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Correlation of Hepatic Steatosis with Hepatic Fibrosis in NAFLD Patients by Fibroscan

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Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) is a significant cause of liver injury in the world. Transient elastography with controlled attenuation parameter (CAP) is now days commonly used as a non-invasive modality to quantify liver steatosis and stage of Fibrosis in the Liver. This study was done to the correlation of hepatic Steatosis with hepatic Fibrosis in NAFLD Patients by fibroscan. **Subjects and Methods**: All NAFLD patients coming to DMCH from 1/1/18 to 30/11/18 were retrospectively analysed for the presence of any correlation between Steatosis and Fibrosis using a controlled attenuation parameter (CAP) and liver stiffness measurement (kPa), respectively by Fibroscan. Patients with a history of significant alcohol intake, viral infection, severe weight loss, on TPN, on drugs like amiodarone, diltiazem, steroids were excluded. Along with this history of hypertension, diabetes and smoking were noted from the available data. **Results**: The mean CAP of all 446 patients was 310.58 ± 53.55 and the mean kPa was 7.14 ± 4.75. Overall there was a significant correlation between CAP and kPa in all NAFLD patients ($p < 0.000$). This was also true in patients who were more than 20 years of age, who have increased levels of triglycerides and were obese. Patients with S0 steatosis had a mean kPa value of 5.33 and as the steatosis stage worsened to S3 mean kPa value also increased to a maximum of 7.63. **Conclusion:** Quantification of Steatosis by CAP has a significant correlation with the stage of Fibrosis, especially in patients with increasing age, obese and who have high triglyceride levels.

Keywords: Fibroscan, Controlled Attenuation Parameter, Non Alcohlic Fatty Liver Disease, Hepatic Steatosis, Liver Stiffness Measurement

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is now the commonest cause of chronic liver disease worldwide. It causes serious hepatic injury and can lead to Fibrosis, cirrhosis, and hepatocellular carcinoma. Worldwide NAFLD is prevalent in 24%-25% of the general population,[1] whereas its prevalence in India is 9-32%.[2] The prevalence of NAFLD ranges from 22.5% to 44% in children with obesity.[3]

The pathogenesis of NAFLD is still unclear. There are multiple theories. According to the famous " Two Hit theory," its natural history includes 2 phases. In the first phase, there is an accumulation of triglycerides due to insulin resistance leading to Steatosis, and in the second phase, oxidative stress causes a hit on the vulnerable liver leading to inflammation and Fibrosis.[4] Then there is a Multiple parallel-hits theory according to which the "first hit" is a sum of multiple factors that brings down liver defenses.[5] Another theory called the Distinct-hit theory describes NAFLD and NASH as two separate entities, which are associated with insulin resistance but are not related to each other.[6]

Whether triglycerides leading to Steatosis are by themselves toxic for the liver or are just innocent bystanders is still uncertain. A study showed a significant correlation between severe Fibrosis and severe Steatosis associated with hypertriglyceridemia and waist circumference in NAFLD patients.[7] On the other hand, studies are suggesting that the accumulation of triglycerides may be a defense mechanism against liver injury, thus causing an inverse relation between Steatosis and Fibrosis.[8,9]

Currently, the routinely used modalities like laboratory tests and ultrasonography are unable to determine the levels of Steatosis and Fibrosis adequately or cannot be applied as a screening procedure (liver biopsy). Among the non-invasive tests, transient elastography with controlled attenuation parameter (CAP) is very accurate in quantifying liver steatosis and staging fibrosis in patients with NAFLD. The

method is fast, reproducible and reliable, thus allowing for population-wide screening and disease follow-up. There is a dearth of data describing the relationship between the severity of hepatic Steatosis and the severity of Fibrosis, hence this study was conducted to find a correlation between the grade of Steatosis and stage of Fibrosis using Fibroscan in patients with NAFLD.

Subjects and Methods

All NAFLD patients coming to DMCH from 1/1/18 to 30/11/18 were retrospectively analysed for the presence of any correlation between Steatosis and Fibrosis using controlled attenuation parameter (CAP) and liver stiffness measurement (kPa), respectively by Fibroscan. Patients with a history of significant alcohol intake, viral infection, severe weight loss, on TPN, on drugs like amiodarone, diltiazem, steroids were excluded. Along with this history of hypertension, diabetes and smoking were noted from the available data.

Liver stiffness measurement:

Liver stiffness was measured using signals acquired by the Fibro Scan M probe or XL probe based on vibration-controlled elastography. The patient lied in dorsal decubitus position with the right arm in maximal abduction and the operator located a liver portion on the right lobe of the liver which is devoid of large vascular structures using time-motion ultrasound image. When the target area had been located, the M probe/X probe button was pressed to start the measurements. The final result was the median value of ten measurements performed between 25 and 65 mm depth and was expressed in kPa. Procedures with at least ten valid shots and interquartile range (IQR) inferior to 30% were considered reliable. All measurements were performed by the same operator. The threshold used (8.7kPa) for severe Fibrosis was the value determined in a study by Wong et al. in 2010 with a sensitivity of 83.9%, the specificity of 83.2% and a negative predictive value of 94.6%. [10]

Controlled attenuation parameter (CAP):

The controlled attenuation parameter measures liver ultrasonic attenuation at 3.5 MHz. [11] It was computed with the same signals as the one used to measure liver stiffness. Therefore both stiffness and CAP were obtained simultaneously in the same volume of liver parenchyma. The final CAP value was the median value of the ten individual measurements and was expressed in dB/m, [Table 1&2].

Statistical analysis:

Variables were expressed as mean ± standard deviations. Pearson correlation was applied to find a correlation between Steatosis and Fibrosis (measured by CAP and kPa respectively) and the effect of hypertriglyceridemia, waist circumference and diabetes status on this correlation was studied. Significance was defined by $p<0.05$.

Table 1: Values used for staging of Fibrosis [based on Wong et al 2010 and reference values provided with Fibroscan (Echosens)]

Stage of Fibrosis	kPa
F0	0-6
F1	6.1-7.2
F2	7.3-8.2
F2-3	8.3-8.7
F3 (severe fibrosis)	8.8-10.5
F3-4 (severe fibrosis)	10.6-11.8
F4 (cirrhosis)	>11.8

Table 2: Values used for grading ofSteatosis [based on reference values provided with Fibroscan (Echosens)]

Grade of Steatosis	CAP (db/m)
S0	<237.7
S1	237.7-259.4
S2	259.4-292.3
S3 (severe steatosis)	>292.3

Results

446 patients were included in the study and out of these, 70% were males. The mean AST was 30.5± 2 and the mean ALT was 35.4 ± 1.5. The mean age was 46.7 years. Out of 446 patients; with the available data 55.1% of patients were obese (BMI ≥ 25), 2.7 % of patients were hypertensive, 19.3% of patients were diabetic and 56.7 % of patients have increased triglyceride levels (≥ 150mg/dl). Baseline characteristics of NAFLD patients. [Table 3].

The mean CAP of all patients was 310.58 ± 53.55 and meant kPa was 7.14 ± 4.75. It was found that those who had aged more than 20 years had higher mean CAP and mean kPa than those who were young. Similarily higher mean CAP and mean kPa was found in patients who were obese or had hypertension or diabetes or hypertriglyceridemia than those who were not obese or normotensive or non-diabetic or had normal triglycerides level respectively. This is shown in [Table 4]. Overall there was a significant correlation between CAP and kPa in all NAFLD patients ($p<0.000$) shown in [Figure 1 & Table 5]. This was also true in patients who were more than 20 years of age, who have increased levels of triglycerides and were obese. [Table 5].

It was tried to find out the percentage of patients with severe fibrosis (≥F3) in each grade of Steatosis and it was found that as the stage of Steatosis worsens there is an increase in several patients with severe Fibrosis (increased from 0% in S0 to 23.38% in S3). It was also found that patients with S0 steatosis had a mean kPa value of 5.33 and as the steatosis stage worsened to S3 mean kPa value also increased to a maximum of 7.63 as shown in [Table 6,7 & Figure 2]. Patients with multiple components of metabolic syndrome were compared using their mean CAP and kPa values, but no particular pattern was observed [Table 8].

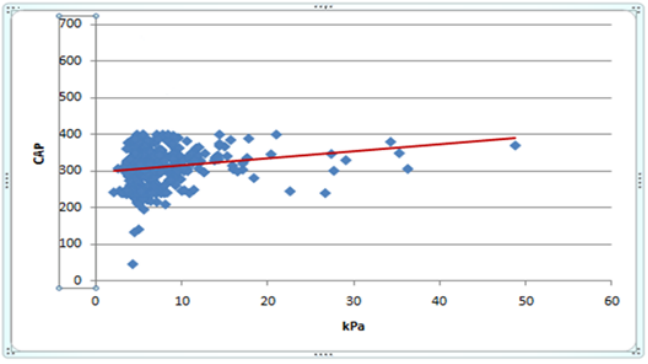

Figure 1: CAP value(y-axis) plotted against values of LSM (x axis)

Overall there was a significant correlation between CAP and kPa in all NAFLD patients (p <0.000) shown in [Figure 1].

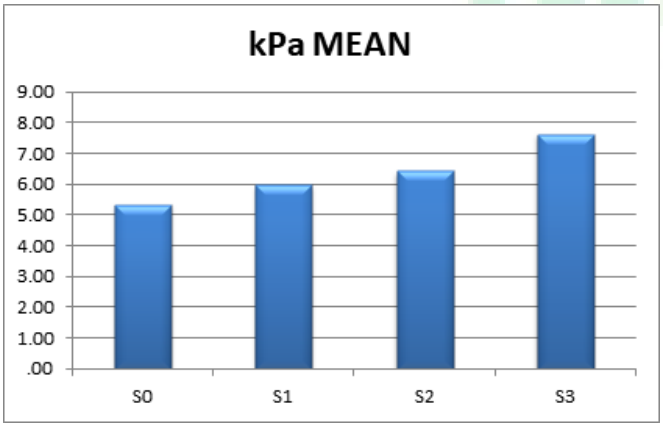

Figure 2: Mean kPa value of patients with each grade of steatosis

Discussion

Our study shows a significant correlation between Steatosis and Fibrosis, especially in patients who were more than 40 years old and have hypertriglyceridemia and obesity. This is expected as Steatosis precedes inflammation and Fibrosis in the natural history of NAFLD.[12]

This is similar to a study conducted by Marty et al. wherein a significant correlation between Steatosis and Fibrosis associated with hypertriglyceridemia and waist circumference was found in diabetic patients.[7] Similarly, a Japanese study conducted by Cho et al found a significant correlation of Steatosis and Fibrosis in the pediatric obese group.[3] However, two experimental studies have suggested that Steatosis protects rather than promote Fibrosis. Listenberg et al. have reported that the incorporation of fatty acids in the hepatic triglyceride pool prevented their pro-apoptotic effects.[8] Yamagushi et al. found more inflammation and Fibrosis in mice whose hepatic triglyceride synthesis was reduced due to DGAT inhibition.[9] If triglycerides protected the liver from Fibrosis, we should have found an inverse relation between Steatosis and Fibrosis.

In the present study, we found a high rate of severe liver fibrosis(19.05 %) and severe Steatosis (69%) which could be expected as all patients were those who came to the hospital for a medical reason like fatigue, right hypochondria heaviness, uncontrolled diabetes etc. Studies using non-invasive methods reported severe Fibrosis in 5.6% of outpatients and up to 15% in hospitalized patients.[13,14] As compared to non-invasive methods, in a study in which biopsy was performed on diabetic patients the rate of severe Fibrosis reached 35%.[15] In another study conducted by Marty et al. using Fibroscan; 41% of diabetic patients had severe Steatosis.[7]

NAFLD and metabolic syndrome (MetS) are associated with insulin resistance (IR) is the common pathogenetic factor. The presence of MetS increases the risk of development of NAFLD and more the number of MetS components present is the risk of severe NAFLD.[16] From the available data, we found that patients those who had higher BMI or high triglyceride levels had higher Steatosis and Fibrosis as compare to those who have normal BMI or normal triglyceride level. Similarily diabetic patients (DM) or patients with hypertension (HTN) had higher Steatosis and Fibrosis and various studies have shown similar results.[3,7] The presence of obesity or hypertriglyceridemia was associated with a significant correlation of Steatosis and Fibrosis. However, the presence of DM or HTN was not associated with any correlation between the two, which could be because of a small number of DM and HTN patients in the present study. We also tried to analyse the relationship between the number of MetS components and the degree of Steatosis and Fibrosis, but we did not find any relation which could be because of the retrospective nature of data.

Table 3: Baseline characteristics of NAFLD patients

		No. of Patients	Percentage
Total		446	-
Male		312	70.00%
Female		134	30.00%
AST		446	Mean = 30.5±2
ALT		446	Mean = 35.4 ± 1.5
Age	0-20	9	2 %
	21-40	141	31.61 %
	> 40	296	66.36 %
BMI	≤ 22.9	14	3.1 %
	23- 24.9	38	8.5 %
	≥25	246	55.1 %
	Data Not Available	148	33.1 %
HTN	Yes	12	2.7 %
	No	304	68.20%
	Data Not Available	130	29.10%
DM	Yes	86	19.30%
	No	230	51.60%
	Data Not Available	130	29.10%
Triglycerides	Increased	253	56.70%
	Normal	27	6.10%
	Data Not Available	166	37.20%

Table 4: Mean CAP and kPa values in NAFLD patients

		No. of Patients	Mean CAP	Mean kPa
Total		446	310.58 ± 53.55	7.14 ± 4.75
Male		312	310.03 ± 53.3	6.98 ± 4.31
Female		134	311.86 ± 54.31	7.49 ± 5.63
Age	0-20	9	258.67 ± 51.03	5.89 ± 1.34
	21-40	141	313.26 ± 59.49	6.31 ± 2.79
	> 40	296	310.88 ± 49.9	7.48 ± 5.18
BMI	≤ 22.9	14	286.14 ± 56.61	5.4 ± 1.15
	23- 24.9	38	298.87 ± 44.73	5.37 ± 1.05
	≥25	246	310.03 ± 50.51	7.22 ± 4.15
HTN	Yes	12	329 ± 41.99	7.82 ± 2.36
	No	304	309.11 ± 54.61	6.94 ± 4.2
DM	Yes	86	319.35 ± 47.97	8.94 ± 6.02
	No	230	306.32 ± 56.12	6.24 ± 2.87
Triglycerides	Increased	253	308.92 ± 51.56	7.15 ± 4.02
	Normal	27	294.15 ± 43.58	5 ± 0.9

Our study does have some limitations as it is a retrospective study, so complete data of all patients was not available. The second limitation is the lack of histological confirmation of our results. It was impossible to perform a liver biopsy in

Table 5: Correlation of CAP and kPa

Correlation of CAP & kPa	N	Pearson Correlation	P-Value
Overall	446	.167**	.000
Age 0-20	9	0.392	.296
Age 21-40	141	.252**	.003
Age more than 40	296	.164**	.005
Only Male	312	.192**	.001
Only Female	134	0.126	.146
Normal Triglycerides	27	0.185	.355
Increased Triglycerides	253	.175**	.005
BMI Normal	14	0.049	.867
BMI Overweight	38	-0.102	.543
BMI Obese	246	.183**	.004
HTN	12	0.206	.520
DM	86	0.149	.172

Table 6: Percentage of patients with a particular stage of Fibrosis in each grade of Steatosis

S Grade	F Stage							Total
	F0	F1	F2	F2-F3	F3	F3-F4	F4	
S0	16	2	1	0	0	0	0	19
	84.21%	10.53%	5.26%	0.00%	0.00%	0.00%	0.00%	100.00%
S1	53	9	4	1	2	2	2	73
	72.60%	12.33%	5.48%	1.37%	2.74%	2.74%	2.74%	100.00%
S2	24	9	3	3	6	0	1	46
	52.17%	19.57%	6.52%	6.52%	13.04%	0.00%	2.17%	100.00%
S3	155	51	20	10	26	10	36	308
	50.32%	16.56%	6.49%	3.25%	8.44%	3.25%	11.69%	100.00%
Total	248	71	28	14	34	12	39	446
	55.61%	15.92%	6.28%	3.14%	7.62%	2.69%	8.74%	100.00%

Table 7: Mean kPa value of patients with each grade of Steatosis

S Grade	kPa Mean	N	SD	P-Value
S0	5.33	19	1.05990	.008
S1	5.96	73	3.64354	
S2	6.42	46	2.56728	
S3	7.63	308	5.25000	
Total	7.1368	446	4.74547	

all patients as it is invasive and has its risks. However, the diagnostic performances of Fibroscan and CAP have been validated in several studies.[17,18] The third limitation is that inflammatory markers were not analysed. However, a large number of patients (446) were analysed and that too by a single operator. Also XL probe was used for obese patients to get accurate values as the use of M probe had resulted in false high values in this subset of patients.[19] We are also conducting a prospective longitudinal study to overcome some of the limitations.

Table 8: Mean CAP and kPa of patients with presence of multiple metabolic syndrome components

Metabolic syndrome components	No. of Patients	Mean CAP	Mean kPa
DM, HTN, Obese, Triglycerides	5	325.4 ± 36.34	9.28 ± 2.52
DM, HTN, Obese	5	325.4 ± 36.34	9.28 ± 2.52
DM, HTN, Triglycerides	6	314.5 ± 42.07	8.8 ± 2.54
DM, Obese, Triglycerides	58	324.84 ± 46.07	9.7 ± 5.76
HTN, Obese, Triglycerides	10	340.4 ± 35.45	8.22 ± 2.37
DM, HTN	6	314.5 ± 42.07	8.8 ± 2.54
DM, Obese	74	320.88 ± 46.27	9 ± 5.5
DM, Triglycerides	66	320.76 ± 49.17	9.17 ± 5.6
HTN, Obese	10	340.4 ± 35.45	8.22 ± 2.37
HTN, Triglycerides	11	333.09 ± 41.46	8.05 ± 2.31
Obese, Triglycerides	211	311.77 ± 51.29	7.47 ± 4.31

Conclusion

Quantification of Steatosis by CAP has a significant correlation with the stage of Fibrosis, especially in patients with increasing age, those who are obese and who have high triglyceride levels. Prevalence of NAFLD is on the rise and by taking efforts at the stage of Steatosis (reversible) like lifestyle modifications or development of drugs, we can prevent Fibrosis which is largely irreversible.

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