Portable Ultrasonography to Assess Adult Hepatosteatosis in Rural Ecuador

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Financial Disclosures: None reported.

Support: None reported.

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Submitted May 13, 2019; accepted June 5, 2019.

Context: Hepatosteatosis (HS) is prevalent worldwide and can be measured via ultrasonographic (US) hepatic-renal (H/R) echo-intensity ratio.

Objective: To examine the incidence of HS in rural communities of the Ecuadorian Chimborazo region and to validate portable US as an effective method of disease screening in rural settings.

Methods: Sagittal right liver/kidney B-mode US was performed in individuals from 4 villages using a portable US scanner equipped with a 3.5-MHz curvilinear probe. National Institutes of Health ImageJ software was used to computerize tissue echogenicity in both renal cortex and hepatic parenchyma offline. Regions of interest of 900 pixels were used for measuring pixel intensity of the right renal cortex and hepatic parenchyma when calculating the H/R ratio. The difference in pixel intensity between liver parenchyma and renal cortex was analyzed using an unpaired *t* test. The intraclass correlation coefficient was used to test intra- and interobserver reliability for computerizing the H/R ratio.

Results: Forty patients were enrolled in the study (32 women and 8 men; mean age, 40 years). The mean (SD) H/R ratio of study patients was 3.61 (2.32), moderately higher than normal (normal, H/R <1.5). A significant difference was found in mean (SD) pixel value between hepatic parenchyma and renal cortex (52.82 [15.34] vs 19.93 [10.39]; $P<.001$). Thirty-four patients (85%) had an H/R ratio greater than 1.5. The intra- and interobserver reliability of computerizing H/R ratio was excellent ($r=0.940$; $P<.01$).

Conclusion: These findings suggest that HS is moderately present in persons in remote communities of Ecuador. The mean H/R ratio was greater than that in the diagnostic criteria for the disease. Portable US imaging may benefit these communities as an efficient method for the HS screening and diagnosis in rural areas.

J Am Osteopath Assoc. Published Ahead of Print
doi:10.7556/jaoa.2020.095

Keywords: grayscale, hepatosteatosis, pixel-intensity, ultrasonography

Hepatosteatosis (HS) encompasses a wide variety of liver diseases and is a common histologic characteristic of both alcoholic fatty liver disease (AFLD) and nonalcoholic fatty liver disease (NAFLD). It is diagnosed by intrahepatic triglyceride (TG) accumulation of more than 5% of total liver weight in the absence of other liver diseases. HS can result in the progression of chronic liver diseases such as steatohepatitis, hepatic cirrhosis, and hepatic cell carcinoma.[1-3] Because of its high prevalence worldwide, HS has become a global concern and has been directly linked to increases in obesity, type II diabetes mellitus, and dyslipidemia.[4] HS from NAFLD is more widespread

in Latin American communities, and research has demonstrated that liver disease is a common illness in rural communities in India and Sri Lanka.[5, 6]

Quantifying hepatic TG accumulation to determine the extent of HS is best conducted by liver biopsy, which is considered the gold standard for HS diagnosis. This process is both costly and invasive. Additionally, sampling variability has been reported.[7] Rural communities rarely have reliable hospital access in which to perform liver biopsies, sampling repeats, and the follow-up care that liver biopsies may require. Although computed tomography and magnetic resonance imaging may be less invasive, they remain inefficient for these populations because of the prohibitive cost, radiation exposure, unavailability, and the lack of transportation for patients living in rural settings. Compared with techniques such as liver biopsy and computed tomography and magnetic resonance imaging, ultrasonography (US)—especially less expensive, noninvasive, and portable US—is an efficient alternative for quantifying HS. Previous studies have shown the validity of using the hepatic/renal (H/R) ratio in diagnosing HS by using state-of-the-art and nonportable US scanners, defining a cut-off diagnostic ratio greater than 1.49 as 100% sensitive and 91% specific for greater than 5% steatosis when compared with liver biopsy.[8]

Liver disease is increasingly becoming a global issue, and the use of US for disease screening and monitoring is crucial to combating disease progression and initiating proper management, especially in rural communities with limited access to healthcare. We did not find any report of US screening for HS in Latin American rural villages in the Ecuadorian Chimborazo region during our literature review.

The purpose of this study was to assess the feasibility of using portable US as a quantitative tool for screening HS in the communities of rural Ecuador.

Methods

The institutional review board of Rocky Vista University College of Osteopathic Medicine approved this study, and written informed consent was waived because it was a retrospective study.

We retrospectively reviewed abdominal US images from 81 patients screened for HS during a medical outreach program with the Hands for Health Foundation between February 18 and 27, 2018, in 4 villages in the Chimborazo region of Ecuador. All 4 villages we visited had limited access to health care, reduced road accessibility, and limited public transportation, and they were located far from hospital and physician services. Patients underwent ultrasound exams of the heart, neck, pelvis (obstetric evaluation), and soft tissue; those with preexisting renal conditions were excluded from the study. The abdominal US results containing images of the liver and right kidney on the remaining 40 patients were included to screen for HS.

All static grayscale imaging was recorded by a US expert with 30 years of experience in abdominal US (J.G.). Standard machine settings for acquiring grayscale images of the liver and kidneys included scanning frequency, 3.5 MHz; dynamic range, 70; image depth, 19.9 cm; total gain, 100; and tissue harmonic imaging. We retrospectively reviewed B-mode sagittal images of the right liver and kidney that were acquired using commercial US scanners (ECO 5, Chison USA, Inc.) equipped with C3-A (multifrequency 2.5-4.5 MHz) curvilinear probes.

Image Analysis

Static US images with Digital Imaging Communication of Medicine (DICOM) format were analyzed on National Institutes of Health ImageJ Software to computerize tissue echogenicity in the hepatic parenchyma and renal cortex offline. A standard region of interest (ROI) of 900 pixels (30 × 30 pixels) was used to count mean pixel intensity in the hepatic parenchyma and renal cortex (**Figure 1**). ROIs excluded nonparenchyma structures in the liver and renal cortex, such as blood vessels, bile ducts in the liver, renal capsule, and renal pelvis of the kidney. ROIs were chosen in the right lobe of the liver and right renal cortex under the same vertical alignment in the center of the image and focal zone (**Figure 1**).

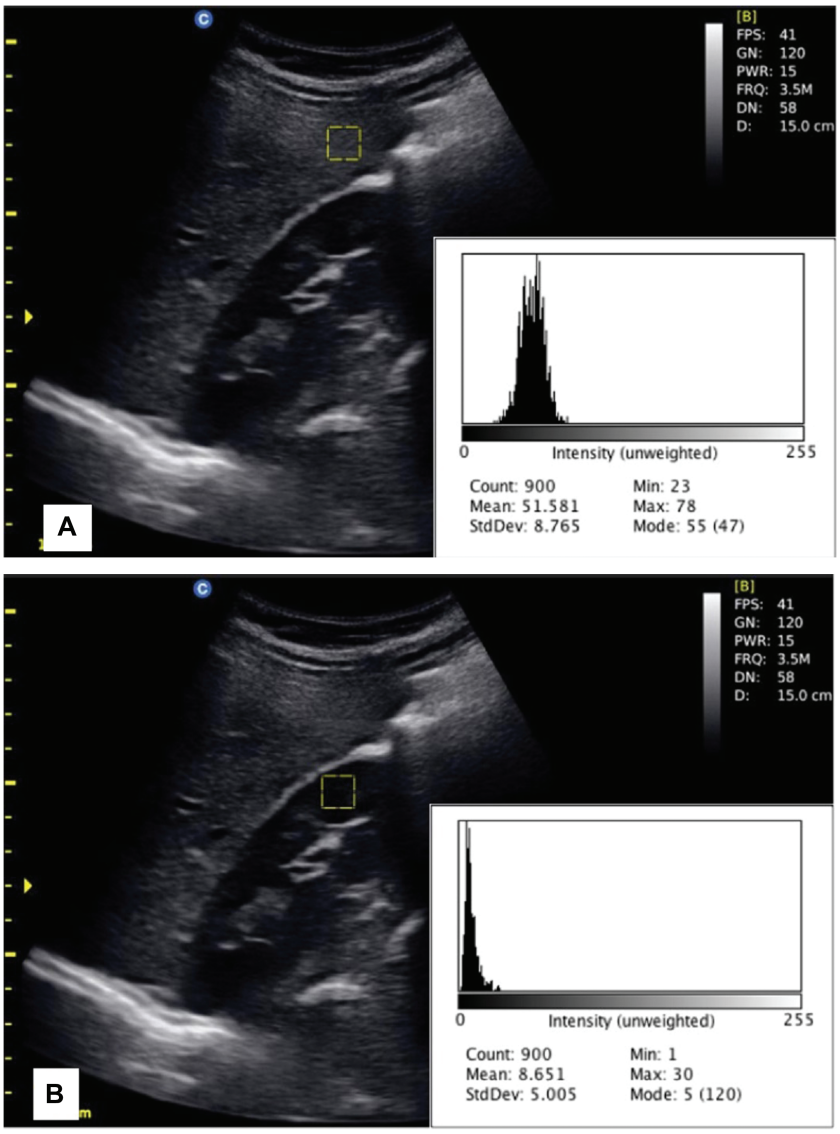

Figure 1.
Sagittal ultrasonographic images of right-side liver parenchyma and right renal cortex acquired from a 46-year-old woman. Using National Institutes of Health ImageJ software analysis, grayscale pixel value was calculated in the regions of interest (ROI, yellow boxes) consisting of 900 pixels (30 × 30 pixels) within the tissues. ROIs excluded structures (capsule, major vessels) that significantly altered tissue echogenicity. Mean (SD) pixel counts are shown in the histograms. A hepatic/renal (H/R) ratio was calculated (mean pixel value of the liver to mean pixel value of right renal cortex); H/R ratio was 5.96 (51.58:8.65) in this case. (A) grayscale pixel counts in the right lobe liver parenchyma. (B) grayscale pixel counts in the right renal cortex.

Mean pixel values between the hepatic parenchyma and renal cortex were computed by 2 observers (BJ and AO) using the previously mentioned ROIs with the ImageJ software (**Figure 1**). Both observers repeated their measurements, the mean values of both data sets were calculated, and then mean values of pixel intensity (the average of 4 pixel-intensity measurements) for hepatic parenchyma and renal cortex

were used for data analysis. All mean calculations and data collections were conducted using Microsoft Excel (Microsoft Inc.).

The H/R ratio was determined by dividing the mean pixel intensity of the hepatic parenchyma by the mean pixel intensity of the renal cortex. An H/R ratio greater than 1.49 signified the presence of more than 5% hepatic parenchyma adipocyte deposition in a previous study,[8] and this measurement was used as a diagnostic criterion for HS.

Statistical Analysis

Pixel intensity of the hepatic parenchyma and renal cortex was expressed by mean (SD). The difference in pixel intensity counted between the hepatic parenchyma and renal cortex was examined using an unpaired samples *t* test and the result was shown using box-and-whisker plots (**Figure 2**). The intraclass correlation coefficient (ICC) was applied to test intra- and interobserver variability of pixel intensity computerization via SPSS software version 25.0 (IBM).

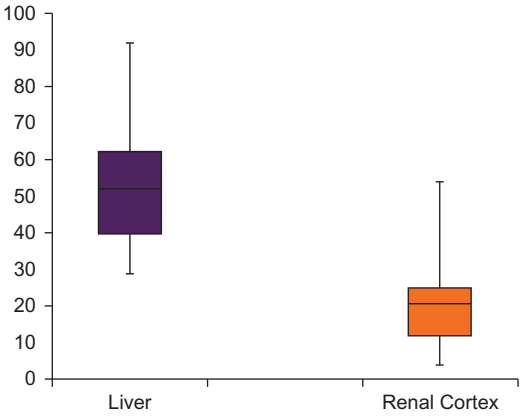

Figure 2.
Box and whisker plots show a significant difference (*P*<.01) in mean pixel intensity between hepatic parenchyma (2D, purple box) and renal cortex (2D, orange box). Both high mean pixel intensity and hepatic/renal ratio represent an increase in fat accumulation in the hepatic steatosis.

Results

The scans of 40 patients were reviewed for the study (32 women and 8 men; mean age, 40 years). As noted previously, patients with preexisting renal conditions were excluded.

The calculated mean (SD) H/R ratio of the 40 patients reviewed for this study was 3.61 (2.32), a moderate increase in value relative to the diagnostic cutoff of greater than 1.49 for HS used in a previous study.[8] Thirty-four patients (85%) had an H/R ratio greater than 1.5, whereas 6 patients (15%) had an H/R ratio less than 1.5. Hepatic parenchyma had a proportionally larger mean (SD) value compared with the renal cortex mean (SD) pixel value (52.82 [15.34] vs 19.93 [10.39]; *P*<.001) after retrospective computerized image analysis. An unpaired *t* test confirmed a significant difference in pixel intensity between tissues of liver and renal cortex (*P*<.001). The computerized H/R ratio of the patients suggested significant presence of HS in the rural communities studied (**Figure 2**). The ICC for inter- and intraobserver reliability in computing the H/R ratio was *r*=0.94 for observer 1 vs observer 2; *r*=0.931 for observer 1 vs observer 1; and *r*=0.998 for observer 2 vs observer 2, respectively (**Table**).

Discussion

This retrospective study demonstrated the extent of HS in rural Ecuador and verified the viability of portable US as a diagnostic screening method for HS in rural settings. In addition, H/R ratio greater than 1.5 was detected in 34 of 40 patients (85%), indicating the prevalence of HS in our study population.

The 34 patients in the study had moderate HS, determined by an elevated H/R ratio. The H/R ratio has been confirmed as an effective tool for HS diagnosis. Studies[8,9] have compared the H/R ratio to that of a liver biopsy specimen; Webb et al[8] and Ferreira de Almeida e Borges V et al[9] determined an US H/R ratio of greater than 1.49 as 100% sensitive and 91% specific for HS. An additional study confirmed the diagnostic accuracy of the H/R ratio when compared with 1H-magnetic resonance spectroscopy.[10]

Table.
Intra- and Interobserver Reliability Tests for Performing H/R Ratio Computerization

Interobserver status	Intraclass correlation	95% CI	*F* test	*P* value
Observer 1 vs observer 1	0.931	0.869-0.963	14.39	<.001
Observer 2 vs observer 2	0.998	0.996-0.996	450.4	<.001
Observer 1 vs observer 2	0.940	0.886-0.968	16.59	<.001

Abbreviations: H/R, hepatic/renal; ROI, region of interest.

A significant difference in pixel intensity between the hepatic parenchyma and renal cortex ($P<.001$) indicates remarkable increase in hepatic echogenicity due to fatty infiltration within hepatocytes (**Figure 2**). Chronic intrahepatic TG accumulation is a structural change that, in line with the osteopathic medical emphasis on the reciprocal correlation between structure and function, leads to decreased liver functionality and to advancement to more complex and severe diseases. For instance, HS from NAFLD has been linked to metabolic dysfunctions, including insulin resistance and cardiovascular disease. Additional long-term advanced complications of liver disease include cirrhosis, liver failure, and the development of hepatocellular carcinoma.[4]

Meta-analyses have estimated global prevalence of NAFLD at 25%, with Latin American communities generally having a greater prevalence of HS and higher hepatic TG content when compared with other ethnic groups.[4,11] A study by Browning et al[12] demonstrated that a higher incidence of HS in Latin Americans correlated with a higher level of obesity and insulin resistance. This relationship may, in part, correspond to the genetic predisposition of the Latin American population to the *PNPLA3* gene, in which a potential I148M polymorphism increases hepatic TG accumulation.[13,14] This increased accumulation increases the risk to these populations of advancing to NAFLD complications.

Early and accurate detection of HS plays an important role in the management of NAFLD. Diet is a major factor that contributes to HS. Increased consumption of soft drinks, fructose, and meat in conjunction with low dietary fiber intake were shown to be more prevalent in patients with NAFLD.[15,16] One study[17] indicated that Ecuadorian adolescents consume high levels of solid fats and added sugars, with a small proportion consuming the recommended levels of fiber, fruit, and vegetables. This aforementioned diet can contribute to the long-term progression of HS. A study by Orces and Gavilanez[18] demonstrated the occurrence of metabolic syndrome in older Ecuadorians to be 66% in women and 47% in men, with hypertriglyceridemia present in 48% of women and 38.4% of men. Sufficient data regarding the cause and prevalence of HS in Ecuadorians are currently lacking, but an increased probability of HS development in the Ecuadorian population is supported by studies indicating a high incidence of metabolic syndrome and poor adolescent diet.[15-18]

These multifactorial predispositions of the Latin American population to HS, both genetic and environmental, make early screening, diagnosis, and disease management important in reducing HS and relevant comorbidities in Latin American communities. With 36.3% of the Ecuadorian population living in rural areas and a low rate of preventive health care use among indigenous or low-income residents, it is crucial to introduce new technology that would increase medical accessibility to these groups.[19,20]

The significant potential for an asymptomatic clinical presentation of NAFLD underlines the necessity of

early screening and detection.[21] Because early-stage HS is a reversible condition, early detection can open the door to patient education on effective lifestyle modification before the onset of irreversible liver damage (fibrosis) and progressive metabolic syndrome. US can guide disease management, as seen in 43% of patient cases in a study conducted in Rwanda.[22] This study also demonstrated a 96% concordance rate of US review between physicians working in the local clinic and those working at major academic institutions, as well as the continuation of US use after the departure of trained physicians. Portable US imaging used in a native Amazonian population with no access to medical equipment exhibited a 68% certainty of diagnosis and a 28% reduction in patient transfer to nearby health clinics.[23] These studies verified the advantages of portable US in narrowing diagnosis, preventing unnecessary travel to health clinics in outlying areas, and guiding disease management in rural settings. These benefits, coupled with the accuracy of the H/R ratio in HS diagnosis, make portable US an efficient, cost-effective, and noninvasive method of HS screening in rural populations.

Importantly, we found excellent intra- and interobserver reliability in computing the H/R ratio (ICC >.94; **Table**).

Several limitations existed in this study. The retrospective aspect of incorporating computer analysis when calculating the H/R ratio can restrict immediate diagnosis when computerized methods to calculate tissue echointensity are not available. Another limitation is that the H/R ratio relies on diffuse liver steatosis. Diseases that present with focal HS may have altered detection when the H/R ratio is used, owing to selection of certain ROIs within the hepatic parenchyma. H/R ratios have been shown to be less accurate in patients with mild steatosis or in populations with high levels of obesity; new detection strategies are needed to provide a more accurate and consistent HS diagnosis.[9]

Future study regarding the H/R ratio should focus on more specific H/R ratio criteria for HS diagnoses to help establish a systematic protocol for HS diagnosis worldwide. For example, categorizing the disease into simple, moderate, or severe HS could mediate standardized approaches to proper treatment initiation.

Conclusion

HS is a consequence of both ALD and NAFLD and is increasingly present worldwide, particularly in Latin American communities. The progression from hepatic steatosis to more chronic diseases places a burden on global healthcare because of its high incidence and correlation to a high incidence of metabolic syndrome. US can benefit remote communities because it is portable, inexpensive, and both specific and selective for diagnosing and monitoring HS. HS is moderately present in rural communities of the Chimborazo region of Ecuador, determined via ultrasonographic H/R ratio. The extent of HS in these rural communities should be examined in order to properly initiate treatment, with the goal of preventing disease advancement.

Author Contributions

All authors provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; all authors gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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