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HYPERINSULINEMIA PREDISPOSES TO NAFLD

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ABSTRACT

Metabolic syndrome contributes to pathogenesis of Type-2 diabetes and CAD. Insulin Resistance is the key factor of metabolic syndrome implicated in development of Non Alcoholic Fatty Liver Disease (NAFLD). In present study we have investigated the prevalence of NAFLD in metabolic syndrome and contribution of metabolic risk factors in causation of NAFLD in non-diabetic North Indian male population. The study was conducted on 495 non-diabetic, nonalcoholic subjects (age 30-65 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, Triglycerides, Cholesterol and its fractions, Insulin, Alanine transaminase, Aspartate transaminase, Gamma glutamyl transferase and free fatty acids. Insulin resistance was estimated by Homeostasis Model and Insulin sensitivity by QUICKI Index. Liver ultrasonographic scanning was used for assessing fatty liver. The prevalence of metabolic syndrome and NAFLD was 24% and 14.8% respectively in non-alcoholic population and 27% of metabolic syndrome had NAFLD which was associated with hyperinsulinemia, insulin resistance, insulin insensitivity along with elevated levels of waist circumference, blood pressure, triglyceride, FFA and decreased HDL- Cholesterol. The prevalence of NAFLD increased with insulin resistance and clustering of metabolic risk factors.

KEY WORDS

Hyperinsulinemia, Insulin resistance, NAFLD, Obesity, Metabolic Syndrome

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a major cause of liver related morbidity and mortality, because of its potential to progress to cirrhosis and liver failure (1). The pathologic 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 occur in patients who do not abuse alcohol (2). Nonalcoholic steatohepatitis that is characterized by the hepatic steatosis, liver cell injury, hepatic inflammation, fibrosis and necrosis is believed to be an intermediate stage of NAFLD. This disease is often associated with obesity (3,4), type 2 diabetes mellitus (5), dyslipidemia (6) and hypertension (7,8) etc. Each of these abnormalities carries a cardiovascular disease (CVD) risk, and together they are often characterized as the insulin resistance syndrome or the metabolic syndrome (9). The third report of the National cholesterol education program expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III) [ATP III] recommended the use of five variables for diagnosing the metabolic syndrome (MetS), namely waist circumference, blood pressure, serum triglyceride, serum high density lipoprotein cholesterol (HDL) level and fasting glucose level (10). Insulin resistance has been identified as one of the important factor for metabolic syndrome (11,12,13) which could have some role in development of NAFLD as Indians are known to be insulin resistant in comparison to other ethnic groups (14, 15,16). With this theme, the present study was undertaken to investigate the prevalence of NAFLD in MetS and contribution of various risk factors in causation of NAFLD in Indian male subjects.

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MATERIALS AND METHODS

The present study was conducted on 495 non-alcoholic male

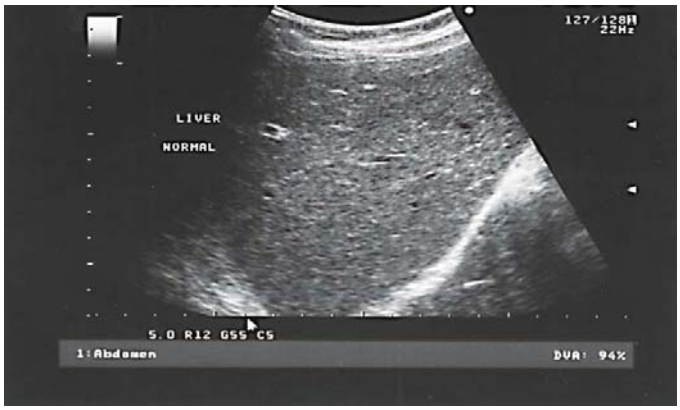

Figure 1 : Liver ultra sound scan of Participant without NAFLD

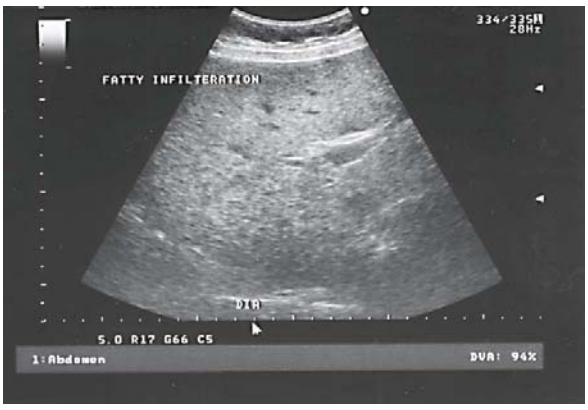

Figure 2: Liver ultra sound scan of participant with NAFLD showing falty infiltration

subjects ranging in age from 30-65 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. The participants were assessed for metabolic syndrome (MetS) as per ATP III guidelines (10). 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 in men(17);2) hypertriglyceridemia (TG): ≥ 150 mg/dl (1.695 mmol/l); 3) low levels of HDL cholesterol: < 40 mg/dl (1.036 mmol/l) in men; 4) high blood pressure (HT): ≥ 130/85 mmHg; 5) high fasting glucose: ≥ 110mg/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. Insulin was estimated by ELISA. Insulin resistance was 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-MHz to 5.0MHz probe (21). Steatosis was observed on the basis of 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 (Figure 1 and 2).

Data are 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 DISCUSSION

Four hundred and ninety five non-diabetic, non alcoholic male subjects free from hepatitis participated in the present study. The prevalence of NAFLD and components of Metabolic Syndrome (MetS) as given in Table 1 show that a large proportion of population under study is predisposed to central obesity and dyslipidemia as almost 32% and 31% participants had WC > 90 cm and TG >150 mg% respectively. When participants were observed for MetS as per ATP III criteria, 24% fulfilled the criteria i.e. having ≥ 3 Risk Factors. NAFLD was prevalent in 14.8% of non alcoholic subjects and 20% of subjects had ALT level above 50 IU/L. Prevalence of NAFLD was two and half times more in Metabolic Syndrome i.e. 11%

Table 1: Prevalence of Risk Factors and Fatty Liver in Relation to Metabolic Syndrome

Variables		Total Subjects	Non-Metabolic Syndrome	Metabolic Syndrome
Subjects		495	376 (76%)	119 (24%)
Sugar (≥ 110 mg%)		105 (21.3%)	64 (17%)	41 (34%)
B.P.	Systolic (≥ 130 mmHg)	118 (23.9%)	84 (22.3%)	34 (28.5%)
	Diastolic (≥ 80 mmHg)	112 (22.6%)	77 (20.4%)	35 (29.4%)
WC (≥90 cm)		158 (32.0%)	109 (28.9%)	49 (41.1%)
TG (≥150 mg%)		149 (30.1%)	102 (27%)	47 (39%)
HDL (≤ 40 mg%)		121 (24.4%)	79 (21%)	42 (35.3%)
ALT (≥ 1.5 X)		99 (20.1%)	45 (11.9%)	54 (45.3%)
NAFLD		73 (14.8%)	41 (11%)	32 (27%)

in those without Metabolic Syndrome and 27% in those with Metabolic Syndrome. Further, the prevalence of risk factors was examined separately in those with and without metabolic syndrome. The prevalence of metabolic risk factors was significantly higher in metabolic syndrome as compared to those without the syndrome. Even 45% of subjects with metabolic syndrome had elevated ALT level as compared to those without this syndrome. When subjects were considered on the basis of presence or absence of NAFLD (Table 2), the prevalence of metabolic risk factors was significantly higher in NAFLD group as compared to Non-NAFLD group. Subjects with NAFLD were more obese, dyslipidemic and glucose intolerant. Almost 70% subjects with NAFLD had metabolic syndrome which is five and half fold higher than those without NAFLD. Prevalence of NAFLD was also examined in relation to prevailing risk factors (Figure 3). With increased clustering of risk factors, the prevalence of NAFLD increased progressively. The prevalence of NAFLD was 3.2% in those without any risk factor and increased to 39.4% in participants with five risk factors. However, the prevalence of NAFLD in metabolic syndrome was 27%.

Table2: Prevalence of Risk Factors and Metabolic Syndrome in Relation to NAFLD

Variables	Total Subjects (n=495)	
Subjects	**Without NAFLD**	**NAFLD**
Participants	422 (85.2%)	73 (14.8%)
Sugar (≥ 110 mg%)	75 (17.7%)	30 (41.09%)
B.P. Systolic (≥ 130 mmHg)	92 (21.8%)	26 (35.6%)
B.P. Diastolic (≥ 80 mmHg)	95 (22.5%)	27 (36.9%)
WC (≥ 90 cm)	106 (25.1%)	52 (71.2%)
TG (≥ 150 mg%)	113 (26.8%)	36 (49.3%)
HDL (≤ 40 mg%)	87 (20.6%)	34 (46.5%)
ALT (≥ 1.5 X)	50 (11.8%)	49 (67.1%)
Metabolic Syndrome	54 (12.8%)	51 (69.8%)

The baseline characteristics of the subjects (Table 3) show that subjects with Metabolic Syndrome had significantly raised ($p<0.001$) fasting blood glucose, Triglycerides, Blood Pressure, Waist Circumference and significant low ($p<0.001$) HDL cholesterol as compared to subjects without Metabolic Syndrome. Besides this, subjects with Metabolic Syndrome had significantly raised ($p<0.001$) serum ALT levels while AST and GGT were marginally raised ($p<0.05$) as compared to those without MetS. Number of prospective studies had shown raised ALT and GGT to predict the development of type 2 diabetes independent of BMI and alcohol intake. Perhaps

Table 3: Baseline Characteristics of Metabolic and Non metabolic Subjects

Variables	Total Subjects	Non-Metabolic Syndrome	Metabolic Syndrome
Subjects	495	376 (76%)	119 (24%)
Age	30-65 48.7 ± 9.25	30 – 58 45.3 ± 8.34	36-65 44.4 ± 10.2
Sugar (mg%)	68 – 126 102 ± 14.2	68 – 110 94.0 ± 12.6	88 – 126 116.0 ± 12.9**
B.P. (mm of Hg) Systolic	120 – 160 138 ± 12.3	120 – 136 126 ± 4.3	120 – 160 148 ± 14.7**
Diastolic	80 – 95 88.0 ± 4.2	80 – 90 86.0 ± 3.0	86 – 96 94.0 ± 3.8**
WC (cm)	70 – 130 88.0 ± 9.2	70 – 96 84.0 ± 8.2	75 – 103 94.0 ± 8.2**
TG (mg%)	76 – 250 158.6 ± 31.3	76 – 165 112.6 ± 24.2	105 – 250 199.0 ± 26.3**
HDL (mg%)	35 – 65 45.2 ± 10.7	44 – 65 51.4 ± 8.2	35 – 55 38.0 ± 7.2**
ALT (IU/L)	16.2 – 75.2 28.9 ± 8.7	16.2 – 40.6 22.6 ± 5.8	35.0 – 75.2 46.0 ± 8.9**
AST (IU/L)	13.6 - 35.8 25.7 ± 6.7	13.6 – 32.6 23.4 ± 5.2	15.2 – 35.8 27.2 ± 7.1*
GGT (IU/L)	18.6-46.2 26.7± 5.9	18.6-39.5 24.4±9.9	27.4-46.2 28.1±10.6*
FFA (mmol/L)	0.1-1.7 0.86±0.20	0.1-1.0 0.66±0.20	0.6-1.7 1.15±0.74**
Insulin (iU/ml)	6.6 – 42 20.7 ± 12.1	6.6 – 29.7 12.6 ± 7.9	12.5 – 42 29.4 ± 8.2**
Insulin Resistance	1.18 – 14.7 6.2 ± 3.4	1.18 – 9.2 3.6 ± 2.4	6.2 – 14.7 9.4 ± 2.3**
Insulin Sensitivity	0.16-4.92 1.01±1.9	0.20-4.92 1.15±1.01	0.16-0.29 0.80±0.08**

Statistical comparison was done using z test between Non-metabolic Syndrome and Metabolic Syndrome. *P<0.05; **P<0.001

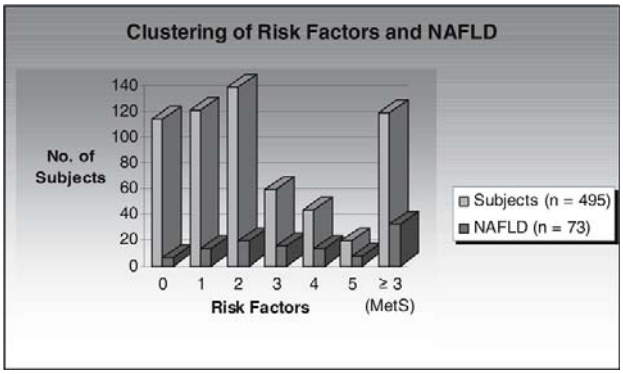

Figure 3 : Prevalence of NAFLD in relation to risk factors 0, 1, 2, 3, 4, 5 and MetS was 3.2%, 10.6%, 14.2%, 26.7%, 32.5%, 39.4% and 27% respectively

Table 4: Association of Insulin Resistance with Metabolic Syndrome and NAFLD

	Insulin Resistance (n=495)			
	1.18-4.41 (n=227)	4.42- 7.64 (n=145)	7.65-10.8 (n=95)	10.9-14.7 (n=28)
MetS (n-119)	6 (2.6%)	22 (14.4%)	63 (66.3%)	28 (100%)
NAFLD % (n-73)	1 (0.4%)	4 (2.7%)	40 (41.7%)	28 (100%)

insulin resistance which is an important key factor for MetS causes hepatic dysfunction which may contribute to development of type 2 diabetes. Further, there are emerging evidences suggesting a strong association between liver enzymes with insulin resistance and MetS (22). On the other hand liver enzymes such as GGT and ALT had been reported to be associated with non-alcoholic fatty liver disease. There are evidences that NAFLD inturn could increase the risk of cardiovascular disease independent of other factors (23). In present study 45% of subjects with MetS and 67.1% subjects with NAFLD were found to have elevated levels of ALT which indicates that involvement of liver dysfunction in metabolic syndrome could lead to NAFLD. Further, subjects with MetS were hyperinsulinemic, insulin resistant and insulin insensitive as compared to those without this syndrome. FFA level was also significantly higher (P<0.001) in those with Metabolic Syndrome. In our earlier study, we found free fatty acid as an important intermediate phenotypic trait in North Indian diabetic subjects as free fatty acid was found to be strongly correlated with insulin resistance (24). Insulin resistance, one of the important component of Metabolic Syndrome (11) was also found to be associated with development of NAFLD as evident from Fig 4. Insulin resistance, estimated using Homeostatic Model, ranged from 1.118 to 14.7 in 495 subjects. This range

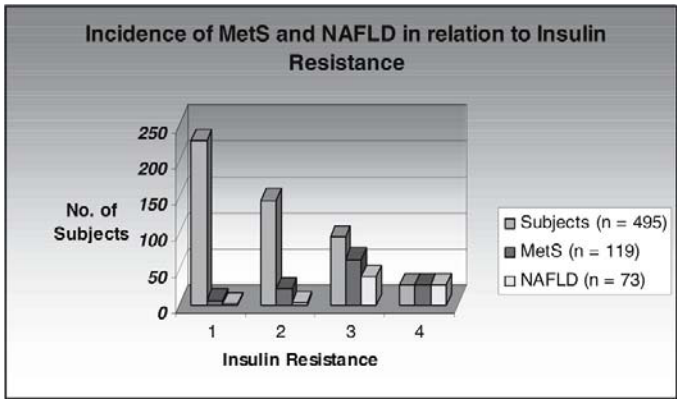

Figure 4 : Incidence of MetS and NAFLD in relation to insulin resistance. Whole range of insulin resistance (1.18 - 14.7) was divided into four quartiles : 1 quartile - 1.8 - 4.41; 2nd quartile - 4.42 - 7.64; 3rd quartile - 7.65 - 10.8 and 4th quartile - 10.9 - 14.7

was distributed into four quartiles to assess the association of Insulin resistance with development of NAFLD (Table 4, Fig 4). All the subjects in highest quartile of Insulin Resistance developed NAFLD indicating that Insulin Resistance is one of the important underlying cause for development of not only CVD but also NAFLD(9). Table 5 gives the results of the univariable analysis of Risk Factors for NAFLD. Prevalence of NAFLD is not related with age, Odds Ratio being 1.12. Normal liver was less likely than NAFLD in subjects with hyperglycemia (OR=0.19), obesity (OR = 0.18), hypertriglyceridemia (OR=0.34), Low HDL (OR=0.23), systolic HT (OR=0.63), diastolic HT (OR=0.52) and raised ALT (OR=0.42). Metabolic Syndrome is a very common clinical condition with approximately 40% of population over 50 years of age in western countries meeting ATPIII diagnostic criteria (11,12). The prevalence of MetS in present study conducted on limited number of subject was 24% after excluding subjects with history of alcohol intake, diabetes and hepatitis. However, in our earlier studies conducted on diabetic families the prevalence of Metabolic Syndrome was investigated to be 60% in diabetic subjects, 42% in first degree diabetic relatives, 33% in first degree non diabetic relatives, and 20% in nondiabetic spouses of Diabetic subjects (25). Metabolic Syndrome is defined as constellation of several metabolic risk factors and each risk factor is independently linked to Insulin Resistance, has also been shown to be associated with clustering of risk

Table 5: Univariable Analysis of Risk Factors for Normal Vs NAFLD

	OR	95% CI
Age	1.12	0.55-1.62
Sugar	0.19	0.07-0.37
B.P.		
Systolic	0.63	0.29-0.86
Diastolic	0.52	0.33-0.79
WC	0.18	0.06-0.42
TG	0.34	0.16-0.62
HDL	0.23	0.12-0.24
ALT	0.42	0.28-0.96
AST	0.86	0.66-1.22
GGT	0.88	0.71-1.20
FFA	0.35	0.12-0.83
Insulin	0.16	0.08-0.61
Insulin Resistance	0.31	0.12-0.76
Insulin Sensitivity	1.09	0.59-2.91

Data are given as ODDS Ratio and 95% Confidence Interval based on Observed Means.

factors (26). Results of the present study supports the hypthesis that the insulin resistance is a key factor in MetS, plays a pivotal role in the pathophysiology of NAFLD (15) as subjects with NAFLD were insulin resistant and prevalence of NAFLD was signifacently higher in those with MetS. Thus, fatty liver can be considered as hepatic consequence of metabolic disease leading to increase prevalence of NAFLD in MetS (15).

Although the mechanism for the development of fatty liver disease is not fully understood, the metabolic syndrome is now proposed to reflect a failure of normal partitioning of surplus fat exclusively into adipose tissue (27,28). The failure leads to ectopic fat storage in the liver, muscle and pancreatic β cells, which leads to hepatic steatosis, dyslipidemia, insulin resistance and insulin secretory failure.

Our study has several limitations. First, indentification/dignosis of subjects for NAFLD was based in ultra sonography which although has relatively high sensitivity (82-94%) and specificity (66-95%) in detecting fatty liver (29), may give an errorneous diagnosis in 10- 30% of cases. Second, self reported information regarding alcohol intake is frequently subjected to under reporting, and misreporting could be a source of bias.

In conclusion, development of NAFLD occurs in a substantial proportion of apparently healthy people with insulin resistance and the prevalence of NAFLD increases with clustering of metabolic risk factors.

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