Controlled Attenuation Parameter for Assessment of Hepatic Steatosis in Indian Patients

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Background/Aims: The gold standard method for measurement of hepatic steatosis is liver histology. Controlled Attenuation Parameter (CAP) can measure hepatic steatosis non-invasively. We aimed to assess the accuracy of CAP for detection of hepatic steatosis. **Methods:** A total of 462 patients (May 2012–January 2017)—89 non-alcoholic fatty liver disease, 182 chronic hepatitis B, 88 chronic hepatitis C and 103 patients with other etiologies who underwent simultaneous liver biopsy and CAP estimation using Transient Elastography (TE) were included. Steatosis was graded as S0: steatosis in 0–5% of hepatocytes, S1: 6–33%, S2: 34–66% and S3: 67–100%. Receiver Operating Characteristic (ROC) curves were plotted to evaluate the accuracy of CAP in detecting hepatic steatosis. Predictors of CAP were assessed by multivariate linear regression model. **Results:** The mean age $\pm$ SD was 33.8 ± 11.6 years; 296 (64.1%) were males. On liver histology, steatosis grades S0, S1, S2 and S3 were seen in 331 (71.6%), 74 (16.0%), 39 (8.4%) and 18 (3.9%), respectively. The median CAP (IQR) values for S0, S1, S2, and S3 steatosis were 206 (176–252) dB/m, 295 (257–331) dB/m, 320 (296–356) dB/m, and 349 (306–363) dB/m, respectively. For estimation of $\geq$ S1, $\geq$ S2, and $\geq$ S3 using CAP, AUROC were 0.879, 0.893, and 0.883, respectively. In multivariate analysis, only BMI (OR 1.18; CI, 1.11–1.26, $P < 0.001$) and grade of hepatic steatosis (grade 1, OR, 3.94; 95% CI, 1.58–9.84, $P = 0.003$; grade 2, OR 42.04; 95% CI, 4.97–355.31, $P = 0.001$ and grade 3, OR 35.83; 95% CI 4.31–297.61, $P = 0.001$) independently predicted CAP. **Conclusions:** CAP detects hepatic steatosis with good accuracy in Indian patients with various etiologies. (J CLIN EXP HEPATOL 2019;9:13–21)

Hepatic steatosis is commonly seen in Non-Alcoholic Fatty Liver Disease (NAFLD), Alcoholic Liver Disease (ALD), and Chronic Hepatitis C (CHC) patients.^{1,2} The presence of hepatic steatosis is associated with treatment failure in Chronic Hepatitis B (CHB) patients,³ progression of hepatic fibrosis⁴ and development of hepatocellular carcinoma in CHC.⁵ In addition, hepatic steatosis is also associated with metabolic syndrome and its complications.⁶ Therefore, estimation of hepatic steatosis is important in the management of patients. Till now, the gold standard for assessment of

hepatic steatosis is liver biopsy. Liver biopsy is an invasive procedure with complications like pain, bleeding, sampling variability, and even a small risk of death (0.01%).⁷ Moreover, it is not feasible to perform repeated biopsies to assess for changes in steatosis on follow-up.

Ultrasonography (US), Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) are some of the imaging methods available for noninvasive assessment of hepatic steatosis.⁸ However, these techniques have limitations, including high operator-dependency of US, radiation exposure during CT, high cost and limited availability of MRI and MRS.^{9–11} Thus, there remains an unmet need for a low cost, easily available, accurate and non-invasive method for detection of hepatic steatosis. Recent data suggests that Controlled Attenuation Parameter (CAP) correlates with steatosis on liver biopsy.¹² It can be used for steatosis detection and quantification. Prior studies have evaluated its accuracy in NAFLD,¹³ ALD, HCV infection¹⁴ and patients with CHB infection.¹⁵ There is a paucity of Indian data on the predictive value of CAP for hepatic steatosis on liver biopsy. Therefore, the objectives of this study were: (i) to assess the diagnostic accuracy of CAP in assessing hepatic steatosis; and (ii) to assess the various factors affecting CAP values.

Keywords: liver biopsy, NAFLD, fibrosis, hepatitis B virus, hepatitis C virus

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Abbreviations: ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; AUROC: Area Under Receiver Operating Characteristics Curves; BMI: Body Mass Index; CAP: Controlled Attenuation Parameter; CHB: Chronic Hepatitis B; CHC: Chronic Hepatitis C; IQR: Interquartile Range; LSM: Liver Stiffness Measurement; NAFLD: Non-Alcoholic Fatty Liver Disease; SD: Standard Deviation

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PATIENTS AND METHODS

Inclusion and Exclusion Criteria

We searched our prospectively maintained database for all patients undergoing liver biopsy and FibroScan after May 2012. The FibroScan touch 502 (Echosens, Paris, France) was available at our center from November 2013. All consecutive patients, who underwent concomitant liver biopsy and CAP estimation in the department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, India between May 2012 to January 2017, were included in this study.

The etiology of underlying liver disease included CHB, CHC, NAFLD, and a miscellaneous group (which included autoimmune hepatitis, non-cirrhotic portal fibrosis (NCPF) and cryptogenic etiology). An adequate liver biopsy sample was defined as one with at least 15 mm of sample tissue and containing at least six portal tracts or more. Patients were excluded from the study if they had co-infection with other hepatotropic viruses or HIV, bilirubin ≥ 5 mg/dl or Aspartate Aminotransferase (AST)/Alanine Aminotransferase (ALT) levels 10 times the upper limit of normal in the last 6 months, ascites, previous liver surgery or liver transplantation or inadequate liver biopsy sample.

Data Collection

The clinical records of all patients were retrospectively retrieved from a prospectively maintained database. The following details were noted in each case: age, sex, alcohol intake, the presence of hypertension and diabetes, Body Mass Index (BMI), various laboratory test results including hemoglobin, platelet count, AST, ALT, fasting blood sugar, fasting lipid profile {total cholesterol, Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), Triglycerides (TG) and High-Density Lipoprotein (HDL)} levels. Tests for hepatitis B surface antigen, antibodies to hepatitis C virus (anti-HCV), and Human Immunodeficiency Virus (HIV) were done using standard commercially available enzyme immunoassays.

Liver Stiffness Measurement and Determination of CAP

Liver Stiffness Measurement (LSM) and CAP measurements were performed on a FibroScan[®] touch 502 (Echosens, Paris, France) by trained personnel who were blinded to the clinical data of patients. All LSM and CAP measurements were done within 4 weeks prior to the liver biopsy. The FibroScan[®] was performed in a fasting state on the right lobe of the liver through the intercostal space. All measurements were done using M/XL probe as per manufacturer's recommendations. As a routine, we used XL probe for measurements in obese patients with BMI above 30 kg/m². Ten successful acquisitions

were performed on each patient. The interquartile range (IQR) was defined as the index of the intrinsic variability of LSM and CAP values corresponding to the interval containing 50% of the valid measurements between the 25th and 75th percentiles. As an indicator of variability, the ratios of the IQR of LSM and CAP values to the median values (IQR/M) were calculated. The measurements were considered reliable if 10 valid acquisitions with IQR/M of LSM less than 0.3 were obtained. Failure of transient elastography was defined as the inability to obtain valid LSM or CAP value.

Liver Biopsy Examination

Percutaneous liver biopsy was performed using a 16G Menghini's liver biopsy needle under local anesthesia and ultrasound guidance. The liver biopsy was done to document the grade and stage of liver disease in hepatitis B, hepatitis C and NAFLD. In other etiologies, biopsies were done to confirm the diagnosis. Liver biopsy was assessed by 2 experienced pathologists who were blinded to the clinical and CAP data. A liver biopsy sample was considered adequate if it was longer than 15 mm and contained six portal tracts or more. Liver fibrosis was evaluated semi-quantitatively according to the METAVIR scoring system as follows: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.¹⁶ Steatosis was categorized by visual assessment as S0, steatosis in 0–5%; S1, steatosis in 6–33%; S2, in 34–66%; and S3, 67–100% of hepatocytes.²

Statistical Analysis

Continuous variables were expressed as mean $\pm$ SD or median (interquartile range), as appropriate. Continuous variables were compared by Student's *t*-test or Mann-Whitney test, as appropriate, or ANOVA test (for more than two group comparisons). Categorical variables were compared by chi-square test or Fisher's exact test. Linear regression (simple—i.e. univariate, and multiple—i.e. multivariate) was used to assess the association between CAP and other relevant variables. The accuracy of CAP in predicting histological steatosis grade was evaluated by plotting area under the receiver operating characteristic curves (AUROC). The threshold cut-offs of CAP values were identified by the ROC curve analysis and, based on these cut-offs, the different diagnostic measures such as sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio were reported. AUROCs were interpreted as follows: 0.90–1.0 = excellent; 0.80–0.90 = good; 0.70–0.80 = fair; <0.70 = poor. All statistical tests were two-sided. Statistical significance was taken as $P < 0.05$. Statistical analysis was performed using SPSS (SPSS version 20.0; SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline Characteristics

Out of a total of 504 patients who underwent Fibroscan® (LSM and CAP) and biopsy during the study period, 42 were excluded—inadequate biopsy in 16, invalid fibroscan® in 13, HIV co-infection in 3, AST/ALT levels 10 times the upper limit of normal in 8, and previous liver surgery in 2 patients.

Finally, a total of 462 patients were included in the analysis. The mean age of these patients was 33.8 ± 11.8 years and 296 (64.1%) were males. The etiologies were NAFLD in 89 (19.3%), HBV in 182 (39.4%), HCV in 88 (19.0%) and miscellaneous in 103 (22.3%). The miscellaneous group comprised of autoimmune hepatitis in 46 patients, NCPF in 13, and cryptogenic in 44. The median BMI was 23.4 kg/m^2 (IQR, 20.8–26.4). The median (IQR) CAP value was 229.5 (189.0–290.0) dB/m, and median LSM was 6.8 (5.3–9.1) kPa. The baseline characteristics, laboratory parameters, liver biopsy (hepatic steatosis and hepatic fibrosis) details, and fibroscan® (LSM and CAP) values are shown in Table 1. Patients with NAFLD were more likely to be older, and as expected had higher BMI, as well as co-morbidities such as hypertension, metabolic syndrome, diabetes mellitus or dyslipidemia (elevated values of total serum cholesterol and serum TGs). On liver histology, higher grades of steatosis and fibrosis were seen in patients with NAFLD as compared to other etiologies (Table 1). The CAP values were higher in patients with NAFLD as compared to other etiologies of CLD (Table 1). The median (IQR) CAP values in patients with NAFLD, CHB, CHC and miscellaneous etiologies were 325.0 (300.0–361.5), 212.5 (184.7–252.0), 202.0 (171.5–249.7) and 231.0 (190.0–274.0), respectively ($P < 0.001$).

Univariate and Multivariate Analysis of Factors Predicting CAP

Older age, female sex, underlying etiology of liver disease, high BMI, metabolic syndrome, low bilirubin, low AST, low ALT, low serum alkaline phosphatase, high total cholesterol, high TG, high LDL, high HDL, high VLDL and higher grade of hepatic steatosis (on liver biopsy) were associated with CAP in univariate analysis. However, only BMI (OR 1.18; CI, 1.11–1.26, $P < 0.001$) and hepatic steatosis grade (grade S1, OR, 3.94; 95% CI, 1.58–9.84, $P = 0.003$; grade S2, OR 42.04; 95% CI, 4.97–355.31, $P = 0.001$ and grade S3, OR 35.83; 95% CI 4.31–297.61, $P = 0.001$) independently predicted CAP on multivariate analysis (Table 2).

CAP Values for Hepatic Steatosis Assessment

The overall CAP values for each grade of hepatic steatosis are shown in Figure 1. Among NAFLD patients, the median (IQR) CAP values in patients with histological

steatosis grades S1, S2, and S3 were 314.0 (281.0–354.0), 334.5 (313.0–359.5) and 360.5 (316.7–363.7), respectively ($P = 0.031$). Among CHB patients, the median (IQR) CAP values in patients with histological steatosis S0, S1, S2, and S3 were 206 (180.0–243.0), 234 (213.7–287.7), 305 (289.7–337.2) and 332 (265.5–368.5), respectively ($P < 0.001$). Among CHC patients, the median (IQR) CAP values in patients with histological steatosis S0, S1, S2, and S3 were 199 (166.7–228.2), 267 (221.0–279.5), 275.5 (208.7–288.2) and 288 (288–288), respectively ($P = 0.006$). Among miscellaneous etiologies of CLD, the median (IQR) CAP values in patients with histological steatosis S0, S1, and S2 were 228.5 (189.7–273.2), 254 (136.0–277.5) and 303 (303–303), respectively ($P = 0.503$). There was no patient with S3 steatosis in the miscellaneous group.

For the overall cohort, AUROC of CAP for prediction of $\geq S1$, $\geq S2$, and S3 grades of hepatic steatosis were 0.879, 0.893, and 0.883, respectively (Figure 2). Overall, the CAP cut-off values for estimation of hepatic steatosis grades $\geq S1$, $\geq S2$, and S3 were 263 dB/m, 287 dB/m, and 296 dB/m, respectively. The CAP cut-off values for differentiating various grades of hepatic steatosis were different among various etiologies. Among NAFLD patients, CAP cut-off values for estimation of hepatic steatosis grades $\geq S2$, and S3 were 324 dB/m and 348 dB/m, respectively. Among CHB patients, CAP cut-off values for estimation of hepatic steatosis grades $\geq S1$, $\geq S2$, and S3 were 232 dB/m, 288 dB/m, and 304 dB/m, respectively. Among CHC patients, CAP cut-off values for estimation of hepatic steatosis grades $\geq S1$, $\geq S2$, and S3 were 252 dB/m, 272 dB/m, and 286 dB/m, respectively.

CAP for Hepatic Steatosis Assessment—Influence of Stage of Fibrosis

There was no significant difference in CAP values in different stages of fibrosis with similar grade of steatosis. Among patients with S0 steatosis, median (IQR) CAP values in patients with histological fibrosis stages F0, F1, F2, F3 and F4 were 200.0 (171.2–252.0) dB/m, 210.0 (178.5–252.0) dB/m, 220.0 (194.5–260.5) dB/m, 202 (167.0–253.0) dB/m, and 206.5 (180.2–234.0) dB/m, respectively ($P = 0.393$). In S1 steatosis, median (IQR) CAP values in patients with histological fibrosis stages F0, F1, F2, F3 and F4 were 271.0 (234.0–315.0) dB/m, 300.5 (254.0–347.5), 343.5.0 (307.5–370.7) dB/m, 297.0 (274.2–348.7) dB/m, and 300.0 (267.0–333.0) dB/m, respectively ($P = 0.151$). In S2 steatosis, median (IQR) CAP values in patients with histological fibrosis stages F0, F1, F2, F3 and F4 were 331.0 (317.0–358.0) dB/m, 338.0 (303.0–363.0) dB/m, 320.0 (292.0–367.0) dB/m, 318.0 (276.2–345.5) dB/m, and 298.0 (278.0–322.7) dB/m, respectively ($P = 0.524$). In S3 steatosis, median (IQR) CAP values in patients with histological fibrosis stages F0,

Table 1 Baseline Demographic, and Laboratory Parameters of Patients With Various Etiologies of Chronic Liver Disease.

Parameter	Overall (n = 462)	NAFLD (n = 89)	CHB (n = 182)	CHC (n = 88)	Miscellaneous (n = 103)	P-value
Age, years (mean ± SD)	33.8 (±11.6)	38.6 (±9.9)	30.2 (±11.2)	34.7 (±11.6)	35.2 (±11.9)	<0.001 ^{*,§,¶,}
Male:female	296 (64.1%):166 (35.9%)	43 (48.3%):46 (51.7%)	147 (80.8%):35 (19.2%)	62 (70.5%):26 (29.5%)	44 (42.7%):59 (57.3%)	<0.001 ^{*,†,¶,}
BMI (kg/m ²)	23.4 (20.8–26.4)	29.2 (25.4–40.3)	21.9 (19.6–24.9)	22.7 (20.5–24.8)	24.2 (21.4–28.4)	<0.001 ^{*,†,§,¶,}
Hypertension (n = 381)	25 (6.6%)	17 (36.2%)	2 (1.1%)	2 (2.3%)	4 (5.6%)	<0.001 ^{*,†,‡}
Diabetes (n = 392)	59 (15.1%)	31 (55.4%)	8 (4.5%)	7 (8.2%)	13 (18.1%)	<0.001 ^{*,†,§,¶}
Metabolic syndrome (n = 432)	62 (14.3%)	43 (50%)	0 (0%)	0 (0%)	19 (20.2%)	<0.001 ^{*,†,‡,¶,}
Bilirubin, mg/dl	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.6 (0.5–0.8)	0.7 (0.5–1.1)	0.002 ^{‡,¶,}
AST, IU/L	40 (28–62)	31.0 (23–57)	34 (28–51)	53 (42–88)	40.0 (23–71)	<0.001 ^{†,§,}
ALT, IU/L	43 (26–84)	31 (22–69)	43 (29–69)	76 (52–133)	36 (21–72)	<0.001 ^{†,§,}
SAP, IU/L	191 (122–262)	125 (83–215)	202 (145–257)	204 (143–269)	197 (109–351)	<0.001 ^{*,§,¶,}
Albumin, g/dl (mean ± SD)	4.4 (±0.6)	4.2 (±0.6)	4.6 (±0.6)	4.6 (±0.5)	4.0 (±0.69)	<0.001 ^{*,†,§,}
Hemoglobin, g/dl (mean ± SD)	13.1 (±2.0)	12.9 (±1.9)	13.7 (±1.8)	13.6 (±1.7)	13.6 (±1.7)	<0.001 ^{*,†,¶}
TLC, /mm ³ (mean ± SD)	7214 (±2231)	8281 (±2470)	7034 (±1828)	7324 (±2102)	7324 (±2121)	<0.001 ^{*,†,‡}
Platelets ×10 ³ , /mm ³ (mean ± SD)	195 (±85)	231 (±92)	182 (±67)	182 (±60)	182 (±60)	<0.001 ^{*,†}
Total cholesterol, mg/dl (mean ± SD)	166.5 (±38.5)	177.4 (±38.1)	158.9 (±37.1)	161.4 (±40.1)	161.4 (±40.1)	0.009 [*]
Triglycerides, mg/dl (mean ± SD)	126.5 (±54.7)	146.6 (±51.8)	112.0 (±47.8)	95.6 (±33.1)	117.4 (±57.9)	<0.001 ^{*,†}
LDL, mg/dl (mean ± SD)	99.0 (±31.8)	104.4 (±32.4)	95.8 (±29.7)	95.6 (±33.1)	95.6 (±33.1)	0.287
HDL, mg/dl (mean ± SD)	42.1 (±8.6)	43.9 (±9.4)	40.3 (±7.8)	42.0 (±4.9)	42.0 (±4.9)	0.032 [*]
VLDL, mg/dl (mean ± SD)	23.8 (±10.3)	28.0 (±9.7)	20.9 (±9.9)	23.5 (±13.2)	23.5 (±13.2)	<0.001 ^{*,‡}
Hepatic fibrosis (METAVIR)						<0.001 ^{*,§,¶,}
0	177 (38.3%)	22 (24.7%)	92 (50.5%)	30 (34.1%)	33 (32%)	
1	114 (24.7%)	26 (29.2%)	48 (26.4%)	24 (27.3%)	16 (15.5%)	
2	59 (12.8%)	15 (16.9%)	23 (12.6%)	12 (13.6%)	9 (8.7%)	
3	51 (11.0%)	11 (12.4%)	9 (4.9%)	14 (15.9%)	17 (16.5%)	
4	61 (13.2%)	15 (16.9%)	10 (5.5%)	8 (9.1%)	28 (27.2%)	
Grade of hepatic steatosis						<0.001 ^{*,†,‡}
0	331 (71.6%)	–	155 (85.2%)	78 (88.6%)	98 (95.1%)	
1	74 (16.0%)	49 (55.1%)	16 (8.8%)	5 (5.7%)	4 (3.9%)	
2	39 (8.4%)	28 (31.5%)	6 (3.3%)	4 (4.5%)	1 (1.0%)	
3	18 (3.9%)	12 (13.5%)	5 (2.7%)	1 (1.1%)	0 (0%)	
LSM, kPa	6.8 (5.3–9.1)	8.2 (6.0–11.9)	6.1 (4.9–7.8)	6.3 (5.0–8.6)	8.8 (6.0–15.1)	<0.001 ^{*,¶,}
CAP, dB/m	229.5 (189.0–290.0)	325.0 (300.0–361.5)	212.5 (184.7–252.0)	202.0 (171.5–249.7)	231.0 (190.0–274.0)	<0.001 ^{*,†,‡,}

Note: All values are expressed as median (IQR) or n (%) unless otherwise specified.

^{*}Significant between NAFLD and CHB.

[†]Significant between NAFLD and CHC.

[‡]Significant between NAFLD and miscellaneous.

[§]Significant between CHB and CHC.

[¶]Significant between CHB and miscellaneous.

^{||}Significant between CHC and miscellaneous.

Abbreviations: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SAP, serum alkaline phosphatase; TLC, total leukocyte count; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; LSM, liver stiffness measurement; IQR, interquartile range; CAP, controlled attenuation parameter.

Table 2 Univariate and Multivariate Analysis of Factors Predicting Controlled Attenuation Parameter.

Variables	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age, years	1.041 (1.024–1.059)	<0.001		
Female sex	1.905 (1.288–2.816)	0.001		
Diagnosis				
Miscellaneous	1			
NAFLD	23.773 (9.918–56.985)	<0.001		
CHB	0.553 (0.322–0.952)	0.033		
CHC	0.486 (0.249–0.949)	0.035		
BMI, kg/m ²	1.239 (1.177–1.304)	<0.001	1.180 (1.111–1.260)	<0.001
Metabolic syndrome present	38.382 (13.587–108.429)	<0.001		
Bilirubin, mg/dl	0.505 (0.322–0.793)	0.003		
AST, IU/l	0.995 (0.990–0.999)	0.024		
ALT, IU/l	0.996 (0.992–0.999)	0.018		
SAP, IU/l	0.994 (0.993–0.997)	<0.001		
Albumin, g/dl	0.754 (0.560–1.016)	0.063		
Total cholesterol, mg/dl	1.012 (1.005–1.019)	<0.001		
Triglycerides, mg/dl	1.009 (1.000–1.014)	<0.001		
LDL, mg/dl	1.012 (1.004–1.020)	0.002		
HDL, mg/dl	1.046 (1.015–1.077)	0.004		
VLDL, mg/dl	1.056 (1.027–1.087)	<0.001		
LSM, mg/dl	1.001 (0.982–1.019)	0.951		
Fibrosis, present	1.051 (0.921–1.200)	0.730		
Grade of hepatic steatosis				
Grade 0	1		1	
Grade 1	10.328 (5.820–18.328)	<0.001	3.941 (1.580–9.841)	0.003
Grade 2	75.708 (17.786–322.260)	<0.001	42.042 (4.971–355.312)	0.001
Grade 3	69.569 (9.092–532.328)	<0.001	35.830 (4.312–297.611)	0.001

Abbreviations: NAFLD, non-alcoholic fatty liver disease; CHB, chronic hepatitis B; CHC, chronic hepatitis C; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SAP, serum alkaline phosphatase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; LSM, liver stiffness measurement.

F1, F2, F3 and F4 were 309.0 (276.0–363.0) dB/m, 330.5 (314.0–347.0) dB/m, 362.0 (324.0–383.0) dB/m, 349.0 (332.0–366.0) dB/m, and 343.5 (325.0–362.0) dB/m respectively ($P = 0.516$) (Figure 3).

The AUROCs of CAP value for various grades of hepatic steatosis— $\geq S1$, $\geq S2$, and $\geq S3$ for various grades of fibrosis are shown in Table 3 and Figure 2. Overall, CAP was a good test for differentiating $\geq S1$, $\geq S2$, and $\geq S3$, irrespective of the underlying fibrosis in the liver. The specificity, sensitivity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio for optimal CAP cut-off values are shown in Table 3.

CAP for Differentiating Various Grades of Hepatic Steatosis

ROC curves were made to assess the ability of CAP to differentiate various individual grades of hepatic steatosis.

The details of AUROC, optimal cut-off values, sensitivity, specificity, PPV, NPV, positive and negative LR are shown in Table 4. CAP was excellent (AUROC > 0.90) in differentiating between S0/S2 (AUROC, 0.936) and S0/S3 grades (AUROC, 0.954) of hepatic steatosis. CAP was good (AUROC > 0.80) in differentiating between S0/S1 grades (AUROC, 0.831) and fair in differentiating between S1/S3 grades (AUROC, 0.715) of hepatic steatosis. CAP was poor in differentiating between S1/S2 grades (AUROC, 0.659) and S2/S3 grades (AUROC, 0.600) of hepatic steatosis.

DISCUSSION

The present study included a large number of patients with different etiologies of chronic liver disease who underwent simultaneous assessment of fibrosis and hepatic steatosis by liver histology and Fibroscan (LSM and CAP). The results indicate that CAP, a novel non-

Figure 1 Controlled attenuation parameter values in different grades of hepatic steatosis in all patients ($n = 462$). *statistically significant (P value < 0.05).

Figure 3 Controlled attenuation parameter value in different grades of hepatic steatosis across the various stages of hepatic fibrosis.

invasive technique, can accurately assess hepatic steatosis in different etiologies with good accuracy. BMI and grade of hepatic steatosis (on liver biopsy) independently influence CAP values.

Hepatic steatosis is present in approximately 30% of the general population, and this percentage increases in patients with obesity and diabetes mellitus.⁶ The presence of steatosis influences the outcome of liver diseases, including NAFLD, ALD, HCV and HBV.³⁻⁵ Liver biopsy is the gold standard for hepatic steatosis estimation, but has its own limitations. Therefore, there is a need for non-invasive tests, which are safe, cheap, reliable, validated, can be done bedside if required, and performed repeatedly to assess the response after interventions. Prior studies have evaluated the role of CAP in patients with various etiologies of CLD.^{13,14,17,18} Our data suggests a high negative predictive value for CAP, which means that it can be used with good accuracy to rule out hepatic steatosis. The

optimal cut-off values for detection of various grades of steatosis in our study were $\geq S1$: 263.5 dB/m, $\geq S2$: 287.5 dB/m and $S3$: 296.5 dB/m. These cut-off values are similar to those reported by Myers et al.¹⁸ In our study, cut-off for steatosis grade $\geq S1$ was higher, whereas cut-off for $\geq S2$, and $\geq S3$ was similar to those reported by Kumar et al.¹⁹ The CAP cut-off values for differentiating various grades of hepatic steatosis were different between the various etiologies of liver disease possibly due to significant differences in the BMI. The median BMI of the NAFLD included in our cohort was higher as compared to those of Kumar et al.¹⁹ BMI is an independent predictor of CAP values along with hepatic steatosis, which explains the higher CAP values in our cohort.^{19,20} In contrast, the CAP cut-offs in other studies were different.^{21,22} These differences in the cut-offs values can possibly be explained by differences in BMI, use of M or XL probe and differences in subcutaneous fat.²³⁻²⁵

Figure 2 Receiver operating characteristic curve of controlled attenuation parameter and different grades of hepatic steatosis. S0: steatosis in 0–5% of hepatocytes, S1: 6–33%, S2: 34–66% and S3: 67–100%, AUROC: area under receiver operating characteristic curve.

Table 3 Diagnostic Accuracy of Controlled Attenuation Parameter for Various Grades of Hepatic Steatosis Across Grades of Hepatic Fibrosis.

	Overall	F0	F1	F2	F3	F4
S0 vs S1S2S3	0.879	0.836	0.867	0.965	0.934	0.898
AUROC	(0.842–0.916)	(0.764–0.907)	(0.790–0.944)	(0.925–1.00)	(0.870–0.998)	(0.79–1.000)
Optimal cut-off	263.5	243.0	262.0	277.5	270.5	263.0
Sensitivity	81.7 (73.9–87.9)	75.6 (59.7–87.6)	78.4 (61.8–90.2)	100.0 (81.5–100.0)	93.8 (69.8–99.8)	89.5 (66.9–98.7)
Specificity	80.4 (75.7–84.5)	73.5 (65.3–80.7)	77.9 (67.0–86.6)	87.8 (73.8–95.9)	85.7 (69.7–95.2)	90.5 (77.4–97.3)
PPV	62.2 (56.5–67.5)	47.3 (38.2–54.5)	63.0 (52.0–72.8)	78.3 (61.3–89.1)	75.0 (56.9–87.2)	80.9 (62.3–91.6)
NPV	91.7 (88.5–94.1)	90.9 (85.3–94.5)	88.2 (80.1–93.3)	100.0	96.8 (81.7–99.5)	95.0 (83.6–98.6)
PLR	4.16 (3.3–5.25)	2.86 (2.05–3.97)	3.55 (2.26–5.58)	8.20 (3.61–18.64)	31.37 (19.11–45.89)	9.39 (3.65–24.17)
NLR	0.23 (0.16–0.33)	0.33 (0.19–0.57)	0.28 (0.15–0.52)	–	0.07 (0.01–0.49)	0.12 (0.03–0.43)
S0S1 vs S2S3	0.893	0.885	0.900	0.906	0.885	0.854
AUROC	(0.855–0.931)	(0.791–0.979)	(0.842–0.958)	(0.832–0.980)	(0.790–0.981)	(0.737–0.971)
Optimal cut-off	287.5	275.5	302.5	296.0	272.5	288.5
Sensitivity	87.7 (76.3–94.9)	87.5 (61.7–98.5)	84.6 (54.6–98.1)	83.3 (51.6–97.9)	100.0 (63.1–100.0)	87.5 (47.4–99.7)
Specificity	82.2 (78.1–85.8)	82.0 (75.2–87.6)	85.2 (76.7–91.4)	83.0 (69.2–92.4)	74.4 (58.8–86.5)	84.9 (72.4–93.3)
PPV	40.9 (35.5–46.7)	32.6 (24.9–41.3)	42.3 (30.3–55.3)	55.6 (38.8–71.2)	42.1 (30.4–54.8)	46.7 (30.5–63.6)
NPV	97.9 (95.9–98.9)	98.5 (94.7–99.6)	97.7 (92.3–99.4)	95.1 (84.5–98.6)	100.0	97.8 (87.8–99.7)
PLR	4.93 (3.92–6.22)	4.86 (3.33–7.09)	5.71 (3.38–9.60)	4.90 (2.48–9.66)	3.91 (2.35–6.51)	5.80 (2.91–11.56)
NLR	0.15 (0.07–0.30)	0.15 (0.04–0.56)	0.18 (0.05–0.65)	0.20 (0.06–0.72)	–	0.15 (0.02–0.92)
S0S1S2 vs S3	0.883	0.852	0.855	0.915	0.918	0.949
AUROC	(0.822–0.944)	(0.735–0.969)	(0.773–0.937)	(0.823–1.000)	(0.810–1.000)	(0.891–1.000)
Optimal cut-off	296.5	275.5	311.0	296.0	331.5	320.0
Sensitivity	83.3 (58.6–96.4)	85.7 (42.1–99.6)	100.0 (15.8–100.0)	100.0 (47.8–100.0)	100.0 (15.8–100.0)	100.0 (15.8–100.0)
Specificity	80.0 (75.9–83.6)	78.2 (71.3–84.2)	81.2 (72.8–88.0)	75.9 (62.4–86.5)	85.7 (72.8–94.1)	93.2 (83.5–98.1)
PPV	14.4 (11.3–18.2)	13.9 (9.7–19.7)	8.7 (6.1–12.3)	27.8 (19.3–38.2)	22.2 (12.6–36.2)	33.3 (16.3–56.3)
NPV	99.2 (97.7–99.7)	99.3 (95.6–99.9)	100.0	100.0	100.0	100.0
PLR	4.16 (3.15–5.49)	3.94 (2.60–5.97)	5.33 (3.63–7.84)	4.15 (2.59–6.67)	7.00 (3.53–13.90)	14.75 (5.73–37.99)
NLR	0.21 (0.07–0.59)	0.18 (0.03–1.12)	–	–	–	–

Abbreviations: AUROC, area under receiver operator characteristic curve; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; S, steatosis.

Classically, hepatic steatosis is graded on histology by pathologists by visual estimation of extent of hepatic steatosis. CAP gives an objective number which can be used to quantify hepatic steatosis and in turn, can be used objectively for following up patients with hepatic steatosis. An ideal test is one which is not be affected by other factors. Our data also suggests that CAP is a good test for differentiating patients with hepatic steatosis of $\geq$ S1, $\geq$ S2, and $\geq$ S3, irrespective of underlying fibrosis in the liver. There was no significant difference in CAP values in a different stages of fibrosis, when compared in patients with similar grade of steatosis. Practically, CAP can be used for assessing hepatic steatosis in all etiologies and stages of fibrosis. The ease of use of this technique at bed-side, availability of immediate results and the overall low cost as compared to other non-invasive techniques make this an important tool in the assessment of hepatic steatosis.

Our data also suggests that CAP performs best in differentiating patients with no steatosis and presence of any steatosis. Furthermore, CAP performs better in differentiating patients with a difference of 2 or more grades of hepatic steatosis (i.e. differentiating between S0/S2 grades and S0/S3 grades) as compared to differentiating 1 grade of hepatic steatosis (i.e. differentiating between S0 vs S1). Our results are similar to those reported in a prior study in HCV patients and another Indian study involving patients with varied etiologies of liver disease.^{14,19}

However, CAP performed poorly in differentiating patients with S1 and S2 hepatic steatosis; S2 and S3 hepatic steatosis. Although the number of patients with S3 hepatic steatosis was less in our cohort, in previous published studies also similar observations were noted,^{14,18,19,21,22,26,27} suggesting the fact that CAP is

Table 4 Diagnostic Accuracy of Controlled Attenuation Parameter for Differentiating Various Grades of Steatosis.

	S0 vs S1	S0 vs S2	S0 vs S3	S1 vs S2	S1 vs S3	S2 vs S3
AUROC	0.831 (0.776–0.886)	0.936 (0.895–0.976)	0.954 (0.910–0.997)	0.659 (0.558–0.759)	0.715 (0.594–0.837)	0.600 (0.434–0.766)
Optimal cut-off	252.5	284.5	287.5	310.5	324.5	331.5
Sensitivity	78.4 (67.3–87.1)	89.7 (75.8–97.1)	88.9 (65.3–98.6)	66.7 (49.8–80.9)	66.7 (40.9–86.7)	61.1 (35.8–82.7)
Specificity	76.4 (71.5–80.9)	89.1 (85.3–92.3)	90.9 (86.9–93.6)	62.2 (50.1–73.2)	71.6 (59.9–81.5)	59.0 (42.1–74.4)
PPV	42.7 (37.2–48.3)	49.3 (41.2–57.4)	34.0 (26.2–42.8)	48.2 (39.2–57.3)	36.7 (25.9–48.2)	40.7 (28.9–53.8)
NPV	0.28 (0.18–0.44)	98.7 (96.7–99.5)	99.3 (97.6–99.8)	77.9 (68.7–85.1)	89.8 (81.9–94.5)	76.7 (63.5–86.1)
LR+	3.33 (2.65–4.18)	8.25 (5.96–11.43)	9.49 (6.54–13.78)	1.76 (1.22–2.54)	2.35 (1.44–3.83)	1.49 (0.88–2.52)
LR–	0.28 (0.18–0.44)	0.12 (0.05–0.29)	0.12 (0.03–0.45)	0.54 (0.33–0.86)	0.47 (0.24–0.91)	0.66 (0.35–1.25)

Abbreviations: AUROC, area under receiver operator characteristic curve; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; S, steatosis.

suboptimal in differentiating between moderate and severe grades of hepatic steatosis. These results gain importance while assessing potential liver donors. Majority of the liver transplants in India are living donor related. Liver graft steatosis is associated with increased complications,²⁸ and presence of significant fat is considered a contraindication for living donation. Therefore, it is essential to quantify fat accurately. Our data suggests that CAP may not be accurate in differentiating S2 and S3 steatosis, hence liver biopsy may still be of use in this subgroup of patients. MRI based proton density fat fraction (MRI-PDFF) has a very good correlation with quantification of steatosis by liver histology. MRI-PDFF is a noninvasive and accurate method for differentiation of different grades of hepatic steatosis.²⁹ MRI-PDFF is presently being used in clinical trials and donor evaluation algorithms. Thus, suboptimal utility of the CAP in differentiating between consequent grades of steatosis can be circumvented by MRI-PDFF.

On multivariate analysis, we found only BMI and hepatic steatosis to be independent predictors of CAP. The presence of metabolic syndrome was not significantly associated with CAP in the overall cohort. Metabolic syndrome is more common in patients with NAFLD as compared to viral hepatitis.³⁰ Our cohort had more patients with viral hepatitis as compared to NAFLD.

Our study has limitations. We could not compare CAP to other non-invasive tools like NAFLD fibrosis score, fatty liver index, enhanced liver function score, and MRI-PDFF for diagnostic accuracy of steatosis and fibrosis, as these tests are not routinely available in our hospital. We also did not take into account the skin-liver capsule distance which could have influenced the results of fibroscan. We included a heterogeneous group of etiologies in our cohort of chronic liver disease, which may have influenced the CAP values in this group.

In conclusion, CAP can quantitatively estimate hepatic steatosis with good accuracy across various etiologies and irrespective of extent of hepatic fibrosis.

AUTHORS' CONTRIBUTIONS

Gyanranjan Rout: acquisition of data; analysis and interpretation of data; drafting of manuscript; critical revision of manuscript.

Saurabh Kedia: acquisition of data; drafting of manuscript.

Baibaswata Nayak: acquisition of data; analysis of blood samples; drafting of manuscript.

Rajni Yadav: analysis of liver biopsy; drafting of manuscript.

Prasenjit Das: analysis of liver biopsy; drafting of manuscript.

Subrat Kumar Acharya: interpretation of data; drafting of manuscript.

Deepak Gunjan: interpretation of data; drafting of manuscript.

Vishwajeet Singh: statistical analysis; interpretation of the data.

Mousumi Mahanta: acquisition of data; drafting of manuscript.

Swatantra Gupta: acquisition of data; drafting of manuscript.

Sandeep Aggarwal: acquisition of data; drafting of manuscript.

Shalimar: study concept and design; interpretation of data; critical revision of manuscript for important intellectual content.

CONFLICTS OF INTEREST

The authors have none to declare.

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