ORIGINAL RESEARCH

The Function of Pluriparametric Ultrasound of the Liver for the Assessment of Non-Alcoholic Steatohepatitis: An Original Research Study

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Received Date: 12 November, 2024 Accepted Date: 15 December, 2024

Abstract

Background: Diagnosed of nonalcoholic steatohepatitis (NASH) is primarily relied on histopathologic examination, which is an invasive process however a noninvasive surrogate markers are preferred for screening of patients who are at high risk of NASH. Attenuation coefficient, dispersion slope and shear-wave speed are Ultrasonographic parameters that assist in estimating inflammation, steatosis and fibrosis and might enable the noninvasive diagnosis of nonalcoholic steatohepatitis within a single examination.

Aim: To evaluate the diagnostic efficacy of attenuation coefficient, dispersion slope and shear-wave speed measurements obtained through two-dimensional (2D) shear-wave elastography (SWE) in evaluating fibrosis, inflammation and steatosis as well as in the noninvasive diagnosis of NASH in individuals suspected of suffering from nonalcoholic fatty liver disease (NAFLD).

Materials and Methods: This prospective analytical study gathered information from 200 consecutive adult patients who have gone for liver biopsy due to suspected NAFLD and were included between January 2021 and December 2023. Three ultrasonographic parameters [attenuation coefficient (dB/cm/MHz), dispersion slope (m/sec/kHz), and shear-wave speed (in meters per second)] were measured using a 2D shear-wave elastography (SWE) system right before the biopsy. The biopsy specimens were evaluated by an expert pathologist bases on the Nonalcoholic Steatohepatitis Clinical Research Network criteria. Diagnostic efficacy was determined using the area under the receiver operating characteristic curve (AUC) for the aspect of steatosis and fibrosis.

Results: All the recorded data were checked at initial stages for presence of any obvious integrated confounders. Post hoc analysis was not attempted so as to ensure data quality with minimal errors. Afterward data was subjected to basic statistical analysis with SPSS statistical package for the Social Sciences version 22 for Windows. Total of 188 adult individuals were selected including both male and female patients. The mean values for age and body mass index were 56 years ± 14 and 28. 3 kg/sqm \pm 5.7 respectively. The AUC of dispersion slope was 0.77 (95% CI: 0.64, 0.89), the AUC of attenuation coefficient was 0.72 (95% CI: 0.59, 0.84), the AUC of shear wave speed was 0.71 (95% CI: 0.59, 0.84), and the AUC of the all three parameters combinedly was 0.82 (95% CI: 0.73, 0.91). When comparing assessment of fibrosis using shear-wave speed, there were significantly differences in shear-wave speed across fibrosis stages f0, f1, f2, f3, and f4

Conclusion: Attenuation coefficient, dispersion slope and shear-wave speed were determined to be beneficial for evaluating lobular inflammation, steatosis, and fibrosis, respectively, in individuals with biopsy confirmed nonalcoholic fatty liver disease (NAFLD).

Keywords: Pluriparametric Ultrasound, Liver, Non-Alcoholic Steatohepatitis, Shear-Wave Elastography, Hepatocellular Carcinoma, Inflammation, Necrosis

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Introduction

Globally, Nonalcoholic fatty liver disease (NAFLD) is ranks among the most prevalent chronic liver condition and is closely linked to metabolic syndrome and obesity [1,2]. Roughly 20%–25% of individuals with NAFLD progress to nonalcoholic steatohepatitis (NASH), resulting in a quicker advancement from fibrosis to cirrhosis and hepatocellular carcinoma, which are recognized risk factors for liver-related mortality [3]. Therefore, diagnosing NASH holds significance importance in today's clinical practice. NASH can solely be diagnosed by assessing histologic characteristics like lobular inflammation, steatosis, ballooning, and fibrosis (4). While histological diagnosis is presently gold standard for diagnosing NASH, liver biopsy possesses several disadvantages, including cost, sampling error, and the potential complications [5]. Consequently, it would be significantly beneficial in clinical practice to discover noninvasive indicators that can act as surrogate markers for these histological characteristic. Liver fibrosis could be the key histologic characteristic linked to increased overall and liver related death and a higher chance of experiencing liver-related complications [6]. At present, the noninvasive evaluation of advanced fibrosis in NAFLD relies partially on ultrasound transient elastography, two dimensional (2D) USG, shear-wave elastography (SWE) and MRI elastography [7-9]. Accurately demonstrating the existence of liver fat is the first diagnostic difficulty for steatosis, which is a beginning cause of nonalcoholic fatty liver disease. There has already been research on the noninvasive evaluation of steatosis in NAFLD using USG [10–12] and Magnetic Resonance Imaging (MRI) [13,14], and these techniques have significantly improved diagnostic performance. As previously mentioned, liver fibrosis and steatosis can now be noninvasively evaluated with a comparatively high degree of accuracy; however, as far as we are aware, noninvasive evaluation of inflammation is still a ways off. The development of hepatic fibrosis is significantly influenced by inflammation [15]. Hence, prompt detection of NASH prior to the development of fibrosis (i.e., steatosis and inflammation) would enable early management to stop the development of end-stage liver disease. Presently, viscosity, which is regarded as a distinct feature from elasticity, can be indirectly measured by analyzing the dispersion properties of shear waves [16]. Although more investigation is required to assess this parameter's potential in tissue characterization, initial data indicates that it might be useful in identifying liver inflammation and initial necrosis [16,17]. The shear-wave dispersion slope may therefore be helpful in determining the extent of inflammation and initial necrosis. Dispersion slope, attenuation coefficient, and shear-wave speed are three US parameters that can be measured by a more recent

version of 2D SWE that has been accessible in India since early 2020. Given this context, we postulated that the combination of these three parameters may enhance the diagnostic performance for NASH and that dispersion slope measurement may be helpful in identifying liver inflammation in NAFLD. This study looked at how well 2D SWE, which combines dispersion slope, attenuation coefficient, and shearwave speed, performed in detecting imaging indicators for NASH diagnosis and determining the severity of the disease in people with biopsy-proven NAFLD.

Material and Methods

This was a cross-sectional prospective investigation of individuals with suspected NAFLD who had both 2D SWE and liver biopsy performed on the same day. From January 2021 to December 2023, consecutive adult individuals with clinical reasons for liver biopsy due to suspected NAFLD were included in the study, which took place entirely in Kanpur city of Uttar Pradesh. All participants provided written informed consent, and the study received approval from our institutional ethical committee. The inclusion criteria consisted of participants who were aged 40 years or older and thought to have NAFLD, who were both willing and capable of giving informed consent. The criteria for suspected NAFLD included (a) indications of hepatic steatosis based on imaging and (b) the lack of secondary factors for hepatic fat accumulation, such as significant alcohol intake, prolonged use of steatogenic drugs, or monogenic inherited conditions. Individuals having comorbidity of cardiac disease were excluded from the study. On the day of the liver biopsy, all participants underwent a uniform clinical assessment, which incorporated medical history, anthropometric evaluation, and biochemical analysis at our facility.

The research aimed to assess the diagnostic efficacy of dispersion slope in relation to histologic assessment of lobular inflammation and the assessment of the diagnostic effectiveness of attenuation coefficient in comparison with histologic evaluation for steatosis along with the the appraisal of the diagnostic proficiency of shear-wave speed in comparison with histologic evaluation for fibrosis. All 2D SWE evaluations were conducted by the same hepatologist using a diagnostic ultrasound scanner equipped with a 3. 5 MHz convex transducer. The ultrasound system automatically presented a twin view, which, after a 5 second cooling period, transitioned to quad view mode, featuring a shear-wave speed map, propagation map, Bmode image, and shear-wave dispersion slope map. Shear-wave speed and dispersion slope data could thus be observed at the same time. A 1-cm circular region of interest was manually placed on the sample box. The activation of shear-wave propagation and the

acquisition of 2D SWE data were carried out 10 times for each participant. Only SWE findings with an interquartile range to median ratio of less than or equal to 0. 3 were utilized (18). The average values of shearwave speed and dispersion slope were employed for analysis. Examination Immediately following the SWE examination, attenuation imaging examination was carried out continuously by the same physician using the same scanner and transducer while the participant maintained breath-holding. First, the attenuation imaging mode was activated, and the examination was conducted at the same intercostal space as the SWE. Comprehensive details regarding the attenuation imaging examination are described in other sources (12,19). The consistency of the results was indicated by the \mathbb{R}^2 value, with \mathbb{R}^2 values classified as poor (\mathbb{R}^2) (0.80) , good $(R^2=0.81-0.89)$ or excellent $(R²\geq 0.90)$. Attenuation coefficient values with $R²$ equal to or greater than 0. 80 were regarded as valid measurements, based on information provided by the manufacturer. Attenuation imaging examinations were conducted until five valid measurements were achieved, and the average value was utilized for analysis. Immediately following the 2D SWE examination, a USguided percutaneous liver biopsy was conducted using an 18- or 16-gauge core needle biopsy kit in accordance with the established protocol. The liver biopsy was executed at a site as near as feasible to the location where the 2D SWE examination was carried out, and two specimens were collected from each participant to ensure that a specimen of adequate size for analysis was obtained and to reduce histologic sampling errors. Tissue specimens were preserved in 10% neutral-buffered formalin and stained with hematoxylin-eosin, azan, and silver impregnation. Histologic scoring was administered using the Clinical Research Network in NASH scoring system (20).

The identification of NASH was established based on the classification outlined by Matteoni et al (21). In summary, type 1 is characterized as having fatty liver only; type 2 is characterized by fat buildup and lobular inflammation; type 3 is characterized by fat buildup and ballooning degeneration; and type 4 is characterized by fat buildup, ballooning degeneration, and either Mallory-Denk bodies or fibrosis. Types 3 or 4 are categorized as NASH. Statistical analysis indicates that continuous variables are represented as means with standard deviations, while categorical variables are presented as absolute numbers alongside percentages. The variations in dispersion slope among lobular inflammation grades, the differences in attenuation coefficient across steatosis grades, and the discrepancies in shear-wave speed within fibrosis stages were evaluated. In this analysis, we refrained from executing multiple testing corrections since this was an exploratory analysis that necessitates validation in an external and/or independent cohort. *P* value less than 0.05 was considered indicative of a statistically significant difference. CIs were reported at the 95% level.

Results

All the gathered data was entered into mater excel sheet for further analysis. Initially, data was checked for any possible incorporated error. Afterwards data was subjected to basic statistical analysis with SPSS statistical package for the Social Sciences version 22 for Windows. Nonparametric test, specifically chi-square test, was used for supplementary data analysis; p-value. A total of 200 adult individuals with clinical indications for hepatic biopsy due to suspected NAFLD were consecutively enrolled at Dr. B. S Kushwah Institute of Medical Science and associated hospitals. Out of these, three participants were excluded due to another medical condition, and 197 participants underwent assessment for ultrasound measurements. From these, nine participants were excluded from the study due to unreliable dispersion slope measurements (inter quartile range–to-median ratio, >0. 3), and one was excluded because of unreliable shear wave speed and dispersion slope measurements (interquartile range–to-median ratio, > 0.3), resulting in 188 participants remaining in the final analytic sample. The mean $(\pm$ standard deviation) values for age and body mass index were 56 years ± 14 and 28. 3 kg/sqm ± 5.7 respectively. Of the 188 participants, 148 (78.7%) were diagnosed with NASH. Out of 188 studied patients, 101 were males and 87 were females [Table 1, Graph 1]. P-value was highly significant for age group 66-70 years. It was 0.01. Maximum 50 patients were noticed in age group of 66- 70 years. Out of 188 participants, 98 (52.1%) exhibited significant fibrosis (i. e. fibrosis stage 2 or higher). The baseline demographic, biochemical, histologic, and imaging data of the participants are summarized in Table 2.

Table 1: Age & gender based statistical explanation of participating patients

Age Group (Years)	Male	Female	Total	P value
42-47	20			0.60
$48 - 53$			30	0.20
54-59			30	0.10
$60 - 65$	41			0.50
66-70	ت			$0.01*$

Table 4: Fundamental statistical explanations with level of significance evaluation using "Pearson Chi-Square" test (for Steatosis Grade)

Assessment of Lobular Inflammation Using Dispersion Slope

Dispersion slope showed significant differences among inflammation grades A0, A1, A2, and A3 (Kruskal-

Wallis test: P \ldots 001; Wilcoxon test: P = 07 between A0 and A1, $P = 02$ between A0 and A2, P, 01 between A2 and A3, $P = 0.04$ between A0 and A3, and P, 0001 in other comparisons). The AUCs and the diagnostic

performance of the dispersion slope cut-off values optimized using the Youden index are outlined. The diagnostic performance was highest at an inflammation grade threshold of A1 or higher, yielding an AUC of 0. 95 (95% CI: 0. 91, 0. 10), a sensitivity of 93%, and a specificity of 99% at the 8.5 (m/sec)/kHz threshold identified by maximizing the Youden index. The diagnostic performance decreased at an inflammation grade threshold of A2 or higher, with an AUC of 0. 83 (95% CI: 0. 71, 0. 88), sensitivity of 87%, and specificity of 67% at the 9.8 (m/sec)/kHz threshold established by maximizing the Youden index, and was also diminished at an inflammation grade threshold of A3, presenting an AUC of 0. 86 (95% CI: 0. 73, 0. 96), sensitivity of 84%, and specificity of 78% at the 12. 5 (m/sec)/kHz threshold determined by maximizing the Youden index. highest at steatosis grade threshold greater than or equal to S2,

Diagnostic Performance of Ultrasound Parameters in the Diagnosis of NASH

The performance of ultrasound parameters for the diagnosis of NASH was also evaluated using a logistic regression statistical tool. The AUC of dispersion slope was 0.77 (95% CI: 0.64, 0.89), the AUC of attenuation coefficient was 0.72 (95% CI: 0.59, 0.84), the AUC of shear wave speed was 0.71 (95% CI: 0.59, 0.84), and the AUC of the all three parameters combinedly was 0.82 (95% CI: 0.73, 0.91).

Assessment of Steatosis Using Attenuation Coefficient

The attenuation coefficient exhibited a significant variation across steatosis grades S0, S1, S2, and S3 (Kruskal-Wallis test: $P < .001$; Wilcoxon test: $P = 0.34$ between S2 and S3, P < 0.001 between S0 and S3, P $=0.01$ between S0 and S1, and P <0.0001 in other comparisons). The AUCs along with the diagnostic performance of the attenuation coefficient cut-off values refined using the Youden index is done. The greatest diagnostic performance was recorded at the steatosis grade threshold of greater than or equal to S1, yielding an AUC of 0. 87 (95% CI: 0. 81, 0. 98), a sensitivity of 76%, and a specificity of 99% at the threshold of 0. 68 dB/cm/MHz determined by maximizing the Youden index. The second highest diagnostic performance was noted at the steatosis grade threshold of greater than or equal to S2, resulting in an AUC of 0. 86 (95% CI: 0. 79, 0. 93), a sensitivity of 91%, and a specificity of 67% at the threshold of 0. 73 dB/cm/MHz identified by maximizing the Youden index.

Assessment of Fibrosis Using Shear-Wave Speed

There were significantly differences in Shear-wave speed across fibrosis stages F0, F1, F2, F3, and F4

(Kruskal-Wallis test: P , 0.001; Wilcoxon test: $P = 0.05$ between F2 and F3, $P = 0.03$ between F1 and F2, P , 0.02 between F0 and F2, *P* , .001 between F3 and F4, and *P* , .0001 otherwise). The AUCs along with the diagnostic efficacy of the shearwave speed cut-off values refined using the Youden index are measured. In summary, the diagnostic efficacy was greatest at fibrosis stage threshold of F4 (i e, cirrhosis), with an AUC of 0.95 (95% CI: 0.92, 0.99), specificity of 82% and sensitivity of 100% at the threshold of 1.55 m/sec determined by maximizing the Youden index. The diagnostic efficacy was second greatest at a fibrosis stage threshold of greater than or equal to F3 (i e, advanced fibrosis), with an AUC of 0.91 (95% CI: 0.84, 0.97), sensitivity of 86%, and specificity of 78% at the threshold of 1.40 m/sec selected by aximizing the Youden index.

Influence of US Parameters on Histologic Parameters

In this study we examined the effect of the ultrasound parameters on each histological parameter. In the analysis of ordinal logistic regression, the notable covariates that impacted lobular inflammation grade were dispersion slope (odds ratio $[OR] = 1.07$; 95% CI: 1.03, 1.10; $P = 0.001$) and attenuation coefficient (OR = 1.51; 95% CI: 1.07, 2.09; *P* = 0.03), the significant covariates that affected steatosis grade were attenuation coefficient (OR = 3.61; 95% CI: 2.38, 5.22; *P* , 0.001) and dispersion slope (OR = 1.06; 95% CI: 1.02, 1.08; *P* $= 0.01$), the significant covariate that impacted fibrosis stage was shear-wave speed (OR = 2.34 ; 95% CI: 1.68, 3.06; *P* , 0.001), and the notable covariate influencing ballooning grade was shear-wave speed (OR $= 1.65$; 95% CI: 1.15, 2.39; *P* = .01).

Discussion

Inflammation is a crucial factor in the progression of liver fibrosis, yet noninvasive evaluation of inflammation has not been accomplished. More ever, diagnosing nonalcoholic steatohepatitis in a noninvasive manner can prove to be difficult. In this research, we utilized ultrasound based markers (attenuation coefficient, dispersion slope and shearwave speed) to predict histological results in nonalcoholic fatty liver disease without invasively procedure. Our findings indicated that the dispersion slope, associated with tissue viscosity, demonstrated strong diagnostic capabilities in discriminating the grade of lobular inflammation. Furthermore, the dispersion slope immerged as a significant factor influencing the grade of lobular inflammation (odds ratio $= 1.07$, Probability $= .001$) and also as a significant factor affecting steatosis (OR = 1.03 , P = $.01$). This observation is of utmost importance because, to our knowledge, no alternative imaging method can allows

Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.12.2024.161

for the assessment of liver inflammation. Furthermore, a good diagnostic distinction between NASH and non-NASH was made possible by the combination of these US-based markers (AUC = 0.81). High diagnostic performance was demonstrated by the attenuation coefficient and shear-wave speed in differentiating the fibrosis stage and the grade of steatosis, respectively. Shear-wave speed and the attenuation coefficient were also important determinants of the extent of liver fibrosis and steatosis, respectively. Because they offer distinct estimates of the three components of the NAFLD Activity Score (the most often used surrogate end point in NASH trials), such as fibrosis, inflammation, and steatosis, which are individually targeted in some experimental monotherapies, these US-based markers may therefore be deemed appropriate for use as surrogate end points in interventional clinical trials. Additionally, even though none of these factors by themselves had adequate power for diagnosing NASH, the combination of these three parameters offered strong diagnostic performance. Numerous investigations carried out in recent times have noted the effectiveness of elastographic techniques in clinical diagnostic imaging [7–9]. These investigations have primarily concentrated on the elastic characteristics of tissues instead of on viscosity. Further mover, the absence of evaluations of tissue viscosity in these researches may have resulted in certain inconsistencies in the assessment of elasticity. In only a few studies, it has the tissue viscosity been evaluated. Deffieux et al. [22] were the first to report on the use of liver viscosity as a marker using an US imaging system (Aixplorer, Supersonic Imagine, Aix-en Provence, France) and Chen et al. (23) reported on the use of shear-wave dispersion US vibrometry technology and US system (iU22, Philips Healthcare, Andover, Mass) to assess liver viscosity as well. These two studies reported that viscosity was not a reliable predictor of both the fibrosis stage, disease activity and even the level of steatosis. On the other hand, our study demonstrated that the dispersion slope, which on the average was also modified by steatosis grade, was a good predictor of inflammation grade. There may be two reasons for this discrepancy. The primary reason is that the individuals involved in the research conducted by Deffieux et al (22) and Chen et al (23) exhibited greater inhomogeneity (i. e. , greater variety in causes) compared to the participants in our study. It is widely recognized that the liver stiffness measurement varies based on the underlying cause (for example, hepatitis C virus infection, hepatitis B virus infection, or NASH) even when the fibrosis stage remains constant (24). Conversely, our study included participants with solely one underlying cause (NAFLD), which might have contributed to the variations in findings. The second reason is that the previous two similar studies

utilized viscoelastic models, like the Voigt model, which is commonly used in MRI elastography to determine viscosity. Rheologic models consist of the Maxwell model and the Zener model. Nevertheless, to our knowledge, there isn't an agreement regarding which model is the most appropriate for assessing liver viscoelasticity. We used the dispersion slope value, a physical quantity that is not reliant on a rheologic model. The reality that this value is independent of the model used may also have contributed to the variations in findings. Our research has several limitations. First, the reliance on liver biopsy as the reference standard for evaluating liver pathologic results presents limitations related to sampling errors (24,25). Nevertheless, a single pathologist assessed all liver specimens. Second, the analysis of US parameters entailed the manual selection of the region of interest, which might lead to intra observer variability. However, a single operator designated all regions of interest as consistently as possible throughout the study. Third, widely accepted confidence criteria for dispersion slope measurements have not yet been defined. We regarded an interquartile range–to-median ratio exceeding 0. 3 as indicative of unreliable measurements, though this standard is founded on the application of vibrationcontrolled transient elastography for shear-wave speed measurement. Fourth, hepatocellular ballooning represents a crucial finding in the pathologic diagnosis of NASH. However, based on our findings, the noninvasive evaluation of the severity of ballooning seemed to be challenging. While shear-wave speed was the only significant covariate affecting ballooning grade, there appeared to be no logical significance. Fifth, the established cut-off values for fibrosis stages above F2 and F3 were identical (1. 40 m/sec), which would impact clinical usage. Furthermore, we did not apply corrections for multiple comparisons as this was an exploratory study. Our findings will necessitate validation in an external cohort to substantiate our results. Finally, this was a preliminary single-center study primarily designed to assess the efficacy of shear-wave dispersion slope for detecting lobular inflammation in only Japanese patients with NAFLD.

Conclusion

In conclusion, the dispersion slope of shear-wave obtained through two-dimensional shear-wave elastography can be utilized to evaluate liver inflammation in individuals with nonalcoholic fatty liver disease (NAFLD). Additionally, the assessed ultrasound parameters may demonstrate their utility for the thorough estimation of steatosis, inflammation, and fibrosis, as well as for the identification of nonalcoholic steatohepatitis in a singular examination. The importance of histological evaluation in liver disease is

indisputable, yet these ultrasound parameters might be capable of noninvasively offering comparable data regarding the condition of the liver parenchyma while circumventing the drawbacks of liver biopsy in NAFLD management. However, additional research featuring prospective, multicenter designs and a greater number of participants is required to confirm our findings, particularly among other racial and ethnic populations.

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