Original Article

Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver disease in Indian patients with normal body mass index: Do they differ from obese or overweight non-alcoholic fatty liver disease?

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ABSTRACT

Background: Obesity is an important risk factor for non-alcoholic fatty liver disease (NAFLD); however, NAFLD does occur in lean subjects. This study was aimed to evaluate the magnitude, clinical, pathological, and metabolic profiles of NAFLD in normal body mass index (BMI) subjects (defined as lean NAFLD) in comparison to overweight or obese NAFLD and lean healthy control. **Materials and Methods:** 336 subjects (205 consecutive NAFLD, and 131 healthy controls) were studied. **Results:** Among 205 NAFLD patients, 27 (13.2%) were lean, while 141 (68.8%) and 37 (18%) patients were obese and overweight, respectively. The lean NAFLD compared to obese NAFLD had significantly lesser degree of fasting hyperinsulinemia ($P < 0.001$), homeostasis model assessment insulin resistance (HOMA-IR, $P < 0.001$), and lower prevalence of diabetes mellitus ($P = 0.01$) and metabolic syndrome ($P < 0.001$). The profiles of serum lipids were similar between all 3 BMI categories, and 89% of lean NAFLD were dyslipidemic. Compared to obese subjects, patients with lean NAFLD had less hepatic necro-inflammation ($P = 0.05$) and fibrosis ($P < 0.001$). However, the proportion of steatohepatitis and advanced fibrosis were similar between all BMI categories. The profiles of overweight NAFLD were similar to those of lean NAFLD, except for higher HOMA-IR, uric acids and male gender in overweight group. Despite being lean, the mean BMI of lean NAFLD were still higher than unselected lean healthy controls ($P = 0.02$). **Conclusions:** Lean NAFLD patients have less severe disease, minor, or no insulin resistance, but are frequently dyslipidemic and have BMI higher than lean healthy control.

Key words: Dyslipidemia, insulin resistance, lean non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease worldwide.[1,2] The prevalence of NAFLD in Indian population ranges from 5 to 28%, which is comparable to the West.[3] The spectrum of NAFLD ranges from simple steatosis, non-alcoholic steatohepatitis (NASH), to cirrhosis. Although NAFLD is more common in subjects with obesity and diabetes mellitus (DM), it does occur in lean and non-diabetic subject.[2,4,5] Furthermore, compared to the West, Indians are known to develop NAFLD at lower degree of adiposity.[2,6,7] In a recent population-based epidemiological study in India, 75% of individuals with NAFLD were non-obese and 54% were neither overweight nor had central obesity.[2] Another recent study revealed that lean, non-alcoholic, non-diabetic, non-smoking ethnic Asian Indians in comparison to matched Caucasians, Hispanics, Black and Eastern Asians had 2- to 3-fold increase in insulin resistance (IR) and 2-fold increase in hepatic triglyceride

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content.[7] Recent concepts also suggest that the magnitude of adipose tissue dysfunction may have more metabolic impact than the severity of adiposity.[8]

Obesity is not only a risk factor for NAFLD but also determine severity of NAFLD.[9,10] The recommended body mass index (BMI) cutoff values for Asians for defining overweight (23-25 kg/m^2) and obesity (>25 kg/m^2) are lesser than those of Western populations.[11] Also, the Asians are known to develop central obesity at lower BMI. Also, lower preponderance of adiposity in Indian NAFLD is well-documented,[2,6,7]; however, data on clinical characteristics, metabolic profiles, and histopathological severity in patients with lean NAFLD in comparison to the overweight or obese NAFLD patients is scant. It is not clear what proportion of lean NAFLD in India has abdominal obesity, IR, and features of metabolic syndrome (MS). Therefore, the aim of this study was to evaluate the magnitude and clinical profiles, metabolic profiles, and histopathological severity of patients with lean NAFLD in comparison to overweight or obese NAFLD, and lean healthy control without fatty liver.

Materials and Methods

The study was conducted between 2009 December to 2011 November. During study period, 205 consecutive patients with NAFLD were included in this study. The diagnosis of NAFLD was made on the basis of characteristics real time ultrasonography features, presence of IR or features of MS, and histologic confirmation whenever possible. NAFLD with BMI of less than 23 kg/m^2 were defined as lean NAFLD. Patients using alcohol >20 g/day, patients with liver diseases of other known causes, patients on certain medications known to induce fatty liver such as estrogens, amiodarone, methotrexate, and tamoxifen were excluded. For comparing characteristics of lean NAFLD patients, we also included 131 lean healthy subjects with normal liver on ultrasonography as control subjects. The consent for including data for the purpose of study was obtained from each patient at the time of enrollment.

Definitions

Patients with BMI of more than 23 kg/m^2 were defined as overweight and those with a BMI of >25 were labeled as obese according to Asian standards.[11] Patients having at least 3 of the following 5 components: Hyperglycemia (fasting Blood sugar >110 mg%), central obesity (waist circumference >90 cm for males and >80 cm for females), hypertension (BP >130/85), hypertriglyceridemia (serum triglyceride >150 mg %), and low HDL cholesterol levels (<50mg/dl for women and < 40mg/dl for men) were labeled to have the metabolic syndrome as per modified Adult Treatment Panel III criteria.[12] Dyslipidemia was defined by presence of one or more than one abnormal serum lipid concentration.

Patients evaluation and procedure

A thorough clinical history and examinations including anthropometric measurements were done in all patients at the initial visit. After an overnight fast, blood sample were collected for a complete blood count and biochemical investigations including a liver function test, lipid profile, fasting serum insulin, and fasting blood glucose. Serum sample from each patient was tested for markers of viral hepatitis A, B, C, and E. Serum ferritin, copper study, thyroid function tests, and autoantibody tests were done using conventional techniques. IR was measured as homeostasis model assessment insulin resistance (HOMA-IR). Value of HOMA-IR more than 2.0 was taken as the presence of insulin resistance.[13]

Liver biopsy

Liver biopsy was performed a day after performance of blood tests. The biopsy was performed using an 18-gauge biopsy gun, after obtaining informed consent and knowing that the coagulation profile was normal. Liver biopsy specimens were fixed in formalin and embedded in paraffin. All biopsy specimens were analyzed by two experienced hepatopathologists (A.R. and C.B.) blinded to clinical data. The classification given by Kleiner *et al*. was used to grade and stage NAFLD.[14] Grade of steatosis was defined: 0 = steatosis <5%, 1 = steatosis 5% to 33%, 2 = steatosis >33%-66%, 3 = steatosis >66%. Fibrosis was staged from 0 to 4: Stage 0 = absence of fibrosis; stage 1 = perisinusoidal or portal; stage 2 = perisinusoidal and portal/periportal; stage 3 = septal or bridging fibrosis; and stage 4 = cirrhosis. NAFLD activity score was calculated in each patient as sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2); which ranged from 0 to 8. Patients with activity score 5 or more were labeled having NASH. The biopsy sample was assessed independently by both pathologists. The consensus between them was good. In case of discrepancies, histological sections were simultaneously reviewed to reach a consensus.

Non-invasive markers of liver fibrosis and FibroScan

The aspartate aminotransferase (AST)-to-platelet ratio index (APRI) was calculated as AST (/upper limit of normal)/platelet count ($\times 10^9$/L) × 100.[15] FIB-4 was calculated as age × AST U/L)/platelet count ($\times 10^9$/L) × √ALT (U/L).[16] The NAFLD fibrosis score was calculated according to the following formula: −1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glycemia/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio-0.013 × platelet ($\times 10^9$/L) − 0.66 × albumin

(g/dL).[17] Transient elastography (TE) was performed using FibroScan (Echosens, France). As suggested by the manufacturer, 10 successful acquisitions were performed on each patient. The results were expressed in kilopascal (kPa). Median value of the successful measurements was kept as representative of liver stiffness. Only TE-results obtained with 10 valid measurements with a success-rate of at least 60% and an interquartile range ≤ 30% were considered reliable.

Data analysis

Normally distributed continuous variables were expressed as mean (SD), and the continuous variables with skewed distribution were expressed as median (range). Categorical data was presented as proportions. For the comparison of normal covariates between BMI categories, one way ANOVA with Bonferroni correction as a post-hoc test was used. Similarly, the comparisons between these groups for skewed data were performed by Kruskal-Wallis followed by Mann Whitney test with adjusted *P* values. Comparisons for categorical variables were done using x2 or Fishers test for discrete variables, wherever applicable. Data were analyzed by using SPSS software version 15.0 (SPSS, Chicago, IL, USA), and a $P < 0.05$ was taken as significant.

Results

Demographic and metabolic characteristics

Among 205 NAFLD patients, obesity was present in 141 (68.8%) patients, 37 (18%) patients were overweight, and 27 (13.2%) patients were lean (BMI < 23 Kg/m^2). The baseline demographic and metabolic profiles of all NAFLD patients in all 3 BMI categories are summarized in Table 1. Majorities (70% to 95%) of the patients were male, and the mean ages at presentation were similar in all 3 BMI groups. Compared to obese NAFLD, patients with lean NAFLD had lower degree of fasting hyperinsulinemia (6.7 [2.3-11.6] vs. 11.9 [3.9-81.6], $P < 0.001$) and HOMA-IR (1.7 [0.5-2.7] vs. 2.72 [0.9-20.1], $P < 0.001$). The IR as indicated by HOMA-IR > 2 was present in only 7.4% ($n = 02$) patients of lean NAFLD, which was significantly lower than that in overweight (40%, $P = 0.05$), or obese NAFLD (61%, $P = 0.001$). Interestingly, 89% ($n = 24$) of lean NAFLD patients were dyslipidemic, and compared to overweight or obese, lean NAFLD patients had the similar serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides. The mean waist circumference of lean NAFL patients was 80.1 ± 5.8, and as per definition, only 2 (7.4%) patient satisfied the criteria for abdominal obesity. The metabolic syndrome tended to be less common in lean NAFLD patients compared to obese NAFLD (22% vs. 64%, $P < 0.001$), but was similar to those with overweight NAFD (22% vs. 27%, $P = 0.77$). However, at least one criterion of metabolic syndrome was seen in majority (89%, 24/27) of lean NAFLD patients. The profiles of lean and overweight NAFLD were similar in terms of the metabolic variables such lipid profiles, levels of fasting blood glucose, and insulin.

Disease severity in lean NAFLD compared to obese and overweight

Laboratory parameters

The median levels of serum transaminases, gamma-glutamyl transpeptidase, and alkaline phosphatase were similar between all 3 BMI categories [Table 2]. The lean compared to obese or overweight NAFLD patients had lower levels of serum uric acid.

Table 1: The baseline demographic and metabolic profiles of NAFLD patients in all 3 BMI categories

Parameters*	Lean (1) *N*=27	Overweight (2) *N*=37	Obese (3) *N*=141	*P* values between groups		
				1 and 2	1 and 3	2 and 3
Age, mean±(SD) years	38 (15.4)	41 (11.9)	40.9 (12.8)	0.51	0.43	0.98
Gender	19:8	35:2	107:34	0.01	0.62	0.01
M:F (%)	(70: 30)	(95: 5)	(76: 24)			
BMI, mean±(SD) Kg/m^2	21.3 (1.9)	24.1 (0.5)	28.3 (3.2)	<0.001	<0.001	<0.001
Waist circumference, mean±(SD) cm	80.1 (5.8)	93.4 (4.5)	96.7 (7.2)	<0.001	<0.001	0.08
Diabetes mellitus, *n* (%)	01 (3.7)	06 (16)	36 (26)	0.22	0.01	0.28
Hypertension, *n* (%)	04 (15)	05 (13)	38 (27)	1.00	0.23	0.13
Hypothyroid, *n* (%)	02 (7)	02 (5.4)	14 (10)	1.00	1.00	0.46
Metabolic syndrome, *n* (%)	06 (22)	10 (27)	91 (64)	0.77	<0.001	<0.001
Fasting blood sugar, mean ±(SD) mg/dl	94.2 (8.4)	96.3 (11.5)	103.6 (21.2)	0.49	0.11	0.25
Serum Insulin, mean ±(SD) IU/mL	6.7 (2.3-11.6)	8.7 (3.8-71.2)	11.9 (3.9-81.6)	0.07	<0.001	0.01
HOMA-IR, median (range)	1.7 (0.5-2.7)	2.07 (1.0-17.2)	2.72 (0.9-20.1)	0.12	<0.001	0.01
High HOMA-IR, *n* (%)	02 (7.4)	15 (40)	86 (61)	0.05	0.001	0.12
Total serum cholesterol, mean±(SD) mg/dl	203 (56.8)	191.5 (53.9)	183.3 (44.6)	0.35	0.16	0.89
Serum triglyceride, mean±(SD) mg/dl	190.4 (97.2)	213.2 (129.8)	177 (100.1)	0.71	0.92	0.31
Serum LDL cholesterol, mean±(SD) mg/dl	120.8 (44.3)	110.8 (34.9)	116.2 (35.6)	0.14	0.65	0.61
Serum HDL cholesterol, mean±(SD) mg/dl	40.2 (9.1)	32.8 (8.4)	34.4 (9.8)	0.68	0.07	0.51

*Normally distributed continuous variables are expressed as mean (SD) and continuous variables with skewed distribution were expressed as median (range), Categorical data are presented as proportions, HOMA-IR: Homeostasis model assessment insulin resistance, BMI: Body mass index, NAFLD: Non-alcoholic fatty liver disease, SD: Standard deviation, LDL: Low density lipoprotein, HDL: High density lipoprotein

Table 2: The biochemical parameters and disease severity (histopathology and non-invasive markers of NAFLD patients in all 3 BMI categories)

Parameters*	Lean (1) (*n*=27)	Overweight (2) (*n*=37)	Obese (3) (*n*=141)	*P* values* between groups		
				1 and 2	1 and 3	2 and 3
Laboratory tests						
Total serum bilirubin, median (range) mg/dl	0.86 (0.6-8.6)	1.17 (0.4-6.0)	0.97 (0.4-4.6)	0.05	0.46	0.04
AST, median (range) IU/L	38 (23-180)	36 (18-114)	39 (15-389)	0.57	0.79	0.81
ALT, median (range) IU/L	45 (11-217)	50 (19-193)	54 (13-312)	0.47	0.76	0.61
GGT, median (range) IU/L	24 (09-241)	33 (10-169)	29.5 (06-358)	0.09	0.13	0.50
SAP, median (range) U/L	84 (55-230)	79 (32-294)	78 (24-358)	0.30	0.28	0.76
Albumin, mean (±SD) mg/dl	4.08 (0.4)	4.2 (0.3)	4.1 (0.3)	0.38	0.73	0.33
INR, mean (±SD)	1.0 (0.1)	1.0 (0.1)	0.9 (0.1)	0.57	0.52	0.21
Uric acid, mean (±SD) mg/dl	5.2 (1.1)	6.1 (1.7)	5.8 (1.1)	0.058	0.05	0.35
Platelets/mm^3, median (range)	180 (80-423)	160 (115-300)	211 (74-431)	0.73	0.17	0.07
TLC/mm^3, median (range)	5800 (6000-9000)	4700 (4000-11400)	5500 (3000-11500)	0.38	0.53	0.82
Neutrophil-lymphocyte ratio, median (range)	1.9 (0.47-10.6)	1.9 (0.6-3.1)	1.8 (0.9-4.3)	0.92	0.51	0.89
Non-invasive markers						
AST/ALT, median (range)	0.84 (0.46-2.6)	0.75 (0.41-1.15)	0.70 (0.86-2.1)	0.08	0.10	0.92
APRI, median (range)	0.47 (0.14-2.5)	0.43 (0.9-1.9)	0.49 (0.9-3.5)	0.85	0.76	0.36
FiB4, median (range)	1.17 (0.75-3.37)	1.08 (0.53-4.25)	1.11 (0.18-5.04)	0.87	0.46	0.45
NAFLD fibrosis score, median (range)	−2.1 (−5.7-2.4)	−1.9 (−4.5-3.8)	−2.1 (−7.2-6.04)	0.42	0.68	0.57
LSM, median (range) KPa	5.9 (3.4-45.7)	6.1 (4.0-17.0)	6.6 (3.1-31.0)	0.68	0.23	0.28
Histological						
Grades, mean (±SD)	3.3 (1.5)	04 (1.5)	4.1 (1.4)	0.31	0.05	0.74
Stage, median (range)	01 (0-04)	01 (01-04)	02 (0-04)	0.11	0.003	0.68
NASH, *n* (%)	05/18 (28)	09/19 (47)	36/73 (38)	0.31	0.12	1.00
Any fibrosis, *n* (%)	09/18 (50)	14/19 (74)	61/73 (84)	0.18	0.01	0.38
Advanced (>F2) Fibrosis, *n* (%)	01/18 (5.6)	06/19 (31)	20/73 (27)	0.09	0.06	0.69

*Normally distributed continuous variables are expressed as mean (SD) and continuous variables with skewed distribution were expressed as median (range). Categorical data are presented as proportions, AST: Aspartate aminitransferase , ALT: Alanine aminotransferase, GGT: Gamma glutamyltransferase, SAP: Serum alkaline phosphatase, INR: International normalized ratio, TLC: Total leukocyte count, APRI: Aspartate aminotransferase-to-platelet ratio index, FiB4: Fibrosis 4 score, NAFLD: Non-alcoholic fatty liver disease, LSM: Liver stiffness measurement, KPa: Kilopascal, NASH: Non-alcoholic steatohepatitis, BMI: Body mass index

Histopathology

Liver biopsy was available in 110 NAFLD patients (18 lean, 19 overweight, and 73 obese patients). The mean NAFLD activity score (NAS) in lean patients (3.3 ± 1.5) was significantly lower in comparison to mean NAS (4.1 ± 1.4) in obese NAFLD patients ($P = 0.01$). However, 28% (5 of 18) lean NAFLD patients met criteria for NASH (NAS > 4), which was statistically similar to the prevalence of NASH in overweight (47%) and obese (38%) patients with NAFLD. The proportion of patients with liver fibrosis was significantly lower in lean compared to obese NAFLD (50% vs. 84%, $P = 0.01$), and advanced fibrosis tended to be lower in lean than in obese NAFLD, but the difference did not reach statistical significance (5.6% vs. 27%, $P = 0.06$).

Non-invasive markers

Because liver biopsy could not be done in all patients, various non-invasive parameters (AST/AST ratio, APRI, FIB-4, and NAFLD fibrosis score) were also used to assess severity of NAFLD in all patients. However, none of parameters were significantly different between patients of 3 BMI categories [Table 2]. Liver stiffness values by measured FibroScan were also similar between lean, overweight, and obese NAFLD patients.

Lean NAFLD versus lean healthy subjects

The characteristics of lean patients with NAFLD were compared to 131 non-selected lean healthy control subjects without ultrasonographic evidence of fatty liver [Table 3]. Despite being in normal range, the mean BMI of lean NAFLD were still higher than of lean healthy controls (21.3 ± 0.9 vs. 22.0 ± 0.76 kg/m^2, $P = 0.02$). Also, the prevalence of dyslipidemia and MS were significantly higher in lean NAFLD than the lean healthy controls.

Discussion

The lean NAFLD comprised approximately 13.2% ($n = 27$) of total NAFLD ($n = 205$) patients coming to our tertiary liver care center. In various study from India, the proportion of lean NAFLD has been reported to vary from 11% to 31.7% [Table 4]. The most important metabolic risk factor among lean NAFLD patients was dyslipidemia, which was present in nearly 90% of them. The IR as indicated by HOMA-IR > 2 was present in only 7.4% ($n = 2$) patients of lean NAFLD, which was significantly lower than that in overweight ($P = 0.05$), or obese NAFLD ($P = 0.001$). Only 2 (7.4%) of lean NAFLD patients had abdominal obesity. However, the mean BMI, hypertension, serum

Table 3: Characteristics of lean NAFLD patients with non-selected lean healthy control subjects

Parameters	Lean healthy controls *N*=131	Lean NAFLD patients *N*=27	*P*
Age, mean±(SD) years	40.1 (13.2)	38 (15.4)	0.78
Gender, M:F	89:42	19:08	1.00
BMI, mean±(SD) Kg/m^2	22.0 (0.76)	21.3 (1.9)	0.02
Diabetes Mellitus, *n* (%)	01 (0.7)	01 (3.7)	0.28
Hypertension, *n* (%)	05 (3.8)	04 (15)	0.03
Dyslipidemia* *n* (%)	10 (7.6)	24 (89)	<0.001
Metabolic syndrome, *n* (%)	0 (0)	02 (22)	<0.001
Fasting blood sugar, mean±(SD) mg/dl	87.2 (12.9)	94.2 (8.4)	0.02
Total serum bilirubin, mean±(SD) mg/dl	1.0 (0.3)	1.3 (1.6)	0.77
AST, mean (±SD) IU/L	23 (5.3)	48.5 (33.6)	<0.001
ALT, mean (±SD) IU/L	22.2 (7.1)	65.5 (49.9)	<0.001
SAP, mean (±SD) U/L	66.4 (20.5)	103.3 (50.8)	<0.001
Serum albumin, mean (±SD) mg/dl	4.2 (0.3)	4.01 (0.4)	0.38
Serum GGT, mean (±SD) IU/L	18.8 (8.1)	38.2 (47.2)	0.005
Platelets/mm^3, mean (±SD)	248 (61)	203 (84)	0.009
TLC/mm^3, mean±(SD)	7200 (2000)	4916 (3257)	<0.001
Total serum cholesterol, mean±(SD) mg/dl	164 (17.5)	203 (56.8)	0.002
Serum LDL cholesterol, mean±(SD) mg/dl	85.4 (13.5)	120.8 (44.3)	0.005
Serum HDL cholesterol, mean±(SD) mg/dl	54.7 (8.8)	40.2 (9.1)	<0.001
Serum triglyceride, mean±(SD) mg/dl	114.4 (24.6)	190.4 (97.2)	0.05
LSM, mean±(SD) kPa	4.1 (0.74)	7.75 (7.85)	<0.001

*Dyslipidemia was defined by presence of one or more than one abnormal serum lipid concentration, BMI: Body mass index, NAFLD: Non-alcoholic fatty liver disease, AST: Aspartate aminotransferase, kPa: Kilopascal, SD: Standard deviation, LDL: Low density lipoprotein, HDL: High density lipoprotein, ALT: Alanine aminotransferase, SAP: Serum alkaline phosphatase, GGT: Gamma glutamyltransferase, TLC: Total leukocyte count, LSM: Liver stiffness measurement

Table 4: Body mass indices of NAFLD patients in Indian studies

Authors	Place	Total NAFLD, *n*	Mean BMI, *N* (±SD) mg/m^2	Lean NAFLD, *N* (%)	Lean+overweight NAFLD, *N* (%)	Obese NAFLD, *N* (%)
Das *et al*.[2]	West Bengal	164	23.06±4.2	52 (31.7)	75 (45.7)	25 (15.2)
Amrapurkar *et al*.[31]	Mumbai	167	26.6±5.1	NS*	80 (48)	87 (52)
Duseja *et al*.[6]	Chandigarh	100	28.7	12 (12)	32 (32)	68 (68)
Bajaj *et al*.[20]	Allahabad	39	26.7±4.4	8 (18)	14 (33)	25 (67)
Madan *et al*.[5]	New Delhi	51	26.7 (21.3-32.5)#	NS	17 (33.3)	34 (66.7)
Agarwal *et al*.[32]	Delhi	71	27.5±3.99	08 (11)	21 (29.5)	50 (70.7)
Uchil *et al*.[33]	Mumbai	225	28.58±4.25	50 (22)	169 (75.1)	56 (24.8)
Das *et al*,[34]	Kerala	105	25.33±2.44	NS	44	56
Viswanathan *et al*.[35]	Chennai	156	29.7±7.0	NS	23 (14.7)	133 (85.3)
Present study	Delhi	205	26.09±3.6	27 (13.2)	64 (31.2)	141 (68.8)

*NS: Not stated, #Data in median (range), BMI: Body mass index, NAFLD: Non-alcoholic fatty liver disease

lipids, fasting blood sugar, and MS among lean NAFLD patients were significantly higher than those in lean healthy control.

Compared to obese NAFLD, the severity of liver histopathology was significantly lower in patients with lean NAFLD in terms of mean NAS (P = 0.01) and liver fibrosis (P = 0.01). Although not statistically significant [Table 2], the proportion of patients with NASH and advanced fibrosis tended to be lower in lean NAFLD compared to obese NAFLD. This may be because of smaller number of patients in lean NAFLD. However, the effect size of difference in disease severity may not be large because histopathology were not available in all patients, and severity based on non-invasive parameters were similar different between patients of 3 BMI categories. The serum level of uric acid, one of the markers of NAFLD severity,[18] was significantly lower among lean NAFLD compared to obese NAFLD. The levels of serum transaminases were similar between all 3 BMI categories. However, transaminases are the poor marker of severity in NAFLD patients.[19]

Although obesity is an important risk factor, NAFLD has been reported in non-obese subjects from developing as well as developed countries.[2,4,5] Furthermore, NAFLD in India had been reported to develop at lower BMI.[2,6,7] In a population-based study from rural India, 52% of individuals with NAFLD were lean (BMI < 23).[2] However, majority (87%) of screened population in this study were lean, and only 7% were obese. The lower preponderance of lean NAFLD (13.2%) in our hospital-based cohort suggests that many of lean NAFLD patients do not seek medical advice. Interestingly, Das *et al*.[2] also found that individuals with normal BMI (18.5-24.9 Kg/m^2) had two-fold increases in risk for NAFLD than those with a BMI < 18.5 Kg/m^2. In

our study also, the mean BMI of lean NAFLD patients were higher than 131 non-selected healthy lean subjects without fatty liver (21.3 ± 0.9 vs. 22.0 ± 0.76 kg/m^2), $P = 0.02$). Also, the prevalence of dyslipidemia and MS were significantly higher in lean NAFLD than the controls. Thus, although obesity is clearly a risk factor for NAFLD, this appears to be modified strongly by ethnicity, genetic predisposition, or environmental factors, which may explain risk of NAFLD in lean subjects. The substantial variability of hepatic fat content varies among individuals with equivalent adiposity supports this view. A study revealed that lean, non-alcoholic, non-diabetic, non-smoking Asian Indians in comparison to similar age, sex, BMI-matched Caucasians, Hispanics, Black and Eastern Asians had 2- to 3-fold increase in IR and 2-fold increase in hepatic steatosis.[8]

Asians are known to have abdominal (visceral) obesity at lower BMI. However, in our study, abdominal obesity measured as waist circumference was not increased in patients with lean NAFLD. This may be explained by the fact that central obesity, which also includes subcutaneous abdominal fat which is relatively inert metabolically, doesn't exactly correspond to visceral adiposity. Another study has also found a poor association of abdominal adiposity with NAFLD in Asian Indian.[20] It is possible that patients with lean NAFLD can have subtle measures of increased adiposity. A study has revealed that lean NAFLD patients have higher subcutaneous skin-fold thicknesses and higher body fat percentage on bioelectric impedance analysis compared to control subjects.[2] Else, the adipose tissues of lean NAFLD may be metabolically more active. There is a growing concept that the quality rather than quantity of adipose tissue may be more important in conferring metabolic risks leading to NAFLD.[8] Under normal condition, adipose tissues are the primary source (70%) of free fatty acids for hepatic triglyceride. Thus, adipose tissue IR may trigger excess release of fatty acids leading to development of hepatic "lipotoxicity" in NAFLD. A study has reported that patients with NASH have severe adipose tissue IR independent of the degree of obesity, and amelioration of adipose tissue IR by pioglitazone is closely related to histological improvement.[21] The lean NSH may have accelerated lypolysis due to IR, mainly at adipose tissues. Notably, majority of lean NAFLD patients in our cohort had dyslipidemia.

In general, IR is believed to be an important trigger for initiation of NAFLD. NAFLD has been shown to be associated with IR independently of BMI,[22] and studies have reported that IR is frequently present in lean NAFLD patients, even without other metabolic disorders.[23,24] However, IR as indicated by HOMA-IR > 2 was present in only 7.4% ($n = 2$) patients of lean NAFLD, and such patients had lower degree of fasting hyperinsulinemia ($P < 0.001$) and HOMA-IR ($P < 0.001$) compared to obese NAFLD. Thus, lean NAFLD patients have minor or no hepatic insulin resistance. A recent study on effect of vitamin E in patients with NAFLD has shown a significant improvement in liver histology without any change in the degree of IR.[25] Furthermore, various genetic factors are known to confer susceptibility to NAFLD in individuals without increasing the level of IR. Patients with mutations in either adipose triglyceride lipase (ATGL) or comparative gene identification-58 (CGI58) have severe steatosis but no IR.[26] Individuals with inactivating mutations in apolipoprotein B (APO B) gene have increased levels of hepatic triglyceride yet no IR.[27] A genetic variant in patatin-like phospholipase 3 gene (PNPLA3) that is associated with hepatic steatosis is not associated with IR.[28] Also, it must be noted that we estimated IR by HOMA, which is an indirect method and has limitation that it reflects only hepatic insulin sensitivity. In lean NAFLD, peripheral (adipose tissues and skeletal muscles) IR may be more important than hepatic IR.[21] Regarding environmental factors, an increased intake of dietary fat has been suggested to lead to increased accumulation of lipids in the liver of lean subjects.[29] A higher intake of soft drinks and meat is associated with an increased risk of NAFLD, independently of age, gender, BMI, and total calories.[30]

Conclusion

The lean subjects with NAFLD are frequently dyslipidemic. Compared to obese or overweight NAFLD, patients with lean NAFLD have minor or no insulin resistance, and appear to have less severe histological disease at presentation. They do not have abdominal obesity, but their BMI was higher than lean healthy control.

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