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## **Research Article**

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# Non alcoholic fatty liver disease and its relationship with hsCRP in type 2 diabetes mellitus

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#### **ABSTRACT**

**Background:** Diabetes mellitus is assuming an epidemic form in India. Non-alcoholic fatty liver disease worldwide is increasing too and is a major cause of liver transplant in the west. Diabetes is a strong risk factor for non-alcoholic fatty liver disease, and some of them go on to develop steatohepatitis which is associated with a more rapid disease progression leading to chronic liver disease including hepatocellular carcinoma. This association of diabetes with fatty liver disease is least investigated. Liver biopsy is not routinely done in clinical practice and various non-invasive markers for fatty liver or steatohepatitis are used frequently to identify patients at risk of fatty liver disease.

**Methods:** 116 Type 2 Diabetics Mellitus on therapy with oral anti-diabetic drugs and atorvastatin for at least 3 months' duration were included and sonologically evaluated for fatty liver after proper exclusion of other causes of fatty liver. Serum hsCRP, an acute phase reactant, was measured in them. Liver function tests, BMI and other necessary investigations were done. 144 healthy controls were also taken.

**Results:** The absolute risk of developing fatty liver was significantly high in T2 diabetics compared to controls. hsCRP was significantly associated with fatty liver and uncontrolled glycemic status. In addition AST/ALT > 1 also showed significant differences amongst the same groups.

**Conclusions:** High hsCRP is a cheap, easily available laboratory marker to suspect fatty liver and possibly steatohepatitis in T2 Diabetics in our region. It can identify a subgroup of diabetic patients in whom liver biopsy may be advisable to confirm steatohepatitis which is important for prognosis and therefore need aggressive intervention.

Keywords: NAFLD, Fatty liver, Diabetes mellitus, NASH, hsCRP, NAFLD markers, Type 2 diabetes

## INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is an increasing health problem, commonly associated with comorbidities like hyperlipidemia, diabetes or metabolic syndrome. The pathogenesis of NAFLD involves a multi-hit process including insulin resistance, oxidative stress, apoptotic pathways, and adipocytokines. NAFLD is a spectrum of liver disease ranging from simple steatosis to steatohepatitis (NASH). Steatohepatitis is characterized by steatosis, lobular inflammation, ballooning and fibrosis. Initially believed to be a benign

disorder, longitudinal studies indicate that fibrosis progression occurs in about a third of NAFLD patients. In NAFLD subjects, patients with biopsy-proven NASH (estimated prevalence of 3-5% in the US) have been convincingly shown to progress to cirrhosis, liver failure and hepatocellular carcinoma.<sup>2</sup> Hence, NASH has a higher risk of disease progression than simple steatosis alone which is relatively benign. Presently, a combination of ongoing global epidemic of diabetes in an aging population is likely to lead to an increasing prevalence of NASH in future since both are established risk factors for hepatic fibrosis.<sup>3</sup>

NAFLD is considered to occur commonly in type 2 diabetes mellitus (T2 DM), with an estimated prevalence ranging from 21% -- 78%. Insulin resistance, obesity and increased concentrations of plasma fatty acids, which are characteristics of T2 DM are considered to increase the risk for development of fatty liver. Many clinical symptoms of fatty liver are nonspecific or silent, although, overall, it has been established that diabetes is an independent risk factor for death in patients with NAFLD 1.0 thus implying that detection of NAFLD in T2 DM may be an important management issue.

Liver produces high sensitivity C-reactive protein (hs-CRP), an acute-phase reactant that rises in bacterial infections, immuno-inflammatory diseases and malignant disorders. Prospective studies have shown that high hs-CRP levels predict the development of type 2 diabetes mellitus (T2DM). More importantly, CRP has also been shown to be significantly associated with histologically proven NASH. There are limited number of studies implicating elevated serum hsCRP in NAFLD. One study from Japan even concluded that high hsCRP could distinguish NASH from simple steatosis and also correlated with the severity of liver fibrosis in NAFLD. There are no Indian studies that we could find in the literature correlating hsCRP with T2DM and NAFLD.

We hypothesized that since hsCRP may correlate with presence of underlying NAFLD in T2 DM or even NASH, it is associated with sonologically proven NAFLD too.

With this background we conducted this study to find out any correlation between raised hsCRP level and ultrasonographically detected NAFLD in diagnosed T2 DM patients.

## **METHODS**

Patients with T2 DM (n:116, M:F 73:43) who attended Assam Medical College and Hospital were studied consecutively after fulfilling the inclusion criteria. Institutional ethics committee approval and informed consent from patients were obtained. T2 DM was diagnosed by ADA (American Diabetic Association) criteria – HbA1C  $\geq$  6.5%, with fasting plasma glucose  $\geq$ 126 mg/dl or 2 hour post meal plasma glucose ≥ 200 mg/dl. All were under treatment with oral anti-diabetic agents and atorvastatin for atleast 3 months. Any case with ongoing infection like respiratory tract infection, urinary tract infection, skin infections/foot ulcers etc., fever, past abdominal surgery, presence of biliary diseases, serious co-morbid states (angina, heart failure, hypertension or past/present myocardial infarction), on medications known to cause fatty liver (calcium channel blockers, corticosteroids, estrogen, amiodarone, etc), and pregnancy were excluded from the study. Hepatitis B and C, and ANA positive cases, alcohol ingestion of >20gm, and any other chronic liver disease if detected during sonography were also excluded. Alcohol intake was assessed by direct questioning of the patients and their relatives separately. Clinical, laboratory and imaging studies were done to find out the status of diabetes. HbA1c level of > 7% was considered to be uncontrolled T2 DM whereas those with < 7% were considered to be controlled (as per American Diabetic Association criteria). Ultrasonography of Abdomen was done to detect the presence or absence of Fatty liver by a sonologist blinded to all clinical/biochemical datas. Body mass index (BMI) was measured by the patient's weight in kilograms divided by the square of his height in meters (kg/m<sup>2</sup>). However, while the WHO accepts a BMI of 25 to be overweight, in 2008 the Health Ministry of India reduced the index-value to 23 in an effort to sensitize the people. Hence, in our study we took a BMI  $\geq$  23 to define obesity. 144 healthy non-diabetic, non-alcoholic controls were evaluated by USG for fatty liver after obtaining consent.

Liver function tests (serum bilirubin, AST, ALT, serum protein and fractions, serum alkaline phosphatise), HbA1c, hsCRP, ANA, hepatitis B and C viral serology were done in all cases. Serum hs-CRP was estimated by Dimension RxL Max autoanalyzer using Particle Enhanced Turbidimetric Immunoassay (PETIA) Technique (Normal Value: 0–3 mg/L). Ultrasonography of abdomen was done by Siemens Acuson Antares 5 Ultrasound System with 3.5MHz transducer by a qualified radiologist.

Statistical analyses for all continuous variables were expressed as mean  $\pm$  SD, whereas categorical variables were expressed as frequencies and their percentages. Student t test was performed for continuous variables, and Fishers exact test for categorical variables. P value of  $\leq 0.05$  was considered as significant.

## **RESULTS**

The mean age was  $43.4\pm13.6$  years and the mean duration of T2DM from diagnosis was  $5.8\pm6.9$  years (range 1.2 to 11.7 years). 39 (34%) T2DM cases and 16 (11%) of controls were detected sonologically to have Fatty liver (Table 1). In this study, this indicates that T2DM increases the risk of NAFLD by 20.43% (at 95% CI of 10.21% - 30.65%).

Table 1: Showing relevant demographic features of T2DM and Controls.

	Total <i>n:116</i>	Controls n:144	P value
Age	43.4±13.6	42.8±9.7	= 0.6788
BMI	24±09	22±07	= 0.0449
Male	73	89	
Female	43	55	
Fatty Liver	39 (34%)	19 (13%)	- 0.0001
No Fatty	77 (66%)	125 (87%)	= 0.0001

Liver			
RAISED CRP	53 (46%)	N/A	
Raised AST < 3 x ULN	64 (55%)	N/A	
Raised AST > 3 x ULN	34 (29%)	N/A	
Raised ALT < 3 x ULN	33 (28%)	N/A	
Raised ALT > 3x ULN	24 (21%)	N/A	

In the diabetics, hsCRP level was raised in 53 cases (46%) showing a mean level of 4.9±1.1 mg/L (Table 2). Further analysis showed that 23 of 39 T2DM cases with fatty liver had raised hsCRP compared to 30 of 77 cases without fatty liver, and the difference was statistically significant. The mean hsCRP level in fatty liver group was also significantly higher. They also showed a higher value when diabetes was uncontrolled (p <0.0001) (Figure 1). The same trend was observed in respect of AST/ALT ratio with a value > 1 in diabetics with fatty liver or uncontrolled diabetes compared to their respective counterparts, showing significant statistical differences (p<0.0001). Raised AST and ALT > 3 times upper normal value were seen in 29% and 21% of all the diabetics respectively.

Table 2: Showing the distribution of hsCRP and AST/ALT ratio in study group in relation to glycemic control and fatty liver.

	n	High hsCRP mg/dl	p value	Mean hsCRP mg/dl	P value	AST/ALT ratio > 1	p value
Total DM	116	53 (46%)		4.9±1.1		47	
Fatty Liver	39	23 (20%)	= 0.0497	4.3±0.8	< 0.0001	30	<0.0001
No Fatty Liver	77	30 (26%)		3.4±0.6	< 0.0001	17	
Controlled DM	71	21 (18%)	< 0.0001	$3.8 \pm 0.3$	< 0.0001	11	< 0.0001
Uncontrolled DM	45	32 (28%)		4.1±1	< 0.0001	28	

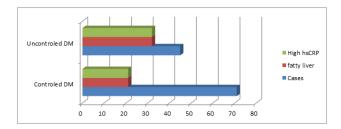


Figure 1: Mean hsCRP level.

#### **DISCUSSION**

The correlation between diabetes mellitus and NAFLD is robust as shown by a study in Japanese adults where it was found that 27% with normal fasting glucose, 43% with impaired fasting glucose and 62% with newly diagnosed diabetes had NAFLD. Hence, NAFLD rises in proportion to blood glucose level. <sup>16</sup>

Our study shows that T2DM has a significantly high incidence of NAFLD compared to non-diabetics with a high calculated risk of 20.43% confirming that diabetes is a major risk factor for NAFLD in our population, even when they are on Atorvastatin. Limited results suggest that the prevalence of NASH is as high as 11% in the general population<sup>3</sup> which is almost same in our control group.

Only a small number of patients with NAFLD eventually ends up with end-stage liver disease and hepatocellular carcinoma. Approximately 5% of NAFLD patients develop cirrhosis over seven years with 1.7% dying from complications of liver cirrhosis and, importantly, they also have a higher risk of all cause mortality than the general population.<sup>9</sup>

The prevalence of NAFLD in T2DM in this study is 34% and is consistent with other reports. 4,5,6 If we consider hsCRP as a marker for NASH, [12] then it is 46%, which means that hsCRP may be raised in T2DM without NAFLD too. However, our study additionally shows a significantly higher hsCRP value in T2DM with fatty liver compared to T2DM without NAFLD and hence hsCRP may indicate that NAFLD in T2DM are at a greater risk of NASH or have already developed it. These observational variations in our study may be explained by the reports that NASH is disproportionately represented in T2DM, and silent but significant hepatic fibrosis and cirrhosis can be present in upto 20% of T2DM. 17

Uncontrolled diabetes with higher insulin resistance leads to hepatic macrosteatosis due to increased lipolysis with dysregulation of free fatty acids. Therefore probably they have a tendency for steatohepatitis compared to controlled T2DM whose insulin resistance presumably

improves with glycemic control. This may explain the significantly higher hsCRP values in uncontrolled T2DM cases in our study. This is also corroborated by the finding that AST/ALT ratio of >1 (commonly used to differentiate NAFLD from alcohol related and other liver diseases), was statistically significantly different between controlled and uncontrolled T2DM, signifying possibly more dominant hepatic inflammation in uncontrolled T2DM. This difference was also seen when we compared T2DM with and without NAFLD. In addition, we found that about 1/3<sup>rd</sup> to 1/5<sup>th</sup> of all T2DM had AST and ALT values > 3 times upper normal limit suggesting that overall hepatic derangement in T2DM is common in our region. Whereas AST/ALT elevation in NAFLD is generally modest (2 to 3 fold), biopsy proven NASH shows higher values. 18

The mean age did not differ significantly between the T2 DM and controls while there was difference in mean BMI between the two groups (Table II). It is known that NAFLD is the commonest cause of asymptomatic hypertransaminesemia. Although NASH occurs in a minority of patients with NAFLD, insulin resistance is the major player in it's pathogenesis as compared to obesity. [19] Hence, we can conclude from our study that is associated with T2DM significant hypertransaminesemia in 20-30% cases. This is important; as it is proved that in NAFLD 20% cases on presentation may demonstrate significant liver disease on biopsy and histology. Therefore we believe that T2DM with high hsCRP and/or significant hypertransaminesemia, may be candidates for a liver biopsy to rule out NASH.

Liver biopsy in NAFLD, (the "gold standard" to differentiate steatosis from NASH), is not routinely done primarily because of sampling error and hence it's value in assesing NAFLD in clinical practice remains uncertain<sup>20</sup> and recent studies have emphasized its sampling variability and inter-observer discordance. Therefore, many non-invasive serum markers or scoring systems to predict NAFLD have been proposed with varying degrees of sensitivity and specificity. Estimation of hsCRP is cheap and easily available. In addition, high hsCRP may distinguish NASH from simple steatosis and also indicate the severity of liver fibrosis in addition, but needs validation in future studies.

The definite limitation of our study was that liver biopsy was not done. Although liver biopsy is currently the best way to confirm NAFLD and distinguish between simple fatty liver and NASH, no guidelines or firm recommendations can be made as for when and in whom it is indicated<sup>24</sup> and we believe that high hsCRP maybe helpful in this regard. However, imaging studies still play a key role in NAFLD diagnosis and ultrasonography is the most widely used, being easily available and least invasive (sensitivity 60-90%, specificity 90%).<sup>25</sup> But in mild cases of NAFLD it's utility may be restricted.

#### **CONCLUSION**

The risk of developing NAFLD in T2DM is significantly more. hsCRP is significantly high in T2DM especially in uncontrolled cases and in those with sonologically detected NAFLD. This may be a sub-group at high risk of having NASH which has a more aggressive disease progression leading to hepatic fibrosis/cirrhosis compared to simple steatosis. Further validation studies in this group to correlate hsCRP alongwith AST/AL > 1 and liver biopsy may be advocated for prognostication, given the rising epidemic of obesity, T2DM and metabolic syndrome across the world and especially in Asians who are phenotypically unique with a greater risk for developing NAFLD. Hence, future studies are needed to address this important aspect of diabetic "complication" which is not often given enough importance in management protocols.

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