Liver ultrasound scanning in the detection of hepatic steatosis and fibrosis in NASH patients

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Background
Nonalcoholic fatty liver disease (NAFLD) has become the most prevalent cause of liver disease in western countries. The development of nonalcoholic steatohepatitis (NASH) and fibrosis identifies the risk group with an increased incidence of liver-related deaths.

Aim
The aim of the present study is to investigate how accurately liver ultrasound (US) can contribute toward the prediction of the severity of liver damage in NAFLD, and to determine whether it may be an easily available, inexpensive, noninterventional, widely used screening method.

Methods
Fifty-four obese patients with variable degrees of BMI were recruited in the present study. Assessment of full medical history, anthropometric measurements, biochemical studies, abdominal US, liver biopsy for histological examination, and determination of the NAFLD activity score (NAS) score were carried out on all patients to identify NASH patients . Liver steatosis was evaluated using liver US, and graded according to a semiquantitative scale from 1 to 4. Liver histological examination was carried out to identify patients with NASH, borderline NASH, or non-NASH according to the NAS score.

Results
According to the NAS score, patients were divided into non-NASH patients (eight patients), borderline NASH patients (24 patients), NASH patients (20 patients), and patients with NASH and fibrosis (two patients). Alanine aminotransferase and γ-glutamyl transpeptidase were significantly higher in NASH patients. Correlating the grading of hepatic steatosis by liver US and NAS score, grade 1 was found in 37.5% of patients with non-NASH, 33.3% of patients with borderline NASH, and only in 5% of patients with NASH, whereas grade 4 steatosis was found in 20% of NASH patients and 4.2% of patients with borderline NASH; none of the non-NASH patients were diagnosed with grade 4 hepatic steatosis. The sensitivity of liver US in detecting grades of steatosis in liver biopsy was 61% in grade 1, 25% in grade 2, and 75% in grade 3. There was a direct correlation between grading of steatosis in the histological examination and the presence of NASH, *P* less than 0.000.

Conclusion
Liver US is not only sensitive in the detection of hepatic steatosis, but also in the prediction of the presence of NASH; therefore, it can be used as a simple, noninvasive, low-cost method for the screening of NAFLD and for the early identification of patients in need of aggressive intervention.

Keywords:
liver steatosis, liver ultrasound, NAS score, nonalcoholic steatohepatitis

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Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of diseases ranging from simple steatosis to inflammatory nonalcoholic steatohepatitis (NASH), with increasing levels of fibrosis and ultimately cirrhosis. NAFLD is strongly associated with obesity and insulin resistance, and is now recognized to represent the hepatic manifestation of the metabolic syndrome. Since the term NASH was first coined by Ludwig and colleagues in 1980 [1], the prevalence of NAFLD has increased rapidly in parallel with the marked increase in of the prevalence of obesity and diabetes [2], resulting in NAFLD, which is currently the most common cause of liver disease in the western world [3]. Among morbidly obese patients undergoing bariatric surgery, the prevalence of simple fatty liver is 47%, the prevalence of NASH is 27–42%, and cirrhosis occurs in 2.9–3.9% [4]. Although simple steatosis is believed to be a relatively benign entity, NASH may potentially result in morbidity and mortality.

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In this context, early identification of patients with NASH before the onset of advanced fibrosis would be useful in guiding aggressive intervention.

The diagnosis of NASH can only be confirmed by liver biopsy, and excess consumption of alcohol can be excluded by clinical enquiry. Hepatic steatosis can easily be determined by imaging studies [ultrasound (US), computer tomography, or magnetic resonance scan]. Transient elastography represents a useful tool for the rapid, noninvasive assessment of liver fibrosis. However, currently, no imaging modality can detect the subtle histological changes in inflammation and ballooning, and thus cannot differentiate simple steatosis from NASH [5].

Real-time US scanning is considered the first-line imaging investigation in patients with suspected liver disorders. Although not sufficiently sensitive to detect liver inflammation and fibrosis, it shows a good correlation with the histological finding of fatty infiltration [6]. The major factor that does not facilitate decision making on the basis of US findings in patients with parenchymal liver disease is the doubt about the accuracy of scanning when performed in a routine clinical setting [7].

The aim of the present study is to investigate how accurately liver US can contribute toward the assessment of the severity of liver damage in NAFLD and to determine whether it can be used as an easily available, inexpensive, noninterventional, widely used screening method.

Patients and methods

Patients

The current study is a prospective study, carried out in Kasr Al-eini hospital, Internal Medicine outpatient clinic (Liver and gastroenterology clinic), faculty of medicine, Cairo University, over a 6-month period (June–November 2011). NAFLD patients had elevated liver enzymes and/or liver US scan showing a picture of fatty liver.

The selection of participants in this study was made on the basis of the following inclusion criteria: men and women, between 18 and 60 years of age, with BMI over 25, with the presence of a bright liver on liver ultrasound scan (picture suggestive of hepatic steatosis), and with no history of alcohol intake. Participants with hepatitis C or B infection, patients with known causes of liver disease (autoimmune, genetic, or drug induced), patients with major systemic conditions, and pregnant women were excluded from the study.

Fifty-four male and female participants (50 women and four men) were subjected to a complete work-up including a detailed medical history, a general physical examination, anthropometric measurements, assessment of serum biochemistry profiles, determination of hepatitis markers, liver US scan, and true-cut US-guided liver biopsy.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Cairo University Hospital Research Ethics Committee (REC) on 28 May 2011 (N-7-2011).

Written informed consents were obtained from all participants in the study.

Methods

All participants were interviewed for their medical history and to obtain information on their life style. The weight and height of each participant were measured while the participant was clothed only in a light gown, and the BMI was calculated as body weight divided by height squared (kg/m^2), obesity defined as a BMI of at least 30 kg/m^2 and BMI between 25 and 29.9 kg/m^2 as overweight. The waist circumference was measured midway between the lowest rib margin and the iliac crest in a standing position by the same examiner.

Laboratory investigations included serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), alkaline phosphatase, total and conjugated bilirubin, prothrombin concentration, fasting blood sugar, and lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides).

Each patient was subjected to a liver US, performed by the same operator (M.H.), using a Toshiba Aplio xv scanner (Toshiba, Japan) equipped with a broad band 2.5–5 MHz curved-array probe to assess the presence of liver steatosis (bright liver), which was defined and graded as follows: (a) a diffuse hyperechoic echo texture (bright liver), (b) increased liver echo texture compared with the kidney, (c) vascular blurring, and (d) deep attenuation [8]. Steatosis was graded using this semiquantitative scale from 1 to 4. Fibrosis, when present with noticeable steatosis, was identified by a coarse echo pattern. Therefore, the grade fibro-fatty indicated the presence of a bright liver, with a coarse texture.

All participants were eligible for liver biopsy on the basis of the presence of hepatic steatosis in liver US with or without elevated liver enzymes and with a normal prothrombin concentration. All the samples were evaluated by the same pathologist (A.M.). They were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks, and then cut into 5-μm-thick sections and stained with hematoxylin & eosin. They were examined under a light microscope for histopathological evaluation.

In a subsequent analysis of liver biopsies, liver damage was assessed using the NAFLD activity score (NAS), which is the sum of steatosis (scale from 0 to 3), lobular inflammation (scale from 0 to 3), and hepatocellular ballooning (scale from 0 to 2), according to Kleiner *et al.* [9]. This scoring system covers the full spectrum of lesions of NAFLD, and allows a diagnostic categorization into NASH, borderline NASH, or no NASH. Fibrosis staging (evaluated separately from NAS) was graded on a scale from 0 to 4, where 0 = no fibrosis and 4 = cirrhosis, according to the classification of Brunt and colleagues [10].

The NAS score was used to correlate the sensitivity of the liver US in predicting the severity of the condition

after dividing our participants into two groups: NASH-positive patients (46 patients in total, 24 patients with borderline NASH and 22 patients with NASH) and patients with no NASH (eight patients).

Statistical methods

Data were statistically described in terms of mean ± SD, and frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of numerical variables between the study groups was carried out using the Kruskal–Wallis test with multiple-group comparisons. For comparison of categorical data, the χ^2-test was carried out. The exact test was used when the expected frequency was less than 5. Agreement between the results of US and biopsy was tested using the κ statistic. A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were carried out using the computer program statistical package for the social science (SPSS Inc., Chicago, Illinois, USA), version 15 for Microsoft Windows.

Results

The present study enrolled 54 obese patients, 50 women (92.6%) and four men (7.4%); all patients had NAFLD, first diagnosed by liver US and then confirmed by liver biopsy. On the basis of the NAS score, they were divided into patients with no NASH (eight patients), patients with borderline NASH (24 patients), patients with NASH (20 patients), and patients with NASH and fibrosis (two patients).

For statistical purposes, we included patients with borderline NASH, NASH, and NASH with fibrosis in one large group that was positive for NASH.

Table 1 shows the anthropometric and biochemical laboratory data of patients positive for NASH; there were 46 patients, 44 women (95.7%) and two men (4.3%), age 42.7 ± 7.5 years. The non-NASH group included eight patients, six (75%) women and two (25%) men, age 45.8 ± 6.3 years.

BMI and waist circumference were higher in NASH patients, but the difference was not significant.

ALT and GGT were significantly higher in patients with NASH. AST and triglycerides were higher in NASH patients, but these levels were not statistically significant.

Table 2 shows the number of patients with NASH and patients without NASH on the basis of the NAS score of the grades of liver steatosis by liver US. Among patients with positive NASH, 31.5% had grade 3 steatosis and among patients negative for NASH, 37.5% had grade 1 steatosis by US as shown in Table 2.

Correlating the grading of hepatic steatosis by liver US and the NAS score, grade 1 was found in 37.5% of patients with non-NASH, 33.3% of patients with borderline NASH, and only in 5% of patients with NASH, whereas grade 4 steatosis was found in 20% of patients with NASH, and 4.2% of patients with borderline NASH; none of the non-NASH patients had grade 4 steatosis. This correlation showed that the greater the advances in the US grading of the liver the more the severity of liver biopsy regarding the presence of NASH, which was significant as shown in Table 3.

Table 1 Clinical and biochemical characteristics of all patients

Characteristics	NASH ($N=46$)	Not NASH ($N=8$)	P value
Age (years)	42.7 ± 7.5	45.8 ± 6.3	0.290
BMI (kg/m^2)	36.0 ± 4.0	33.6 ± 2.2	0.643
Waist circumference (cm)	106.7 ± 15.5	103.1 ± 11.8	0.487
ALT (IU/l)	35.8 ± 19.4	18.6 ± 7.4	0.005*
AST (IU/l)	34.4 ± 21.4	25.0 ± 6.2	0.223
GGT (IU/l)	51.6 ± 36.6	24.1 ± 14.6	0.006*
FBS (mg/dl)	120.7 ± 30.6	121.6 ± 23.8	0.679
Total-C (mg/dl)	203.0 ± 31.5	224.3 ± 38.2	0.161
HDL-C (mg/dl)	47.4 ± 15.0	51.6 ± 10.8	0.176
LDL-C (mg/dl)	103.5 ± 18.7	110.1 ± 33.7	0.990
TGs (mg/dl)	173.5 ± 44.0	160.3 ± 44.2	0.465

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; GGT, γ-glutamyl transpeptidase alkaline; NASH, nonalcoholic steatohepatitis; TGs, triglycerides; Total-C, total cholesterol.
*$P<0.05$.

Table 2 Correlation between ultrasound finding and the presence or absence of nonalcoholic steatohepatitis in liver biopsy

	NASH			
Liver US	No ($N=8$)	Yes ($N=46$)	Total ($N=54$)	P for trend
Grade 1	3 (37.5%)	9 (19.6%)	12 (22.5%)	–
Grade 2	1 (12.5%)	12 (26.1%)	13 (24.1%)	–
Grade 3	1 (12.5%)	16 (34.8%)	17 (31.5%)	0.099
Grade 4	0 (0%)	5 (10.9%)	5 (9.3%)	–
Fibro-fatty	3 (37.5%)	4 (8.7%)	7 (13%)	–

Grade; grade of steatosis; NASH, nonalcoholic steatohepatitis; US, ultrasound; %, mean percentage % within NASH.

Table 4 shows the correlation between the amount of steatosis detected in liver biopsy and the presence or absence of NASH by the NAS score; when the fat content increased, the incidence of NASH increased. None of the patients with grade 1 steatosis (less than 5% fat) had NASH, whereas all patients with grade 2 and 3 steatosis had NASH.

Correlation of the grades of steatosis in liver biopsy (grades 1, 2, and 3) with the results of liver US (steatosis grades from 1 to 4, and fibro-fatty liver) was difficult because we had to compare three parameters with five parameters. Therefore, we considered fibro-fatty and grade 1 steatosis in US as mild steatosis, grade 2 as moderate steatosis, and grades 3 and 4 as severe steatosis (Table 5).

The sensitivity of liver US in detecting grades of steatosis in liver biopsy was 61% in grade 1, 25% in grade 2, and 75% in grade 3.

Discussion

The term NAFLD is used to describe a wide spectrum of changes in fatty liver ranging from simple steatosis to NASH. Early identification of patients with NASH before

Table 3 Correlation between finding in ultrasound and the presence of nonalcoholic steatohepatitis by the NAS score in liver biopsy

	NASH				
Liver US	No (*N*=8)	Borderline (*N*=24)	Yes (*N*=20)	+ fibrosis (*N*=2)	*P* for trend
Grade 1	3 (37.5%)	8 (33.3%)	1 (5.0%)	0 (0.0%)	–
Grade 2	1 (12.5%)	7 (29.2%)	5 (25.0%)	0 (0.0%)	–
Grade 3	1 (12.5%)	8 (33.3%)	8 (40.0%)	0 (0.0%)	0.002*
Grade 4	0 (0.0%)	1 (4.2%)	4 (20.0%)	0 (0.0%)	–
Fibro-fatty	3 (37.5%)	0 (0.0%)	2 (10.0%)	2 (100.0%)	–

NASH, nonalcoholic steatohepatitis; US, ultrasound.
*Mean *P* significant.

Table 4 Correlation between grades of steatosis in liver biopsy and the presence and absence of nonalcoholic steatohepatitis by the NAS score

	NASH			
Liver biopsy	No (*N*=8)	Yes (*N*=46)	Total (*N*=54)	*P* for trend
Grade 1	8 (100%)	18 (39.1%)	26 (48.2%)	–
Grade 2	0 (0%)	12 (26.1%)	12 (22.2%)	0.000*
Grade 3	0 (0%)	16 (34.8%)	16 (29.6%)	–

Grade, grade of steatosis in liver biopsy; NASH, nonalcoholic steatohepatitis; %, percentage % within NASH.
*Mean *P* significant.

Table 5 Correlation between grading of steatosis by ultrasound and liver biopsy

	Grade of steatosis in liver biopsy			
Liver steatosis by US	Grade 1	Grade 2	Grade 3	*P* for trend
Mild	16 (84.2%)	2 (10.5%)	1 (5.3%)	–
Moderate	7 (53.8%)	3 (23.1%)	3 (23.1%)	0.000*
Severe	3 (13.6%)	7 (31.8%)	12 (54.5%)	–

%; percentage in the US group, (mild, moderate, severe); grade of hepatic steatosis by ultrasound.
US, ultrasound.
*Mean *P* significant.

the onset of fibrosis would be useful in guiding aggressive intervention [11].

Obesity has been reported in 70–100% of patients with NASH, and most patients are 10–40% above the ideal body weight [12]. Numerous reports have documented the resolution of fatty liver following gradual weight loss (which improves insulin resistance and hepatic histology) [13].

Percutaneous liver biopsy is the gold standard method for the definitive assessment of parenchymal liver disease. However, the ethical and medical considerations represent limitations of its use in many cases of NAFLD.

Real-time US scanning is considered as the first-line imaging investigation in patients with suspected liver disorder. However, the difficulty faced in decision making on the basis of US in cases of fatty liver (diffuse parenchymal liver disease) lowers the accuracy of the diagnosis.

In the present study, we attempted to define the clinical value of routine US examination in patients with NAFLD by prospectively comparing the results of scanning with the histological diagnosis on the basis of liver biopsy. We classified the grades of steatosis detected by US according to a semiquantitative scale from 1 to 4 [8], and if the bright liver showed a coarse texture, we defined it as fibro-fatty.

The present study showed that liver US is not only highly sensitive for the diagnosis of NAFLD (100%) but also for the detection of different grades of steatosis correlated with liver biopsy (steatosis in biopsy directly correlates with the progression of the disease and the presence of NASH), with a sensitivity of 61, 25, and 75% in grades 1, 2, and 3, respectively.

Fibrosis was detected less reliably; it was identified in seven patients, and only two (28.6%) of these were proven by the biopsy, 61% in grade 1, 25% in grade 2, and 75% in grade 3.

Use of the combination of the previous semiquantitative scale for the grading of hepatic steatosis by US allowed the diagnosis of fatty liver in a previous retrospective study with a sensitivity of 89% and a specificity of 93%, and in the same study, fibrosis was correctly identified in 77% of patients with a sensitivity of 100% [6]. Another older study published in 1986 showed that US scanning carried out during the course of the routine clinical workload was 87% sensitive and 89% specific in detecting fatty infiltration that had been proved by histology, and the sensitivity to detect moderate and severe steatosis was 100% [14].

On correlating the grade of steatosis in liver biopsy (by its severity 'increasing in amount') with the presence or absence of NASH by the biopsy NAS score, it was found that none of the eight patients negative for NASH had grade 2 or 3 steatosis (100% of non-NASH patients had grade 1 steatosis), whereas among the 46 patients with NASH, 39.1, 26.1, and 34.8% had grades 1, 2, and 3 steatosis, respectively. These findings indicate that the presence of grade 1 steatosis may or may not indicate the presence of NASH; however, the presence of grade 2 and 3 steatosis almost indicate NASH.

In the present study, we also correlated the presence of steatosis in biopsy and US grading of steatosis; we found that mild steatosis was detected by US in 19 patients (84.2% of these patients had grade 1 steatosis on biopsy), moderate steatosis was diagnosed in 13 patients (53.8% had grade 1 steatosis and 46.2% had grade 2 and 3 steatosis on biopsy), and severe steatosis was diagnosed in 22 patients (54.5% had grade 3 steatosis, 31.8% had grade 2 steatosis, and only 13.6% had grade 1 steatosis); these

correlations were highly significant, *P* less than 0.000. US had a sensitivity of 61, 25, and 75% in detecting grades 1, 2, and 3, respectively, in steatosis confirmed by liver biopsy.

According to the previously mentioned results and the highly significant direct correlation between the US grading of steatosis, grading of liver steatosis by histology, and the presence or absence of NASH by the NAS score, we can conclude that grades 3 and 4 steatosis based on US are almost indicative of the presence of NASH, grade 2 steatosis based on US is highly predictive of NASH, and grade 1 steatosis has an almost 50% chance of the presence or ongoing progression to NASH.

The present study showed that BMI and waist circumference were higher in patients proved to have NASH, borderline NASH by the NAS score in biopsy when compared with patients with NAFLD (non-NASH), but the difference was not significant. Harnois and colleagues [15] reported that BMI was the only predictive factor for NASH, whereas Arvaniti and colleagues [16] did not find any difference in BMI and waist circumference between NASH and non-NASH patients.

ALT, GGT, and AST were higher in patients with NASH in comparison with simple NAFLD patients; of these, only AST did not reach a significant value. In 2009, Shi *et al*. [17] reported that ALT and AST levels of NASH patients were higher than those of non-NASH patients. ALT level is also an independent predictor of the degree of inflammation associated with NASH [17,18].

Some limitations of the study should be noted: first, the unequal distribution of sexes in the study group; second, the unequal distributions of NASH and non-NASH patients; and finally the relatively limited number of patients included. We believe that all these limitations exist because most NAFLD Egyptian patients believe that fatty liver is a common benign condition and hence they refused to undergo a liver biopsy.

This study had several strengths. First, it showed that a liver US is highly sensitive in the detection of NAFLD, Second, it also showed a direct correlation between the degree of steatosis in liver US and the presence of NASH. Finally, we could reduce the sources of interobserver errors because all the scans and histological examinations were performed by the same examiner.

The identification of precirrhotic NASH liver disease may be more important than previously believed. Although hepatic steatosis is believed to be reversible with gradual weight loss in obese patients, the degree of steatosis is a major prognostic factor for the development of cirrhosis. The present study concluded that liver US is not only sensitive in the detection of hepatic steatosis but can also predict the presence of NASH; therefore, it can be used as a simple, noninvasive, low-cost method for screening of NAFLD, aiding in the early identification of patients in need of aggressive intervention.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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