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# A Study of Carotid Atherosclerosis in Patients with Non-alcoholic Fatty Liver Disease

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**Abstract** Non-alcoholic fatty liver disease shares many features of metabolic syndrome and its presence could signify a substantial cardiovascular risk above and beyond that conferred by individual risk factors. This study is an attempt to investigate the association of non-alcoholic fatty liver disease with carotid intima-media thickness and plaque as surrogate measures of increased cardiovascular risk. The study was conducted on 645 non diabetic, non alcoholic subjects in the age range of 20–60 years. Metabolic syndrome was assessed by using ATP III and ADA (2005) criteria. Anthropometric factors—waist circumference and blood pressure were measured. Fasting serum samples were analyzed for glucose, triglyceride, cholesterol and its fractions, insulin, alanine and aspartate transaminases, gamma glutamyl transferase and free fatty acids. Insulin resistance and secretion were calculated by homeostasis model and insulin sensitivity by QUICKI index. Liver ultrasonographic scanning was used for assessing fatty liver. Carotid atherosclerosis was assessed by B-mode ultrasonography of common carotid artery and internal carotid artery. The prevalence of non-alcoholic fatty liver disease was 15.6 % in non alcoholic population and 68.5 % of non-alcoholic fatty liver disease had metabolic syndrome, which was associated with hyperinsulinemia, insulin resistance, insulin insensitivity along with elevated levels of waist circumference, blood pressure, triglyceride, FFA and decreased HDL cholesterol. NAFLD patients had markedly greater carotid intima media thickness than non NAFLD subjects with MCIMT of $591.6 \pm 108$ and $489.5 \pm 132.4$ μm ($P < 0.001$) and plaque prevalence of 19.2 and 2.2 %, respectively, thus the carotid intima media thickness is associated with NAFLD.

**Keywords** NAFLD · CIMT · Metabolic syndrome · Carotid atherosclerosis

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) is now recognized as one of the most common form of chronic liver diseases in developed countries with an estimated prevalence of 10–24 % in the general population. It is a major cause of liver related morbidity and mortality because of its potential to progress to cirrhosis and liver failure [1]. The pathological picture of non-alcoholic fatty liver disease, ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis, resembles that of alcohol induced liver disease, but it occurs in persons who do not abuse alcohol [2]. Among the important risk factors that are considered responsible for hepatic manifestation of metabolic syndrome (MetS), a highly atherogenic condition, are: obesity, type 2 diabetes mellitus (DM), and hyperlipidemia. Further, the studies have shown that insulin resistance (IR) is the pathogenic link underlying the metabolic

abnormalities that constitute the MetS [3] and play a major role in the pathogenesis of NAFLD [4]. Though NAFLD shares many features of the MetS and its presence could signify a substantial cardiovascular risk above and beyond that conferred by individual risk factors [5], however its potential cardiovascular risk is yet to be investigated. This study is an attempt to investigate the association of NA-FLD with carotid intima-media thickness (CIMT) and plaque as surrogate measures of increased cardiovascular risk [6].

## Materials and Methods

The study was carried out at the Department of Biochemistry, SMS Medical College, Jaipur and Department of Radiology, Mahatma Gandhi University of Medical Sciences and Technology, Jaipur. The study included 645 non-alcoholic, non diabetic male subjects, ranging in age from 20 to 40 years. Non alcoholism was decided on the basis of history given by subjects; those who had never consumed alcohol were included in the study. Persons with history of alcohol intake, diabetes (serum glucose >126) and hepatitis were excluded from the study. A written informed consent was obtained from each selected subject after explaining the purpose of study. The participants were assessed for metabolic syndrome (MetS) as per ATP III guidelines [7]. A participant was considered to have the metabolic syndrome if he had three or more of the following risk factors: (1) abdominal obesity: waist circumference (WC) >90 cm [8]; (2) hypertriglyceridemia (TG): ≥150 mg/dl (1.695 mmol/l); (3) low levels of HDL cholesterol: <40 mg/dl (1.036 mmol/l); (4) high blood pressure (HT): ≥130/85 mmHg; (5) high fasting glucose: ≥110 mg/dl (=6.1 mmol/l). The waist circumference was measured at the highest point of the iliac crest at minimal respiration to the nearest 0.1 cm. Three readings of systolic and diastolic blood pressure were obtained from each participant and the average of the last two measurements was used. The current use of antihypertensive medication was also considered as an indication of high blood pressure. Fasting blood samples of subjects were collected and analyzed for sugar, triglycerides, cholesterol and its fractions, alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT) and free fatty acid (FFA) on Olympus AU 400 analyzer using appropriate kits [9–16]. Insulin was estimated by ELISA [17]. Insulin resistance and secretion were calculated by homeostasis model [18, 19] and insulin sensitivity by QUICKI INDEX [20]. Liver ultra sound scanning was performed to assess steatosis using an ALOKA apparatus equipped with a convex 3.5 to 5.0 MHz probe [21]. Steatosis was observed according to abnormally intense, high level echoes arising from the hepatic parenchyma, liver–kidney

difference in echo amplitude, echo penetration into deep portion of liver, and clarity of liver blood vessel structure along with exclusion of other known etiologies of chronic liver disease. The diagnosis was not confirmed by liver biopsy for ethical reasons. High resolution B mode ultrasonography of both the common and internal carotid arteries was performed using 10 MHz linear array transducer. The maximum CIMT (MCIMT) was measured and the average measurement was used [22]. The anterior and posterior walls of the common carotid arteries, internal carotid arteries, and carotid bulbs were evaluated for determination of presence of carotid plaque, defined as a focal thickening >1.2 mm of the intima-media complex, measured from the media-adventitia interface to the intima–lumen interface.

Data were expressed as mean ± SD for continuous variables. Student's $t$ test for unpaired data were used for the comparison of mean values. Group comparisons were performed by the use of ANOVA.

## Results and Discussions

NAFLD is a widely prevalent condition characterized by fatty infiltration of hepatic cells resembling that of alcohol-induced liver injury, occurring even in persons who do not abuse alcohol [23]. The spectrum of this ranges from simple fatty liver to steatohepatitis, a condition histologically similar to alcoholic hepatitis and may progress to end-stage liver disease [24]. NAFLD often shares many features of MetS such as obesity [25], hypertension [26, 27], dyslipidemia [28], type 2 diabetes mellitus [29], and importantly IR a key feature of the syndrome. Since, the components of MetS have implications in the development of cardiovascular disease i.e. CVD [30] and as reported Indians have relatively greater IR, hence prone to CVD as compared to other ethnic groups [31–33]. This study was undertaken to assess cardiovascular risk in 645 NAFLD randomly selected subjects ranging in age 20–40 years, with no history of alcohol abuse and having fasting blood glucose level <126 mg/dl. Further evaluation of subjects was carried out on the basis of presence or absence of fatty liver changes in ultrasonographic study, which confirmed the presence of fatty liver in 15.6 % of the total subjects who were certainly elderly, less active with high calories consumption but had never, abused alcohol. These subjects were separately grouped as NAFLD (Table 1).

The fatty liver changes in NAFLD subjects were associated with significant elevation in liver enzymes. Further these subjects showed appreciable impairment in fasting glucose level as compared to those without fatty liver changes (non NAFLD). Analysis of data also indicated that subjects with NAFLD were insulin resistant as evident from raised fasting glucose, insulin, HOMA β levels with

**Table 1** Characteristic of subjects with and without NAFLD

|  | Non-NAFLD | NAFLD |
|---|---|---|
| No. of subjects ($n$ = 645) | 544 (84.4) | 101 (15.6) |
| Age (years) | 27.1 ± 16.4 | 31.6 ± 18.4 |
| Physical activity |  |  |
|  Sedentary | 0 | 18 (17.8) |
|  Moderate | 372 (68.4) | 72 (71.3) |
|  Active | 172 (31.6) | 11 (10.9) |
| Calorie intake |  |  |
|  1,800–2,200 KCals | 211 (38.8) | 19 (18.8) |
|  >2,200 KCal | 333 (61.2) | 82 (81.2) |
| SBP (mm of Hg) | 118 ± 8.8 | 148 ± 14.7*** |
|  | (102–136) | (118–156) |
| DBP (mm of Hg) | 86.0 ± 3.0 | 94.0 ± 3.8** |
|  | (72–94) | (88–102) |
| WC (cm) | 85.0 ± 8.2 | 92.9 ± 8.2*** |
|  | (69.5–91.8) | (75–99.1) |
| Sugar (mg/dl) | 94.0 ± 12.6 | 115.0 ± 11.2** |
|  | (68–112) | (88–126) |
| Insulin (μm/ml) | 12.6 ± 8.2 | 29.4 ± 4.1*** |
|  | (4.42–28.9) | (12.5–49.8) |
| HOMA IR | 3.8 ± 2.2 | 9.9 ± 3.1*** |
|  | (0.42–6.9) | (3.4–19.88) |
| QUICKI IS | 1.09 ± 0.21 | 0.82 ± 0.09** |
|  | (0.20–6.89) | (0.19–4.92) |
| HOMA β | 208.0 ± 98.4 | 289.2 ± 106.4** |
|  | (32.7–298.4) | (68.1–467.6) |
| TC (mg/dl) | 162.8 ± 51.4 | 198 ± 46.1*** |
|  | (130.6–208.4) | (140–255) |
| TG (mg/dl) | 120.9 ± 28.6 | 192.4 ± 7.6*** |
|  | (64.2–215.8) | (72–270.9) |
| HDL (mg/dl) | 51.0 ± 9.8 | 38.1 ± 6.4** |
|  | (44.1–56.2) | (37.2–51.4) |
| LDL (mg/dl) | 101.88 ± 48.4 | 122.6 ± 28.2* |
|  | (58–130.6) | (62.7–135.6) |
| VLDL (mg/dl) | 20.66 ± 4.8 | 32.4 ± 5.2* |
|  | (12.5–48.2) | (14.9–54.6) |
| FFA (mmol/L) | 0.66 ± 0.20 | 1.15 ± 0.74** |
|  | (0.1–1.0) | (0.6–1.7) |
| ALT (IU/L) | 22.9 ± 6.6 | 46.2 ± 9.8*** |
|  | (12.8–40.6) | (32.7–82.9) |
| AST (IU/L) | 22.4 ± 5.8 | 26.2 ± 7.4* |
|  | (13.9–27.6) | (15.2–28.2) |
| GGT (IU/L) | 22.2 ± 8.9 | 36.4 ± 10.6** |
|  | (18.6–35.4) | (26.7–44.3) |
| Plaque ($n$) | 12 (2.2) | 20 (19.2) |
| MCIMT (μm) | 489.5 ± 132.4 | 591.6 ± 108.6** |

Values are number and mean ± SD; values in parenthesis are range and percentage

* ($P < 0.05$: significant), ** ($P < 0.01$: very significant); *** ($P < 0.001$: highly significant). Rest not significant

significant reduction in insulin sensitivity as compared to those without NAFLD. Insulin resistance calculated as HOMA IR was 2.6-fold higher than that of non NAFLD group. Besides significantly higher SBP, DBP and WC in comparison to non NAFLD they were having dyslipidemia with significantly raised TC, TG, LDLC, VLDLC and FFA with decreased HDLC. Further when assessment was done according to ATPIII criteria NAFLD group was found to be more obese (73.4 %), dyslipidemic (50.9 %) and hypertensive (36.9 %) in comparison to non-NAFLD group (Table 2) and alarmingly 68.5 % subjects of NAFLD had MetS while only 13.3 % of non NAFLD subjects had MetS (Tables 1, 2). Thus the present study also supports that the insulin resistance is a key factor in MetS, and may substantially contribute in the pathophysiology of NAFLD which is a hepatic consequence of metabolic disease [4, 32].

In view of the strong relationship between CIMT and the risk of myocardial and cerebral infarction, an evaluation of carotid atherosclerosis was also done in this study by measuring CIMT as an indicator of cardiovascular risk in ultrasonographically confirmed fatty liver [34, 35]. MCIMT in subjects with NAFLD was significantly higher than in gender-matched and age-matched control group ($P = 0.001$). On looking for plaque prevalence 19.2 % NAFLD had plaques in comparison to 2.2 % in non-NAFLD subjects (Table 1). Further it was also observed that clustering of risk factors was found to be associated with not only IR and MCIMT but also with prevalence of NAFLD and plaques (Table 3). On Pearson analysis, a strong positive correlation was observed between MCIMT and fasting blood sugar ($r = 0.18$, $P = 0.0001$), HT ($r = 0.18$, $P = 0.0001$), WC ($r = 0.39$, $P = 0.0001$), age ($r = 0.44$, $P = 0.0001$), TC ($r = 0.19$, $P = 0.0001$) and TG ($r = 0.18$, $P = 0.0001$), while a strong negative correlation was found between HDLC and MCIMT ($r = -0.32$, $P = 0.0001$). Since both metabolic syndrome and increased MCIMT occur frequently among NAFLD patients screening for both the conditions might be beneficial for assessment of future atherosclerotic complications [36].

**Table 2** Comparison of risk factors defined by ATP III criteria

|  | Non-NAFLD | NAFLD |
|---|---|---|
| Obesity | 147 (26.9) | 74 (73.4) |
| Hypertension | 129 (23.8) | 37 (36.9) |
| Dyslipidemia |  |  |
| ↑ TG | 148 (27.2) | 51 (50.9) |
| ↓ HDL | 116 (21.4) | 47 (46.5) |
| Metabolic syndrome | 72 (13.3) | 69 (68.5) |

Values are number

Values in parenthesis are percentage

**Table 3** IR, NAFLD and CIMT in relation to clustering of risk factors ($n = 645$)

| Risk factor | 0 | 1 | 2 | $\geq 3$ |
|---|---|---|---|---|
| No. of subjects | 128 | 178 | 180 | 159 |
| Age (years) | 24.1 ± 7.9 | 28.7 ± 11.4 | 32.5 ± 8.6 | 36.9 ± 7.2 |
| IR | 2.24 ± 0.11 | 4.0 ± 0.12 | 9.66 ± 1.04 | 32.8 ± 6.6 |
| MCIMT (μm) | 462.4 ± 46.8 | 484.9 ± 59.3 | 522.6 ± 61.4 | 564 ± 72.5 |
| Plaque ($n$) | 0 | 0 | (8) | (20) |
| NAFLD ($n$) | 0 (0) | (4) | (20) | (40) |

Values are number and mean ± SD

Values in parenthesis are percentage

Although the underlying cause for the development of fatty liver disease and its association with CVD is not fully understood, the metabolic syndrome is now proposed to reflect a failure of normal partitioning of surplus fat exclusively into adipose tissue which leads to ectopic fat storage in the liver, muscle and pancreatic β cells, causing hepatic steatosis, dyslipidemia, insulin resistance and insulin secretary failure [37, 38]. Based on the "lipotoxic" hypothesis, the free fatty acid flux from the excessive amount of adipose tissue toward the peripheral tissues could induce the development of insulin resistance especially when the triglyceride storage or the concentration of intermediate fat metabolites such as diacylglycerides, ceramides become excessive within the cytoplasm of these cells. Potential mechanism by which NAFLD may increase cardiovascular risk beyond that imposed by MetS is abnormal lipoprotein metabolism with reduced hepatic synthesis of apoB and its response to increase in triglycerides [39–41].

As the Metabolic Syndrome and its individual component factors are linked with insulin resistance, which may contribute to impaired liver function. Thus NAFLD without other cardiovascular risk factors may be associated with increased CIMT and increased risk of cardiovascular events in patients with incidentally diagnosed fatty liver on abdominal ultrasonography, which may serve as a trigger for assessment of cardiovascular risk factors, and such patients should be evaluated for cardiovascular risk.

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