

Correlation between ultrasound and histologic findings of fatty liver changes among non-alcoholic obese Patients

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of liver disease in western countries. We aim to evaluate sensitivity, specificity and predictive values of ultrasound in diagnosing NAFLD's patterns among morbidly obese patients.

Methods: 45 morbidly obese patients undergoing sleeve gastrectomy were prospectively studied. They underwent true cut liver biopsy during the surgery. Classification of histological patterns was based on the NIH-sponsored NASH Clinical Research Network NAFLD Activity Score (NAS). Prior to surgery, patients had an assessment for fatty liver changes by ultrasound (4 grades). The findings from histology (being the gold standard test) and ultrasound (being the test in question) were compared.

Results: 71% were females (32/45). Mean age was 35 (range: 17-58), mean BMI was 43 (range: 35-52). The prevalence of NAFLD histologically was 91.8%. 19/45 patients (39%) had non-alcoholic steatohepatitis (NASH) on histology. Ultrasound's sensitivity, specificity and positive predictive value in diagnosing NAFLD were 85%, 50% and 95%; respectively. While its sensitivity, specificity and positive predictive value in diagnosing NASH were 100%, 28% and 43%; respectively. There was significant correlation between ultrasound grades and each of steatosis histologic grades ($P < 0.001$), NAS (score) ($P < 0.001$) and the presence of NASH ($P < 0.001$).

Conclusion: US has demonstrated a good sensitivity for NAFLD and very high sensitivity for NASH in morbidly obese patients, making it a good screening test for hepatic steatosis in obese individuals. Because of its high negative predictive value, US can be completely relied on to rule out NASH, maybe with no need for further confirmatory investigations.

Keywords: obesity, steatosis, NASH, NAFLD, NAFL.

(*J Med J 2021; Vol. 55(2):84-95*)

Received

Accepted

April, 30, 2019

December, 13, 2020

Introduction

Nonalcoholic fatty liver disease (NAFLD) is increasingly recognized as the most common cause of chronic liver disease in children and adults¹. Over the last two decades, the increased awareness of obesity and its' related morbidities contributed to the

perception of NAFLD in bariatric patients as well as in general population. Since obese patients are more likely to experience fat deposition in their livers, they have been investigated by multiple studies screening for NAFLD, using a variety of blood tests and imaging modalities^{2,3}.

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Patients with NAFLD have increased overall mortality compared to healthy individuals². Although the most common cause of death in patients with NAFLD, nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) is still cardiovascular disease, patients with NASH (a subtype of NAFLD) have an increased liver-related mortality rate³. In addition, bridging fibrosis is seen in 25–33% of NASH patients at diagnosis, including cirrhosis in 10–15%⁴. Hence, establishing the diagnosis of NASH to distinguish it from simple steatosis is of utmost importance. However, diagnosis of fatty liver is challenging because of the silent nature of the disease and the invasiveness of liver biopsy, which is the current gold standard for diagnosis¹. In adults, a morbidity rate of 3% and a mortality rate of 0.03% were associated with liver biopsy (prior to the more recent practice of ultrasound (US) guidance^{5, 6}, which prohibited its routine use as a screening method for hepatic steatosis in the general population⁷.

Abdominal US has been widely used as a rapid, inexpensive and noninvasive study for liver evaluation in patients with elevated hepatic enzymes. Despite its widespread use, US has certain limitations, especially in obese patients.

In this study, we included a cohort of morbidly obese patients undergoing sleeve gastrectomy along with laparoscopic guided true cut liver biopsy, between July 2016 and January 2017⁸. In this analysis, we compared radiologic to histologic findings and tested the validity of US in predicting NAFLD and its patterns in morbidly obese patients.

We aim to validate the use of conventional US in diagnosing NAFLD's patterns among morbidly obese patients, of both genders, undergoing sleeve gastrectomy using liver biopsy as a reference method.

Materials and methods

We protected Patients' confidentiality in accordance with the declaration of Helsinki provisions. The Research Ethics Committee and Institutional Review Board (IRB) at our

university approved the study. It is registered under a grant number of (20160209). Study-specific informed consents were obtained from all participating patients.

Our patients' demographic data, including age, gender as well as anthropometric measurements (weight, height, body mass index (BMI)) were reported. Details of comorbidities, alcohol intake and hepatotoxic drugs were also collected. Preoperative laboratory testing included alkaline phosphatase (ALP), total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TGs), total cholesterol, low-density lipoprotein (LDL), high density lipoprotein (HDL), hepatitis C antibodies (anti-HCV) and hepatitis B surface antigen (HBsAg).

Preoperative sonography was performed by one experienced radiologist for all study patients. The operator was blinded to the patient clinical and laboratory data. A curved linear 1-6 MHz transducer was used (LOGIQ E9, GE Healthcare, USA). Standard liver images were obtained including a parasagittal scan plane to view the right lobe of the liver and right kidney, transverse scan plane to view the left lateral segment of the of liver and spleen, and transverse scan plane to view the left liver lobe and pancreas. Focal areas of altered echotexture were further imaged in both sagittal and transverse planes.

The severity of echogenicity was graded as follows: grade 0, normal echogenicity (figure 1); grade 1, slight, diffuse increase in fine echoes in liver parenchyma with normal visualization of diaphragm and intrahepatic vessel borders; grade 2, moderate, diffuse increase in fine echoes with slightly impaired visualization of intrahepatic vessels and diaphragm (figure 2); grade 3, marked increase in fine echoes with poor or nonvisualization of the intrahepatic vessel borders, diaphragm, and posterior right lobe of the liver^{9, 10}.

Sleeve gastrectomy was indicated for a BMI more than 40 or a BMI of 35–40 with presence of one or more significant comorbidities attributable to obesity. Exclusion criteria included history of alcohol

intake, hepatotoxic drugs intake (i.e.; corticosteroids, diltiazem, nifedipine, amiodarone, high dose estrogen, or any other hepatotoxic medicine), chronic liver disease or seropositive testing for viral hepatitis. Operations were carried out by the laparoscopic approach in all patients by one surgeon. All subjects provided specific written informed consent to undergo a liver biopsy as part of their bariatric operation.

Liver biopsy of the left hepatic lobe was performed under laparoscopic guidance using an 18-gauge Tru-Core™ II biopsy Instrument (Argon medical devices, TX, USA). Area of biopsy was cauterized and hemostasis was confirmed. No wedge biopsies were obtained. All biopsies were submitted with hematoxylin and eosin (H&E) and Masson trichrome stains and studied by two experienced pathologists.

NAFLD's essential histologic features, including steatosis, lobular inflammation, and ballooning were explored in each biopsy and scored using the NIH-sponsored NASH Clinical Research Network NAFLD Activity Score (NAS). NAS score is defined as the unweighted sum of the scores for lobular inflammation (0-3), ballooning (0-2) and steatosis (0-3); thus ranging from 0 to 8¹¹.

The term **NAFLD** includes all patterns of fatty liver disease in patients without significant alcohol consumption, which ranges from simple steatosis (NAFL) to steatohepatitis (NASH) and cirrhosis. **NAFL** refers to the presence of hepatic steatosis (NAS score ≥ 1) with no evidence of hepatocellular injury in the form of ballooning (figure 3). Biopsies with **NASH** scored ≥ 5 in NAS; hepatic steatosis and inflammation with hepatocyte injury (ballooning) (with or without fibrosis) is essential for this diagnosis (figure 4) [3]. Biopsies that scored 3 and 4 with steatosis grade 1 and ballooning were included in the NASH category. If steatosis was $< 5\%$ with no inflammation, ballooning, or fibrosis (corresponding to a NAS of 0), a biopsy was declared **normal**.

The findings from histology (being the gold standard test) and US (being the test in question) were compared. Based on this

assumption, sensitivity, specificity and predictive values were calculated. Moreover, US grades were analyzed for association with NAS, NAFLD and its histological categories NAFL, NASH and fibrosis. Pearson's correlation coefficient was used to assess the relationship between histologic patterns and sonographic grades of fatty liver disease. Analysis was performed with the IBM SPSS v.20.0 (Chicago, IL, USA) software. A p value < 0.05 was considered as statistically significant. Data was expressed as mean and range for parametric data.

Results

In a high-volume bariatric surgery center, consecutive 50 morbidly obese patients were enrolled in this study. Five patients were excluded from the study due to missing data. None of our patients had history of alcohol intake. Table 1. demonstrates demographic and clinical characteristics of study participants. No morbidity or mortality associated with liver biopsy was reported in this study.

The prevalence of NAFLD, NASH and NAFL by histology among all liver samples was 91.8%, 39% and 53%, respectively. Only 8% of biopsies were reported as normal. The prevalence of steatosis by US in all patients was 82% (37/45). Table 2. illustrates the steatosis grades by US in NAFLD and NASH patients.

US's sensitivity, specificity and predictive values in diagnosing NAFLD, NASH and NAFL are shown in table 3. US was 100% successful in detecting all patients with NASH, which means that US can be used as a screening test for NASH, however, its' specificity in differentiating NASH patients from others was low (28%).

Since negative predictive value of US for NASH is found to be 100%, if US is reported as normal (no steatosis), further investigations (i.e., biopsy) may not be required to exclude this pathology. There was significant correlation between US grades and each of steatosis histologic grades ($P < 0.001$), NAS (score) ($P < 0.001$) and the presence of NASH ($P < 0.001$).

Discussion

Abdominal US is the most widely used radiologic investigation for hepatic steatosis, owing to its availability, low-cost and safety in screening for liver pathologies. Nevertheless, histopathologic diagnosis of fatty liver changes using biopsy is still considered the gold standard, which is best-attempted -in obese patients- during bariatric surgery.

Multiple reports evaluated the reliability of liver US to diagnose NAFLD in general population as well as in obese subjects. Different US parameters and grading scales were used. Most studies measured parenchymal echogenicity of the liver (compared to right kidney cortex brightness), the ability to visualize intrahepatic vessels and the degree of diaphragm reflectivity^{1, 7, 10, 12, 13}. The diagnostic performance of US in detecting fatty changes varies depending on the extent of steatosis⁷; ranging from fairly accurate diagnosis in moderate-to-severe steatosis (sensitivity 81.8%-100.0%) [9, 10] to low accuracy in mild steatosis (sensitivity 53.3% - 66.6%)^{9, 14-16}. Our study showed an US sensitivity of 85% and a positive predictive value of 95% for NAFLD. It also revealed a reasonable sensitivity of 76% for NAFL and a very high sensitivity of 100% for NASH. In this study, US was not meant to diagnose NASH or NAFL, since these were histopathologic descriptions; US was used to assess grades of steatosis according to the grading system mentioned above, then US measurements were correlated with histologic diagnoses.

Multiple studies disclosed variable sensitivity and specificity rates for US. Almeida et al involved 105 morbidly obese patients and reported US sensitivity and specificity of 64.9% and 90.9%, respectively, with a positive predictive value of 98.4%¹⁵. In another study by Mottin et al, the US yielded sensitivity, specificity and positive predictive value of 49.1%, 75% and 95.4%, respectively, suggesting its use as a diagnostic tool¹⁷. The variable results of US sensitivity and specificity reported in the literature may be explained by using different parameters and

criteria by different US operators.

Previous studies demonstrated higher US sensitivity and specificity in detecting fatty liver changes in non-obese than obese patients^{18,19}. The low sensitivity values obtained for US in obese people may be attributed to technical difficulties encountered in such patient¹⁷. The increased thickness of the abdominal wall results in poor penetration of the US beam; for example, in an obese patient who has an extra-peritoneal fat layer of 8 cm thickness, only 6% of the initial beam intensity penetrates the peritoneal cavity. Moreover, thick body parts increase the attenuation of the beam as it passes through subcutaneous fat²⁰. At the frequency of 7 MHz, 50% of the beam intensity (watts/cm²) is attenuated per centimeter of fat and the signal strength drops by 3 decibels (dB). Of all the radiologic modalities, US examinations are the most limited by body habitus²¹. Although US is limited by obesity, image quality varies with the distribution of fat. Obese patients with predominately subcutaneous fat will have lower quality images compared to obese patients with minimal subcutaneous fat but more intraperitoneal fat^{21, 22}.

In this study, we utilized NAFLD Activity Score (NAS) suggested by the NIH-sponsored NASH Clinical Research Network as an objective scale, measuring the extent of fatty changes affecting the liver. Our study is one of few reports that correlate both NAFLD and NASH with steatosis diagnosis, and grades on US¹.

US ability to differentiate between NASH and NAFL has not been evaluated thoroughly in the literature. A small number of studies reported different outcomes^{10, 23, 24, 25}. Saadeh et al concluded that none of the radiological modalities (US, computerized tomography (CT) and magnetic resonance imaging (MRI)), could detect the hepatocyte features necessary for NASH diagnosis¹⁰. On the contrary, Liang and colleagues, used a different histologic and US grading systems to test the correlation between US fatty score (FS) (involving parenchymal echogenicity, fat gain attenuation, gallbladder wall blurring, portal

vein wall blurring, and hepatic vein blurring), modified FS(MFS) and histologic diagnosis of simple steatosis and NASH (based on classification proposed by Bunt, 1999). They revealed high US sensitivity of 72% and specificity 86% for the detection of NASH²³. In our study, US demonstrated low specificity (28%) in differentiating NASH, however, the negative predictive value was 100%. In other words, we can completely rely on US to rule out NASH. We encountered difficulties in comparing our results to others because of different diagnosis criteria in both histology and US.

It is well known that the radiologic assessment of hepatic steatosis by US depends mainly on the subjective impression of hepatic echogenicity and posterior attenuation of the ultrasound beam. The reproducibility of sonographic findings by the same operator and the agreement between operators determine the reliability of this test. Cengiz and colleagues, in their review of 113 hepatic US examinations reported an intra-observer agreement of 51% and 68% for two operators, while the inter-observer agreements in the initial and second readings were 39% and 40%²⁶. Strauss et al showed inter-observer and intra-observer agreements of 72% and 76%, respectively²⁷. Younossi *et al.* have also found inter- and intra-observer variability in the pathological diagnosis of NAFLD²⁸. The results of these studies and others demonstrate that radiologists, as well as histopathologists, may vary significantly with respect to their evaluation of hepatic steatosis.

To minimize confusion associated with terms and definitions used in literature, we recommend using the terms NASH & NAFL to describe findings by histology- based on NAS(score)-, and the terms normal, mild, moderate and high to describe steatosis grades by US based on standard echogenicity

parameters. The term NAFLD can be used to describe any fatty changes detected by US or histology.

Our study has some limitations. First, the sample size is fairly small. Second, only one radiologist interpreted the ultrasound images; due to the known interobserver variability, a second interpreter could have led to different sensitivity, specificity, and predictive values than reported in this study. Third, this study has limited generalizability since it applies to a specific population of morbidly obese patients and lastly, the radiologist was not blinded to the body habitus of the patient which could influence the radiologist scoring given the high prevalence of steatosis in morbidly obese patients.

Conclusion

US has demonstrated a good sensitivity for NAFLD and very high sensitivity for NASH in morbidly obese patients, which makes it a good screening test for hepatic steatosis in obese individuals. Because of its high negative predictive value, US can be completely relied on to rule out NASH, maybe with no need for further confirmatory investigations.

Compliance with Ethical Standards:

- **Conflict of Interest:** Authors Abdel Rahman Al manasra, Haitham Qandeel, Mohammed Bani Hani, Ruba Khasawneh, Mohammad Alqudah, Asra. Al-rhamani, Nabil Al-zoubi, Jihad Z. Fataftah declare that they have no conflict of interest.

- **Funding:** This research was funded by the deanship of research at our university (Jordan university of science and technology), and registered under grant number 20160209.

- **Informed consent:** Informed consent was obtained from all individual participants included in the study.

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Table 1: patients' demographic and clinical characteristics.

Variable	n	%
Age (years), mean (range)	34 (17 – 58)	
Body mass index (Kg/m ²), mean (range)	43 (35 - 52)	
Gender:		
Male	13	26.5
Female	36	73.5
Comorbidities:		
Diabetes mellitus	11	22
Hypertension	7	14.3
Metabolic syndrome	14	25.6
NAFLD^a patients with abnormal values^b:		
Triglycerides	14	31
Cholesterol	12	27
Reduced High Density Lipoprotein	10	22
Raised Low Density Lipoprotein	7	16
Glucose	11	24
Aspartate aminotransferase	7	16
Alanine aminotransferase	12	27
a: Nonalcoholic fatty liver disease, b: Reference normal values: Triglycerides (0–1.7mmol/l), Cholesterol (\geq 6.2mmol/l), High Density Lipoprotein (M:0.9– 1.45, F:1.15–1.68), Low Density Lipoprotein (\geq 0–4.4mmol/l), Glucose (3.9–5.5mmol/l), Aspartate aminotransferase (0– 34u/l), Alanine aminotransferase (0–31u/l).		

Table 2: steatosis grades by ultrasound in NAFLD, NASH and NAFL patients.

US ^a steatosis grade (45 patients)	NAFLD ^b (41 patients)	NASH ^c (16 patients)	NAFL ^d (25)
Normal 8 (18%)	6	0	6
Grade-1 28 (62%)	26	9	17
Grade-2 9 (20%)	9	7	2
a:Ultrasound, b:non-alcoholic fatty liver disease, c:non-alcoholic steatohepatitis, d: non-alcoholic fatty liver.			

Table 3: Ultrasound sensitivity, specificity and predictive values in diagnosis of NAFLD, NASH and NAFL.

US ^a	NAFLD ^b	95%CI ^c	NAFL ^d	NASH ^e	95%CI ^e
Specificity	50%	6.76%- 93.24%	10%	28%	12.73% - 47.24%
Sensitivity	85%	70.83%- 94.43%	76%	100%	79.41%- 100.00%
Negative Predictive value	25%	8.90%- 53.22%	25%	100%	
Positive Predictive value	95%	86.69%- 97.52%	51%	43%	37.83%- 48.82%

a:Ultrasound, b:non-alcoholic fatty liver disease, c: confidence interval, d:non-alcoholic fatty liver, e: non-alcoholic steatohepatitis.

Figure 1: Normal liver echogenicity (similar to adjacent right kidney parenchyma)

Figure 2: Grade II hepatic steatosis showing moderate diffuse increase echogenicity of the liver with mildly impaired visualization of the intrahepatic vessels and diaphragm.

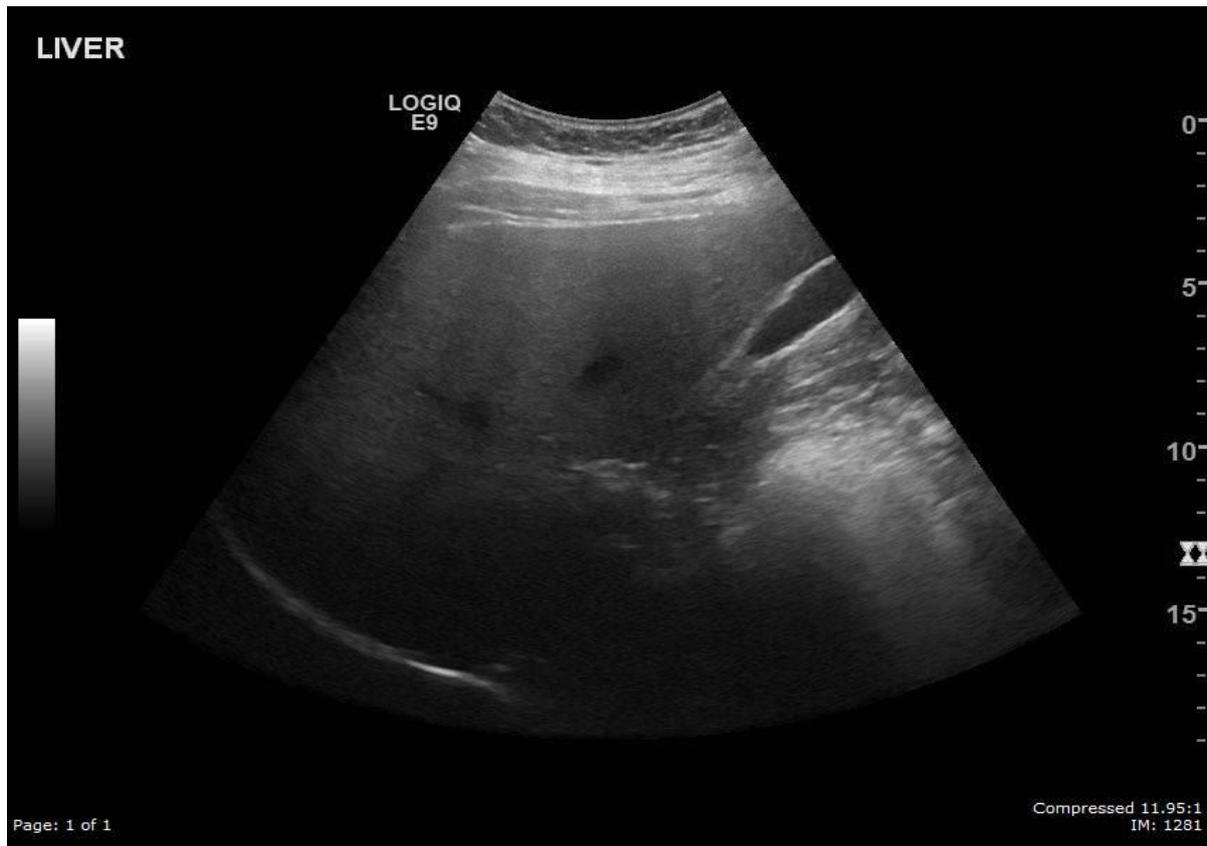


Figure 3: Nonalcoholic fatty liver

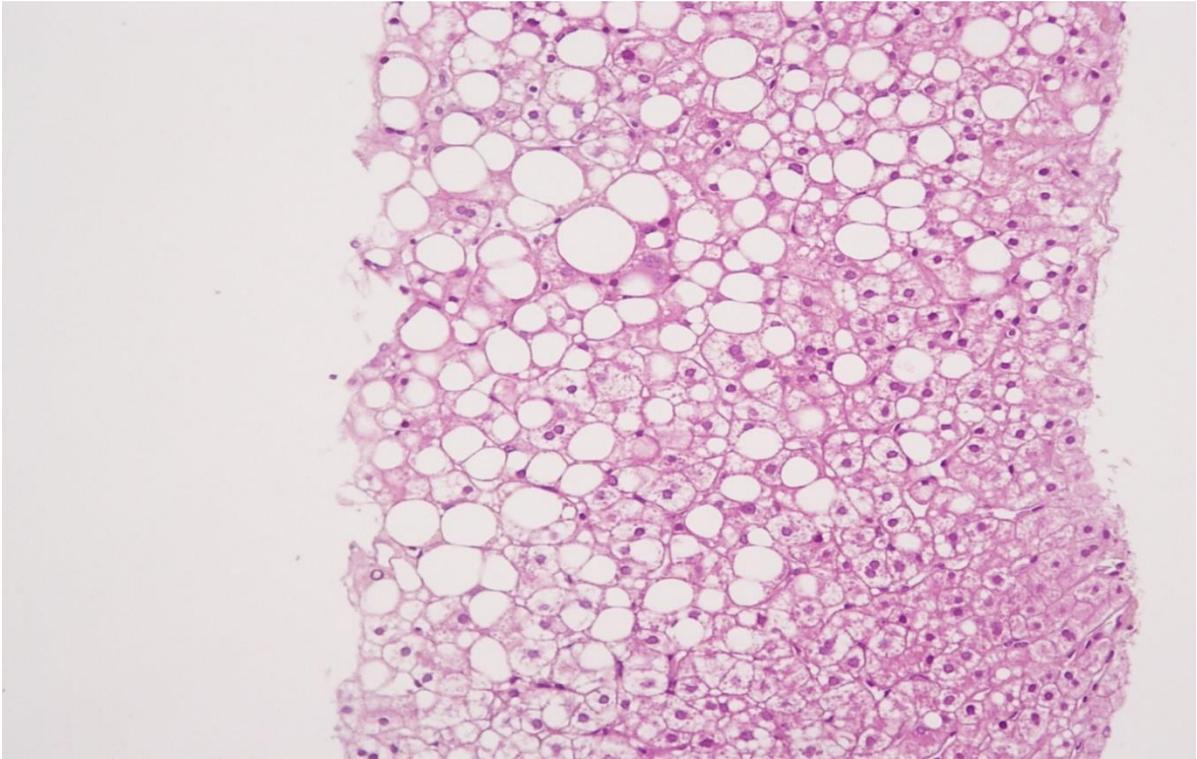
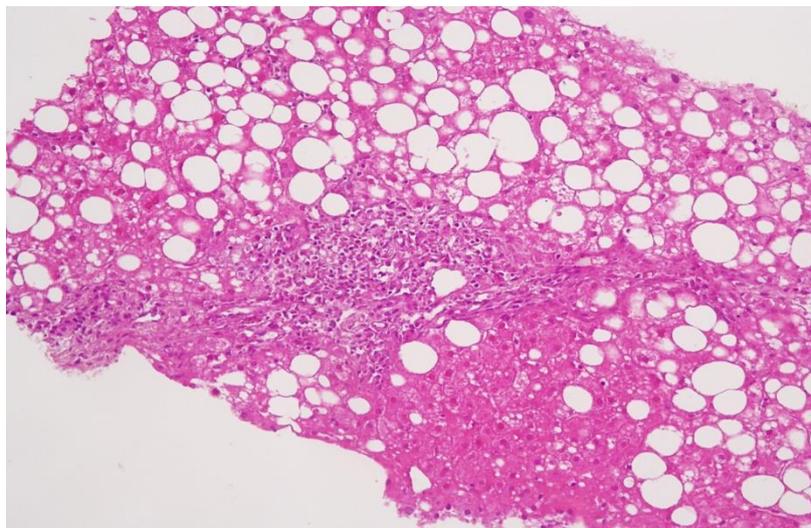


Figure 4: Nonalcoholic steatohepatitis (hepatic steatosis and inflammation with hepatocyte injury, ballooning)



العلاقة بين تقييم تغيرات الكبد الدهنية بواسطة التصوير بالموجات فوق الصوتية والتحليل النسيجية لدى المرضى الذين يعانون من السمنة المفرطة غير الكحولية

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الملخص

الخلفية: مرض الكبد الدهني غير الكحولي (NAFLD) هو السبب الأكثر شيوعاً لمرض الكبد في الدول الغربية، ونحن نهدف إلى تقييم الحساسية والنوعية والقيم التنبؤية للموجات فوق الصوتية في تشخيص أنماط NAFLD بين المرضى الذين يعانون من السمنة المفرطة. **الأسلوب والأدوات:** لقد تمت دراسة 45 مريضاً ممن يعانون من السمنة المفرطة والذين خضعوا لعملية تكميم المعدة جميعهم خضعوا لخزعة الكبد أثناء العملية الجراحية، واستند تصنيف الأنماط النسيجية إلى مقياس نشاط NAFLD (NAS) لشبكة NASH التي ترعاها NIH. وقبل الجراحة، خضع المرضى لتقييم تغيرات الكبد الدهنية بواسطة التصوير بالموجات فوق الصوتية (4 درجات). وتمت مقارنة النتائج من الأنسجة (كونه اختبار المعيار الأساسي) بنتائج فحص الموجات فوق الصوتية.

النتائج: (71٪) كانوا من الإناث (45/32)، وكان متوسط العمر 35 (النطاق: 17-58)، وكان متوسط مؤشر كتلة الجسم 43 (النطاق: 35-52)، وكان معدل انتشار NAFLD نسيجياً 91.8 ٪، وكان (45/19) مريضاً (39 ٪) لديهم التهاب الكبد الدهني غير الكحولي (NASH) حسب تحليل الأنسجة، وكانت حساسية الموجات فوق الصوتية، والنوعية والقيمة التنبؤية الإيجابية في تشخيص NAFLD 85 ٪، و 50 ٪ و 95 ٪ على التوالي، في حين أن الحساسية والنوعية والقيمة التنبؤية الإيجابية في تشخيص (NASH) كانت 100 ٪، و 28 ٪ و 43 ٪ على التوالي، وكان هناك ارتباط كبير بين درجات الموجات فوق الصوتية وكل من الدرجات النسيجية للتنكس الدهني (P<0.001)، NAS (P<0.001) ووجود NASH (P<0.001).

الخلاصة: لقد أظهرت الموجات فوق الصوتية حساسية جيدة لل NAFLD وحساسية عالية جداً لل NASH في مرضى السمنة المفرطة؛ مما يجعلها اختباراً جيداً للكشف عن التنكس الدهني الكبدي لدى المصابين بالسمنة، ونظراً لقيمتها التنبؤية السلبية العالية، فإنه يمكن الاعتماد على التصوير بالموجات فوق الصوتية تماماً لاستبعاد NASH، ربما دون الحاجة إلى مزيد من الفحوصات التأكيديّة.

الكلمات الدالة: السمنة، تنكس دهني، NASH، NAFLD، NAFL.