ORIGINAL ARTICLE

Grading of Hepatic Steatosis by Ultrasound Quantification

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Received: 18 March 2022 / Accepted: 25 April 2022

Abstract

Purpose: Fatty liver is the most common chronic liver disease, and finding the appropriate method for detecting the problem is necessary. The current study aimed to quantity liver steatosis using Computed Tomography (CT), ultrasound images, and Alanine Aminotransferase (ALT) blood test.

Materials and Methods: In this work, 163 Non-Alcoholic Fatty Liver Disease (NAFLD) patients implemented CT and ultrasound images on their abdomen regions. The liver and spleen density were calculated using CT images (as the standard method), and then the patients were divided into mild, and moderate to severe groups. During the sonography, an M-value histogram of the liver and the right kidney was drawn and their ratio (liver/kidney) was considered as a Hepatorenal Index (HRI). Mann-Whitney test was used to evaluate the relationships between HRI and ALT values.

Results: The mean and standard deviation of the liver density in CT scans were obtained as 51 ± 4 HU. The HRI had better performance (Area Under the Curve, AUC: 0.94) than the ALT (AUC: 0.88) in determining liver steatosis. In addition, there was a significant difference between the mild, and moderate to severe groups ($P < 0.001$) in HRI and ALT values.

Conclusion: Based on the results, HRI is an excellent factor to distinguish between mild, and moderate to severe fatty liver. Notably, HRI is reproducible and operator-independent.

Keywords: Liver Steatosis; Computed Tomography; Ultrasound; Hepatorenal Index.

DOI: https://doi.org/10.18502/fbt.v10i2.12215

1. Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most prevalent liver diseases and affects more than 30% of the western adult population, which is defined as the accumulation of more than 5% fat in the liver [1, 2]. The prevalence of fatty liver in the general population of Iran has been reported to be 33.9%. Patients suffering from the moderate and severe fatty liver are about 8% of the population, equal to 23% of all NAFLD patients [3].

Liver steatosis is classified into simple steatosis, steatohepatitis, fibrosis, and hepatocellular carcinoma [4, 5]. It is also associated with endothelial dysfunction, metabolic syndrome, insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular events [6, 7]. Previous studies revealed that NAFLD has increased overall mortality whereas cardiovascular diseases, various extra hepatic malignancies, and other liver diseases contribute to 28%, 25%, and 13% of total deaths, respectively [8]. Therefore, early diagnosis and staging of NAFLD are critical in preventing irreversible inflammatory development and fibrotic changes in the liver [9, 10].

Liver biopsy is considered the gold standard for diagnosing and staging NAFLD [11]; however, biopsy is an invasive method with potentially severe complications such as infection, bleeding, and bile leakage. Furthermore, concerning the population-based studies, liver biopsy is not recommended for healthy individuals [12]. There are three non-invasive methods for NAFLD diagnosis. First of all, Non-contrast Enhanced Computed Tomography (NECT) scan is a reliable technique to assess steatosis; however, the use of this method is limited due to the side effects of ionization radiations [13,14]. Second, Magnetic Resonance Spectroscopy (MRS) and/or Magnetic Resonance Imaging (MRI) is the most accurate non-invasive method for quantification of fatty changes in the liver; nevertheless, these modalities are expensive and require standardization [15]. Last but not least, ultrasound imaging is easy to perform, completely safe, and inexpensive; therefore, it has become a widely accepted and valuable tool in clinical settings and extensive population studies [16–18]. However, the disadvantages of the ultrasound method are the qualitative grading, operator dependency, and subjective criteria [19].

Efforts on sonographic quantification of liver fat content have led to the introduction of the Hepatorenal Index (HRI) and elastography [19–21]. HRI was introduced in 1996 by Osawa and Mori [22], who investigated the ratio of the liver and the right kidney brightness using the sonographic method. Afterward, several studies compared HRI with liver biopsy and other imaging modalities [12, 19, 23–25].

The present study aimed to quantity the liver steatosis using Computed Tomography (CT) and ultrasound techniques for the patient samples. In addition, the results of HRI values were compared with the blood test, Alanine Aminotransferase (ALT) as the liver enzyme which can show liver disorders and is a reliable sensitive marker of liver diseases.

2. Materials and Methods

A summary of the present study methods as the "study flowchart" is drawn in Figure 1.

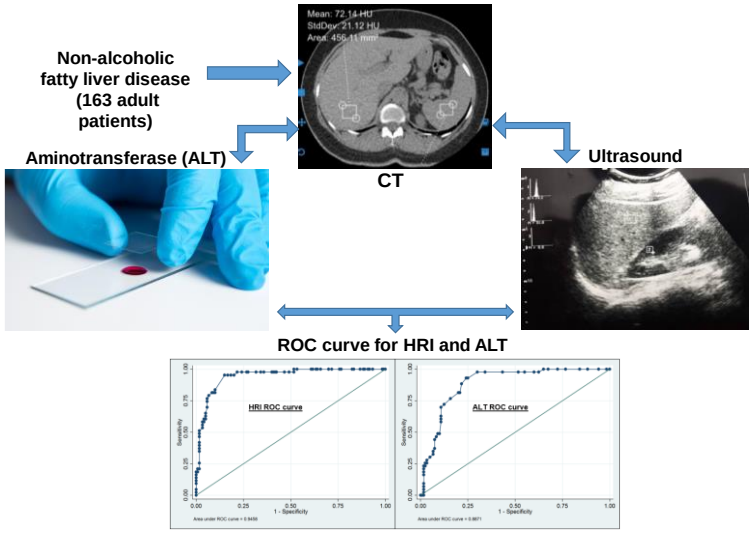


Figure 1. Study flowchart

2.1. Study Population and Measurements

The study protocol was reviewed and approved by National Ethical Committee. This cross-sectional research was performed on 163 NAFLD adult patients (male = 86 and female = 77) who were referred to the radiology center. The exclusion criteria were alcohol consumption, confirmed or suspicious hepatitis or other liver diseases, non-homogenous fat infiltration of liver, focal lesion of liver, hemangioma, ascites, renal disease, ectopic kidney, and absence of kidney. To evaluate and rule out imaging-related exclusion criteria, all participants underwent a baseline conventional B-mode liver ultrasound. The blood test result, ALT, was performed for all the participants.

2.2. CT Scan Protocol

A multislice CT device (Asteion; Toshiba Medical Co., Ltd., Tokyo, Japan) was used for scanning, and all of the scans were performed without contrast-enhanced materials. The tube voltage and tube current-time were selected at 120 kVp and 250 mAs, respectively. To minimize radiation exposure, we used a validated CT protocol to evaluate liver fat content. The slice thickness, interval, pitch, and rotation time were selected as 2mm, 1s, 1, and 2mm, in that order, as well as the scan type was helical.

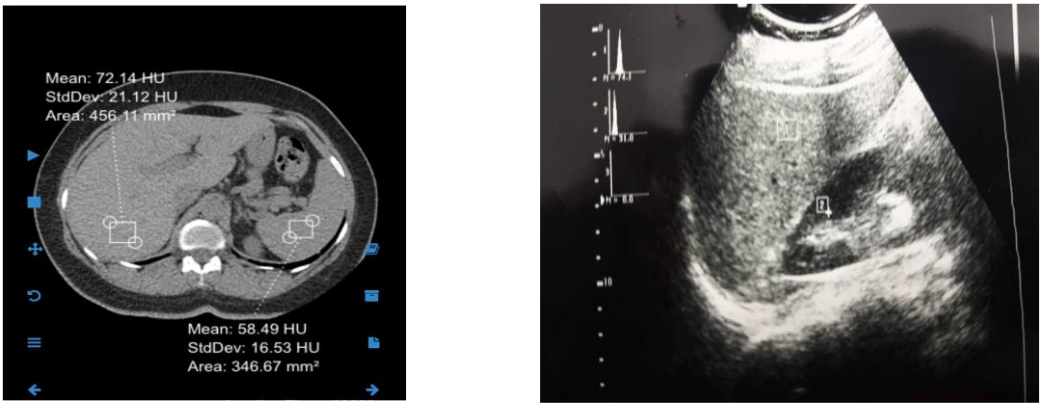
According to Davidson *et al.*'s [26] study, we have used a cross-sectional image at T12-L1 intervertebral space to provide a homogenous image of the liver for measurement of fat content and also spleen would be visible. Liver and spleen attenuation numbers in Hounsfield Unit (HU) were obtained using a validated Region-Of-Interest (ROI) [12]. Homogenous parts on the posterior aspects of the liver and spleen, which were the same depth have been selected as the ROI (Figure 2). Notably, we were careful to avoid blood vessels, bile ducts, and marginal surfaces.

Previous studies [12, 27] have shown that absolute liver attenuation less than or equal to 40 HU is the most accurate measurement in diagnosing moderate to severe NAFLD disease. Since the participants of the current study were at greater risk of developing liver inflammation and fibrosis, we chose the criteria mentioned above for CT (as the reference method), which was compared with HRI values. Another index in CT scan is Liver to Spleen attenuation ratio (L/S index), when it is less than or equal to 1, it indicates a mild degree of steatosis.

2.3. Ultrasound Protocol

Ultrasound imaging was performed by an experienced radiologist using a Medison Accuvix V20 system equipped with a 5-MHz phased-array convex transducer. The parameters such as frame rate (frame per second), gain, power (W), and dynamic range (dB) were chosen as 5, 60, 70, and 130, respectively. It is also worth mentioning that the radiologist was blind to the patient's CT scan results and laboratory findings.

To obtain HRI, for each patient, a coronal image for the liver and the right kidney was obtained in the mid or anterior axillary line, where the liver/kidney contrast was most clearly displayed. Then, an echo intensity histogram was drawn within the ROI. The ROI in the liver had a rectangular area (3.5-4 cm^2) on the superficial aspect of the liver. For the right kidney, the ROI was set as the cortical area between the pyramids and was at the same



(a) (b)

Figure 2. A sample of Computed Tomography (CT) (a) and ultrasound (b) images with fatty liver disease representing the region of interest

depth as the liver ROI. The measurement protocol and the size of the ROI were selected according to the guidelines described in Webb *et al.*'s study [23]. The selected ROI allowed obtaining an average histogram measurement while avoiding vessels and bile ducts (Figure 2). The mean brightness levels of the liver and right renal cortex were obtained on the same longitudinal sonographic plane. HRI was calculated manually by using the ratio between the M-value histogram of the liver and right kidney.

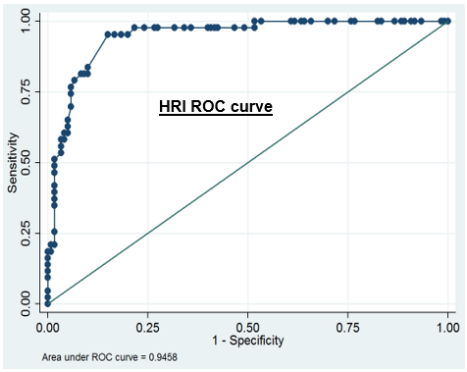
2.4. Statistics

Statistical analyses were performed using SPSS software version 16 (IBM, USA). The Kolmogorov-Smirnov test was used to evaluate the distribution of continuous variables. A t-test was used to evaluate the relationship between age and the severity of fat infiltration. Additionally, the χ^2 test for categorical variables (gender), and the Mann-Whitney test for continuous variables were used to evaluate the potential relationships among the para-clinical variables (HRI and ALT). Sensitivity, specificity, accuracy, Receiver Operating Characteristic (ROC) curve, and the Area Under the Curve (AUC) were used to measure the power of ultrasound HRI for detecting hepatic steatosis compared to NECT, as the standard method in the present study. A p-value of less than 0.05 was considered significant in all analyses.

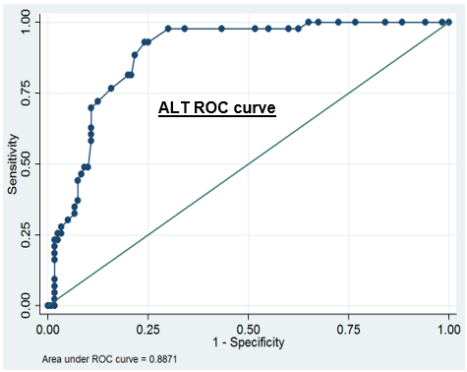
3. Results

Table 1 demonstrates the probable significant variation of sociodemographic factors, and paraclinical characteristics of ALT and HRI. The attenuation of normal liver parenchyma at NECT was obtained, ranging from 55-65 HU, and the two groups (mild, and moderate to severe steatosis) were classified based on CT criteria (as the standard method).

The mean echogenicity value for the liver and renal was obtained at 72 ± 12 and 38 ± 7, respectively. M-value histograms of the liver and the right kidney were plotted using the ultrasound images, and then the HRI values were calculated and reported in Table 1 for mild, and the moderate to severe steatosis. In order to facilitate the detection and differentiation between the two groups, an appropriate cut-off point was obtained for HRI using the ROC curve. According to Figure 3, the HRI has better performance (AUC: 0.94) compared to the ALT (AUC: 0.88) in determining liver steatosis. In addition, there was a significant difference between the two groups (P < 0.001) in HRI and ALT values, in a



(a)



(b)

Figure 3. Receiver Operating Characteristic (ROC) curve for Hepatorenal Index (HRI) (Area Under the Curve, AUC = 0.95) and Alanine Aminotransferase (ALT) (AUC = 0.88) in the two groups of fatty liver (mild, and moderate to severe steatosis)

Table 1. Comparison of demographic and paraclinical information of patients in the two groups of fatty liver

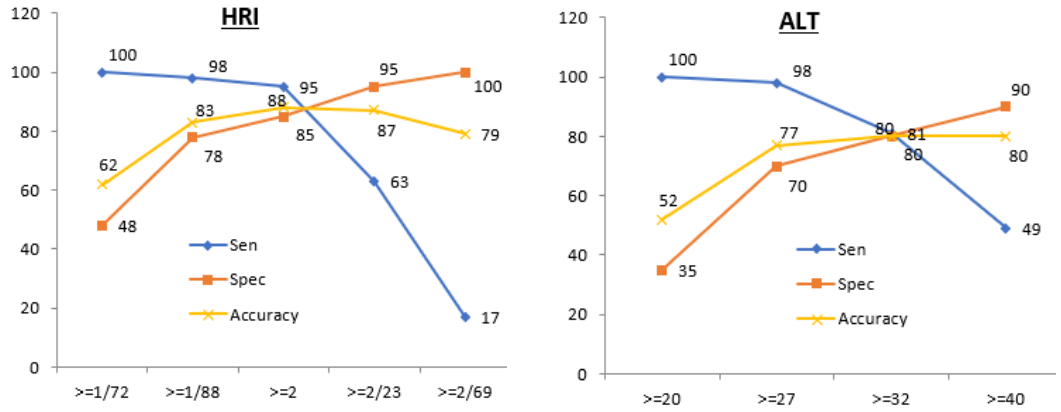
Baseline characteristic	Mild steatosis	Moderate to severe steatosis	P-value (between the two groups)
Age range	48 ± 12	47 ± 11	0.61
Male (number)	65	21	0.55
Female (number)	55	22	0.55
HRI	1.76 ± 0.21	2.34 ± 0.31	< 0.001
ALT (mg/dl)	26 ± 19	45 ± 18	< 0.001

way that, an increase of HRI and ALT in the moderate to severe steatosis was observed compared to mild steatosis.

Table 2 depicts the sensitivity, specificity, accuracy, Positive Predictive Values (PPV), and Negative Predictive Values (NPV) of different HRIs. For example, if we use HRI ≥ 1.53, we will be able to differentiate between the two groups of fatty liver with 100% sensitivity and 82.5% specificity. The diagnostic parameters of different values of HRI and ALT in predicting moderate to severe steatosis are shown in Figure 4. It is also worth mentioning that the HRI and ALT values were selected based on the ROC analysis. Regarding the findings from Figures 4 and 5, the accuracy and PPV on HRI shifts have a better ascent in comparison to the ALT diagram.

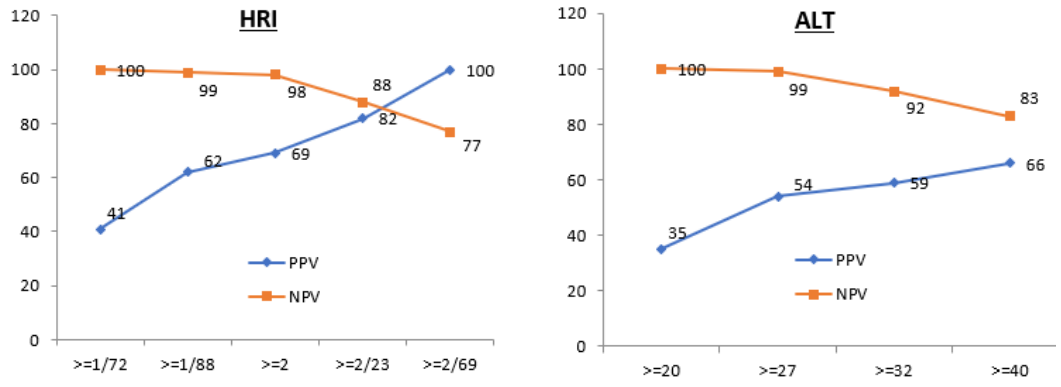
Table 2. Diagnostic parameters (True Positive: Sick people correctly identified as sick, False Positive: Healthy people incorrectly identified as sick, True Negative: Healthy people correctly identified as healthy, False Negative: Sick people incorrectly identified as healthy) of different values of Hepatorenal Index (HRI) for distinguishing between the two groups of patients with fatty liver

HRI Value	Sensitivity (%) = $\frac{TP}{TP+FN}$	Specificity(%) = $\frac{TN}{TN+FP}$	Predictive value of positive test (%) = $\frac{TP}{TP+FP}$	Predictive value of negative test (%) = $\frac{TN}{TN+FN}$
≥ 1.53	100.00	82.50	67.19	100.00
≥ 1.72	100.00	48.33	40.95	100.00
≥ 1.88	97.67	78.33	61.76	99.95
≥ 2	95.35	85.00	69.49	98.08
≥ 2.23	62.79	95.00	81.82	87.69
≥ 2.69	18.60	100.00	100.00	77.42



(a) (b)

Figure 4. Sensitivity (Sen), Specificity (Spec) and accuracy of HRI and ALT in predicting moderate to severe steatosis



(a) (b)

Figure 5. Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of HRI and ALT for determining moderate to severe steatosis

4. Discussion

The current work aimed to quantity the liver steatosis using CT as the standard method, and the results related to HRI from ultrasound were compared with ALT. Although conventional ultrasound imaging is still the most available, safe, and practical method for the liver, it suffers from subjective criteria resulting in intra- and inter-observer variability. Hence, it seems that sonographic HRI overcomes these limitations.

Normally, the liver has higher attenuation than the spleen which is attributed to the presence of glycogen in the liver. Hepatic steatosis increases the fat content of the liver and decreases liver parenchyma's attenuation; however, it does not affect spleen attenuation. Therefore, the spleen is a reliable internal reference for steatosis measurement. According to the previous studies, NECT indicates a good performance for diagnosing moderate to severe fat infiltration with a sensitivity of 82% and specificity of 100% [28, 29]. In addition, several studies used CT scans to examine and diagnose liver steatosis using absolute liver attenuation and L/S attenuation [19, 28, 30].

In CT modalities, the liver with HU ≤ 40 has been shown to have 100% specificity [27, 28]. Several studies used different criteria of HU and L/S to show the degree of liver steatosis. For instance, Kodama *et al.* [14] reported that absolute liver attenuation of ≤ 40 HU and ≤ 42 HU correspond to ≥ 30% and ≥ 25% liver fat content, respectively. In another study by Park *et al.* [28] , they expressed that L/S ≤ 0.8 has a high specificity (100%) for distinguishing/diagnosis of grade II and III hepatosteatosis with a sensitivity of 82%. Zeb *et al.* [27] demonstrated that L/S ≤ 0.8 and liver with HU ≤ 40 have similar diagnostic values. On the other hand, it has been reported that a liver with HU ≤ 48 or L/S < 0.9 is considered the threshold for liver steatosis, and a liver with HU < 51 or L/S < 1.1 could indicate a mild degree of steatosis. In the current work, to have the highest sensitivity and specificity, we have used two criteria for diagnosing the two levels of fatty liver: liver attenuation ≤ 40 HU for diagnosis of moderate to severe steatosis, and L/S ≤ 1 for detection of mild steatosis. According to the results, the mean and standard deviation of the liver density in CT scans were obtained at 51 ± 4 HU (21-64 HU).

The current study showed a positive association between HRI and fatty liver disease. We tested the diagnostic accuracy of HRI with NECT, and it has been shown that HRI ≥ 2 differentiates moderate to severe liver steatosis (≥ 30% fat accumulation) from mild degree with 90% sensitivity and 91% specificity. Previous studies have determined that the characteristics of the study population, such as the history of previous liver or liver-affecting diseases, play a major role in HRI cut-off values. Therefore, we excluded patients with known chronic liver diseases such as hepatitis and cancer.

Webb *et al.* [23] compared HRI with liver biopsy and reported that HRI > 1.86 had a 90% sensitivity and specificity in distinguishing moderate to severe degree fat infiltration from mild degree. Since the selected NECT criteria are 100% specific for > 30% steatosis, all patients with HRI > 2 certainly had more than 30% fat infiltration [19]. The difference between the findings of the current study and Webb *et al.* [23], could be attributed to the different choice of criteria, in the way that, in Webb's study, steatosis greater than 25% was considered as an indication of moderate fatty liver. Additionally, in their study, CT criteria had 73-82% sensitivity; some borderline cases were possibly filtered out from this category. In a survey by Goulart *et al.* [31], HRI has been examined with NECT results. They tested HRI > 1.5 against CT criteria of the liver with HU < 48. This criterion does not detect some mild cases of fatty liver; as a result, HRI did not show a good performance compared with the findings of the current study. Based on ultrasound findings, liver fibrosis has led to an increase in liver echogenicity, which is hard to distinguish with liver steatosis [32]. However, liver fibrosis does not affect the liver attenuation on NECT.

There are several limitations to our study. First of all, we did not compare our findings with other techniques like MRI and biopsy due to the lack of accessibility. In addition, the blood test (ALT) results can be compared/verified with other tests such as aspartate aminotransferase and SteatoTest, a simple and non-invasive quantitative estimate of liver steatosis.

5. Conclusion

HRI seems to be a convenient and easy method for diagnosing and quantifying liver steatosis without additional investigations. The differences in the obtained

values can be due to different techniques and criteria in getting the liver histogram.

Acknowledgments

This article was supported by Kashan University of Medical Sciences [grant number: 93051]; [ethical committee consent number: 29/5/1/1679].

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