"Normal" Liver Stiffness Measure (LSM) Values Are Higher in Both Lean and Obese Individuals: A Population-Based Study From a Developing Country

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The liver stiffness measure (LSM) needs to be explored in ethnically and anthropometrically diverse healthy subjects (to derive an acceptable normal range) and also in patients with liver disease. In view of this objective, LSM was performed by transient elastography (TE) using FibroScan in 437 healthy subjects with normal alanine aminotransferase (ALT) levels, recruited from a free-living population of the Birbhum Population Project (BIRPOP; www.shds.in), a Health and Demographic Surveillance System (HDSS), and from 274 patients with liver disease attending the Hepatology Clinic of the School of Digestive and Liver Diseases (SDLD; Institute of Post Graduate Medical Education & Research [IPGME&R], Kolkata, India) including 188 with nonalcoholic fatty liver disease (NAFLD) and 86 with chronic hepatitis of viral and other etiologies. Liver biopsy was performed in 125 patients. The range of normal values for LSM, defined by 5th and 95th percentile values in healthy subjects, was 3.2 and 8.5 kPa, respectively. Healthy subjects with a lower body mass index (BMI; < <18.5 kg/m^2) had a higher LSM compared with subjects who had a normal BMI; this LSM value was comparable to that of obese subjects (6.05 ± 1.78 versus 5.51 ± 1.59 and 6.60 ± 1.21, $P = 0.016$ and 0.349, respectively). Liver disease patients without histologic fibrosis had significantly higher LSM values compared with healthy subjects (7.52 ± 5.49 versus 5.63 ± 1.64, $P < 0.001$). Among the histologic variables, stage of fibrosis was the only predictor for LSM. LSM did not correlate with inflammatory activity and ALT in both NAFLD and chronic hepatitis groups. *Conclusion:* LSM varies between 3.2 and 8.5 kPa in healthy subjects of South Asian origin. Both lean and obese healthy subjects have higher LSM values compared with subjects with normal BMI. Liver stiffness begins to increase even before fibrosis appears in patients with liver disease. (HEPATOLOGY 2012;55:584-593)

The liver stiffness measurement (LSM), which is performed using transient elastography (TE), is an increasingly popular, noninvasive method for assessment of hepatic fibrosis.[1] Precise estimation of the degree of liver fibrosis provides useful information in prognostication, therapeutic planning, and assessment of the impact of treatment in chronic liver diseases.[2] Significant liver fibrosis can exist in otherwise asymptomatic individuals, often without evident abnormal biochemical liver function tests.[3] TE has shown excellent correlation with histological fibrosis, especially advanced fibrosis (METAVIR F3/F4), across

Abbreviations: AILD, autoimmune liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under receiver operating characteristics; BMI, body mass index; CCl_4, carbon tetrachloride; FBG, fasting blood glucose; HAI, histological activity index; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; HS, healthy subject; IDF, International Diabetes Federation; IQR, interquartile range; LB, liver biopsy; LD, liver disease patients; LSM, liver stiffness measure; MS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; ROC, receiver operating characteristics; TE, transient elastography; ULN, upper limit of normal.

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Received April 23, 2011; accepted September 14, 2011.

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View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24694

Potential conflict of interest: Nothing to report.

Additional Supporting Information may be found in the online version of this article.

different etiologies of chronic liver diseases, and can be potentially useful in screening for chronic liver disease in the community.[4-6]

Technical performance of the procedure improves with increasing operator experience. In addition, use of the strength of concordance of TE with other noninvasive modalities like FibroTest can identify predictors of variability as well as limitations and thereby can improve the utility of the technique.[7,8]

TE provides quantification of the complex biological phenomenon of liver stiffness. Establishment of a set of normative values and setting a cutoff for "normalcy" in different populations are important for its clinical application. Although the exact biophysical bases of liver stiffness are not yet clear, graded deposition of extracellular matrix in progressive chronic liver disease makes the liver stiffer, and TE measures this reproducibly in a dynamic frame.[9] However, the relationship of stiffness to the body mass index (BMI) suggests that body composition is an important determinant of the viscoelastic property of the liver.[10,11]

Most of the validation and normative studies on TE have been carried out in developed countries.[6,8,10] We have earlier shown that, in contrast to the developed countries, significant liver disease can exist in developing countries at lower BMI, often in the presence of undernutrition.[12] Asians, particularly Indians, develop metabolic syndrome and significant liver disease at BMIs that are lower than those of Caucasians/Europeans.[12-14] Moreover, in such developing countries, chronic viral infections also occur mainly in poor agricultural workers, who have different anthropometric correlates compared with those of the developed nations.[15,16] Because TE is being increasingly used in the South Asian and Far Eastern population, in view of the high burden of liver disease here, it is important that normative values be determined in such populations across the different BMI ranges.

We report here a population-based study for determination of "normal" LSM values in healthy individuals of a community cohort in India and compare the values with those of a clinically asymptomatic liver disease cohort to delineate the cutoffs in a South Asian population.

Patients and Methods

Study Design and Subjects. The present study involved two sets of subjects: a healthy subjects (HS) cohort selected from a systematically maintained population laboratory and an asymptomatic liver disease cohort (LD) attending our Institute for evaluation. Informed consent was obtained before participation, and the study was approved by the institutional ethics committee.

Healthy Subjects. Healthy subjects were recruited from the Birbhum Population Project (BIRPOP; www.shds.in), a Health and Demographic Surveillance System (HDSS) organized and maintained since August, 2008. Included in the project were 59,395 free-living individuals residing in 13,053 households in 333 villages of four community development blocks (the apex of the local self-governance system in India) in Birbhum district, West Bengal, India. The operational unit of the HDSS is a cluster, and there are 40 such geographical clusters in BIRPOP, each comprising approximately 300 households. In this longitudinal population cohort, baseline and periodic demographic as well as health information is collected by trained surveyors. The initial census and generation of the baseline data of BIRPOP was complete by July, 2009. We initiated the healthy subject recruitment for this study in August, 2009. Using the BIRPOP database, we randomly selected 15 individuals from each cluster (by computer-generated random numbers), from those older than 18 years, making a total of 600 individuals.

After informed consent, these 600 individuals were subjected to rigorous screening to assess eligibility for the study based on the following inclusion and exclusion criteria (Fig. 1):

Inclusion criteria: (1) age >18 years, and (2) willingness to comply with study protocol.

Exclusion criteria: (1) any degree of fatty liver on transabdominal ultrasonography (US)[17]; (2) alanine aminotransferase (ALT) >40 IU/L, regardless of sex; (3) evidence of metabolic syndrome (MS) as defined by the criteria proposed by the International Diabetes Federation (IDF)[18]; (4) any amount of alcohol use; (5) positivity for HBsAg/IgG anti-HBc/anti-HCV/anti-HIV antibodies; (6) clinical evidence of heart disease; and (7) any illness/hospitalization within the past 6 months. A history of alcohol intake was rigorously sought, as described.[12] Whereas the clinical evaluation, history, and anthropometry were done by physicians (S.M., K.C., and A.C.) in the field, the subjects were brought to the Institute in batches for subsequent investigations (fasting blood glucose [FBG], fasting serum insulin, triglyceride and high-density lipoprotein [HDL] levels, liver function tests, serum urea and creatinine, viral serology, US, and TE). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated.[19] The study was carried out between August, 2009 and September, 2010. Out of 600 initially screened, 437 were enrolled as HS.

Liver Disease Subjects. The LD subjects were recruited from patients presenting to the Hepatology Clinic of the School of Digestive and Liver Diseases

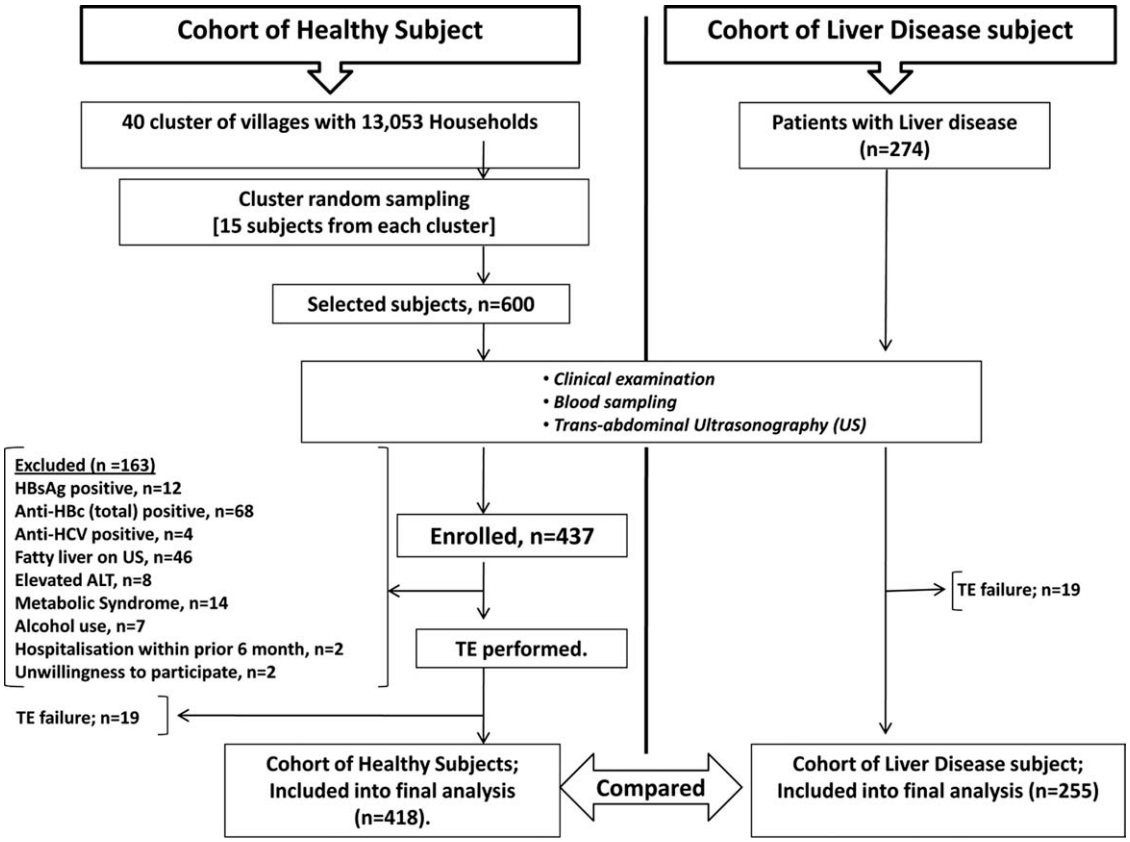

Fig. 1. Method of selection of healthy subjects. Population densities in 40 clusters were comparable. TE, transient Elastography; US, ultrasonography.

(SDLD), Institute of Post Graduate Medical Education & Research (IPGME&R), Kolkata, India, with asymptomatic transaminitis and/or incidental detection of positive viral serology or incidental detection of fatty liver on US. The primary purpose of the LD group was to provide a comparison between the group with possibly no liver disease and therefore healthy livers versus a possibly less advanced liver disease group, based on clinical and laboratory evaluation.

Their inclusion and exclusion criteria were:

Inclusion criteria: (2) age >18 years; (2) no previous treatment for any liver disease; (3) first specialty consultation for possible liver disease; and (4) willingness to comply with the study protocol.

Exclusion criteria: (1) present/past symptoms of jaundice/ascites/gastrointestinal bleeding/unexplained fatigue/weight loss/pedal edema/pruritus, and so forth; and (2) presence of clinical (splenomegaly), endoscopic (varices) and/or US evidence of portal hypertension.[20]

All the LD subjects were interviewed and their previous medical records checked. After anthropometric and blood pressure measurements, detailed investigations, as described in for the HS group, were done.

NAFLD and MS were diagnosed based on the criteria proposed by the Asia-Pacific Working Party on NAFLD and the IDF, respectively.[18,21] Fatty liver on US was defined by the presence of increased echogenicity of the liver along with the presence of any two of three features (liver-kidney contrast, vascular blurring, and deep-attenuation of echo-beam).[17,18] Chronic hepatitis B/C and autoimmune liver disease (AILD) were diagnosed based on the standard criteria.[22-24] None had alcoholic liver disease. Cryptogenic liver disease was diagnosed by exclusion of viral, alcoholic, Wilson's disease, and autoimmune etiologies. BMI categories proposed by the World Health Organization were used.[13]

LSM and Liver Histology. TE was done with FibroScan (Echosens, Paris) in compliance with the technical recommendations.[1] A reliable result was defined as at least 10 valid shots, a success rate of at least 60%, and interquartile range <30% of the median LSM value. Results were considered unreliable if these criteria were not met. Failure of the procedure was defined as no valid shot after at least 10 attempts.[1,7]

Liver biopsy was performed using the method described by Menghini and read by a single

pathologist (A.R.M.) blinded to the clinical, laboratory, and TE data.[25] Histology of NAFLD and non-NAFLD etiologies of chronic hepatitis (CH) was described by using the scoring systems proposed by Kleiner et al. and METAVIR, respectively.[4,26]

Considering the clinical perspective, stages of fibrosis were grouped as minimal/no fibrosis (stages 0/1A/1B/1C for NAFLD and METAVIR F0/F1 for CH), moderate fibrosis (stage 2 for NAFLD and METAVIR F2 for CH), and advanced fibrosis (stages 3/4 for NAFLD and METAVIR F3/F4 for CH). LB and TE were done on the same day in subjects who provided consent.

Statistical Analysis. Mean ± SD, median, range, 5th and 95th[h] percentiles, and absolute number with percentages were calculated wherever applicable. We defined the "normal" LSM values as those between the 5th and 95th[h] percentiles and upper limit of normal (ULN) in HS, as the 95th percentile value.[27] Chi-square or Fisher's exact test and Student *t* test were used appropriately to compare variables. Correlations between LSM and continuous variables were assessed by Pearson's test.

The receiver-operating-characteristics (ROC) curves were plotted, and areas under the curves (AUROC) with 95% CI were calculated to explore the diagnostic efficacy of the calculated ULN of LSM in HS, to differentiate between those with no fibrosis versus any fibrosis and also between those with minimal/no fibrosis versus significant fibrosis (stage $\geq$2) in the cohort of subjects with LD. Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy were also calculated. Diagnostic accuracy was defined as percentage of true observations (sum of true positive and true negative) among total number of patients.

Multivariate analysis (multiple linear regressions) was done to determine the histological predictors of LSM. Histological fibrosis stage, steatosis score, lobular inflammation, and hepatocyte ballooning in case of NAFLD and METAVIR fibrosis stage and histological activity index (HAI) in case of CH were used as independent variables for regression analysis. Coefficient of regression (δ) and squared partial correlation coefficient (r^2) were computed. An α level of <0.05 was adopted for statistical significance. All statistical analyses were performed by using SPSS version 13 for Windows (SPSS, Chicago, IL).

Results

Subjects

Healthy Subjects. Our inclusion and exclusion criteria were satisfied by 437 individuals (64% men) (Table 1, Fig. 1). After excluding 19 because of technical failure, 418 individuals were used for the final analysis. Their mean (±SD) ages and BMIs were 37 (±12) years and 21.20 (±3.53) kg/m^2, respectively; 21% had a BMI $<$ 18.5 kg/m^2, 65% had normal a BMI (18.5-24.9 kg/m^2), 13% were overweight (BMI 25.0-29.9 kg/m^2), and only 7 (1%) were obese (BMI $>$ 30 kg/m^2). Abdominal obesity was present in 18%. Mean (±SD) ALT was 26.23 (±7.79) IU/L. (Table 2)

HS who were excluded (n = 163; Fig. 1) were comparable to study subjects in terms of mean age (36 versus 37 years, P = NS), sex distribution (men 65% versus 64%, P = NS), and mean BMI (20.7 versus 21.2 kg/m^2, P = NS), but those with failed TE (n = 19) included more women (74% versus 34%, P = 0.003), higher BMI (25.7 ± 3.83 versus 20.9 ± 3.37 kg/ m^2, P = 0.001), and waist circumference (88.2 ± 9.6 versus 76.7 ± 9.8 cm, P = 0.001) with similar age (39.2 ± 9.3 versus 37.1 ± 12 years, P = 0.068) and ALT (28.6 ± 6.5 versus 26.2 ± 7.8 IU/L, P = 0.21) in comparison with those included.

Liver Disease Patients. LD of different etiology was seen in 274 individuals (NAFLD 188 [68.6%], chronic HBV 54 [19.7%], chronic HCV 9 [3.3%], autoimmune hepatitis 12 [4.4%] and cryptogenic disease 11 [4.0%]). After excluding 19 because of technical failure, 255 individuals were used for the final analysis. NAFLD subjects had a significantly higher mean (±SD) BMI (25.66±3.71 kg/m^2), a higher prevalence of abdominal obesity (66.5% versus 16.9%), a higher prevalence of MS (44.1% versus 1.4%), and higher HOMA-IR values compared with those with CH. Liver biopsies were performed in 125 patients (45.6%; 22.9% of NAFLD versus 95.3% of the CH group). Mean ± SD length of the liver tissue obtained was 2.73 ± 0.35 cm. The median number of portal tracts per fragment of tissue was 9 (range 8-12).

Patients with failed TE had mostly NAFLD (n = 18) and included more women (79% versus 59%, P = 0.001), higher BMI (29 versus 24 kg/ m^2, P = 0.001), and higher prevalence of abdominal obesity (95% versus 47%, P = 0.001) with similar age (44 ± 10 versus 42 ± 41 years, P = NS) and ALT (58.4 versus 54.7 IU/L, P = NS) in comparison with those with valid LSM.

The distribution of patients who underwent liver biopsy (n = 125), in different stages of fibrosis, stratified by the etiology of the liver disease is presented in Table 5.

Performance Characteristics of TE

A total of 711 TE studies were done with a median success rate of 100% (range 70%-100%). Overall, median IQR was 0.5 (range 0.0-6.1). There were 38 failures and no unreliable results. The failures were due to the presence of thick subcutaneous fat (n = 29 [76%]) and inadequate intercostal space (n = 9 [24%]).

Table 1. Baseline Characteristics of the Study Population*

Parameter	Healthy Subjects[a] (n = 437)	NAFLD[b] (n = 188)	CH[c] (n = 86)	*P*
Age, years	37 ± 12	46 ± 47	35 ± 12	a vs b 0.021 b vs c 0.004 a vs c 0.063
Men, n (%)	281 (64)	110 (58.51)	59 (68.60)	a vs b 0.171 a vs c 0.445 b vs c 0.111
BMI (kg/m^2)	21.20 ± 3.53	25.66 ± 3.71	21.15 ± 3.81	a vs b < 0.001 b vs c < 0.001 a vs c 0.986
Prevalence of abdominal obesity, n (%)	78 (18.0)	125 (66.5)	12 (16.9)	a vs b < 0.001 a vs c 0.467 b vs c < 0.001
Systolic blood pressure, mm Hg	119 ± 6	131 ± 13	124 ± 10	
Diastolic blood pressure, mm Hg	70 ± 7	79 ± 12	71 ± 10	
ALT, IU/L	26.23 ± 7.79	59.91 ± 44.69	58.57 ± 40.98	a vs b < 0.001 a vs c < 0.001 b vs c 0.888
FBG, mg/dL	81.71 ± 7.81	98.21 ± 30.87	83.82 ± 13.98	
TG, mg/dL	118.77 ± 19.58	174.83 ± 80.98	110 ± 30.19	
HDL, mg/dL	47.87 ± 5.8	42.67 ± 9.25	42.50 ± 13.82	
HOMA-IR	1.15 ± 0.84	1.31 ± 1.1	1.19 ± 1.7	a vs b 0.245 a vs c 0.333 b vs c 0.244
Prevalence of metabolic syndrome, n (%)	0 (0)	83 (44.15)	1 (1.41)	
LSM	5.4 (2.2-10.4)	5.9 (2.2-73.5)	7.70 (3.50-73.50)	
IQR	0.5 (0.1-1.8)	0.5 (0.0-6.1)	0.5 (0.0-6.0)	
Liver biopsy performed, n (%)	0 (0)	43 (22.87)	82 (95.34)	
Failure rate for TE, n (%)	19 (4.35)	18 (4.12)	1(1.16)	

Abbreviations: BMI, body mass index; CH, chronic hepatitis; FBG, fasting blood glucose; HDL, high-density lipoproteins; HOMA-IR, homeostasis model assessment-insulin resistance; IQR, Interquartile range; LSM, liver stiffness measure; NAFLD, nonalcoholic fatty liver disease; TE, transient elastography.

$P < 0.05$ is taken as significant.

*Continuous variables are presented as mean ± SD except for LSM and IQR, which are presented as median (range). *P* values were obtained by performing Student *t* test and Chi-square test in continuous and categorical variables respectively.

In HS, failures increased from 1% (n = 1/91) in those with BMI < 18.5 kg/m^2, 1.8% (n = 5/283) in those with BMI of 18.5-24.9 kg/m^2 to 20.6% (n = 13/63) in those with BMI > 25.0 kg/m^2. Similarly, in LD subjects, the failures were significantly higher in those with NAFLD versus those with CH (4.1% versus 1.2%, respectively; $P = 0.01$).

LSM did not correlate with ALT in either of the liver disease groups (correlation coefficient and *P* values were 0.044 and 0.701 versus 0.148 and 0.063 for CH and NAFLD, respectively)

LSM in HS

The mean (SD), median (range), and 5th and 95th percentile values of LSM values in this cohort of 418 subjects were 5.63 (1.64), 5.4 (2.2-10.4), 3.2, and 8.5 kPa, respectively. The normal range of LSM in our population was 3.2-8.5 kPa. Men had a higher mean LSM value than women (mean ± SD: 5.74 ± 1.65 kPa versus 5.41 ± 1.63 kPa, respectively; $P = 0.04$). LSM did not correlate with age (r = −0.038; $P = 0.44$) but did so with BMI (r = 0.150; $P < 0.001$).

BMI had a significant influence on LSM values in these healthy individuals, with significantly higher values toward the extremes of BMI categories, giving a U-shaped distribution (Fig. 2). The mean ± SD of LSM of the underweight (6.05 ± 1.78 kPa) and obese (6.60 ± 1.21 kPa) subjects were significantly higher compared with those who had normal (5.51 ± 1.59 kPa) BMI ($P = 0.02$ and $P = 0.01$, respectively).

There was no correlation between LSM and biochemical parameters, including markers of metabolic syndrome (FBG, fasting serum insulin level, HOMA-IR, triglycerides, high-density lipoproteins (HDL), ALT, and AST) in these subjects.

LSM and Histology in LD

The medians (ranges) of LSM in subjects with NAFLD and CH were 5.9 (2.2-73.5) kPa and 7.70 (3.50-73.50) kPa, respectively. LSM values increased in a graded fashion with increasing stages of fibrosis in both NAFLD (n = 43) and CH (n = 82) patients (Fig. 3). Thus, the mean ± SD LSM values for those with minimal/no fibrosis versus moderate versus advanced fibrosis were: 7.79 ± 5.37, 11.73 ± 5.17,

Table 2. Comparison Among Different BMI Categories in Healthy Subjects*

Parameter	WHO BMI categories (kg/m^2) Underweight[a] (BMI < 18.5) (n = 90)	Normal weight[b] (BMI 18.5-24.9) (n = 278)	Preobese[c] (BMI 25-29.9) (n = 46)	Obese class I[d] (BMI 30-34.9) (n = 4)	*P*
Age, years	35 ± 12	37 ± 12	39 ± 12	44 ± 17	a vs b 0.164 a vs d 0.077 b vs d 0.257 d vs c 0.692
Men, n (%)	63 (70)	182 (65.47)	26 (56.52)	2 (50.00)	a vs b 0.538 a vs d 0.154 b vs d 0.210 d vs c 0.708
Prevalence of abdominal obesity, n (%)	1 (1.11)	31 (11.15)	27 (58.70)	4 (100)	a vs b 0.002 a vs d < 0.001 b vs d < 0.001 d vs c 0.178
ALT, IU/L	25.05 ± 7.76	26.39 ± 7.81	27.33 ± 7.50	26.86 ± 9.39	a vs b 0.127 a vs d 0.557 b vs d 0.909 d vs c 0.103
FBG, mg/dL	81.69 ± 7.80	81.65 ± 7.80	81.42 ± 8.19	86.71 ± 4.53	a vs b 0.745 a vs d 0.054 b vs d 0.041 d vs c 0.991
LSM, kPa	6.05 ± 1.78	5.51 ± 1.59	5.43 ± 1.59	6.60 ± 1.21	a vs b 0.016 a vs d 0.349 b vs d 0.014 d vs c 0.098

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LSM, liver stiffness measure; WHO, World Health Organization.

*Continuous variables are presented as mean ± SD. *P* values are derived from Student *t* test and Chi-square test in continuous and categorical variables respectively.

and 24.88 ± 13.0 kPa for NAFLD and 7.25 ± 2.78, 13.20 ± 4.64, and 36.07 ± 20.82 kPa for those with CH, respectively. Absolute numbers of patients and mean ± SD values of LSM and inflammatory activity in individual stages of fibrosis stratified by the etiology of liver disease are presented in Table 5.

In NAFLD subjects, median (range) value of steatosis was 30% (0%-90%). Steatosis involving more than 30% of the hepatocytes was found in 55.8% (n = 24). No patient had either biochemical or histological features of cholestasis.

Although an overall increasing trend of LSM was observed toward the higher stages of fibrosis, stage 1A/F1 had LSM comparable to the stage of no fibrosis ($P = 0.687$ and 0.138 for NAFLD and CH, respectively) despite having significantly higher inflammatory activity ($P = 0.033$ and 0.002 for NAFLD and CH, respectively) (Table 5).

Inflammatory activity did not correlate with LSM in either NAFLD (correlation coefficients and *P* values were 0.054 and 0.739, respectively) or CH group (correlation coefficients and *P* values were 0.319 and 0.12, respectively).

Multivariate analysis showed that neither inflammatory activity nor degree of steatosis, but stage of fibrosis was the only independent variable predicting LSM (data not shown).

Comparison Between HS and Patients With No Fibrosis

Among patients with liver disease who underwent liver biopsy, 57 had no fibrosis (METAVIR F0/NAFLD stage 0; Table 3). Patients in this subgroup were comparable to HS in terms of sex distribution, BMI, and prevalence of abdominal obesity.

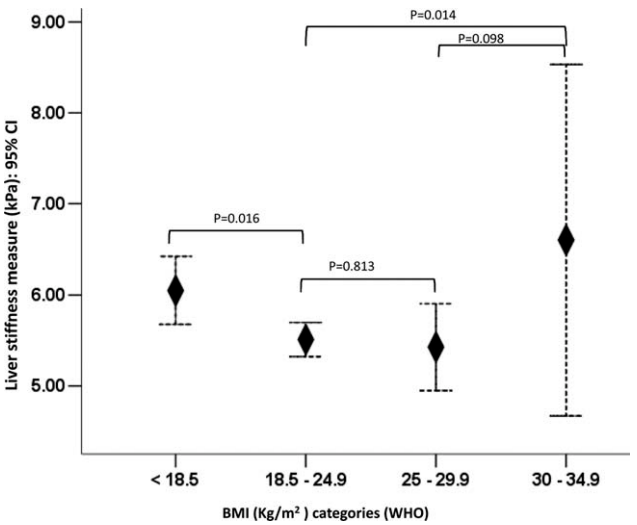

Fig. 2. Distribution of LSM (mean with 95% CI) in different categories of BMI of healthy subjects. BMI, body mass index; WHO, World Health Organization.

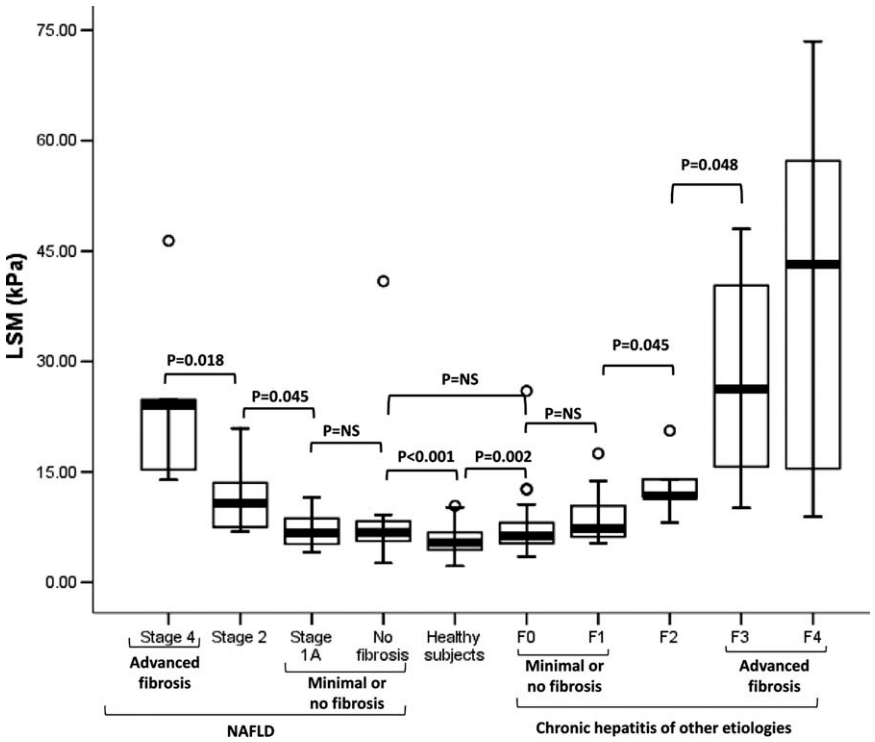

Fig. 3. Box-plot showing LSM at different stages of fibrosis and in healthy subjects. NS, not significant; NAFLD, nonalcoholic fatty liver disease; LSM, liver stiffness measure.

These subjects had a significantly higher mean ± SD LSM values than HS (7.52 ± 5.49 kPa versus 5.63 ± 1.64 kPa, $P < 0.001$; Fig. 3). Although they had a higher mean ALT than HS, they still had significantly higher LSM values than HS regardless of ALT (7.12 ± 2.58 kPa in those with no fibrosis and normal ALT versus 5.63 ± 1.64 kPa in HS; $P = 0.01$). They also had a higher LSM regardless of etiology (7.15 ± 3.70 kPa in METAVIR F0 and 8.37 ± 8.34 kPa in NAFLD stage 0 versus 5.63 ± 1.64 kPa in HS; $P = 0.002$ and $P < 0.001$, respectively).

ROC Curve for LSM (Tables 4 and 5, Supporting Fig. 1

Using our derived ULN of LSM, i.e., 8.5 kPa, the AUROC of this value in differentiating those with no fibrosis versus those with any fibrosis (i.e., ≥stage 1A/F1) in our cohort of LD subjects was 0.7 (95% CI 0.56-0.93) in NAFLD and 0.8 (95% CI 0.76-0.93) in CH, respectively. Similarly, the AUROC in differentiating those with no/minimal fibrosis versus those with clinically significant fibrosis (i.e., ≥stage 2/F2) was 0.9 (95% CI 0.91-1.02) in NAFLD and 0.9 (95% CI 0.91-0.99) in CH, respectively. AUROCs for adjacent stages are presented in Table 5.

Discussion

The main strength of our study is its prospective population-based approach toward the development of a "normal" range of LSM and the strict selection criteria used to identify subjects who probably did not harbor liver disease. Recruiting healthy individuals from a health and demographic surveillance system eliminates many biases that emerge in studies including people volunteering for health checkups.

Our most remarkable finding was the demonstration that, in healthy individuals, undernutrition and leanness, manifested by lower BMI, increase liver stiffness values in a similar way as obesity does, providing a U-shaped distribution of normal LSM values. This novel finding, not reported so far, is likely to add a

Table 3. Comparison Among Healthy Subjects and Patients With Liver Disease Having No Fibrosis*

Parameter	Healthy Subjects (n = 418)	Patients With Liver Disease and No Fibrosis (n = 59)	P
Age, years	37 ± 12	34 ± 12	0.055
Men, n (%)	276 (66.03)	44 (74.58)	0.119
BMI, kg/m^2	21.20 ± 3.53	22.26 ± 4.07	0.062
Prevalence of abdominal obesity, n (%)	74 (17.71)	13 (22.03)	0.186
ALT, IU/L	26.23 ± 7.79	61.26 ± 51.96	0.01
FBG, mg/dL;	81.61 ± 7.81	89.19 ± 18.95	0.54
LSM, kPa	5.63 ± 1.64	7.52 ± 5.49	<0.001

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LSM, liver stiffness measure.

*Continuous variables are presented as mean ± SD. *P* values are derived from Student *t* test and Chi-square test in continuous and categorical variables, respectively.

Table 4. Performance Characteristics of 95th Percentile LSM Value (8.50 kPa) as Cutoff to Exclude Fibrosis in Persons With Liver Disease

	No Fibrosis vs fibrosis of Any Stage		No/Minimal fibrosis vs Significant Fibrosis (stage ≥ 2)	
Etiology	NAFLD	CH	NAFLD	CH
AUROC, 95% CI (range)	0.722 (0.556-0.889)	0.847 (0.763-0.931)	0.965 (0.909-1.021)	0.953 (0.912-0.994)
Sensitivity, %	88.90	80.50	70.60	75.00
Specificity, %	60.00	71.10	99	95.7
Positive likelihood ratio	2.22	2.79	70.6	17.44
Negative Likelihood ratio	0.19	0.27	0.297	0.26
Positive predictive value, %	66.67	75.00	100	97.72
Negative predictive value, %	85.71	77.14	33.33	62.86
Diagnostic accuracy, %	73.68	75.95	74.36	82.28

Abbreviations: AUROC, area under receiver operating characteristics curve; CH, chronic hepatitis of viral and other etiologies; LSM, liver stiffness measure; NAFLD, nonalcoholic fatty liver disease.

further dimension to the standardized interpretation of LSM values across populations. Liver disease is fast emerging as important public health burden in countries in epidemiological transition.[28,29] Tools useful in screening for liver disease are being actively sought, and TE has been shown to be effective in detecting subclinical liver disease in the community.[6]

Although obesity is an important global problem and contributor to liver disease, the vast majority of the world's population lives with undernutrition or in countries in nutritional transition.[30] We have earlier shown that significant liver disease is often present in people from developing countries who have low (<18.5 kg/m^2) or normal BMI (18.5-24.9 kg/m^2).[12] It should be mentioned here that the exploratory as well as validation studies of TE have mostly been conducted in the European population, with anthropometric profiles different from those of Asians and citizens of underdeveloped countries. The mean BMIs of the subjects in the two available large normative studies were 25.6 and 26.4 kg/m^2, respectively, compared with 21.2 kg/m^2 in the present study.[10,31] Despite this background difference, the mean value of LSM in the present study (5.63 ± 1.64 kPa) is similar to that of the French study in healthy subjects (5.49 ± 1.59 kPa).[10]

It is noteworthy that the demonstration that leanness and undernutrition are determinants of LSM values in a healthy liver adds to the basic, as yet unresolved issue of putative contributors to the viscoelastic properties of a normal liver. Cellular components and scaffolding materials along with the influence of Glisson's capsule are more relevant in a healthy liver than collagen tissue as in fibrotic disease.[32,33] Although liver

Table 5. Distribution of Patients in Different Histological Categories Stratified by Type of Liver Disease and AUROC for Detection of Different Stages of Fibrosis by LSM

Histological Category in Liver Disease Patients	No. of patients (%)	Inflammatory Activity* (mean±SD)	LSM (kPa) (mean±SD)	AUROC (95% CI)
NAFLD (n = 43)				
Fibrosis stage:				
No fibrosis	18 (41.86)	2 ± 2	8.37 ± 8.34	0.745 (0.558-0.901)
Stage 1A	11 (25.58)	4 ± 2	7.21 ± 2.41	0.590 (0.388-0.792)
Stage 2	09 (20.93)	6 ± 1	11.73 ± 5.17	0.657 (0.475-0.838)
Stage 4	05 (11.63)	4 ± 2	24.88 ± 13.00	0.951 (0.886-1.016)
Chronic hepatitis (n = 82)				
METAVIR grade:				
A0	11 (13.41)		6.71 ± 1.55	
A1	33 (40.24)		11.05 ± 13.13	
A2	23 (28.05)		14.61 ± 13.88	
A3	15 (18.30)		17.00 ± 16.76	
METAVIR stage:				
F0	39 (47.56)	2 ± 2	7.15 ± 3.65	0.913 (0.865-0.960)
F1	15 (18.29)	5 ± 2	8.86 ± 3.78	0.731 (0.635-0.827)
F2	07 (8.54)	6 ± 2	13.20 ± 4.64	0.929 (0.886-0.973)
F3	08 (9.76)	7 ± 4	27.77 ± 14.80	0.971 (0.952-0.990)
F4	13 (15.85)	5 ± 3	40.22 ± 22.67	0.987 (0.977-0.998)

Abbreviations: AUROC, area under receiver operating characteristics curve; LSM, liver stiffness measure; NAFLD, nonalcoholic fatty liver disease.

*Stage 1A/F1 had comparable LSM (*P* values 0.687 and 0.138 for NAFLD and chronic hepatitis, respectively) and significantly higher inflammatory activity (*P* values 0.033 and 0.002 for NAFLD and chronic hepatitis, respectively) in comparison with stages of no fibrosis.

stiffness in pathological states correlates well with the degree of hepatic fibrosis, portal hypertension, passive venous congestion, extrahepatic cholestasis, and inflammation have also been described to increase LSM values.[1,34-37] Debate continues over the influence of steatosis on LSM.[38,39] Exploration into the biophysical properties of the liver will probably unravel the determinants of tissue elasticity at physiologic state in the future and this might explain its geographic and ethnic variability.

The usefulness of different noninvasive tests for assessment of liver fibrosis has been shown in studies. FibroTest, in particular, has been found to predict advanced fibrosis efficiently in the diabetic population as well as in otherwise healthy adults in a community at large.[40,41] Moreover, FibroTest is the only noninvasive modality, having extensive prospective evaluation, against which TE can be compared.

Thus it is relevant that both FibroTest and TE showed concordance with liver biopsy in prospective evaluation of patients with liver disease, with only a possible risk of overestimation during the early follow-up period in the case of TE.[42] However, a recent study assessed the feasibility of TE as a screening tool for liver disease in the community and emphasized its value in this setting.[6]

In this context, our study, involving adults of all ages, was done in two stages: first, "normal" values and ULN of LSM in healthy individuals of a community-based cohort were determined by using accepted standards[27]; and second, we demonstrate that even healthy individuals have significantly lower LSM values than clinically asymptomatic liver disease subjects having no fibrosis on histology. Finally, the accuracy of this newly developed ULN of LSM in distinguishing no fibrosis from those with more advanced fibrosis was assessed by ROC. The ULN value in the present study was 8.5 kPa, compared with 8 kPa in the French study.[10] Moreover, when a ULN of 8.5 kPa was used, the AUROC of this value in differentiating those with no fibrosis versus those with any fibrosis in our cohort of LD subjects was 0.7 in NAFLD and 0.8 in CH, respectively.

A gray zone between adjacent stages of fibrosis is inherent to any noninvasive modality of assessment. The utility of LSM for detection of early/intermediate stages of fibrosis is as yet unclear.[5,43] Although uniformity of efficiency over different stages of fibrosis, assessed by AUROC, is more evident with a biochemical modality like FibroTest than with a biophysical modality like TE, the potential clinical applicability of TE should drive scientific endeavors to improve its performance.[44,45]

Our study provides an important contribution to that endeavor by probing the "gray zone" of the early/intermediate stage of fibrosis. Subjects with liver disease without histological fibrosis had significantly higher LSM compared with healthy subjects. Even comparison of healthy subjects with the subgroup of patients with liver disease having normal ALT and no histological fibrosis showed similar results, eliminating the possibility of a confounding effect of hepatic inflammation.[37] The relatively smaller number of patients belonging to subgroups of different fibrosis stages is a limitation of our study. This explains the inability of this study to demonstrate a significant difference in LSM between the F0 and F1 stages.

However, based on our results, we suggest that liver stiffness probably begins to increase before fibrosis sets in, as has been found in an animal model of liver fibrosis in which increase in liver stiffness preceded activation of hepatic stellate cells and deposition of fibrous material.[8] Cell culture experiments show that alterations in matrix character and cellular microenvironment are the prerequisites for transformation of hepatic stellate cells into a fibrogenic phenotype, corroborative clinical evidence of which is lacking.[46,47] We hypothesize that similar changes in the hepatic microenvironment, *in vivo*, before onset of fibrosis, alter tissue elasticity and could also play a role in activation of stellate cells. Our observation provides a preliminary clinical correlate for that basic research question.

Finally, we propose 8.5 kPa as the cutoff for differentiating healthy liver from that with significant fibrosis.

The two main disadvantages of our study were the small sample size of healthy subjects and the inability to perform liver biopsy in them because of ethical constraints. However, our sample size is equivalent to that of the French study of healthy individuals in whom TE was done.[10]

In conclusion, we demonstrate that the healthy range of LSM in our population is 3.2-8.5 kPa; LSM values have a U-shaped distribution in healthy individuals; healthy subjects have lower LSM than those with liver disease without fibrosis; and the ULN of LSM of 8.5 kPa has a good ability to differentiate those with no fibrosis from those with any degree of fibrosis.

References

1. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis by transient elastography. J Hepatol 2008;48:835-847.

2. Vergniol J, Foucher J, Terrebonne E, Bernard PH, Bail BL, Merrouche W, et al. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. Gastroenterology 2011; 140:1970-1979.

3. Kumar M, Sarin SK, Hissar S, Pande C, Sakhuja P, Sharma BC, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology 2008;134:1376-1384.
4. Bedossa P, Poynard T; The French METAVIR Cooperative Study Group. An algorithm for grading activity in chronic hepatitis C. Hepatology 1996;24:289-293.
5. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960-974.
6. Roulot D, Costes JL, Buyck JF, Warzocha U, Gambier N, Czernichow S, et al. Transient elastography as a screening tool for liver fibrosis and cirrhosis in a community-based population aged over 45 years. Gut 2011;60:977-984.
7. Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828-835.
8. Poynard T, Ingiliz P, Elkrief L, Munteanu M, Lebray P, Morra R, et al. Concordance in a world without a gold standard: a new non-invasive methodology for improving accuracy of fibrosis markers. PLoS One 2008;3:e3857.
9. Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, et al. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. Am J Physiol Gastrointest Liver Physiol 2007; 293:G1147-G1154.
10. Roulot D, Czernichow S, Le Clésiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol 2008;48:606-613.
11. Corpechot C, El Naggar A, Poupon R. Gender and liver: is the liver stiffness weaker in weaker sex? Hepatology 2006;44:513-514.
12. Das K, Das K, Mukherjee PS, Ghosh A, Ghosh S, Mridha AR, et al. Nonobese population in a developing country has a high prevalence of nonalcoholic fatty liver and significant liver disease. Hepatology 2010; 51:1593-1602.
13. WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157-163.
14. Misra A, Khurana L. Obesity and the metabolic syndrome in developing countries. J Clin Endocrinol Metab 2008;93:S9-S30.
15. Chowdhury A, Santra A, Chaudhuri S, Dhali GK, Chaudhuri S, Maity SG, et al. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. Hepatology 2003;37: 802-809.
16. Chowdhury A, Santra A, Chakravorty R, Banerji A, Pal S, Dhali GK, et al. Community-based epidemiology of hepatitis B virus infection in West Bengal, India: prevalence of hepatitis B e antigen-negative infection and associated viral variants. J Gastroenterol Hepatol 2005;20: 1712-1720.
17. Yajima Y, Ohta K, Narui T, Abe R, Suzuki H, Ohtsuki M. Ultrasonographical diagnosis of fatty liver: significance of the liver-kidney contrast. Tohoku J Exp Med 1983;139:43-50.
18. Alberti KGMM, Zimmet P, Shaw J, for the IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. Lancet 2005;366:1059-1062.
19. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and B-cell function from fasting glucose and insulin concentrations in man. Diabetologia 1985;28:412-419.
20. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet 2008;371:838-851.
21. Farrell GC, Chitturi S, Lau GKK, Sollano JD, for the Asia-Pacific Working Party on NAFLD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol 2007;22:775-777.
22. Ghany MG, Strader DB, Thomas DL, Seef LB. AASLD Practice Guidelines. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335-1374.
23. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2007;45:507-539.
24. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929-938.
25. Menghini G. One-second needle biopsy of the liver. Gastroenterology 1958;35:190-199.
26. Kleiner DE, Brunt EM, Natta MV, Behling C, Contos MJ, Cummings OW, et al. for the Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321.
27. Green R, Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002;123:1367-1384.
28. Williams R. Global challenges in liver disease. Hepatology 2006;44: 521-526.
29. Quigley MA. Shifting burden of disease—epidemiological transition in India. Int J Epidemiol 2006;35:1530-1531.
30. Food and Agriculture Organization of the United Nations. The double burden of malnutrition: case studies from six developing countries. FAO Food Nutr Pap 2006;84:1-334.
31. Sirli R, Sporea I, Tudora A, Deleanu A, Popescu A. Transient elastographic evaluation of subjects without known hepatic pathology: does age change the liver stiffness? J Gastrointest Liver Dis 2009;18:57-60.
32. Orescanin M, Qayyum MA, Toohey KS, Insana MF. Dispersion and shear modulus measurements of porcine liver. Ultrason Imaging 2010; 32:255-266.
33. Roan E. The effect of Glisson's capsule on the superficial elasticity measurements of the liver. J Biomech Eng 2010;132:104-105.
34. Vizzutti F, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, et al. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. Hepatology 2007;45:1290-1297.
35. Millonig G, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, et al. Liver stiffness is directly influenced by central venous pressure. J Hepatol 2010;52:206-210.
36. Millonig G, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Buchler MW, et al. Extrahepatic cholestasis increases liver stiffness (FibroScan) regardless of fibrosis. Hepatology 2008;48:1718-1723.
37. Sagir A, Erhardt A, Schmitt M, Haussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. Hepatology 2008;47:592-595.
38. Yoneda M, Fujita K, Inamori M, Nakajima A, Tamano M, Hiraishi H. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). Gut 2007;56:1330-1331.
39. Gaia S, Carenzi S, Barilli AL, Bugianesi E, Smedile A, Brunello F et al. Reliability of transient elastography for the detection of fibrosis in non-alcoholic fatty liver disease and chronic viral hepatitis. J Hepatol 2011;54:64-71.
40. Jacqueminet S, Lebray P, Morra R, Munteanu M, Devers L, Messous D, et al. Screening for liver fibrosis by using a noninvasive biomarker in patients with diabetes. Clin Gastroenterol Hepatol 2008;6:828-831.
41. Poynard T, Lebray P, Ingiliz P, Varaut A, Varsat B, Ngo Y, et al. Prevalence of liver fibrosis and risk factors in a general population using non-invasive biomarkers (FibroTest). BMC Gastroenterol 2010;10:40.
42. Poynard T, Ngo Y, Munteanu M, Thabut D, Massard J, Moussalli J, et al. Biomarkers of liver injury for hepatitis clinical trials: a meta-analysis of longitudinal studies. Antivir Ther 2010;15:617-631.
43. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol 2011;54:650-659.
44. Bedossa P, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003;38:1449-1457.
45. Poynard T, Benhamou Y, Thabut D, Ratziu V. Liver biopsy: the best standard when everything else fails. J Hepatol 2009;50:1267-1268.
46. Rockey DC, Boyles JK, Gabbiani G, Friedman SL. Rat hepatic lipocytes express smooth muscle actin upon activation in vivo and in culture. J Submicrosc Cytol Pathol 1992;24:193-203.
47. Rockey DC, Housset CN, Friedman SL. Activation-dependent contractility of rat hepatic lipocytes in culture and in vivo. J Clin Invest 1993; 92:1795-1804.