratio and the degree of steatosis were compared using linear regression. The sensitivity and specificity of opposed-phase for diagnosing steatosis were defined by ROC analysis. Furthermore, a correlation between visual signal intensity variation and the degree of steatosis was assessed using Pearson correlation coefficient.

Results — Histology demonstrated fatty liver infiltrations in 81 % of specimens. The percentage of fatty hepatocytes was 28 +/- 30 %. Fat/water ratio was significantly correlated with the pathologic grading of steatosis (r = 0.816, P < 0.001). The opposed phase MR imaging sensitivity and specificity for the diagnosis of hepatic steatosis were respectively 80 % and 71 %. We obtained a statistically significant correlation between visual SIV and fatty liver grading (P = 0.017).

Conclusion — We demonstrated a significant correlation between fat/water ratio and histological findings for the detection and grading of fatty liver.

Hepatic steatosis is one of the most common morphological abnormalities identified on liver biopsy [1]. Detection and quantification of fat within the liver is of interest in groups of suspected non-alcoholic steatohepatitis [2-4], live liver donors or liver transplants [5]. Until recent publications, fat infiltration was considered most of the time as a benign pathology with no clinical or biochemical signs. Although percutaneous liver biopsy should be the standard investigation to determine accurately the fat fraction, it is an invasive technique with sampling errors. Thus, a non invasive method could be useful to appreciate the degree of steatosis, and so to estimate further potential risk in a specific group of patients. Magnetic resonance imaging has already been demonstrated to be a non invasive method of quantification in liver iron overload [6, 7]. Clinical cases using variable magnetic resonance (MR) methods, including conventional pulse sequences, chemical shift imaging, and MR spectroscopy have been previously published in the assessment of fatty liver [8, 9]. Based on their difference in resonant frequency, Dixon discriminated fat and water spins using a dedicated magnetic resonance sequence [10]. More recently, use of fast gradient echo technique in a breath hold examination can produce alternating signals in and out of phase for voxels containing fat and water. However, no clinical study has correlated
the fraction of fat in human liver biopsy with a commonly available opposed-phase gradient-echo imaging. The objective of this work was to appreciate if the signal intensity variation of the human liver between the in-phase and opposed-phase images reflects the histological results for the detection and the grading of steatosis.

Materials and methods

Study population

We cross-referenced the database of the Department of liver disease at the University of Herriot at Lyon with the MR imaging database to identify patients who had undergone liver biopsy and MR imaging in a time frame of less than one week. Institutional review board approval was obtained, who did consent for this retrospective study. A total of 25 patients who had undergone MR imaging of the liver between March 2001 and January 2002 were identified (14 women and 11 men). The average age was 51 years +/- 13.5. Two groups of patients were defined as follows: patients with diffuse liver disease followed up for hepatitis B virus (N = 2), hepatitis C virus (N = 1), alcoholic hepatitis (N = 6), nonalcoholic hepatitis (N = 4), and patients with focal malignant or benign hepatic lesions (N = 11) or hepatic biochemical abnormalities (N = 1).

Histological study

Liver biopsy was obtained from the right lobe of the liver by the subcostal approach under ultrasound guidance using a semi-automated 16-gauge needle either percutaneously (N = 19) or at laparatomy (N = 6). Biopsy samples were fixed in Bouin’s fluid, processed according to conventional histological techniques and stained with hematoxylin-eosin–saffron, Perls’solution (equal volumes of 20% potassium ferrocyanide and 2% anular grade hydrogen chloride) and chromotopoepe and retrospectively evaluated histologically for grading of fat deposition by a single pathologist using a visual quantification. Recording items were: presence or absence of steatosis, type of steatosis (macro- or micro-vesicular), topography (centrolobular, periporal or diffuse) and evaluation of steatosis by the percentage of hepatocytes with fat infiltration. Furthermore, the presence of inflammatory infiltrates, presence and extent of fibrosis were also evaluated according to the METAVIR score (F0 (no fibrosis) to F4 (cirrhosis)) for patients with hepatitis, and the presence of iron deposition was assessed by the Perls reaction.

MR Imaging

All MR studies were performed at 1.5 T clinical MR imaging system (Magnetom Symphony, Siemens Erlangen) and a phased-array body coil. All MR imaging examinations were performed with the following protocol: transverse dual fast in and out-phase T1-weighted gradient-echo sequences of the upper abdomen (repetition time msec/echo time msec, 202/2.3–4.6; flip angle, 70°; 22 sections acquired in a 23-second breath hold). Section thickness was 8 mm and matrix size was 256 x 112 (phase x frequency encoding). All protons exposed to the magnetic field of a 1.5 T machine precess at 64 MHz, so that, the chemical shift difference between water and fat is about 3.5 parts per million or about 225 Hz at 1.5 Tesla. This means that lipid protons precess at a velocity 225 Hz, which is slower than water protons. By varying the time between excitation and signal acquisition in fast gradient echo imaging with a fast low angle shot, mixture of lipid and water within the voxel can be identified.

Imaging interpretation

All MR images were retrospectively reviewed by the same experienced radiologist (G.C). At the time of review, we performed a qualitative analysis to assess image quality. Image quality was considered excellent when the liver border was well defined and no motion artefacts were present. Analyses of signal intensity within regions of interest placed centrally in the right lobe of the liver at the same location in both fat and water images using a standard software displayed on an independent workstation. The regions of interest were always 2-2.5 cm² for both the liver parenchyma’s regions of interest were obtained on the same location, taking care to avoid regions with signal intensity variation by partial-volume effects, chemical shift artefact and motion artefact. Measurement was made at the external part of the right lobe where liver biopsy was performed, avoiding vessels. The hepatic signal intensities in the out-of-phase images (fat images) were divided by the hepatic signal intensities in the in-phase images (water images) [10].

Signal intensity variation between in phase and in opposed-phase MR images were qualitatively evaluated. Hepatic steatosis was considered present when the signal intensity variation diffusely decreased between in phase and in opposed-phase images and was scored with use of the following four-point severity scale: “0” as none if the signal intensity variation was absent; “1,” as mild if the signal intensity on opposed phase was slightly less than that on in phase; “2,” as moderate if the signal intensity on opposed phase was moderately less than that on in phase signal intensity of the liver; and “3,” as severe if the signal intensity on opposed phase was markedly less than that on in phase signal intensity of the liver.

Statistical analysis

Descriptive statistical analyses with simple contingency tables were performed to characterize the data. Histological findings have been arbitrarily chosen as the gold standard and more than 10% of hepatocytes with fat infiltration has been considered as liver steatosis [16]. Fat liver infiltration was correlated with the fat/water ratio on in and out-of-phase imaging using Pearson’s coefficient of correlation. The relation between the fat/water ratio of the liver and the degree of steatosis was studied with a linear regression. Sensitivity and specificity of dual fast gradient echo imaging for the diagnosis of steatosis were defined by a ROC analysis. Because the fat/water ratio was a quantitative value, the composite ROC curve has been used to define the threshold between positive and negative results of steatosis. For all tests, P<0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with SPSS 10.0 for Windows (SPSS, Chicago, Ill) and Microsoft Excel 2000 (Microsoft, Redmond, Wash).

Results

Descriptive analysis

MR images were homogeneous in all cases, with some shading from magnetic field heterogeneity at the bottom or top sections. Motion artefact was minor due to short echotime and breath-hold sequences.

Histological specimens demonstrated a fatty liver infiltration in 81% of patients. Macrovesicular steatosis was present in all cases, associated with microvesicular steatosis in 19% (figure 1).

Fig. 1 – Mixt macrovesicular (asterisk) and microvesicular (arrow) steatosis after hematoxylin-eosin coloration of liver sample.

Stéatose combinée macrovésiculaire et microvésiculaire sur une pièce de biopsie de foie après coloration hématoiylxine-éosine.
Fatty infiltration was heterogeneous in 85% of patients. The average percent of fatty hepatocytes was 28.7 +/- 30.3%, with a median of 10% (minimum 0 - maximum 90%). Other parenchymal abnormalities were cirrhosis (N = 7), cholestasis (N = 1), alcoholic hepatitis (N = 1) and siderosis (N = 1). Presence of fibrosis was identified in 15 cases (mean METAVIR score was 1.6 +/- 1.7).

Relationship between histological features and fat/water ratio

Distribution of patients according to the degree of hepatic steatosis and the fat/water ratio is described on figure 2. We demonstrated a significant correlation between fat/water ratio and fatty liver infiltration (r = -0.816, P < 0.001). Presence of fatty hepatocytes higher than 10% within the parenchyma of the liver defined steatosis [8]. For this percentage of fat, the sensibility and the specificity for the diagnosis of hepatic steatosis was respectively 80% and 71%.

We also obtained a statistically significant correlation between the visual signal intensity variation (absent or mild, moderate or important) and the quantitative signal intensity variation (P = 0.014) and the grading of fatty liver (P = 0.017).

Discussion

Fatty liver is characterized by the accumulation of triglycerides within hepatocytes. Fatty infiltration of the liver is a nonspecific response of the liver tissue to various kinds of injury or systemic disorders, such as pregnancy, diabetes, obesity, ingestion of hepatotoxins, drug therapy, intravenous hyperalimentation, high-fat diet, or hepatic resection [11]. Research on fatty liver and its role in the progression of chronic liver disease and other metabolic disorders has seen a recent rapid growth. This is likely due, in part, to an increased incidence of fatty liver, which has occurred because of a combination of rich diet and lack of exercise in the general population, resulting in a rise in the prevalence of obesity and hepatic steatosis. Liver steatosis has become an increasingly frequent diagnosis since the introduction of ultrasoundography and computed tomography scan in the routine assessment of patients with suspected liver disease. In a cohort study of an entire population, the presence of steatosis was found in 15% of the total [12]. In the vast majority of cases, steatosis was totally asymptomatic. Although the natural history of steatosis has been reported to be benign, recent data indicate that non-alcoholic steatosis may progress to cirrhosis in more than 3% of patients [13]. This percentage is much higher if alcohol consumption is present [2]. These data emphasize the importance of a correct diagnosis of steatosis and the need for careful follow-up. Furthermore, technical improvement of liver transplanation with use of living donor allows an increasing number of patients with end-stage liver disease the opportunity for effective treatment. In the meanwhile, presence of steatosis decreases functional graft mass and may contribute to graft dysfunction. However, the diagnosis is currently based on a qualitative rather than quantitative test, except when an invasive biopsy is performed. Liver biopsy is the gold standard for accurately determining the amount of fat within the liver. As an added bonus, it allows the detection of subclinical hepatic pathologies such as fibrosis and hepatitis. However, percutaneous liver biopsy is an invasive procedure and can have serious complications to an ostensibly normal individual. Furthermore, multiple biopsy samples are generally necessary and fatty quantification may be unduly influenced by error resulting from heterogeneity of the pathological changes. A reliable non-invasive method without sampling error would offer obvious advantages in assessments of fatty infiltration in these patient populations. Computed tomography (CT) scan and ultrasonography (US) have been used in clinical practice to identify hepatic fat [14-16]. On ultrasound, it may be difficult to differentiate diffuse hepatic steatosis from other diffuse liver disease such as fibrosis. Although, unenhanced CT scan allows the detection of hepatic steatosis with 99% specificity when density difference between the liver and the spleen is up to 10 Hounsfield units [15]. Otherwise, quantitative criteria of fatty liver infiltration is more complex and required protocol specific and depended on the type of CT scan used, contrast volume, injection rate and the type of contrast used [17, 18]. Finally, classic CT methods, either in terms of absolute Hounsfied units of the liver or as liver-to-spleen ratio, may suffer from fluctuations related to the volume, the “noise” of the patients and the shape of both the liver and spleen. Accordingly, it would be desirable to have a reproducible and reliable method to quantify fat infiltration of the liver. Because of long time examination, magnetic resonance (MR) has been proscribed in the quantitative assessment of steatosis on routine examination. However, several studies have indicated that MRI techniques can be used to evaluate the degree of liver steatosis in selected patients [19]. The MR images of the liver show an increased signal intensity on conventional T1-weighted images in case of moderate to severe fatty infiltration due to the short T1 of the fat. In the present study, liver steatosis has been evaluated using the chemical shift results between in and out of phase T1 weighted gradient echo compared to quantitative histological grading score of hepatic fatty deposition. Mitchell used three different techniques to correlate MR signal intensity variation of normal liver, fatty liver on rats with pathology results such as water saturation, fat saturation and opposed phase on spin echo [20]. Then, good correlation between liver signal variation and the degree of steatosis has been found except for fat saturation sequence.

The aim of our study was to validate a non invasive test which could be used in MR clinical study for an assessment of the amount of fat in the liver. Dedicated MR sequences accurately measured the degree of steatosis when compared to morphometric analysis of fatty infiltration provided by the liver biopsy. Liver biopsy was the gold standard for this evaluation. A significant correlation was found between the degree of steatosis on histology and the fat/water ratio on MR imaging. To our knowledge, few articles with such results have been published using gradient echo on MRI. Fishbein et al. [21] described the utility of fast gradient echo technique MRI to quantify hepatic fat content. Because of long time acquisition of sequences, preliminary studies of fat quantification on MR demonstrated significant motion artefacts [20]. The use of fast gradient technique allowed shorter TR (repetition time) and TE (echo time), and than reducing acquisition time.
sufficiently to make breathholding technique. One recent study found a high sensitivity when extensive steatosis (> 33% hepatocytes) was present, but confirmed that imaging can not distinguish between the different types of steatosis [22]. Our series confirmed these results without possibilities to distinguish between micro- and macrovesicular steatosis, with a sensitivity of 80% for a threshold value of 10% of fatty deposition. Although the study population was heterogeneous with variable liver pathology, we found significant results between fat/water ratio on MR images and degree of fatty infiltration. These good results have been found when we compared the signal intensity value between in phase and out of phase sequences on the same location of interest. Furthermore, the presence of fibrosis classified by the METAVIR score did not interfere with the correlation between fatty infiltration and the fat/water ratio. Although CT diagnosis of hepatic steatosis is established when the liver-spleen attenuation difference is greater than - 10 Hounsfield units, the correlation between the hepatic signal relative to spleen signal with the degree of steatosis was heterogeneous with variable liver pathology, we found significant results between fat/water ratio on MR images and degree of fatty infiltration. These good results have been found when we compared the signal intensity value between in phase and out of phase sequences on the same location of interest. Furthermore, the presence of fibrosis classified by the METAVIR score did not interfere with the correlation between fatty infiltration and the fat/water ratio. Although CT diagnosis of hepatic steatosis is established when the liver-spleen attenuation difference is greater than - 10 Hounsfield units, the correlation between the hepatic signal relative to spleen signal with the degree of steatosis on MR technique was not statistically significant.

In addition, we demonstrated significant correlation between visual signal intensity variation of the liver, objective value of fat/water ratio and histological grading of fat deposition in the liver. With advances in MR imaging in the evaluation of liver transplantation for the segment morphology, vascular and biliary anatomic variation, an accurate and non invasive method to detect and quantify steatosis should have further interest (figures 3 a et b). Indeed, the number of living donor liver transplantations has increased six-fold in the last 4 years. One of the main difficulties with living liver transplant is the high degree of steatosis which may increase the severity of the ischemia-reperfusion lesion producing an initial poor function in the recipient. However, only severe macrosteatotic grafts (> 60%) are rejected for transplantation, and donor livers with moderate to severe macrosteatosis (30-60%) have a relative risk of primary non-function and should be considered for transplantation. Thus, dedicated MR sequences may provide accurate and definitely less invasive evaluation of fatty content of the liver. In addition, the method used allowed a quantitative rather than qualitative measure of the hepatic fat content. With this technique hepatic fat content is expressed as a percentage of the entire liver, thus facilitating the evaluation of its variation over time.

In conclusion, comparison of T1-weighted gradient echo in-phase and out of phase images seems to be a possible method of diagnosis liver steatosis with only one short TR series. Image quality is more reproducible than previous series with long TR acquisition. Estimation of liver steatosis can rely on visual inspection, although location of interest signal intensity measurements provides accurate information of the percent of fat within the hepatocytes. This suggests that further study in a homogeneous group should be done. In addition, this approach to the diagnosis of fatty liver may offer in a subgroup of transplant patients the possibility of making a more accurate assessment of donor livers prior to transplantation [23].

REFERENCES

Fatty infiltration of the liver


