Title:

Measurement of Controlled Attenuation Parameter, a Surrogate Marker of Hepatic Steatosis in Patients of Nonalcoholic Fatty Liver Disease on Lifestyle Modification- A prospective follow-up study

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Running title: Measurement of CAP in Patients of Nonalcoholic Fatty Liver Disease on Lifestyle Modification

Abstract:

Background

Liver biopsy is a gold standard method for steatosis assessment. However, liver biopsy is an invasive and painful procedure and can cause severe complications therefore it cannot be frequently used in case of follow-up of patients. Non-invasive assessment of steatosis and fibrosis is of growing relevance in non-alcoholic fatty liver disease. To evaluate hepatic steatosis, transient elastography is an optimal technique now days.

Objective

Aim of this study is to evaluate role of measurement of controlled attenuation parameter, a surrogate marker of hepatic steatosis in patients of nonalcoholic fatty liver disease on lifestyle modification.

Methods

In this study, 37 participants were included who were followed up after 6months with transient elastography, blood biochemical tests and anthropometric measurements. The results were analyzed by Multivariate linear regression analysis and paired samples t-test (Dependent t-test) with 95 % confidence interval. Correlation is calculated by Pearson correlation coefficients.

Results

Mean CAP value for assessing hepatic steatosis during 1st consultation (278.57 ± 49.13 dB/m) was significantly improved (P value = 0.03) after 6 months of lifestyle modification (252.91± 62.02 dB/m). Only fasting blood sugar (p value = 0.008), weight (p value =0.000), BMI (p value = 0.000) showed significant positive correlation with CAP. BMI (p value = 0.034) and weight (p value = 0.035) were the independent predictor factors of CAP.

Conclusion

CAP can be used to detect the improvement of hepatic steatosis during follow-up in patients with NAFLD on lifestyle modification.

Introduction:

Non-alcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and is the most prevalent chronic liver disease in both developed and developing countries ranging from simple steatosis and steatohepatitis to fibrosis and long-term complications like cirrhosis and hepatocellular carcinoma [1, 2, 3]. Therefore, early assessment of extent of hepatic steatosis and monitor during therapy are important. The gold standard method to detect hepatic steatosis and steatohepatitis is liver biopsy but it has some limitations like sampling errors, intra- and inter-observer variability, invasiveness of the procedure and major complications like bleeding [4]. To avoid these limitations of liver biopsy and to quantify the hepatic steatosis, controlled attenuation parameter (CAP) being implemented on Fibroscan has been introduced as a non-invasive method in order to evaluate both steatosis and fibrosis simultaneously [5], and is reported to be highly sensitive in detecting low grade steatosis as fat deposition $\geq$ 10% [6]. Several biopsy-controlled clinical studies showed that CAP can adequately detect and quantify hepatic steatosis [7, 8, 9]. Lifestyle modification including weight loss and increased physical activity is vital in managing NAFLD patients irrespective of their underlying liver histology. There is no research using CAP values to assess effect of lifestyle modification, on hepatic steatosis in Indian population with non-alcoholic fatty liver disease.

Therefore, the aim of this study is to evaluate role of measurement of controlled attenuation parameter, a surrogate marker of hepatic steatosis in patients of nonalcoholic fatty liver disease on lifestyle modification

Methods and Materials:

Study design and Study population:

In this prospective follow up study, initially there were 45 NAFLD patients diagnosed by USG abdomen, blood report and history included, but only 37 participants came after 6 months of their 1st consultation. Out of 37 patients, 2

patients were excluded from our study, since they did not follow the life style modification as advised to them. So our study was concentrated on 35 patients. .

Inclusion criteria:

Age > 18 years, Elevated liver enzyme: Alanine aminotransferase (ALT) levels >1.5 times of the upper limit of normal. The upper limit of normal for ALT will be defined as 35 U/L in males and 19 U/L in females [(10)], Fatty liver in USG abdomen

Exclusion criteria:

Alcohol consumption, smoking, Medications & toxins causing fatty liver, Autoimmune & inflammatory disease, Malnutrition, total parenteral nutrition, Severe weight loss, Viral hepatitis and metabolic liver disease (Wilson's disease, Hemochromatosis, etc.), Ascites, Cirrhosis, Chronic illness: e.g. Tuberculosis, Renal failure, Pregnancy, Gastroplasty / bowel resection / bowel bypass surgery.

Study site: Department of Gastroenterology, Manipal Hospital, Bangalore, India.

Study duration: one years and three months. Follow up evaluation (2nd consultation) has been done after six months of first consultation

Methodology:

Anthropometry measurements:

- Weight in kilograms, Height in meters,
- BMI (Body Mass Index): BMI was calculating with the using of weight in kilograms /height in meters^2,
- Blood pressure: Hypertension was diagnosed when a patient had received medicine for hypertension or had systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after taking 5 min rest [(11)].

Others biochemical tests: Blood sample was collected after an overnight fast of > 12 hours.

- Fasting blood sugar

- Total cholesterol: Patients who used cholesterol lowering medication or had a total serum cholesterol level ≥200 mg/dl were classified as having hypercholesterolemia [12].
- Serum fasting Insulin
- HOMA-IR (Homeostatic model assessment of insulin resistance) = {Fasting insulin ((μU/m) × [Fasting glucose (mmol/l) /22.5]} was calculated to assess the insulin resistance. A HOMA-IR value of ≥ 2.5 is taken as an indicator of insulin resistance (IR) in adults [13]
- Alanine aminotransferase (ALT)

CAP and liver stiffness measurement:

The Fibroscan equipped with the M probe placing on the skin between the ribs over the right lobe of the liver was used to capture both CAP and liver stiffness measurement (LSM) values simultaneously. CAP values and LSM values were expressed in units of decibels per meter (dB/m) and kilopascal (kPa) respectively.

During measurement, patients were in supine position with the right arm in abduction. At least 10 valid measurements were obtained in one particular site from each participant. A success rate of ≥60% and the ratio of the interquartile range (IQR) of liver stiffness to the median (IQR/M_{LSM}) ≤30% were considered reliable and used for the final analysis [14].

Steatosis was graded depending on CAP value according to a previous study [5]: S1≥ 238 dB/m, S2≥ 260 dB/m, and S3≥ 293 dB/m.

Hepatic Steatosis	CAP value
S1	≥ 238 dB/m
S2	≥ 260 dB/m
S3	≥ 293 dB/m

CAP: Controlled attenuation parameter; dB/m: Decibels per meter

Trans-abdominal ultrasonography:

Fatty liver was diagnosed based on standard criteria from trans-abdominal ultrasonography (USG) findings using a 3.5 MHz probe. The USG findings of fatty liver are hepatorenal echo contrast, liver brightness, and vascular blurring,

[15], hyper echogenic liver tissue with fine, tightly packed echoes [16]. Trans abdominal Ultrasonography was done by single consultant radiologist who was not aware of the subjects' clinical details or laboratory findings.

Physical activity:

In our study, physical exercise was assessed by self-reported questionnaires which include questions about duration, frequency, and intensity of exercise.

We advised to do regular moderate physical activity which is defined as doing physical exercise of at least moderate intensity at least 3 days per week, for at least 45 minutes each time (The University of California San Diego approach) for an uninterrupted duration of 6 months.

Physical activity of moderate intensity is defined by World Health Organization as requiring a metabolic equivalent task (MET) score of 3.0–6.0 and a typical activity of moderate intensity is ''brisk'' walking at 5.6 km/h on a flat surface requiring 3.8 MET [17]

Metabolic equivalent of functional capacity [18]; 3 MET: Brisk Walking; 4 MET: Raking leaves, gardening, jogging; 5 MET: Climbing 1 flight of stairs, dancing, bicycling, Badminton, swimming; 6 MET: Plying golf, carrying clubs

Diet:

Restriction of caloric intake to 25 -30 kcals/kg/day [19] was advised to our study population. Patients were advised to avoid saturated fats, simple carbohydrates and sweetened drinks, 'fast food diet' and monitored after six months with self-reported questionnaires.

Statistical analysis:

The Statistical Package for Social Sciences (SPSS) is used for statistical analysis. The results were analyzed by Multivariate linear regression analysis and paired samples t-test (Dependent t-test) with 95 % confidence interval. Correlation is calculated by Pearson correlation coefficients. A probability value (P value) below 0.05 is considered significant. Data were expressed as means ± SD (Standard Deviation).

Results and Analysis:

Demographic and Clinical data:

Out of 35 participants, 24 participants (68.57%) were male and 11 participants (31.43%) were female. The mean age of our study population was 34.03 ± 6.28 years. Mean age of male and female participants were 34.25 ± 6.47 years and 33.54 ± 6.14 years respectively.

10 (28.57%) and 4 (11.42%) participants during their 1^{st} consultation and 2^{nd} consultation were insulin resistance respectively. Mean HOMA-IR value for detection of insulin resistance of our study population during 1^{st} and 2^{nd} visit were 2.13 ± 0.63 and 1.75 ± 0.45 respectively.

Out of 35 NAFLD patients, 10 (28.57%) and 8 (28.85%) were hypertensive during their 1^{st} consultation and 2^{nd} consultation respectively. During 1^{st} visit, 17 participants (48.7%) and during 2^{nd} consultation, 11 (31.42%) participants had hypercholesterolemia.

Overall mean BMI of study population at the time of 1^{st} consultation was 27.58 ± 2.56 kg/m^2 but Mean BMI of male participants (26.95 ± 2.29 kg/m^2) was lower than the female participants (28.95 ± 2.70 kg/m^2).

During follow up visit after 6 months of lifestyle modification, overall BMI of study populations was 25.65 ± 2.31 kg/m^2 but BMI of male participants and female participants were 25.27 ± 2.26 kg/m^2 and 26.46 ± 2.31kg/m^2 respectively.

Mean fibroscan value for liver stiffness during 1^{st} consultation and after 6 months of lifestyle modification were 8.35 ± 0.73 kPa and 7.92± 0.76 kPa respectively.

Mean Alanine aminotransferase (ALT) value before and after 6 months of lifestyle modification were 99.57± 14.37 U/L and 68.51 ± 14.70 U/L respectively.

Mean CAP value for assessing hepatic steatosis during 1^{st} consultation (278.57 ± 49.13 dB/m) was significantly improved (P value = 0.03) after 6 months of lifestyle modification (252.91± 62.02 dB/m) (Table 1).

Correlations of CAP with different parameters during First consultation:

Fibroscan (p value = 0.91), ALT (p value = 0.62), height (p value = 0.17), systolic blood pressure (p value = 0.53), diastolic blood pressure (p value = 0.90), serum fasting insulin (p value = 0.37), HOMA IR value (p value = 0.08), Total cholesterol (p value = 0.15) and age (p value = 0.16) were not significantly correlated with CAP. But fasting blood sugar (p value = 0.008), weight (p value =0.000), BMI (p value = 0.000) showed significant positive correlation with CAP (Table 2) (Figure 1).

Multivariate linear regression analysis of CAP with other parameters during first consultation:

Only BMI (p value = 0.034) and weight (p value = 0.035) were the independent predictor factors of CAP (Table 3).

Discussion:

Liver biopsy, the gold standard for assessing hepatic steatosis, is rarely performed due to its limitations. Recently hepatic fat quantification by non-invasive methods has gained importance for the diagnosis and monitoring of hepatic steatosis. Although for detecting hepatic steatosis ultrasound is a useful technique but it cannot be used for grading of hepatic steatosis.

CAP, a recently used novel and noninvasive method for both diagnosing and quantifying hepatic steatosis as it has accuracy, convenience, and economic feasibility.

As far as my knowledge perhaps this is the first study where the effect of lifestyle modification (including diet restriction and moderately intense physical exercise) on CAP in patients with NAFLD has been studied.

In our study, we have seen that after 6 months of lifestyle modification CAP value, a surrogate marker of hepatic steatosis has improved significantly (*p* value = 0.03). All the previous studies involving liver biopsy for the detection of hepatic steatosis in NAFLD patients has showed that lifestyle modification has helped to reduce the hepatic steatosis significantly **[20, 21, 22, 23].** Same result was also found in another study using abdominal ultrasonography for detection of hepatic steatosis in NAFLD patients **[24]**.

Significant improvement in fasting blood sugar and HOMA-IR index (p value = 0.001) were noted in our study. These findings are compatible with those of previous reports [21, 25].

Like the other previous studies [21, 26], in our present study it has been demonstrated that there is reduction in alanine aminotransferase level (p value < 0.001) in NAFLD patients after 6 months of lifestyle modification.\

Conclusion:

Lifestyle modification improves the hepatic steatosis and CAP can be used to detect the improvement of hepatic steatosis during follow-up in patients with NAFLD on lifestyle modification. There is no relation between CAP and Fibroscan score.

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Table 1: Demographic characters and clinical data of study population before and after of 6 months lifestyle modification

Demographic character and clinical data	Before 6 months of lifestyle modification	After 6 months of lifestyle modification	P value
Mean Age (Years)	34.03 ± 6.28		
Mean Weight (kg)	82.42 ± 9.27	74.92 ± 8.60	-----
Number of patients	N =35		
Male: Female	N= 24 (68.57%): N= 11 (31.43%)		
Insulin resistance (HOMA-IR > 2.5)	N= 10 (28.57%)	N= 4(11.42%)	-----
HOMA-IR value	2.13 ± 0.63	1.75 ± 0.45	0.001
Hypertension	N= 10 (28.57%)	N= 8 (28.85%)	-----
Hypercholesterolemia	N= 17 (48.7%)	N= 11 (31.43%)	-----
Mean BMI (kg/m^2)	27.58 ± 2.56	25.65 ± 2.31	<0.001
Mean BMI of Male (kg/m^2)	26.95 ± 2.29	25.27 ± 2.26	-----
Mean BMI of Female (kg/m^2)	28.95 ± 2.70	26.46 ± 2.31	-----
ALT (U/L)	99.57± 14.37	68.51 ± 14.70	<0.001
FBS	96.28 ± 10.59	89.71 ± 9.08	0.002
Fibroscan value (kPa)	8.35 ± 0.73	7.92± 0.76	0.019
CAP (dB/m)	278.57 ± 49.13	252.91± 62.02	0.03
Hepatic steatosis (S0) $^{\Psi}$	N= 7 (20%)	N= 15 (42.85%)	-----
Hepatic steatosis (S1) $^{\Psi}$	N= 5 (14.28%)	N= 4 (11.43%)	-----
Hepatic steatosis (S2) $^{\Psi}$	N= 12 (34.29%)	N= 8 (22.86%)	-----
Hepatic steatosis (S3) $^{\Psi}$	N= 11 (31.43%)	N= 8 (22.86%)	-----

HOMA IR: Homeostatic model assessment of insulin resistance; BMI: Body Mass Index; ALT: Alanine aminotransferase; FBS: Fasting blood sugar; CAP: Controlled attenuation parameter
Ψ: Staging of Steatosis done on the basis of CAP value (Sasso M, et al. Ultrasound Med Biol 2010; 36: 1825-1835)

Table 2: Correlation between the Controlled Attenuation Parameter (CAP) and Clinical Parameters

Parameters	Correlation	P value
Fibroscan	-0.018	0.91
ALT	0.085	0.62
Height	0.232	0.17
Weight	0.733	<0.001
BMI	0.754	<0.001
Systolic blood pressure	0.110	0.53
Diastolic blood pressure	-0.021	0.90
Fasting blood sugar	0.442	0.008
Fasting Insulin	0.154	0.37
HOMA IR value	0.297	0.08
Age	0.239	0.16
Total Cholesterol	0.247	0.15

ALT: Alanine aminotransferase; HOMA IR: Homeostatic model assessment of insulin resistance; BMI: Body Mass Index; Correlation is significant at p value < 0.05 level

Table 3: multivariate linear regression analyses between controlled attenuation parameter (CAP) and different parameters

Parameters	95% CI Lower bound	95% CI Upper bound	P value
Fibroscan	-18.259	18.655	0.98
Age	-1.191	3.177	0.35
Weight	0.171	4.344	0.035
BMI	0.798	18.112	0.034
Fasting blood sugar	-6.008	6.827	0.90
Fasting Insulin	-52.540	53.397	0.98
HOMA IR	-245.098	239.123	0.98
ALT	-1.231	0.826	0.68
Total cholesterol	-0.787	0.094	0.11

BMI: Body Mass Index; ALT: Alanine aminotransferase; HOMA IR: Homeostatic model assessment of insulin resistance; CI: Confidence Interval; P value <0.05 is considered as statistically significant

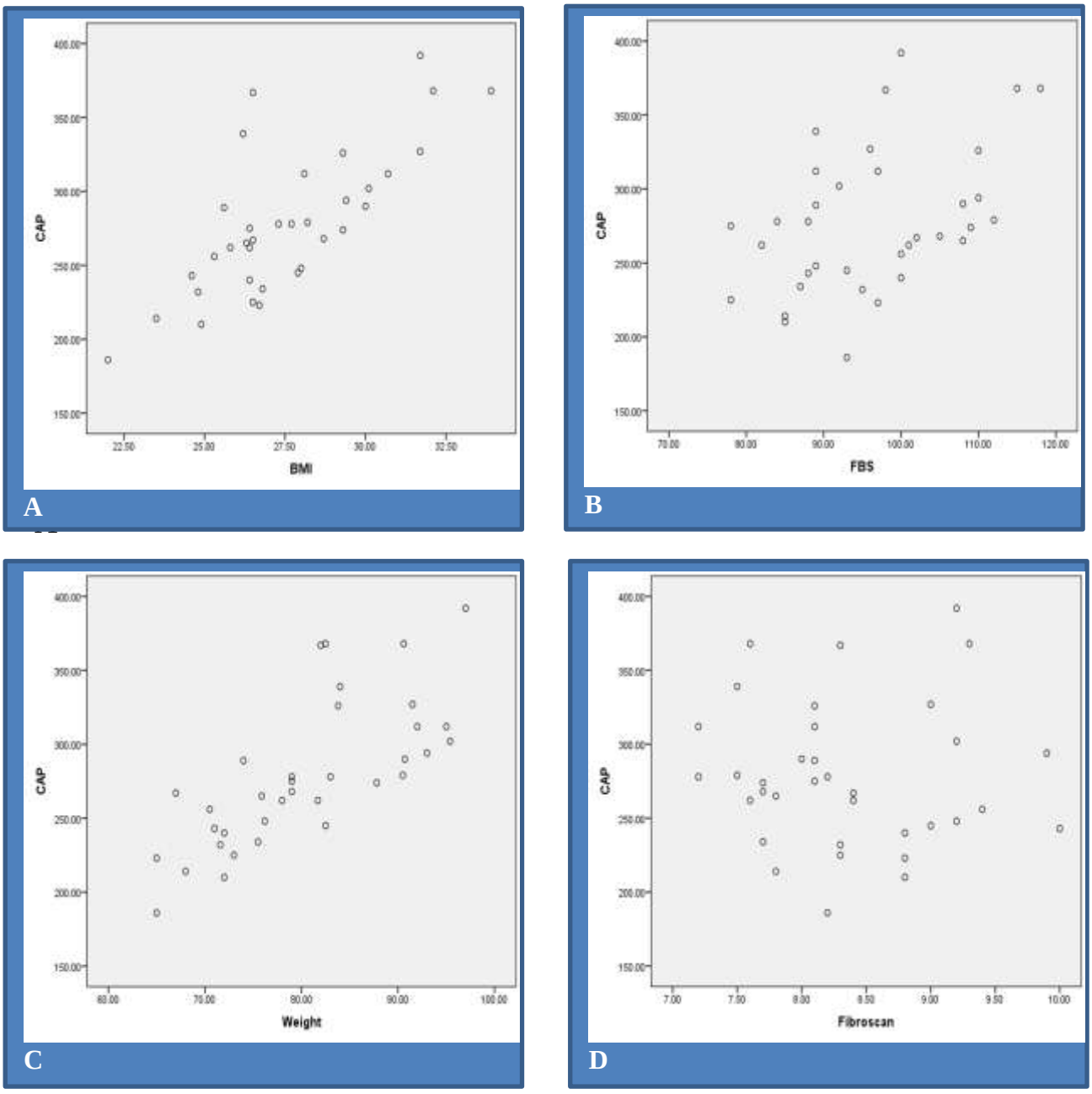


Figure 1: Correlations of CAP (Controlled Attenuation Parameter) with different parameters during First consultation. A: Correlation of CAP with BMI (Body Mass Index); B: Correlation with FBS (Fasting blood sugar); C: Correlation of CAP with Weight; D: Correlation of CAP with Fibroscan