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Original Research Article

Magnitude of nonalcoholic fatty liver disease (NAFLD) and concomitant risk factors in patients with type 2 diabetes mellitus

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a distinct hepatic condition that is strongly associated with insulin resistance and type 2 Diabetes Mellitus (T2DM). This study was designed to assess the magnitude of NAFLD and its concomitant risk factors among patients with T2DM.
Methods: In a hospital based observational descriptive study, 150 patients with T2DM were submitted to a complete clinical and laboratory evaluation; abdominal ultrasonography for NAFLD detection and grading. Patients with known chronic liver disease and history of alcohol intake were excluded. They were divided into fatty and non-fatty liver group: evaluated, compared and statistical analysis done.
Results: Out of 150 patients with T2DM, 104 (69.33%) had fatty liver on USG. 42.67% had grade 1, 24% had grade 2, and the remaining 2.67% had grade 3 fatty changes in liver. The severity of NAFLD increased with increasing age (r-value = +0.554, p<0.001). Statistically significant difference in Body Mass Index (29.64±4.36 v/s 25.94±3.25 kg/m2, p<0.001) and triglycerides (175.47±24.82 v/s 128.53±26.66 mg/dl, p<0.001) was observed in the fatty liver group as compared to non-fatty liver group.
Conclusions: The magnitude of NAFLD is higher in type 2 diabetic patients. Older age group, Body Mass Index (BMI) and triglyceride (TG) had significant relationship with the presence of fatty liver. Ultrasonographic evidence of fatty liver with older age, elevation of triglyceride level and increasing BMI should be taken seriously as a predictor of severity of NAFLD.

Keywords: Age, BMI, NAFLD, T2DM, Triglycerides

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a distinct hepatic condition characterized by abnormal fat accumulation in liver cells, mostly in the form of triglycerides, which exceed 5% of liver weight: histologically resembling alcohol induced liver damage. Nonalcoholic fatty liver disease (NAFLD) appears to be the hepatic manifestation of metabolic syndrome, and is increasingly recognized as a major contributor to the burden of chronic liver disease world-wide.[1] The term NAFLD is used to describe a wide array of fatty liver changes from simple steatosis to steatohepatitis, cirrhosis and hepatocellular carcinoma (HCC), in the absence of excessive alcohol intake.[2] Epidemiological studies suggest the prevalence of NAFLD to be around 9-32% in general Indian population, with a higher incidence amongst overweight/obese and diabetic/prediabetic patients.[3-9] The prevalence of NAFLD in T2DM patients in India is reported to be in range of 12.5-87.5%.[5-7, 9]

Dyslipidemia and insulin resistance both are strongly associated with the prevalence of NAFLD. Prevalence of hypertriglyceridemia in different NAFLD case series is

quite variable, but all evidence linked it to NAFLD.[10,11] NAFLD is usually either discovered incidentally during routine laboratory investigation or work-up of conditions such as diabetes, hypertension, or morbid obesity. Elevated ALT levels or sonographic evidence of fatty liver is sometimes noted during work-up of suspected gall stone disease.

NAFLD has a wide clinical spectrum, and its severity can be measured by several methods. Liver biopsy represents the best diagnostic test for staging liver steatosis, inflammation and fibrosis, but medical and ethical considerations limit its use in subjects with non-progressive fatty liver conditions.[12,13] Liver ultrasonography which had a sensitivity and specificity of 83% and 100%, respectively, as compared with histologic finding as the gold standard method demonstrates a good correlation with histological findings of fatty infiltration.[14] The association of T2DM with microvascular and macrovascular complications is well established, but the association of T2DM with NAFLD as a major complication has been recently recognized. There is evidence that T2DM patients with NAFLD are at higher risk of developing cirrhosis compared to non-diabetic patients.[15,16]

Although cardiovascular disease is the major cause of excess morbidity and mortality in type 2 diabetes, liver failure may also be a threat to patients with type 2 diabetes NAFLD.[17,18] An observational descriptive study was conducted to find out the magnitude of NAFLD in patients with type 2 DM and to find the risk factors predicting the same.

METHODS

The present study is a hospital based observational descriptive study conducted at S.M.S. Medical College and Hospital in Jaipur. 150 subjects with type 2 DM were enrolled in the study to assess the magnitude of NAFLD among them. Cases with known hepatic disease, HBs antigen or Anti-HCV positivity, alcoholic, type 1 DM, pregnancy, ingestion of hepatotoxic drug(s) were excluded. All patients gave informed consent and the study protocol was approved by the ethics committee of the hospital.

Out of 150 subjects with type 2 diabetes mellitus, 104 subjects (69.33%) were detected to have fatty liver ultrasonographically and 46 subjects (30.67%) without fatty liver served as a control. The baseline physical characteristics and investigations were compared in these two groups using statistical tests of significance.

Detailed physical examination was carried out with emphasis on blood pressure, height and weight. Blood samples were collected after at least twelve hours overnight fasting for complete blood counts, blood glucose (fasting and postprandial), glycated haemoglobin (HbA1c), blood urea, serum creatinine, liver functions (total bilirubin, direct and indirect bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transferase, serum total protein, serum albumin, serum globulin), thyroid hormone levels (TSH, fT3, fT4), HBsAg, Anti-HCV antibody, lipid profile (including total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C] and triglycerides [TG]) in all participants.

Abdominal ultrasound scanning was performed in all participants by a trained radiologist using a Hitachi EZU-MT28-S1 scanner, convex EVP-C-514 probe with a 2.5-3.5 MHz linear transducer. Each subject was examined in the supine and 60° left lateral positions during quiet inspiration. Fatty liver was defined by the presence of at least 2 out of 3 abnormal findings on abdominal ultrasonography: diffusely increased echogenicity ("bright") of liver with liver echogenicity greater than kidney, with vascular blurring, and deep attenuation of ultrasound signal.

Three grades are defined for assessment of fatty liver

- Grade 1: Mild fatty liver (mild increase in echogenicity of liver and normal view of diaphragm and vascular margins in the liver).
- Grade 2: Moderate fatty liver (moderate increase in echogenicity of liver and blurring of the vascular margins in the liver).
- Grade 3: Severe fatty liver (severe increase in echogenicity of liver and severe blurring of diaphragm and the vascular margins in the liver).[14]

According to NIH19 (National Institute of Health 2000, India) overweight was defined as a body mass index (BMI) between 25 and 29.99 kg/m^2, and obesity as BMI equal or above 30 kg/m^2. Patients with one of the criteria: LDL-C >100 mg/dL, total cholesterol >200 mg/dL, triglycerides >150 mg/dL, or HDL-C < 40 mg/dL in males and <50 mg/dL in females were considered to have dyslipidemia. Type 2 DM was defined according to guidelines of American Diabetes Association.[20]

Data was analyzed by SPSS version 16 using student 't' test, chi square test, logistic regression, analysis of variance and study of correlation; p value <0.05 will be considered as significant.

RESULTS

150 patients suffering from T2DM were included in our study; out of which 48 (32%) cases were males and 102 (68%) were females. Majority of subjects were between 45-54 years age group accounting for 42.67%; 10% and 15.33% subjects were between 25-34 and 35-44 years age group respectively. On ultrasonography, 104 (69.33%) patients were detected to have fatty liver and 46 (30.67%) subjects were without NAFLD (Table 1).

Table 1: Age wise distribution according to presence or absence of NAFLD among patients of type 2 diabetes mellitus.

Age group (in years)	Non NAFLD (n=no. of patients)	Non NAFLD (%)	NAFLD (n=no. of patients)	NAFLD (percentage)	Total (n=no. of patients)	Total (%)
25-34	9	6%	6	4%	15	10%
35-44	15	10%	8	5.33%	23	15.33%
45-54	17	11.33%	47	31.33%	64	42.67%
55+	5	3.33%	43	28.67%	48	32%
Total	46	30.67%	104	69.33%	150	100%

Out of the patients with NAFLD, 33 (31.73%) were males and 71 (68.26%) were females. However, this prevalence of NAFLD in total number of males (68.75% of the total males) and females (69.60% of the total females) was found to be comparable in the study.

The mean age among type 2 diabetic patients with NAFLD was 52.30±8.50 years and those without NAFLD had mean age of 43.85±8.46 years (p<0.001). Out of 150 subjects, 42.67% (n=64) patients had grade 1 fatty liver with majority belonging to 45-54 age group. 24% subjects (n=36) and 2.67% subjects (n=4) had grade 2 and grade 3 fatty liver respectively with majority belonging to 55+ age group. There is higher degree of positive correlation found between age of the patient and grading of fatty liver (FLG) (r-value=+0.554, p<0.001). (Table 2) (Figure 1).

Table 2: Mean±SD. of age according to FLG (Fatty Liver Grade).

FLG (Fatty liver grade)	n (no. of subjects)	Mean ± S.D. of age (years)
0	46	43.85±8.46
1	64	49.06±8.57
2	36	57.25±5.41
3	4	59.50±3.11

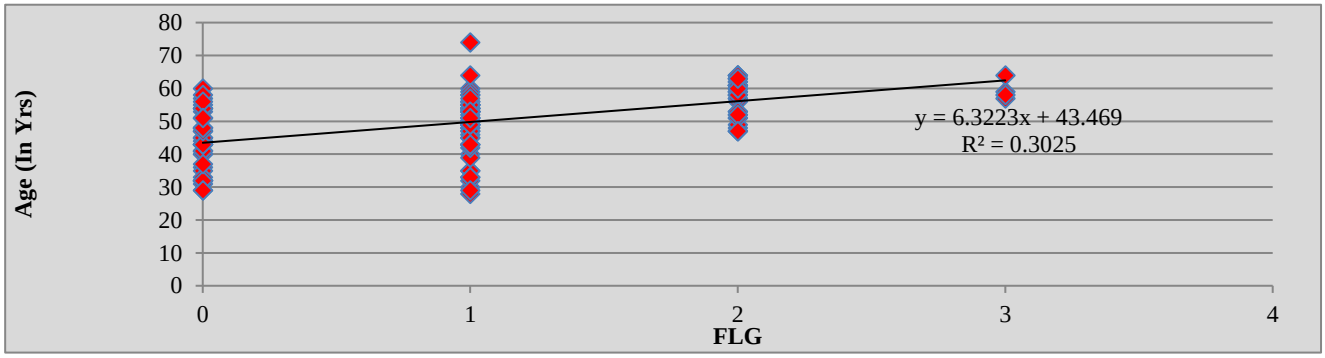

Figure 1: Correlation between age and severity of fatty liver disease.

BMI and NAFLD

Mean BMI among type 2 diabetic patients without NAFLD and with NAFLD was 25.94±3.25 and 29.64±4.36 kg/m2 respectively (p<0.001). None of the patients with BMI<18.5 kg/m2 had fatty liver. Majority of the patients with grade 1 fatty liver were overweight (34%, n=51) followed by 8.67% subjects with normal BMI. All patients with grade 2 (24%) and grade 3 (2.67%) fatty liver was obese with BMI ≥ 30 kg/m^2.

There is higher degree of positive correlation found between BMI and grading of fatty liver (FLG) (r value=+0.899, p<.001) (Table 3, Figure 2).

Table 3: Mean±SD. of BMI according to FLG.

FLG (Fatty liver grade)	n (no. of subjects)	Mean±SD. of BMI
0	46	25.94±3.25
1	64	26.76±2.03
2	36	33.48±1.67
3	4	41.12±2.02

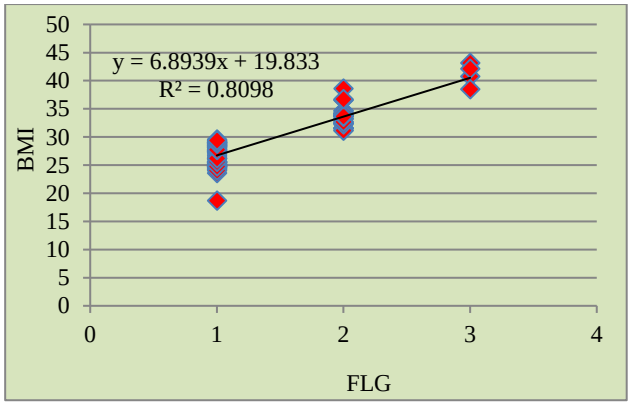

Figure 2: Correlation between BMI and fatty liver disease.

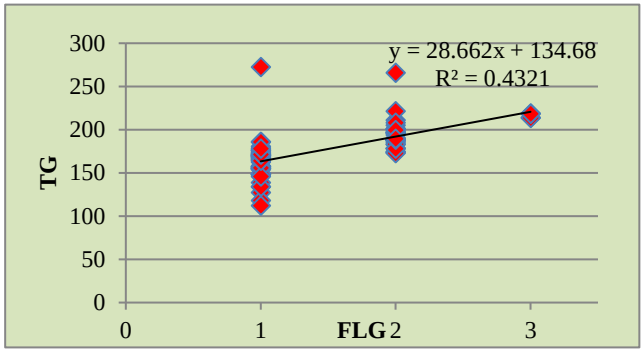

Figure 3: Correlation between TG levels and fatty liver disease.

Table 4: Mean±SD. of TG (Triglyceride) according to FLG.

FLG (Fatty liver grade)	n (no. of subjects)	Mean±SD. of TG (mg/dl)
0	46	128.53±26.66
1	64	163.07±20.38
2	36	192.99±16.68
3	4	216.25±2.71

Lipid profile and NAFLD

Out of 150 subjects, 64.67% (n=97) patients had hypertriglyceridemia with 35.33% subjects (n=53) had triglyceride levels within normal limit.

Mean TG among type 2 diabetic patients without NAFLD and with NAFLD was 128.53±26.66 and 175.47±24.82 mg/dl respectively (p<0.001).

The mean triglyceride level of the patients with grade 1, grade 2 and grade 3 fatty liver was 163.07±20.38, 192.99±16.68, 216.25±2.71 mg/dl respectively with a high degree of positive correlation (Table 4, Figure 3).

There was no significance found between severity of fatty liver and other parameters of lipid profile like HDL and LDL and total cholesterol.

Table 5: Comparison of parameters studied between the two groups of patients, with and without NAFLD.

Parameter	Type 2 D.M. with NAFLD (n=104, 69.33%) Mean±SD.	Type 2 D.M. without NAFLD (n=46, 30.67%) Mean±SD.	'p' value
Age (years)	52.30±8.50	43.85±8.46	< 0.001 (HS)
B.M.I. (kg/m^2)	29.64±4.36	25.94±3.25	< 0.001 (HS)
S.B.P. (mm of Hg)	123.69±6.00	122.78±5.17	>0.05 (NS)
D.B.P. (mm of Hg)	80.85±3.75	79.91±8.86	>0.05 (NS)
FBS (mg/dl)	132.50±5.08	132.33±4.52	>0.05 (NS)
BS (PP) (mg/dl)	237.35±23.68	235.72±29.85	>0.05 (NS)
HbA1c (%)	7.73±0.56	7.06±0.42	>0.05 (NS)
AST (U/L) SGOT	36.60±5.48	34.30±6.04	>0.05 (NS)
ALT (U/L) SGPT	42.80±5.89	41.30±3.82	>0.05 (NS)
Bilirubin (T) (mg/dl)	0.79±0.21	0.77±0.15	>0.05 (NS)
Bilirubin (D) (mg/dl)	0.41±0.15	0.41±0.13	>0.05 (NS)
ALP (IU/L)	100.81±20.66	97.67±15.30	>0.05 (NS)
Urea (mg/dl)	29.19±4.55	30.19±5.00	>0.05 (NS)
Creatinine (mg/dl)	0.89±0.25	0.91±0.20	>0.05 (NS)
T.S.H. (uIU/ml)	2.34±0.49	2.33±0.45	>0.05 (NS)
fT4 (ng/dl)	1.21±0.19	1.19±0.19	>0.05 (NS)
TC (mg/dl)	165.36±21.33	162.73±15.46	>0.05 (NS)
LDL (mg/dl)	100.95±18.94	96.27±21.19	>0.05 (NS)
HDL (mg/dl)	46.11±4.11	47.12±5.03	>0.05 (NS)
TG (mg/dl)	175.47±24.82	128.53±26.66	< 0.001 (HS)
Albumin	4.35±0.39	4.43±0.24	>0.05 (NS)

Glycemic control and NAFLD

Blood sugar (fasting and post prandial) as well as HbA1c were comparable among patients with and without NAFLD (p value >0.05).

Twenty clinical and biochemical characteristics were compared between the patients with and without fatty liver (Table 5).

The differences in age, BMI and TG level were statistically significant between two groups.

No significant relationship was found between fatty liver and variables like sex, blood pressure, blood sugar (Fasting, post prandial), HbA1c, bilirubin, AST, ALT, ALP, urea, creatinine, TSH, fT4, TC, LDL, HDL and albumin. The difference in clinical and biochemical characteristics was statistically significant for age, B.M.I. and triglycerides (TG) levels.

DISCUSSION

The prevalence of NAFLD in patients with T2DM was 69.33% in our study. The prevalence was found to be higher than those found in SPRINT study and Mohan V. et al study which was 56.5% and 54.5% respectively.

Angulo P et al, observed a prevalence of NAFLD in T2DM patients of approximately 50% in the US.[15]

Insulin resistance is responsible for disturbance in lipid storage and lipolysis in insulin sensitive tissues which increases the flow of fatty acids from adipose tissue to liver and leads to steatosis.[22,23]

Mild fatty liver (61.53%) was predominant in our study followed by moderate (34.61%) and severe fatty liver (3.84%) among type 2 diabetic patients with NAFLD.

George Boon-Bee Goh et al studied that among those type 2 diabetes mellitus patients who had fatty liver, 36% had grade 1, 33.5% had grade 2 and 28.4% had grade 3 fatty liver. The prevalence of grade 3 fatty liver was higher in this study as compared to our study.[24]

Prevalence was comparable among the males (68.75% of the total males) and females (69.60% of the total females) in our study in concordance to the findings shown by Ashutosh M et al.[25]

Highly significant variation was found in mean age group between type 2 diabetes mellitus patients with and without NAFLD (p<.001) statistically with the majority of cases occurring between the age of 45 and 54 years as evident in a study by Sanjay Kalra et al.[21]

As the mean age of the subjects' increases, severity of fatty liver also increases. There is higher degree of positive correlation found between age and grading of fatty liver (FLG). (Correlation coefficient= +0.554) Angulo P et al in their study supported the progression of NAFLD with increasing age.[15]

There is higher degree of positive correlation found between BMI and grading of fatty liver (FLG). (Correlation coefficient= +0.899).

It was clear that as the BMI increases, grade of fatty liver also increases. Morbid obesity places patients with type 2 diabetes at particularly high risk of severe fatty liver according to Silverman JF et al.[26] Virtually 100% of these patients were found to have at least mild steatosis.

In this study, Mean TG among type 2 diabetic patients without NAFLD was 128.53±26.66 and patients with fatty liver had mean TG of 175.47±24.82 mg/dl (p value <0.001) with high statistical significance between TG and presence or absence of fatty liver. Singh SP et al and Hajieh Bibi Shahbazian et al showed mean TG levels considerably higher in type 2 DM patients with NAFLD as compared to non-NAFLD patients.[22,27]

As the level of TG increases, the severity of fatty liver also increases. This positive correlation was comparable to the study done by Hajieh Bibi Shahbazian et al.[27]

In our study, 20 known risk factors for hepatosteatosis (sex, age, BMI, blood pressure, blood sugar (Fasting, post prandial), HbA1c, bilirubin, AST, ALT, ALP, urea, creatinine, TSH, fT4, TC, LDL, TG, HDL and albumin) were statistically analyzed. Between diabetic patients with and without fatty liver, only age, BMI and TG had significant relationship with steatosis. Surprisingly the difference was not significant for the ALT in contrast to majority of the previous studies.

The findings of this study indicate that the prevalence of NAFLD among subjects with type 2 DM is continuously on rise especially with increasing age. Ultrasonography and increasing age, BMI, elevation in triglyceride level can be used as reliable indicators of severity of NAFLD. Our study also makes a note that increasing age, BMI and high TGs are becoming stronger predictors of NAFLD among Type 2 DM patients which needs to be explored in further studies.

The results from this study establish a prevalence pattern of NAFLD in Indian type 2 Diabetes Mellitus Population. This study should alarm us of the need for frequent evaluation of BMI and Triglyceride levels in type 2 DM patients, as even mild elevation of these may be a strong predictor of occurrence of steatosis and severity of fatty liver.

CONCLUSION

The results indicate the high magnitude of fatty liver (69.33%) in type 2 diabetic patients in our population and

ultrasonographic evidence of fatty liver with old age, elevation of Triglyceride level and increasing BMI should be taken seriously as a predictor of severity of hepatic steatosis. Therefore, it is necessary to follow patients with Type 2 DM for the incidence of fatty liver.

Patients with T2DM should always be assessed for NAFLD to ensure early diagnosis, intensive blood glucose and obesity control, and effective dyslipidemia correction to prevent and minimize the occurrence of NAFLD.

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