Original Article

# Study of Association of Non Alcoholic Fatty Liver Disease (NAFLD) with Micro and Macro Vascular Complications of Type 2 Diabetes Mellitus (T2DM).

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**Abstract**

**Aims**:

Nonalcoholic fatty liver disease (NAFLD) is a common liver disorder that is strongly associated with insulin resistance and Type 2 Diabetes Mellitus (T2DM). This study was designed to determine the prevalence of NAFLD among T2DM patients and to evaluate whether there is an association between NAFLD and diabetic micro-and macro vascular complications.

**Methods**:

In a cross-sectional design study, 120 type-2 diabetic patients were submitted to a complete clinical and laboratory evaluation and abdominal ultrasonography for NAFLD detection and grading. They were divided

into fatty liver group and non fatty liver group and various laboratory and clinical variables were compared in these two groups.Statistical analysis included bivariate tests, chi square test, univariate and multivariate logistic regression.

**Results**:

Out of 120 type 2 diabetic patients,68(56.66%) had fatty liver on ultrasonography. An increase in the waist circumference, BMI,systolic blood pressure, and systolic blood pressure and levels of HBA1c,AST, ALT,Total Cholesterol,Triglycerides and a decrease in HDL was observed in the fatty liver group as compared to non fatty liver group. NAFLD.NAFLD group had higher prevalence of retinopathy (67.67% vs. 17.30%, $P < 0.001$), neuropathy (52.94% vs. 19.23%, $P = 0.0002$), nephropathy (83.82% vs.53.84%, $p = 0.0003$). The prevalence of CAD (70.58% vs. 21.11%, $P<0.0001$) and POVD (10.25% vs 0%,$P <0.05$ ) was higher in NAFLD patients. All patients with severe fibrosis had raised BMI,HbA1c and hypertension.The results of multiple logistic regression analysis showed that NAFLD was associated with BMl, HbA1c,Trigyceride and CAD.Univariate analysis showed significant association between retinopathy,neuropathy,CAD,POVD and NAFLD.

**Conclusion**:

The prevalence of NAFLD is higher in type-2 diabetic patients. Obesity, dysglycemia,dyslipidemia,elevated liver enzymes and coronary artery disease are seen to be significantly associated with fatty liver than non fatty liver type-2 diabetic patients.

**Keywords :** Type-2 diabetes mellitus, NAFLD, micro and macro vascular complications

## Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as the most common cause of chronic liver disease world wide,probably related to the increasing incidence of obesity and type-2 diabetes[1].Incidence of type 2 DM is increasing throughout the world, reaching levels of a pandemic in countries like India and China[2].Only recently has liver disease been recognized as a major complication of type 2 DM with standard mortality rates for cirrhosis greater than that for cardiovascular disease[3]

The prevalence is reported higher among patients with diabetes mellitus and obesity ranging from 35% to 75% in various studies[1].The prevalence of NAFLD is on rise in Asian countries which may be attributed to the improvement of life style , the change of dietetic structure and the application of new diagnostic techniques[1].Chitturi *et al.* highlighted the potential burden of the disease in the Asia-Pacific region, with estimated 1.8 million Asians with NASH[4]. Some patients with NAFLD develop necro inflammatory changes in the liver called nonalcoholic steatohepatitis (NASH) and a fraction of those will develop cirrhosis, This progressive fibrotic disease can progress to end stage liver disease[4].

Much work has been done on NAFLD and NASH in the western world but its presentation and aetiology has not been well studied in Asian population because of its asymptomatic presentation. There are few studies addressing specifically NAFLD in diabetic patients. Hence, new data are still required in order to better clarify the prevalence and clinical spectrum of NAFLD in this specific population.

This study was conducted to know the frequency of NAFLD in type 2 diabetic patients and to see whether the presence and severity of NAFLD are related to diabetic metabolic status and to the occurrence of chronic micro-

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and macro vascular degenerative complications. With the rising incidence and prevalence of T2DM a close estimate of the prevalence of NAFLD, as well as its clinical risk factors, is important for predicting the number of patients that require monitoring for more advanced liver diseases, or those who may benefit from future disease modifying agents.

**Material and Methods**

An observational cross sectional study was conducted with 120 type 2 DM consecutive adult patients of both sexes diagnosed by standard criteria attending tertiary diabetes care center, GMCH, Nagpur during the period between December 2011 and November 2013. Ethics committee of the institution approved the study. Informed consent was taken from the subjects and they were evaluated by abdominal ultrasonography.

Patients giving a history of alcohol abuse (>20g/day), pregnancy, hepatitis/jaundice or other liver diseases, hepato-toxic medication, serious concominant disease (severe cardio-respiratory disease, cancer etc.) and patients with type 1 diabetes mellitus were excluded.

The patients were divided into fatty liver and non fatty liver group and were further evaluated by the measurement of Body Mass Index (BMI), Glycosylated haemoglobin (HbA1c), alanine aminotransferase (ALT), aspartate aminotransferase(AST), alkaline phosphatase(AKP), total cholesterol (TC) triglycerides (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL).

Each patient's baseline demographic data, age, sex, duration of diabetes was recorded. Waist circumference (WC) was measured in a standing position at the level of the umbilicus. Blood pressure was measured with a standard cuff sphygmomanometer in a seated position after a minimum rest period of 5 min and patient was considered hypertensive if systolic blood pressure (SBP) was above 120 mmHg or diastolic blood pressure (DBP) reading above 80mmHg in more than three occasion or if patients were receiving antihypertensive drug therapy. Diagnosis of diabetes was made if the fasting plasma glucose was ≥126 mg/dl or a non fasting glucose >200 mg/dl or a self-reported physician diagnosis, or on treatment for diabetes. Serology for viral hepatitis B and C was also assessed in all participants.

Overweight was defined as a BMI between 23 and 25 $kg/m^2$, and obesity as BMI equal or above 25 $kg/m^2$.Patients were considered centrally obese if the waist circumference was greater than 80 cm in females and 90 cm in males. 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. Metabolic syndrome was defined according to guidelines of IDF.

Diabetic Nephropathy (DN) was diagnosed by a positive proteinuria and serum creatinine >130 μmol/l. Diabetic Peripheral Neuropathy (DPN) was diagnosed in the presence of persistent numbness, paresthesia, loss of sense of vibration of the tuning fork and failure to elicit a knee and/or ankle jerk. Diabetic Retinopathy (DR) was assessed by dilated fundus examination by an ophthalmologist. Retinopathy was considered to be present if it was noted in any form either nonproliferative diabetic retinopathy or proliferative diabetic retinopathy by the ophthalmologist during dilated fundoscopy. DR was diagnosed in the presence of retinal hemorrhage, exudates, and macular edema. Presence of coronary artery disease (CAD) was defined by any history of CAD in the past or positive Electrocardiographic changes. Peripheral Occlusive vascular disease (POVD) was diagnosed using ultrasound arteriovenous doppler.

Ultrasound imaging of the liver was done by single blinded experienced radiologists. Diagnosis of steatosis was established based on liver brightness (evident sonographic contrast between hepatic and renal parenchyma), together with high posterior attenuation and reduced vessel wall and diaphragm distinction. The severity of steatosis was graded as follows : slight (diffuse increase in fine echoes in liver parenchyma), moderate (diffuse increase in fine echoes with impaired visualization of the intrahepatic vessel borders and diaphragm), and severe (diffuse increase in fine echoes with non-visualization of the intrahepatic vessel borders and diaphragm). Ultrasonography has a sensitivity of 89% and a specificity of 93% in detecting moderate-to-severe hepatic steatosis.

**Statistical analysis**

The analysis was performed using STATA statistical software Version 10.00.Significant differences between groups were evaluated using the Student t-test, chi-square test and univariate analysis wherever appropriate. Multiple logistic regression analysis was done using NAFLD as the dependent variable. Independent variables included were age, gender, duration of DM, BMI, nephropathy, neuropathy, retinopathy, POVD, and CAD. A *P* value of less than 0.05 was considered significant.

**Results**

The study included 120 patients of type 2 diabetes mellitus. The average age of the patients was 58.08±6 years (Ranging from 35 to 72). Fifty one (42.5%) were males and 69 (57.5%) were females. Sixty eighth (56.66%) patients had fatty liver whereas fifty two (43.33%) had no fatty liver on ultrasonography. Subjects with positive NAFLD

(Group-1, $n$ = 68) were compared with a group without NAFLD (Group 2, $n$ = 52).

The mean age of patients in Group 1 and Group 2 were similar (55.73 ±6.08 vs. 54.51 ±.5.46). The duration of diabetes was found to be longer in patients with NAFLD(11.83±4.07,P<0.0001).

Group 1 patients had a higher BMI (26.97 ± 1.78 vs. 22.98 ± 2.62) and systolic and diastolic blood pressure when compared with Group 2 [**Table 1].** $HbA_{1c}$ was significantly higher in Group 1 compared to Group 2. Subjects in Group 1 had higher Triglyceride,Total cholestrol and low HDL levels than Group 2.

ALT and AST levels were significantly higher in patients with NAFLD **[Table 1**]. Prevalence of obesity and hypertension were significantly higher among subjects with NAFLD than in subjects without NAFLD.

The prevalence of diabetic micro- and macrovascular complications in the study groups. NAFLD patients had significantly higher prevalence of retinopathy (67.67 % vs. 17.30%, P < 0.001), neuropathy (52.34 vs. 19.23%, $P$ < 0.05), and nephropathy (83.83 vs 53.84 %,p<0.05).The prevalence of CAD was high among NAFLD patients (70.58% vs. 21.11%, $P$ < 0.001). Prevalence of POVD was significantly higher among NAFLD patients (10.29% vs 0%, *P<0.05*). Univariate analysis showed significant association between retinopathy, nephropathy, neuropathy, CAD, POVD and NAFLD. (P<0.05) **(Table 2)**

Results of multiple logistic regression analysis revealed Body Mass Index (OR = 1.61, 95% CI: 1.17-2.21,P <0.05), HbA1c (OR 14.48,95% CI: 3.03-69.13,P <0.05), Trigycerides (OR 1.07,95% CI: 1.03-1.11,P <0.05) and CAD (OR 5.13,95% CI: 1.01-26.00,P<0.05) were significantly associated with NAFLD**. (Table 3)**

**Discussion**

NAFLD is more commonly seen in type 2 diabetic patients and is clearly now an important public health issue. Prevalence reports on NAFLD are available both from India and abroad, but there is lack of data on the association of NAFLD with diabetic micro- and macrovascular complications from India[4]. The important finding of this study was that NAFLD, as diagnosed by liver ultrasound, which is the most widely used imaging test for detecting hepatic steatosis was associated with micro- and macrovascular complications among type 2 diabetic subjects.

This study revealed that the prevalence of NAFLD in male hospitalized T2DM patients was 49.01% whereas in female patients (62.31%),but without statistical significance. The average age of the patients was 58.08±6 years (Ranging from 35 to 72).Targher *et al* reported that the prevalence of NAFLD increased with age (*i.e.*, 65.4% among participants aged 40-59 years and 74.6% among those aged ≥ 60 years; $P$ < 0.001)[5]. Duvnjak *et al*, which reports that the highest prevalence of NAFLD occurs in those aged 40-60 years[6]. Our study revealed that prevalence rates of NAFLD increased significantly with the prolonged course of diabetes.

Banerjee *et al*, found that a longer duration of T2DM was significantly associated with NAFLD[7].

It is known that NAFLD is an integral part of the metabolic syndrome which comprises a cluster of abnormalities such as dysglycemia, dyslipidemia, hypertension, and obesity with insulin resistance as a central pathogenic factor[4].As has been reported in other studies[8,9] ,our study also showed raised systolic and diastolic blood pressure in patients with NAFLD. It has been demonstrated that insulin resistance leads to higher free fatty acid load to the liver, consequently higher triglyceride synthesis and increased secretion of triglyceride from the liver. In fact, hypertriglyceridemia have been strongly correlated with liver fat accumulation[1]. Our study showed increased triglyceride levels (mean-177.40±18.91) in diabetic fatty liver group as compared to non fatty liver group (mean 140.47 ± 18.63) and the results were statistically significant (P value <0.0001).In addition, we also found that HbA1c levels in fatty liver disease group were higher then non-fatty liver group, which confirmed the obvious dysglycemia in these patients (P value < 0.0001)(1). Diabetes mellitus and obesity may lead to increased liver fibrosis through different mechanism, the effect of these two conditions may be additive when they both exist in the same individual[1]. The levels of ALT AST were also elevated in diabetic fatty liver patients in our study. This is also reported in other studies as well[10,11].

As shown in many studies, our results also showed that obesity, hypergycemia and hyperlipidemia had an association with NAFLD. The prevalence of obesity as indicated by high BMI and waist circumference was significantly higher among subjects with NAFLD in our study. In a recent report, the prevalence of most of the cardiometabolic risk factors was significantly higher in NAFLD subjects[12]. In a hospital-based study from North India, it was shown that 20% of NAFLD patients were overweight and 68% had obesity. Abnormal cholesterol, triglycerides, and HDL-cholesterol were present in 36%, 53%, and 66%, respectively in this study[13].The above abnormalities were also seen in our study which was found to be statistically significant(P<0.0001).

Univariate analysis showed statistically significant association between neuropathy, retinopathy nephropathy and POVD with NAFLD in our study.Recent study by

Targher ***et*** al reported that NAFLD patients had higher age- and sex-adjusted prevalence of both retinopathy and chronic kidney disease[14]. Another study from the same group of researchers also showed that NAFLD was associated with a higher prevalence of CVD[15] and with increased risk for future CVD events[16]. Our study also showed significant association of CAD with NAFLD.

In conclusion, our study reported an association among NAFLD and retinopathy, neuropathy, nephropathy and CAD by univariate analysis. The prevalence of obesity, hypertension, and dyslipidemia were significantly higher in subjects with NAFLD compared to subjects without NAFLD. Given that hepatic fat content can be reversed with lifestyle changes and drugs, NAFLD should be included in future preventive public health initiatives, and the affected individuals should be motivated to adopt a healthier lifestyle[17]. Patients with T2DM should be always assessed for NAFLD to ensure early diagnosis intensive blood glucose and blood pressure control, and effective dyslipidemia correction to prevent and minimize the occurrence of NAFLD.

**Table.no.1**. Showing Demographic, Hemodynamic and Biochemical details of the study groups.

| Variables | Group 1 with NAFLD (n = 68) | Group 2 without NAFLD (n = 52) | P value |
|---|---|---|---|
| Age ( yrs) | 55.73 ± 6.08 | 54.51 ± 5.46 | 0.2594 |
| duration of diabetes in years | 11.83±4.07 | 7.21 ±2.06 | <0.0001* |
| waist circumference (cms) | 93.66± 6.66 | 83.19± 5.01 | <0.0001* |
| BMI (KG/M²) | 26.97 ± 1.78 | 22.98 ± 2.62 | <0.0001* |
| SBP (mmHg) | 147.58 ± 13.40 | 132.88 ± 9.02 | <0.0001* |
| DBP (mmHg) | 92.05 ± 8.82 | 79.48 ± 6.76 | <0.0001* |
| HbA1c (%) | 7.86 ± 0.59 | 6.71 ± 0.26 | <0.0001* |
| Triglycerides (mg/dl) | 177.40 ± 18.91 | 140.47 ± 18.63 | <0.0001* |
| Total Cholesterol (mg/dl) | 255.23 ± 31.80 | 173.27 ± 36.73 | <0.0001* |
| HDL (mg/dl ) | 37.05 ± 5.74 | 45.56 ± 5.68 | <0.0001* |
| LDL (mg/dl) | 111.66 ± 9.83 | 108.61 ± 7.35 | 0.0641 |
| AST (IU/L) | 35.79 ± 6.27 | 22.07 ± 5.11 | 0.0007* |
| ALT (IU/L) | 40.70 ± 7.06 | 22.90 ± 6.03 | <0.0001* |
| ALP (IU/L) | 79.67 ± 5.31 | 78.11 ± 4.91 | 0.1025 |
| Urea (mg/dl) | 62.98 ± 35.53 | 32.13 ± 10.73 | <0.0001* |
| Creatinine (mg/dl) | 2.82 ± 1.54 | 1.55 ± 0.70 | <0.0001* |

**Table.2.** Showing the prevalence of diabetic micro- and macrovascular complications in the study groups.

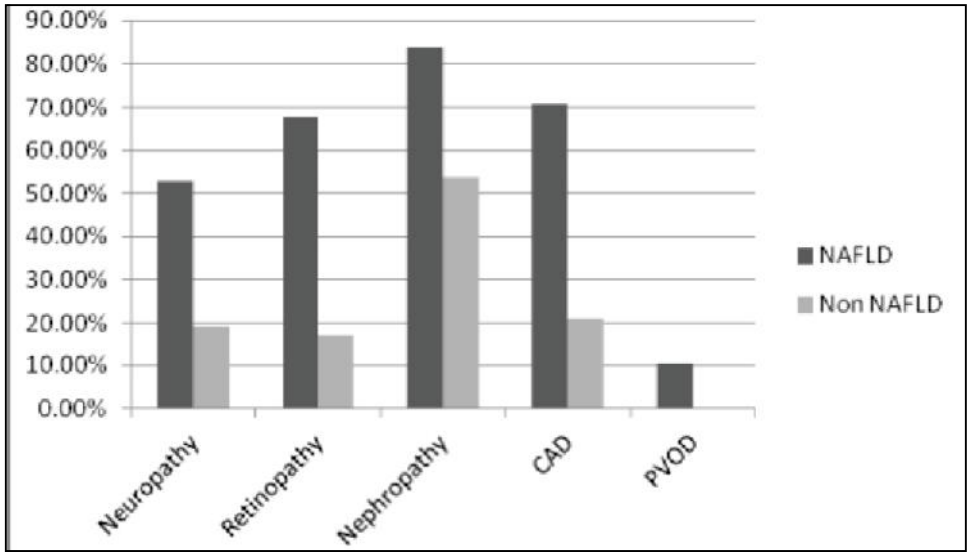

**Table 3.:**Results of Multiple logistic regression analysis

| Significant variable | ODDS RATIO (OR) | 95 % CONFINDENCE INTERVAL | P Value |
|---|---|---|---|
| BMI | 1.61 | 1.17-2.21 | 0.003* |
| HbA1c | 14.48 | 3.03-69.13 | 0.001* |
| Coronary Artery Disease | 5.13 | 1.01-26.00 | 0.048 * |
| Triglyceride | 1.07 | 1.03-1.11 | <0.001* |

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