



# Distribution of Normative Percentiles of Liver Stiffness Measurement Using Ultrasound Shear Wave Elastography in an Adult Asian Indian Population

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Indian J Radiol Imaging 2024;34:596–602.

## Abstract

**Objective** The aim of this study was to determine the normative percentiles for liver stiffness measurement (LSM) using shear wave elastography in an adult Asian Indian population as part of the preventive radiology initiative of the Indian Radiological and Imaging Association (IRIA).

**Methods** LSMs were ascertained by two-dimensional (2D) shear wave elastography using the Mindray Resona series of ultrasound machines. The image quality was assessed using the motion stability index (M-STB) and reliability (RLB) map. Ten acquisitions were documented, and an interquartile range-to-median (IQR/M) ratio  $\leq 30\%$  kilopascal (kPa) units was considered a good-quality measurement. A subgroup of the study population without comorbidities was chosen to derive the normative percentile distribution of LSM using a generalized least squares multivariable fractional polynomial regression model that adjusted for sex and body mass index (BMI). The effectiveness of the estimated percentiles was assessed on the entire study population using the greater than 90th percentile value of the LSM as the cutoff for abnormality.

**Results** The study included 852 people who underwent shear wave elastography from June 2022 to July 2023. The magnitude of compensated advanced chronic liver disease (cACLD) and clinically significant portal hypertension (CSPH) was 6.81% (95% confidence interval [CI]: 5.30–8.7) and 4.91% (95% CI: 3.67–6.60), respectively. The normative percentiles were estimated from 282 persons without associated

## Keywords

- ▶ ultrasound elastography
- ▶ shear wave elastography
- ▶ liver stiffness
- ▶ percentiles
- ▶ chronic liver disease

article published online  
April 25, 2024

DOI <https://doi.org/10.1055/s-0044-1782163>  
ISSN 0971-3026.

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comorbidity and risk factors. The mean age (standard deviation [SD]) of the normal individuals was  $40.90 \pm 12.92$  years, and 210 (71.47%) were males. The mean age (SD) of the 570 persons excluded from the normative percentiles analysis was 47.94 (12.49) years and 72.11% were males. The sex- and BMI-adjusted age-specific 90th percentiles of LSM were 8.76, 8.78, 8.96, 8.97, 9.25, and 9.45 kPa for 18 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, and 61 to 70 years, respectively.

**Conclusion** The sex- and BMI-adjusted age-specific 90th percentiles for LSM using shear wave elastography in Asian Indian adults are almost similar to the greater than 9 kPa cutoff proposed by the Society of Radiologists in Ultrasound Liver Elastography Consensus Statement guidelines to discriminate cACLD and CSPH from normal individuals.

## Introduction

More than half of the global population resides in the Asia-Pacific region and accounts for 62.6% of global deaths due to liver diseases, 54.3% of global deaths due to cirrhosis, 72.7% of global deaths due to hepatocellular carcinoma, and over two-thirds of the global burden of acute viral hepatitis.<sup>1</sup> The global prevalence of nonalcoholic fatty liver disease (NAFLD) is reported to vary between 15 and 25% with the prevalence in Asian countries reportedly varying between 15 and 49.8%.<sup>1-6</sup> The prevalence of NAFLD on ultrasonography in urban areas in India increased from 18.9% in 2007 to 32.0% in 2009 and 8.7% in 2010 to 30.7% in 2016 in rural areas.<sup>1</sup> The prevalence of NAFLD on ultrasonography was reported as 16.6% in Mumbai, 32% in Chennai, and 49.8% in Thiruvananthapuram in India.<sup>4,6</sup> The Indian government's initiative to integrate NALFD with noncommunicable and preventive disease strategies reported the magnitude of NAFLD in India to vary between 9 and 32%.<sup>7</sup>

Elastography measures and displays biomechanical properties associated with the elastic restoring forces in the tissue that act against shear deformation. The measurement of tissue stiffness or elasticity is useful as these changes usually precede structural changes and can be used for early diagnosis of disease and progress.<sup>8,9</sup> Shear wave elastography (SWE) generates shear waves using dynamic stress and provides qualitative and quantitative estimates of tissue elasticity. The speed of the generated shear waves inside the tissue is measured using Young's modulus  $E$  in either meter per second (m/s) or kilopascal (kPa) units. The shear wave techniques have less operator dependence and better depth penetration, are less susceptible to decay, and offer a more accessible, available, and affordable alternative to computed tomography (CT) and magnetic resonance imaging (MRI) studies. SWE also provides better-quality images than conventional ultrasound studies.

The Indian Radiological and Imaging Association (IRIA) has initiated a preventive radiology program that aims to utilize imaging-based biomarkers for early identification of chronic diseases including metabolic disorders, to assess endothelial dysfunction, to identify imaging-based criteria for therapeutic interventions, and to monitor the progress of

diseases and impact of interventions on the structural and functional integrity of target end organ systems in the body. The program also aims to estimate appropriate normative percentile distributions for various parameters of interest for an Asian Indian population. Normative age-specific percentiles adjusted for sex and body mass index (BMI) for two-dimensional (2D) ultrasound SWE-derived liver stiffness measures are currently not available in India. In this study, we present the distribution of sex- and BMI-adjusted age-specific percentiles of LSM using SWE estimated in normal adult Asian Indian population as part of a larger study on NAFLD in India by the preventive radiology initiative of IRIA.

## Methods

The study utilized a cross-sectional design and consecutive selection of eligible individuals to recruit the study population from the radiology departments of the three participating institutes. The study included persons that were aged 18 years or older, either self-referred as part of wellness checkup programs or referred from other medical specialties for a liver assessment and provided informed consent for participation in the study. The study excluded persons in whom a reliable elastogram or a good acoustic window was not acquired. The sample size for the study was determined based on an anticipated proportion of 50% for any grade of NAFLD, 95% confidence interval, an absolute precision of 5%, a design effect of 2, and a response rate of 90%. The anticipated proportion was input as 50% as that will provide the maximum sample size for the desired precision and confidence when the prevalence estimates are unknown or have a wide range in the available scientific literature. The sample size for the study was estimated as 800 to 854 people based on these assumptions.

Eligible, enrolled individuals were allocated a unique individual identification number. Clinical and demographic details collected from all enrolled individuals included age, sex, occupation, height and weight, self-reported or medical record-based history of hypertension, diabetes mellitus, ischemic heart disease, metabolic syndrome, thyroid disorders, hepatitis, sleep apnea, daytime drowsiness, and dyslipidemia for both sexes and polycystic ovarian disease and

menopause in females. The BMI of each study individual was estimated and details of blood sugar levels, glycosylated hemoglobin, alanine transaminase (ALT), and aspartate transaminase (AST) were collected from the medical records. Details of personal risk behaviors including alcohol, tobacco, and junk/unhealthy food consumption, exercise, and daily coffee consumption were recorded based on a self-recall history.

The 2D SWE of the liver was performed using the Resona series of MindRay ultrasound machines (M/s Mindray Medical India Pvt Ltd) by three radiologists with a minimum of two decades of experience with imaging assessments of the liver. Study participants were advised to fast for at least 4 hours before the SWE.<sup>10,11</sup> An intercostal approach with the patient in the left lateral position, at 30 degrees, and with the right arm raised above the head to increase the width of the intercostal space was used to survey the liver in B mode.<sup>12</sup> The B mode was optimized to show the liver capsule as a white line without shadowing of the rib or lungs in the liver parenchyma.<sup>12</sup> The probe was positioned searching for the best acoustic window with the transducer held at 90 degrees perpendicular to the liver capsule and the upper edge of the sampling box (region of interest [ROI]) at least 1 to 2 cm below the liver capsule avoiding any large vessels, and small vessels as well whenever possible. An ROI of 2 to 3 cm in size was considered good to qualitatively assess the stiffness of the targeted liver parenchyma area possibly without artifacts.<sup>12</sup> The sound touch elastography (STE) ROI box was placed in the middle center of the B-mode image in a homogeneous area of the liver parenchyma, avoiding ligaments, vessels, or bile ducts. Holding the probe steady, the patient was asked to hold the breath for a few seconds at mid-expiration while placing the targeted segment at the center of the image and the STE was activated on the touchscreen, and both B-mode and SWE displays were observed.<sup>12</sup> Consecutive frames for 3 to 5 seconds were generated and captured as sequential STE images in a cine loop. The best possible frame of STE image was indicated with both the five green stars motion stability index (M-STB) and reliability (RLB) map. A full green color was considered as good shear wave quality and reliable indicators for better reproducibility of the stiffness measurement. The M-STB helps monitor the degree of motion interference and a star number less than 4 indicates the presence of high motion interference and a number greater than 4 indicates minimal or no motion interference.<sup>12</sup> The low and high star numbers are color coded as orange and green, respectively, for easy interpretation. An M-STB index that was green for several consecutive frames was considered a stable SWE.<sup>12</sup> The RLB map is related to the STE shear wave intensity and indicates the areas where a reliable measurement is possible and is colored green. Areas that are associated with unreliable measurements are colored purple. STE measures were taken avoiding purple coded areas and ensuring at least 4 green stars in the 5-star stability index.<sup>12</sup>

The measurement key was used to place the “circle” (15 mm in diameter) within the ROI box in a homogenous color area. Only one measurement for each acquisition either

in meter per second (speed of the shear waves) or kilopascal (stiffness value derived from the speed of the shear wave by using the Young module) was performed for better accuracy. The diameter of the “circle” was reduced to 10 mm in case of artifacts within the ROI. Ten acquisitions were documented to judge the variability between measurements using the interquartile range-to-median (IQR/M) ratio. An IQR/M  $\leq 30\%$  when the median value is given in kilopascal and  $\leq 15\%$  when the median value is given in meter per second was considered as good quality.<sup>13,14</sup> The criteria for elimination of measures included the quality of image, image stability, and reliability indices, extreme values or outliers that were at least 3 SD outside of the mean. A linear high-resolution probe was used to assess the liver surface for irregularities and the presence or absence of micronodules. A micronodule was considered as a nodule less than 2 mm in size and an irregular liver surface was defined as the loss of regular echogenic surface and the presence of wavy margins.

Liver fibrosis was graded based on the system proposed by Barr et al in 2020.<sup>15</sup> A liver stiffness value less than 5 kPa was considered a high probability of being normal. A value of less than 9 kPa was considered as the absence of compensated advanced chronic liver disease (cACLD) in the absence of associated clinical signs. A value of 9–13 kPa was considered suggestive of cACLD that needs further evaluation. A value greater than 13 kPa ruled in cACLD and a value greater than 17 kPa indicated clinically significant portal hypertension (CSPH).

The data were entered into an online Google form and stored in a password-protected Google Drive folder. The data were subsequently exported to the statistical software STATA v 14.0 (StataCorp LLC, College Station, TX, United States) for further checks and analysis. The distribution of categorical data was presented as proportions and continuous data were presented as mean (SD). The percentiles of LSM were estimated on a normal subgroup of the study population without comorbidity. Individuals with a history of hypertension, diabetes mellitus, ischemic heart disease, metabolic syndrome, hepatitis, dyslipidemia, polycystic ovarian disease, prior hepatitis, and history of alcohol consumption were excluded for the estimation of the percentiles. Individuals with abnormal levels of ALT and AST were also excluded. The normality of LSM was assessed using skewness, kurtosis, and the Shapiro–Wilk test. A generalized least squares multivariate fractional polynomial regression model was used to determine the distribution of LSM by age groups and adjusted for sex and BMI. The goodness of fit of the model was assessed using a visual inspection of the percentile charts, Akaike information criteria (AIC), Bayesian information criteria (BIC), and Q-Q plots. The 10th, 25th, 50th, 75th, and 90th percentiles were estimated for each age category. The effectiveness of the estimated percentiles was assessed on the entire study population using a greater than 90th percentile value of the LSM as the cutoff for abnormality. The area under receiver operator characteristic (AUROC) curve, sensitivity, specificity, positive and negative predictive values, diagnostic odds ratios, and likelihood ratios were ascertained to determine the effectiveness of the greater

than 90th percentile value to identify liver fibrosis of any stage (suggestive of cACLD) and advanced liver fibrosis (ruled in cACLD and CSPH).

## Results

The study included 852 people who underwent SWE from June 2022 to July 2023. Most ( $n = 732$ , 85.92%) of the persons who underwent SWE of the liver were referrals from other clinical disciplines for liver assessments. The clinical and demographic distribution of patients in the study is presented in ►Table 1. Most ( $n = 780$ , 91.55%) of the liver assessments showed a smooth liver surface, and nodular or irregular surfaces were present in 36 (4.23%) persons each and micronodules were present in 40 (4.69%) persons. An irregular surface of the liver was not observed in any of the persons with a grade 1 liver; however, it was observed in 0.81% grade 2 livers and 5, 24.14, and 19.05% grade 3, 4, and 5 livers, respectively. A nodular liver surface was present in 10, 10.34, and 23.81% grade 3, 4, and 5 livers, and in none of the grade 1 and 2 livers. Micronodules were present in 1.08% of grade 1 and 2 livers and 4, 24.14, and 28.57% of grade 3, 4, and 5 livers, respectively.

Based on the LSM-derived grading of the liver, 58 (23.47%, 95% CI: 5.30, 8.70) persons had cACLD, 42 (4.91%, 5% CI: 3.67, 6.60) had CSPH, and LSM was considered suggestive of cACLD, but further confirmatory tests were required in 200 (23.47%, 95% CI: 20.75, 26.44) persons (►Table 2). The pairwise correlation of the LSM with grades of liver fibrosis was 0.70.

Five hundred and seventy people with hypertension ( $n = 194$ ), diabetes mellitus ( $n = 274$ ), ischemic heart disease ( $n = 42$ ), abnormal lipid profiles ( $n = 182$ ), metabolic syndrome ( $n = 28$ ), prior hepatitis ( $n = 132$ ), polycystic ovarian disease ( $n = 70$ ), history of alcohol consumption ( $n = 354$ ), abnormal AST ( $n = 244$ ), abnormal ALT ( $n = 234$ ), irregular or nodular liver surface ( $n = 36$ ), and micronodules ( $n = 40$ ) were excluded from the analysis to derive the normative percentiles. The mean age (SD) of the normal individuals was

**Table 1** Clinical and demographic details of the study population ( $n = 852$ )

Parameter	Value
Mean age (SD), range	46.36 (13.04), 19–78 y
Males	612 (71.83%)
Females	240 (28.17%)
Body mass index >30	244 (28.64%)
Hypertension	194 (22.77%)
Diabetes mellitus	274 (32.16%)
Ischemic heart disease	42 (4.93%)
Abnormal lipid profile	182 (51.12%)
Irregular liver surface	36 (4.23%)
Nodular liver surface	36 (4.23%)
Micronodules	40 (4.69%)

**Table 2** Distribution of the grades of the liver fibrosis based on liver stiffness measurements in shear wave elastography in 852 subjects

Parameter	N (%) 95% CI	Interpretation
< 5 kPa	56 (6.57%) 5.10, 8.44	High probability of being normal
< 9 kPa	496 (58.22%) 54.87, 61.48	In the absence of other clinical signs, rules out cACLD. Needs further tests based on clinical signs
9–13 kPa	200 (23.47%) 20.75, 26.44	Suggestive of cACLD but needs further evaluation
> 13 kPa	58 (6.81%) 5.30, 8.7	Rules in cACLD
> 17 kPa	42 (4.91%) 3.67, 6.60	Suggestive of CSPH

Abbreviations: cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension.

40.90 (12.92) years and 210 (71.47%) were males. The mean age (SD) of the 570 persons excluded from the normative analysis was  $47.94 \pm 12.49$  years and 72.11% were males. The median LSM was 7.09 (IQR: 6.31–8.08) kPa in the normal individuals and 8.29 (IQR: 6.67–10.11) kPa in individuals with comorbidity and risk factors. The 10th, 25th, 50th, 75th, and 90th percentiles of LSM by age groups were estimated using a generalized least squares multivariable fractional polynomial regression model, adjusted for sex and BMI, on 282 persons with grade 1 or 2 liver (►Table 3 and ►Fig. 1). AST and ALT showed suboptimal effectiveness compared with the greater than 90th percentile LSM category to identify liver changes suggestive of cACLD and ruled in cACLD (►Table 4) and CSPH (►Table 5).

## Discussion

It is important to establish a set of normative values for LSM and derive cutoff values that discriminate between normal and abnormal in different populations for clinical application and to monitor the progress of the disease. The normative age-specific 90th percentile values of LSM derived in the study population are almost similar to the greater than 9 kPa cutoff used to discriminate between the presence and absence of cACLD and CSPH in the grading system proposed by Barr et al,<sup>15</sup> indicating that the grading system can be applied for clinical use in the Indian population. Understanding the distribution of normative percentiles is important from a preventive radiology perspective. The 90th percentile provides a cutoff to discriminate between normal and abnormal and to initiate focused therapeutic options. The 50th to 90th percentiles provide a window of opportunity to initiate early or preventative lifestyle modifications and early initiation of more optimal management of risk factors that are present. The 50th to 90th percentiles can also be used to monitor

**Table 3** Distribution of liver stiffness value percentiles in the normal study population ( $n = 282$ )

Age group (y)	<i>n</i>	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile
18–20	8	4.94	5.89	6.88	7.87	8.76
21–30	44	4.97	5.89	6.89	7.88	8.78
31–40	94	4.98	5.92	6.97	8.02	8.96
41–50	64	4.98	5.93	6.98	8.03	8.97
51–60	52	4.99	5.98	7.11	8.24	9.25
61–70	20	5.00	6.01	7.19	8.38	9.45

Note: Percentile values presented as kPa units were estimated using a generalized least squares multivariable fractional polynomial regression model adjusted for sex and body mass index.

**Fig. 1** Sex and body mass index (BMI) adjusted age-specific liver stiff measurement percentiles in an adult Asian Indian population.**Table 4** Effectiveness of estimated 90th percentile liver stiffness measurement with shear wave elastography using the Resona series, AST and ALT with liver changes suggestive of compensated advanced chronic liver disease (cACLD)

Parameter	90th percentile cutoff LSM value, % (95% CI)	AST value, % (95% CI)	ALT value, % (95% CI)
Sensitivity	90% (85, 93.8%)	43.9% (36.2, 51.9%)	36.6% (29.2, 44.5%)
Specificity	96.4% (94.5, 97.8%)	70.5% (66, 74.7%)	68.2% (63.6, 72.5%)
Positive predictive value	90% (85, 93.8%)	35.6% (29, 42.7%)	30% (23.7, 36.9%)
Negative predictive value	96.4% (94.5, 97.8%)	77.1% (72.7, 81.1%)	74.3% (69.7, 78.5%)
Positive likelihood ratio	24.8 (16.1, 38.3)	1.49 (1.19, 1.86)	1.15 (0.90, 1.47)
Negative likelihood ratio	0.10 (0.07, 0.16)	0.79 (0.68, 0.92)	0.93 (0.81, 1.06)
AUROC	0.93 (0.91, 0.95)	0.57 (0.52, 0.61)	0.52 (0.48, 0.56)
Diagnostic odds ratio	239 (126, 454)	1.87 (1.29, 2.7)	1.24 (0.85, 1.8)

Abbreviations: AUROC, area under receiver operator characteristic curve; CI, confidence interval.

trends of radiology biomarkers of metabolic dysfunction and target organ damage like LSM to stratify risk and adjust risk factor management before significant fibrosis sets in.

Histopathological examination of liver biopsy specimens is the diagnostic gold standard for chronic liver diseases but is not a viable pragmatic clinical option especially in early stages of the disease. Liver biopsy is a painful, invasive

procedure, and histopathology of the liver could not be done in this study due to ethical concerns.

Noninvasive assessments for chronic liver disease include imaging modalities like ultrasound, CT, and MRI.<sup>16</sup> B-mode ultrasound is widely available, affordable, and accessible, and is used as a noninvasive portable test with minimal radiation; however, intracellular fat deposits in the hepatocytes



**Table 5** Effectiveness of estimated 90th percentile liver stiffness measurement with shear wave elastography using the Resona series, AST and ALT, with liver changes ruling in clinically significant portal hypertension

Parameter	90th percentile cutoff LSM value, % (95% CI)	AST value, % (95% CI)	ALT value, % (95% CI)
Sensitivity	90% (85–93.8%)	51.2% (39.9–62.4%)	46.3% (35.3–57.7%)
Specificity	96.4% (94.5–97.8%)	70.5% (66–74.7%)	68.2% (63.6–72.5%)
Positive predictive value	90% (85–93.8%)	24.4% (18.2–31.5%)	21.3% (15.6–28.1%)
Negative predictive value	96.4% (94.5–97.8%)	88.6% (84.8–91.7%)	87.2% (83.2–90.6%)
Positive likelihood ratio	24.8 (16.1, 38.3)	1.73 (1.34, 2.24)	1.46 (1.11, 1.91)
Negative likelihood ratio	0.10 (0.07, 0.16)	0.69 (0.55, 0.87)	0.78, (0.63, 0.97)
AUROC	0.93 (0.91, 0.95)	0.60 (0.55, 0.66)	0.57 (0.51, 0.63)
Diagnostic odds ratio	239 (126, 454)	2.5 (1.55, 4.03)	1.85 (1.15, 2.98)

Abbreviations: AUROC, area under receiver operator characteristic curve; CI, confidence interval; LSM, liver stiffness measurement.

may reduce visibility of deeper structures like the portal and hepatic veins and diaphragm. Intra- and inter-operator variability and difficulty in assessing the liver in obese patients are additional limitations of routine ultrasound studies.<sup>17–19</sup> SWE offers an affordable alternative as a first-line imaging modality to screen for chronic liver diseases including NAFLD. An affordable, accessible, and easily available painless noninvasive screening strategy is important when we consider the high magnitude of NAFLD in India.

Graded deposition of extracellular matrix in progressive chronic liver disease makes the liver stiffer and can be impacted by several factors including age, sex, and BMI. The association of LSM with BMI suggests that body composition is an important determinant of the viscoelastic property of the liver.<sup>20,21</sup> Significant liver disease occurs in developing countries at a lower BMI even in the presence of undernutrition.<sup>22</sup> Asians, particularly Indians, develop metabolic syndrome and significant liver disease at BMIs that are lower than those of Caucasians/Europeans.<sup>23,24</sup> The percentiles reported in this study are adjusted for the effects of sex and BMI.

A previous study from India reported the lack of correlation between LSM and biochemical parameters, including fasting blood glucose, fasting serum insulin level, homeostasis model assessment of insulin resistance (HOMA-IR), triglycerides, high-density lipoproteins (HDL), ALT, and AST.<sup>25</sup> An animal model of liver fibrosis has shown that an increase in liver stiffness preceded the activation of hepatic stellate cells and deposition of fibrous material and may probably precede biochemical changes. Both AST and ALT had poor diagnostic and discriminatory ability for cACLD and CSPH in this study. The use of LSM might provide a pathway for early diagnosis and lifestyle and risk mitigation interventions.

The inability to perform liver biopsy studies, due to ethical concerns, to confirm the histopathological status of the liver is a weakness of our study. The selection of individuals through an opportunistic screening process is a limitation that can introduce a selection bias that can underrepresent certain demographics. We used a sex- and BMI-adjusted model to derive age-specific percentiles to minimize the impact of any potential selection bias. In the next phase, we

aim to study the comparative effectiveness of SWE with magnetic resonance elastography. All SWE measurements were performed by three senior, experienced radiologists who are designated trainers of trainers in the preventive radiology initiative of the IRIA. The sex- and BMI-adjusted model and age-specific percentiles are another strength of our study.

In conclusion, the results of the study provide the distribution of sex- and BMI-adjusted age-specific percentiles for LSM in an adult Asian Indian population.

#### Note

This work is attributed to Preventive Radiology Program of IRIA, Indian Radiological & Imaging Association, IRIA House, New Delhi, India.

#### Funding

The work was supported in part by a research grant provided by M/s Mindray Medical India Pvt Ltd to the Indian Radiological and Imaging Association. The grant agency did not have any inputs into data collection, analysis, or preparation of the manuscript.

#### Conflict of Interest

None declared.

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