Quantitative measurement of ultrasound attenuation and Hepato-Renal Index in Non-Alcoholic Fatty Liver Disease

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Abstract

Objective: The aim of this study was to non-invasively explore new methods of ultrasound attenuation measurements in livers of patients with Non-Alcoholic-Fatty-Liver-Disease (NAFLD) and to measure the liver tissue elasticity. **Material and method:** Sixteen patients with NAFLD, twelve patients with liver fibrosis and fifteen healthy subjects were included. Echo Levels (ELs) in dB were measured at 2 and 7cm depths in the right liver to calculate the attenuation. ELs were measured in liver and right kidney tissue to calculate the Hepato-Renal Index (HRI). This index was calculated both as a difference, HRI-diff; (EL Liver –EL Kidney) and HRI-ratio; (EL Liver / EL Kidney) using built-in software of the ultrasound scanner. Liver tissue elasticity was measured using transient elastography (TE, Fibroscan®). NAFLD and liver fibrosis were confirmed by liver biopsy. **Results:** We found that HRI- diff was significantly higher in the NAFLD group compared with healthy subjects, 6.2 dB (0.8-11.4) vs. 1.9 dB (0.0-6.1), $p=0.012$. HRI- ratio was significantly lower between the same two groups, 0.9 dB (0.8-1.02) vs. 1.01 dB (0.9-1.12), and $p<0.0001$. TE, ELs and liver size showed significant differences between NAFLD patients and healthy controls. Between patients with fibrosis and NAFLD the differences were significant for TE, liver size and attenuation. Intra- and interobserver correlation and agreement of ELs were good. **Conclusion:** Measurements of liver tissue using HR-Indexes, ultrasound attenuation, and tissue elasticity may be useful methods to differentiate objectively between steatosis and healthy and quantify the differences.

Keywords: ultrasound, NAFLD, steatosis quantification, attenuation

Introduction

The prevalence of fatty liver disease increases in the Western world. It is often caused by alcohol consumption (AFLD) or more frequently by Non-Alcoholic Fatty-Liver Disease (NAFLD) [1]. Steatosis is defined by accumulation of triglycerides in the liver parenchyma. In ultrasound imaging, fatty liver infiltration is characterised by hyperechogenicity of the liver parenchyma

with increased attenuation of the ultrasound waves in the deeper parts compared to healthy liver tissue. The prevalence of NAFLD is varying between 13 and 23 %, but amongst obese patients as high as 74 % [1,2].

Ultrasound (US) is a widely available and non-invasive method to examine the liver tissue. In most patients the whole organ may be evaluated in one single examination [3]. Liver biopsy is the gold standard when investigating the fat infiltration in the liver, but is an invasive method. It only evaluates approximately 1/50.000 part of the liver parenchyma with a high risk of sampling error. A non-invasive method providing similar accuracy as provided by liver biopsy is wanted [4-6]. To establish the presence and grading of fatty infiltration based on a subjective visual examination using ultrasound substantial experience of the examiner is required.

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A method to quantify liver steatosis by using the liver echogenicity or the increased US attenuation in fatty liver tissue would be valuable. Previous studies have compared the echogenicity of the liver with the echogenicity of the kidney calculating a Hepato-Renal Index (HRI) [7-9]. The aim of the present study was to examine non-invasive methods measuring the attenuation of ultrasound in the liver in NAFLD patients and compare with measurements in patients with established fibrosis and healthy subjects. Secondly, we compared the liver tissue elasticity in these three groups using liver elasticity measurements. Thirdly, we wanted to assess the intra- and interobserver agreement of the US measurements of liver tissue ultrasound attenuation.

Materials and methods

Study population

This case-control study was conducted at Haukeland University Hospital.

Sixteen patients, 11 females, 5 males, age 52 ± 7 years with NAFLD, twelve patients

3 females, 9 males, age 44 ± 11 years, with histologically proven liver fibrosis and fifteen healthy subjects, 9 females, 6 males, age 45 ± 9 years. The patients in the NAFLD group had all ultrasonographic homogeneous distribution of fatty infiltration in the liver. Patients with focal fatty sparing were not included. The NAFLD patients were recruited from the Department of Gastroenterology after standard work-up due to elevated liver tests.

In the group where the patients had established liver fibrosis, the majority (11/12) had their fibrosis due to chronic virus (B or C) - infections. One of the patients with fibrosis had a histopathological feature of hepatitis C, but the blood test did not confirm this. For the sake of simplicity, we chose to call this group "the fibrosis group".

The healthy subjects were recruited from the Department of Gastroenterology and were nurses or physicians. They had no history of liver disease and had normal blood tests. The inclusion followed when the sonographic examination of the liver was normal.

No liver biopsy or virus markers were taken from the healthy controls. The biopsied patients in the NAFLD and fibrosis group were recruited based on a previous liver biopsy, and not as a result of the examination.

All participants had normal kidney function and no history of kidney disease or malfunction. Patients or controls with pacemaker, defibrillator, pregnancy or alcohol consumption more than 20 g daily were not included.

The study was conducted according to the Helsinki declaration and with the approval of the Regional Ethics Committee for medical research, Bergen, Norway. Written informed consent was obtained from all patients.

Ultrasonography

The same gastroenterologist (HLvV) performed all examinations. The scanner used was GE Logiq 9 (Milwaukee, Wisconsin, USA) with a curvilinear transducer 3.5-5.0 MHz. The liver was described as normal when the ultrasound examination showed a homogenous liver parenchyma and isoechoic echogenicity. The affected parenchyma in the NAFLD group was described as hyperechoic compared with the right kidney.

Regions of interest (ROI) were identified with a set diameter and depth avoiding visible bile ducts or vessels. The ROI was circular or oval in configuration with a diameter of 1-2 cm both in the liver and in the right kidney cortex. The measurements were performed at the same level of depth in the two organs.

EchoLevel (EL) measures the mean intensity of pixels within a user defined area. The scanner utilizes the intensity data from raw data per pixel and calculates the average:

Average = $\text{sum (intensity per pixel)} / \text{pixels}$. Raw data pixel measurements imply that there is no influence of gain, dynamic range or other scanner parameters. EL is measured in dB and is linear to the intensity. Zero dB equals max intensity i.e. white with gray level 255. Minimum intensity equals -99 dB, i.e. black with gray level zero. $Y = 255 + (255/99) * EL[\text{dB}]$; $EL[\text{dB}] = 20 * \log_{10}(E)$, where EL denotes Echo Level.

We measured the following ultrasound parameters: EL in anterior and lateral positions, liver tissue elasticity using transient elastography, and the size of the liver in the right medio-clavicular line with measurements from the hepatic dome to the frontal inferior hepatic tip [10].

Attenuation

The attenuation was estimated using EL. Steatosis was visualised by Ultrasound in all 16 NAFLD patients. EL measurements were made in regions of interest in the right liver lobe at 2 cm and 7 cm depth in the medio-clavicular line (EL ant) and lateral (EL lat) in right intercostal spaces 9-12. Subsequently, attenuation was calculated by subtracting the EL level at 7 cm from the EL level at 2 cm below the capsule. The frequency of the scanner was set to 4 MHz for all measurements.

Hepatorenal Index

Hepato-Renal Index Difference (HRI-diff: EL Liver - EL Kidney) or Hepato-Renal Index Ratio (HRI-ratio: EL Liver / EL Kidney) were estimated using built-in software on the scanner enabling local measurement of attenuation in dB. When the right kidney cortex was

Fig 1. Ultrasound image showing Echo Level measurements in the right liver lobe and in the right kidney cortex. The ROI was circular or oval in configuration with a diameter of 1-2 cm both in the liver and in the right kidney cortex. It is important to measure at the same level of depth in the two organs.

clearly visualized, ELs were measured in the liver and in kidney cortex at the same distance from the US probe (fig 1).

Transient elastography (TE)

Liver stiffness measurements (LSM) were performed by TE using Fibroscan® (Echosens, Paris, France); M Probe with transducer frequency 3.5 MHz. The probe of the Fibroscanner has a small piston that creates a vibration of low amplitude and frequency. This generates a shear wave from the skin into the liver parenchyma. The US measures the traveling –time of this shear-wave. This is proportional with liver tissue elasticity [11]: the higher velocity of the shear wave, the stiffer the liver. The measurements are expressed in kilo Pascal (kPa). For each examination ten consecutive TE measurements in the right liver lobe through intercostal space 9-11 are required.

In previous studies with TE in patients with hepatitis, Fibroscan showed a good ability to separate between different stages of liver fibrosis, particularly between cirrhosis and non cirrhosis [6;12].

Histopathology

Liver biopsies had been obtained from the patient groups with NAFLD and fibrosis. The NAFLD and fibrosis patients were selected based on a recent liver biopsy.

The histological assessment of fatty livers were graded according to criteria by Brunt and Kleiner et al in accordance with percentual fatty infiltrated hepatocytes as grade 0 (<5 %), grade1 (5-33%), grade 2 (33-66%) and grade 3 (> 66%) [13]. Fibrosis were staged as

0 (no fibrosis), 1 (perisinusoidal or periportal fibrosis), 2 (perisinusoidal and periportal fibrosis), 3 (bridging fibrosis) or 4 (cirrhosis) [14]. The histological examinations were performed by three experienced pathologists.

The histological grading of the NAFLD patients were performed as a consensus between two pathologists experienced with using classification by Brunt and Kleiner. The fibrosis classification from the patients with liver fibrosis was performed by a third pathologist using Batt's and Ludwig's classification.

Statistical analysis

ELs are presented as median and range. Liver elasticity levels are shown as the median of 10 consecutive measurements with standard deviation for a 95% confidence interval. Shapiro Wilk's test was used to evaluate the distribution of the measurements. Not all groups had normally distributed data according to this test. We used the Mann-Whiney test to compare groups for statistical significance. P-values <0.05 was selected as the level of statistical significance.

Intra-and interobserver agreements were made from 12 random subjects from the three groups. The examinations were performed blinded on images recorded on a standardized set up aiming to estimate the observer variation of this new attenuation measurement on the images per se, not the total procedure including the ultrasound acquisition. We used EL as the expression for attenuation in the anterior position and Pearson's correlation coefficient was calculated. Limits of agreement using the difference between observations vs. mean value of the two observations were calculated according to Bland and Altman [15]. Statistical analysis was performed using SPSS 17.0 Software.

Results

The histological classifications are presented in Table I. In the fibrosis group two patients had fibrosis grade 1, five had grade 2 and 3.

The results of the measurements and comparison between the three groups are presented in Table II. The HRI- difference between NAFLD patients 6.2 dB (0.8-11.4) and the healthy controls 1.9dB (0.0-6.1) was significant, $p=0.012$. The HRI- ratio also showed differences between the same two groups, NAFLD 0.9 (0.8-1.02) vs. healthy 1.0 (0.9-1.12), $p<0.001$. However, between the NAFLD and Fibrosis patients we did not find any differences either in the HRI-diff nor in the HRI- ratio. Between the fibrosis patients and healthy controls there were also no significant differences in the measurements of the HRI.

For the measurements of attenuation in the anterior and lateral positions of the liver we found significant differences between the NAFLD and healthy controls, NAFLD EL anterior 3.9 dB (0.0-8.0) vs. healthy, EL anterior -1.0 dB (-4.0-4.2), $p=0.002$, NAFLD EL lateral, 4.6 dB (2.5-10.7) vs. healthy EL lateral 0.6 (-3.0-8.7), $p=0.005$. There were no significant differences in the attenuation between fibrosis and healthy controls.

Between the NAFLD and fibrosis group there were also significant differences in the anterior and lateral positions, NAFLD EL anterior 3.9 dB (0.0-8.0) vs. fibro-

sis EL anterior -0.4 dB (-4.8-7.2), $p=0.001$, NAFLD EL lateral 4.6 dB (2.5-10.7) vs. fibrosis group, EL lateral 2.0 dB (-7.2-7.10) $p=0.007$. Tissue elasticity measurements using transient elastography showed significant difference between the three groups. The healthy group scored low, 4.35 kPa (3-6), indicating low liver stiffness, and small measurement variation. The NAFLD group, 8.5 kPa (3-21) scored less than the healthy and the fibrosis group who scored 23.6 kPa (6-66). TE measurements could be obtained from all but three NAFLD patients and one liver fibrosis patient who all had BMI greater than 30.

The liver size was significantly enlarged in the NAFLD group compared to healthy controls, 15.9 cm (12.3-17.5) vs. 13.4 cm (11.2-15.5), $p<0.001$, NAFLD and fibrosis patients 16.1 cm (12.3-17.5) vs. 13.7 cm (9.0-18.6), $p=0.003$, but not between the healthy controls and the patients with fibrosis 13.4 cm vs. 13.7 cm (9.0-18.6), $p=0.414$.

Sub analyses were performed only in the NAFLD group. We did not find any significant differences in the measurements of the liver stiffness between the different grades of steatosis. There were also no correlations between the different indexes in this small group.

Intra- and interobserver agreement

The limits of agreement for intraobserver varied from -0.41 to 0.25 (mean-0.08) and for interobserver from -2.78 to 2.16 (mean-0.31). The results of the intraobserver and interobserver analyses of measurements from EL at 2 cm and 7 cm beneath the liver capsule are displayed as correlation plots and as Bland-Altman plots (fig 2-5). The correlations and agreements were very good and indicate high reproducibility of the method.

Table I. The histological classifications of NAFLD patients.

NAFLD patients	Steatosis	Inflammation	Fibrosis
1	1	1	0
2	2	1	1
3	3	1	0
4	2	0	-
5	2	0	0
6	3	0	0
7	3	0	1
8	3	1	2
9	3	1	0
10	1	0	0
11	1	2	1
12	1	1	0
13	1	2	1
14	3	1	3
15	2	1	1
16	1	0	1

NAFLD- Non-Alcoholic Fatty-Liver Disease

Table II. The results of measurements and comparisons between the three groups

	NAFLD median (min- max)	Fibrosis median (min- max)	Healthy Controls Median (min-max)	NAFLD vs. Healthy controls (p)	NAFLD vs. Fibrosis (p)	Fibrosis vs. Healthy controls (p)
HR Diff	6,2 (0.8-11.4)	2,7 (1.1-10.3)	1.9 (0.0-6.1)	0.012	0.558	0.064
HR Ratio	0.9 (0.8-1.02)	0.9 (0.82-1.20)	1,0 (0.9-1.12)	0.00	0.212	0.140
Liver size	16.1 (12.3-17.5)	13.7 (9.0-18.6)	13.4 (11.2-15.5)	0.001	0.032	0.414
Liver elasticity	8.5 (3-21)	23.6 (3-66)	4.35 (3-6)	0.000	0.011	0.001
EL-lateral	4.6 (2.5-10.7)	2.0 (-7.2-7.10)	0.6 (-3.0-8.7)	0.005	0.007	0.892
EL anterior	3.9 (0.9-8.0)	- 0.4 (-4.8-7.2)	- 1.0 (-4.0-4.2)	0.002	0.002	0.663

NAFLD- Non-Alcoholic Fatty-Liver Disease; HR Diff- Hepato-Renal Index Difference; HR Ratio- Hepato- Renal Index Ratio; EL- Echo-Levels

Fig 2. Intraobserver correlation for observer A, 2 measurements of ultrasound attenuation between Echo Level measurements in the right liver lobe at 2 cm and 7 cm depth. Attenuation was calculated by subtracting the EL level at 7 cm from the EL level at 2 cm below the capsule.

Fig 4. Interobserver correlation between observer A and B for ultrasound attenuation measurements between 2 cm and 7 cm depth. Attenuation was calculated by subtracting the EL level at 7 cm from the EL level at 2 cm below the capsule.

Fig 3. Intraobserver agreement for observer A, 2 measurements of ultrasound attenuation between Echo Level measurements in the right liver lobe at 2 cm and 7 cm depth. Attenuation was calculated by subtracting the EL level at 7 cm from the EL level at 2 cm below the capsule. Intraobserver agreement between 2 measurements made by observer A was calculated according to Bland and Altman.

Fig 5. Interobserver agreement between observer A and B for ultrasound attenuation between Echo Level measurements in the right liver lobe at 2 cm and 7 cm depth. Attenuation was calculated by subtracting the EL level at 7 cm from the EL level at 2 cm below the capsule. Intraobserver agreement between observer A and B were calculated according to Bland and Altman.

Discussion

We found that HRI- diff was significantly higher in the NAFLD group compared with healthy subjects, and that HRI- ratio was significantly lower in the same groups.

Webb et al [8] presented a high correlation between Hepato-Renal sonographic Index and steatosis, inflam-

mation and fibrosis using the ratio between liver and kidney parenchyma. The Hepato-Renal index had a sensitivity and specificity of 90 % for moderate to severe steatosis to predict the degree of steatosis.

Soder et al [7] compared the Hepato-Renal difference between normal-weight and obese children. Kidney measurement values were subtracted from liver values, showing significant differences.

The only significant difference using HRI Diff or Ratio was observed when comparing NAFLD livers with livers from healthy subjects. This suggests that comparing ELs in the liver and in the right kidney can be a method to distinguish between fatty and healthy livers and quantifying the difference objectively. Further studies regarding the impact of inflammation and/or fibrosis are warranted.

The difference in EL in the lateral and anterior positions showed significantly higher US attenuation in NAFLD patients than in both healthy controls and in patients with liver fibrosis. Significant differences were demonstrated in US attenuation both between NAFLD and healthy controls and between NAFLD and liver fibrosis. Adding this method to the HRI indexes may provide further diagnostic confidence in identifying fatty infiltrated livers. However, no difference in liver tissue attenuation was observed between fibrosis and healthy livers.

Liver stiffness measurements using TE, demonstrated significant differences between all three groups (median values for NAFLD 8.5 kPa, healthy subjects 4.3kPa and fibrosis patients with 23.6 kPa). Interestingly, the majority of NAFLD patients had low levels of fibrosis and low-grade inflammation in biopsy evaluation.

NAFLD patients had significantly enlarged liver compared to fibrosis patients and healthy controls. However, no significant difference was observed between the fibrosis group and healthy controls. This information may be of interest when performing sonographic examination of patients with liver steatosis.

The small number of patients in each group and the fact that the healthy subjects did not undergo liver biopsy constitute limitations of our study. The healthy group was not examined of chronic virus hepatitis. The healthy subjects had no history of hepatitis, no sign of steatosis upon ultrasonography, no obesity and all had normal liver enzymes. A liver biopsy in healthy subjects was considered to be unethical.

The fibrosis patients were a heterogeneous group with inflammation, steatosis and fibrosis. Therefore, these data need to be carefully interpreted. The fibrosis group had significantly higher liver stiffness compared to NAFLD and healthy subjects. The attenuation was also different compared to the NAFLD group. The methods tested in this small study do not allow us to draw firm conclusions regarding discrimination between fibrosis and fatty livers.

EL measurement is an integrated function of the scanner which makes it easy to use and it is independent of B-mode scanner settings such as gain and focus. The patients in this study were recruited on the basis of

previous liver biopsies to fit into one of three groups; healthy liver, liver fibrosis and NAFLD. The examiner was not blinded to the disease category at the time of US scanning. Using the described Echo Level, size and elasticity criteria in consecutive patient examinations in a clinical setting may yield different results and will require further study.

Conclusion

External ultrasound examination measuring attenuation at different depths in the liver combined with local measurement of attenuation between liver and the right kidney cortex may be a useful and easy method to discriminate NAFLD patients from healthy subjects.

Ultrasound attenuation can easily be quantified by Echo Level measurements with acceptable intra- and interobserver reproducibility. Further studies are required to confirm these findings in a prospective clinical study.

Conflict of interest: none

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