

# Association of non-alcoholic fatty liver disease with diabetic microvascular and macrovascular complications in South Indian diabetic subjects

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**Aims:** Nonalcoholic fatty liver disease (NAFLD) is a common liver disorder that is strongly associated with insulin resistance and type 2 diabetes. This study was designed to evaluate whether there is an association between NAFLD and diabetic micro- and macrovascular complications among diabetic subjects. **Materials and Methods:** The subjects were selected from 2161 (M:F; 1187:974) type 2 diabetic patients who had undergone ultrasound of abdomen for assessment of fatty liver. A total of 156 patients with evidence of NAFLD (group 1) were compared with 142 (group 2) patients with normal liver ultrasound and the presence of micro- and macrovascular complications of diabetes were recorded. Multiple logistic regression analysis was performed using NAFLD as the dependent variable. Independent variables included were age, gender, duration of diabetes mellitus (DM), body mass index (BMI), nephropathy, neuropathy, retinopathy, peripheral occlusive vascular disease (POVD), and coronary artery disease (CAD). **Results:** Prevalence of obesity, hypertension, and dyslipidemia were significantly higher in subjects with NAFLD. They had higher prevalence of retinopathy (29.4% vs. 9.8%,  $P < 0.001$ ), neuropathy (27.5% vs. 10.5%,  $P < 0.001$ ), nephropathy (32% vs. 25%,  $P = 0.2$ ). The prevalence of CAD among NAFLD (11.5% vs. 1.4%,  $P = 0.01$ ) was higher and POVD was similar in both the groups. The results of multiple logistic regression analysis showed that NAFLD was associated with BMI, retinopathy, neuropathy, and CAD. **Conclusions:** NAFLD as diagnosed by ultrasound was associated with micro- and macrovascular complications of diabetes. The prevalence of obesity, hypertension, and dyslipidemia

were significantly higher in subjects with NAFLD.

**KEYWORDS:** Indian subjects, micro- and macrovascular complications, non-alcoholic fatty liver disease, type 2 diabetes

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder which is closely associated with insulin resistance and type 2 diabetes, and it is characterized by fat accumulation in the liver.<sup>[1-3]</sup> The prevalence of NAFLD has been reported to be in the range of 15-20% in the general population, whereas in type 2 diabetic population the prevalence was as high as 50-75%.<sup>[4-6]</sup> Some patients with NAFLD develop necroinflammatory 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.<sup>[7]</sup>

The diagnosis of NAFLD requires a high index of suspicion, especially in obese patients over the age of 45 years who have diabetes, because these patients are at greatest risk of developing cirrhosis. Primary NASH is associated with metabolic syndrome-related conditions, such as obesity, type 2 diabetes and hyperlipidemia. Chitturi *et al.* highlighted the potential burden of the disease in the Asia-Pacific region, with estimated 1.8 million Asians with NASH.<sup>[8]</sup>

Type 2 diabetic patients appear to have an increased risk of developing NAFLD than non-diabetic subjects and certainly have a higher risk of developing fibrosis

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and cirrhosis.<sup>[1,2,7]</sup> One study reported the prevalence of abnormal aminotransferase levels, which are a poor proxy measure of NAFLD.<sup>[9-11]</sup> Recent data suggest that the presence of NAFLD in type 2 diabetes may also be linked to increased cardiovascular disease (CVD) risk.<sup>[12-14]</sup> A very recent study showed that NAFLD was independently associated with an increased prevalence of chronic kidney disease and retinopathy in type 2 diabetic patients.<sup>[15]</sup> Currently, there is a lack of information on association between NAFLD and macrovascular complications in Indian scenario. The aim of this study was to assess the association between NAFLD and micro- and macrovascular complications of diabetes.

## Materials and Methods

The study subjects were selected from 2161 (M:F 1187:974) consecutive adult outpatients with type 2 diabetes attending a tertiary diabetes care center in India during the period between December 2006 and July 2007. All the patients were referred to ultrasonography scanning and were told that they were entering a study designed to examine the association of NAFLD with diabetic micro- and macrovascular complications. Informed consent was obtained from all the patients who agreed to participate in the study. Ethics committee of the institution approved the study. Patients giving a history of alcohol abuse, hepatitis/jaundice or other liver diseases, obesity-related intestinal surgery, rapid weight loss in the obese and patients who are on hepato-toxic medication were excluded. Patients with ultrasound evidence of NAFLD were ascertained to group 1 ( $n = 156$ , M:F 101:55), and a comparative group of 142 (M:F, 90:52) patients (group 2) were randomly selected from patients with normal liver ultrasound. The study subjects were age and duration of diabetes matched groups.

Each patient's baseline demographic data, age, sex, location, and duration of diabetes, history of previous illness, medication they were currently taking as well as anthropometric measurements including height and weight were recorded. Body mass index (BMI) was calculated. 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 reading  $\geq 130$  mmHg or diastolic blood pressure reading  $\geq 85$  mmHg in more than three occasions or if patients were receiving antihypertensive drug therapy.

Diagnosis of diabetes was made if the fasting plasma

glucose was  $\geq 126$  mg/dl or a nonfasting glucose  $> 200$  mg/dl or a self-reported physician diagnosis, or on treatment for diabetes.

Fasting lipid profile, HbA<sub>1c</sub>, urea, creatinine and liver function tests were done by standard enzymatic procedures. LDL cholesterol and VLDL cholesterol were calculated by using Friedewald's equation. HbA<sub>1c</sub> was estimated by immunoturbidimetric method. Subjects were considered to have nephropathy if they had persistent microalbuminuria as determined by urinary albumin to creatinine ratio ( $\geq 30$   $\mu$ g albumin/mg creatinine) using the immunoturbidimetry method.

Neuropathy was diagnosed by biothesiometry.<sup>[16]</sup> Vibration perception threshold more than 25 V as measured by the biothesiometer was considered abnormal. Retinopathy was assessed by dilated fundus examination by an experienced 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 funduscopy.

The presence of coronary artery disease (CAD) was defined by any history of CAD in the past or positive stress test (treadmill test). Peripheral occlusive vascular disease (POVD) was diagnosed using ankle brachial index (ABI) (ABI less than 0.8 was considered as significant POVD).

Ultrasound imaging of the liver was done to diagnose NAFLD. Hepatic ultrasonography scanning was performed for all participants by a single experienced radiologist to avoid interobserver variation, who was blinded to subjects' details. Hepatic steatosis was diagnosed by characteristic hyperechogenicity of liver relative to kidneys, ultrasound beam attenuation, and poor visualization of intrahepatic structures.<sup>[7,17]</sup> Ultrasonography has a sensitivity of 89% and a specificity of 93% in detecting moderate-to-severe hepatic steatosis.<sup>[18]</sup>

## Statistical analyses

The analysis was performed using SPSS 10.0 Version software. Mean and standard deviation and proportions are reported as relevant. Significant differences between groups were evaluated using the Student *t*-test,  $\chi^2$  test 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

Out of the total 2161 patients screened for the presence of NAFLD, 156 (7.2%) were found to be positive for NAFLD. A flow chart showing the selection of study subjects was given in Figure 1. Subjects with positive NAFLD (Group-1, *n* = 156) were compared with a group without NAFLD (Group 2, *n* = 142). The mean age of patients in Group 1 and Group 2 were similar (50 ± 10 vs. 49 ± 11). The duration of diabetes was similar in both the groups. Group 1 patients had a higher BMI (29.7 ± 7 vs. 26.4 ± 4) and diastolic blood pressure when compared with Group 2 [Table 1].

HbA<sub>1c</sub> was significantly higher in Group 1 compared to Group 2. Subjects in Group 1 had higher triglyceride and low HDL levels than Group 2. ALT and AST levels were significantly higher in patients with NAFLD [Table 1].

Table 2 shows the prevalence of individual abnormalities among the study groups. Prevalence of obesity and hypertension were significantly higher among subjects with NAFLD than in subjects without NAFLD. Hypertriglyceridemia, low HDL-Chol, and abnormal aminotransferase levels were more common in subjects with NAFLD.

Figure 2 shows the prevalence of diabetic micro- and macrovascular complications in the study groups. NAFLD patients had significantly higher prevalence of retinopathy (29.4 vs. 9.8%, *P* < 0.001), neuropathy (27.5 vs. 10.5%, *P* < 0.0001), compared to patients without NAFLD. The prevalence of nephropathy was higher in patients with NAFLD group, but no significant difference

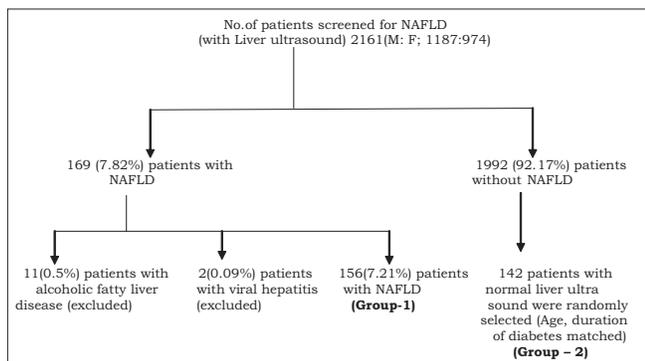


Figure 1: Selection of study subjects

was found between the groups (32% vs. 25%, *P* = 0.2). Prevalence of NPDR and PDR in NAFLD patients was 27.5% and 1.9%, while among patients without NAFLD it was 7.7% and 2.1%, respectively [NPDR; Group 1 vs. Group 2;  $\chi^2 = 18.4$ ; *P* < 0.0001; PDR; Group 1 vs Group 2;  $\chi^2 = 0.008$ , *P* = 0.8].

The prevalence of CAD was high among NAFLD patients (11.5% vs. 1.4%, *P* = 0.001). Prevalence of POVD as assessed by Doppler was similar in both the groups (6% vs. 7%, *P* = 0.82). None among the two groups had known cerebrovascular accidents in the form of transient ischemic attack or stroke.

Table 3 shows the results of multiple logistic regression analysis. Body Mass Index (OR = 4.16, 95% CI: 2.01–8.6), neuropathy (OR 5.89, 95% CI: 2.1–16.2), retinopathy (OR 3.46, 95% CI: 1.6–7.5) and CAD (OR 3.58, 95% CI: 1.66–7.72) were significantly associated with NAFLD.

Antidiabetic medication intake like sulphonylurea, metformin, thiozolidinediones, and alphaglucoisidase inhibitor were similar in both the groups, but insulin

Table 1: Demographic, hemodynamic, and biochemical details of the study groups

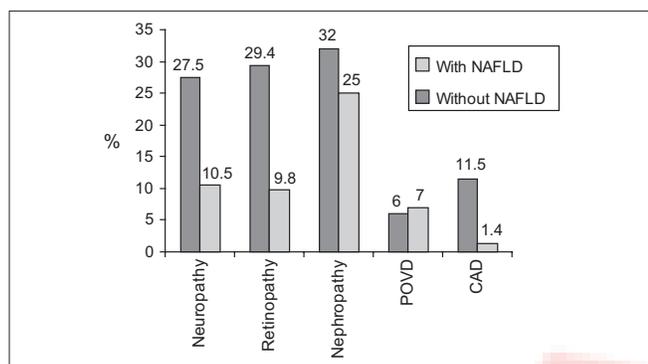
Variables	Group 1 with NAFLD (n = 156)	Group 2 without NAFLD (n = 142)	<i>P</i> value
Age (years)	50 ± 10	49 ± 11	0.4
Duration of diabetes (years)	9 ± 6.7	8.6 ± 6.9	0.6
BMI (kg/m <sup>2</sup> )	29.7 ± 7	26.4 ± 4	<0.0001
SBP (mmHg)	132.6 ± 14.5	130 ± 15.3	0.13
DBP (mmHg)	83.4 ± 6.9	80 ± 7.8	<0.0001
HbA <sub>1c</sub> (%)	9.7 ± 2.1	8.5 ± 2.1	0.01
Total cholesterol (mg/dl)	182 ± 43.7	190.5 ± 39.2	0.09
Triglycerides (mg/dl)	213 ± 138	157 ± 77	<0.0001
HDL- C (mg/dl)	40.8 ± 10.3	42 ± 11.1	0.001
LDL-C (mg/dl)	101.4 ± 34.5	104.4 ± 28.8	0.4
VLDL-C (mg/dl)	34.8 ± 13.2	30.6 ± 14.4	0.016
Bilirubin (mg/dl)	1.0 ± 0.26	0.93 ± 1.8	0.1
Total protein (g/dl)	7.7 ± 0.6	7.2 ± 0.4	<0.0001
Albumin (g/dl)	4.3 ± 0.4	4.4 ± 0.36	0.11
Globulin (g/dl)	3.3 ± 0.5	2.7 ± 0.5	< 0.0001
ALT (IU/L)	29.3 ± 17.9	22.4 ± 15.4	<0.0001
AST (IU/L)	37.6 ± 24.9	27.6 ± 17.3	<0.0001
Alk. phosphatase (IU/L)	225 ± 99	208 ± 59	0.07
Urea (mg/dl)	27.6 ± 18.1	24.4 ± 11.2	0.08
Creatinine (mg/dl)	0.88 ± 0.4	0.9 ± 0.4	0.63

Values are mean ± SD.

**Table 2: Prevalence of individual abnormalities among the study groups**

Variables	Group 1 with NAFLD (n = 156)	Group 2 without NAFLD (n = 142)	$\chi^2$ , P value
BMI ( $\geq 25$ kg/m <sup>2</sup> )	133 (85.3)	82 (57.7)	19.0, 0.0001
Hypertension (SBP/DBP) ( $\geq 130/85$ mmHg)	101 (64.7)	58 (40.8)	9.9, 0.001
Cholesterol $\geq 200$ mg/dl	43 (27.6)	45 (31.7)	0.6, 0.44
Triglycerides $\geq 150$ mg/dl	92 (58.9)	54 (38)	12.5, 0.0001
Low HDL cholesterol (Male: <40 mg/dl) (Female: <50 mg/dl)	84 (53.8)	59 (41.5)	3.8, 0.039
ALT ( $\geq 35$ IU/L)	35 (22.4)	11 (7.7)	10.9, 0.001
AST ( $\geq 40$ IU/L)	46 (29.5)	21 (14.8)	8.2, 0.003

Values are n (%).



**Figure 2:** Prevalence of diabetic micro and macro vascular complications in the study groups. \* indicates  $P < 0.001$ .

usage was higher in patients with NAFLD (54.4% vs. 21.1%,  $P < 0.01$ ).

## Discussion

NAFLD is more commonly seen in type 2 diabetic patients and is clearly now an important public health issue. It is now well documented in most countries, and the geographic variations are noted in the prevalence of NAFLD. The overall prevalence of NAFLD was 32% in urban South Indian population.<sup>[19]</sup> The prevalence of 87% on histology with 62.6% steatohepatitis and 37.3% fibrosis has been reported in patients with type 2 diabetes from North India.<sup>[20]</sup> NASH is the histological diagnosis in 7–11% of patients undergoing liver biopsy in the USA and Canada. The prevalence figure of 10–29% has been reported from large surveys in China, Japan, and Korea. 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. The important finding of this study was that NAFLD, as diagnosed by patient history and liver ultrasound, which is the most widely used imaging test for detecting hepatic steatosis was associated with micro- and macrovascular

**Table 3: Results of multiple logistic regression analysis**

Significant Variables	$\beta$	SE $\beta$	P value	OR (95% confidence interval)
BMI (kg/m <sup>2</sup> )	1.43	0.37	<0.0001	4.16 (2.01– 8.6)
Neuropathy	1.77	0.52	0.001	5.89 (2.1–16.2)
CAD	1.27	0.39	0.001	3.58 (1.66–7.72)
Retinopathy	1.24	0.39	0.002	3.46 (1.6–7.5)

Nonsignificant variables: age, gender, duration of diabetes, nephropathy, POVD. Dependent variable: NAFLD.

complications among type 2 diabetic subjects.

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.<sup>[21]</sup> As shown in many studies, our results also showed that obesity, hypertension, and hyperlipidemia had an association with NAFLD. The prevalence of obesity as indicated by high BMI, hypertension, and hyperlipidemia 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.<sup>[19]</sup>

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.<sup>[22]</sup> The prevalence of above abnormalities was almost similar in our study population.

In this study approximately only 25% of the NAFLD subjects had abnormal liver enzymes. It is evident that normal liver enzymes will provide little diagnostic or prognostic value when assessing NAFLD patients. They appear to be insensitive markers for NAFLD.

The prevalence of retinopathy, neuropathy and coronary

artery disease in our population was significantly higher in patients with NAFLD than patients without NAFLD. In confirmation of this association, even results of multiple logistic regression analysis showed an association of body mass index, neuropathy, retinopathy, and CAD with NAFLD in this study. Nephropathy was higher in NAFLD patients, but was not statistically significant.

The higher OR was for neuropathy (5.89) followed by body mass index (4.16), CAD (3.58) and retinopathy (3.46). Recent study by Targher *et al.*<sup>[15]</sup> reported that NAFLD patients had higher age- and sex-adjusted prevalence of both retinopathy and chronic kidney disease. Another study from the same group of researchers also showed that NAFLD was associated with a higher prevalence of CVD<sup>[13]</sup> and with increased risk for future CVD events.<sup>[12]</sup> Our study also showed an association of CAD with NAFLD.

In conclusion, our study reported an association among NAFLD and retinopathy, neuropathy and CAD. The prevalence of obesity, hypertension, and dyslipidemia were significantly higher in subjects with NAFLD compared to subjects without NAFLD. Future experimental and follow-up studies are needed to elucidate the possible molecular mechanisms linking NAFLD and diabetic complications and to determine whether NAFLD predicts the development and progression of these complications.

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