Nonobese Population in a Developing Country Has a High Prevalence of Nonalcoholic Fatty Liver and Significant Liver Disease

Kausik Das,[1] Kshaunish Das,[2] Partha S. Mukherjee,[5] Alip Ghosh,[4] Sumantra Ghosh,[4] Asit R. Mridha,[3] Tapan Dhibar,[6] Bhaskar Bhattacharya,[6] Dilip Bhattacharya,[1] Byomkesh Manna,[7] Gopal K. Dhali,[2] Amal Santra,[4] and Abhijit Chowdhury[1,5]

There is a paucity of community-based epidemiological data on nonalcoholic fatty liver (NAFL) among nonaffluent populations in developing countries. Available studies are radiological and/or biochemical and lack histological assessment, limiting their strength. We conducted a prospective epidemiological study comprising a 1:3 subsample of all adult (>18 years) inhabitants of a rural administrative unit of West Bengal, India. Subjects positive for hepatitis B virus and/or hepatitis C virus infection and consuming any amount of alcohol were excluded. Diagnosis of NAFL was by dual radiological screening protocol consisting of ultrasonographic and computed tomographic examination of the liver. Transient elastographic examination and liver biopsy were performed in a subset to identify significant liver disease. The risk factors of having NAFL were analyzed. A total of 1,911 individuals were analyzed, 7% of whom were overweight and 11% of whom had abdominal obesity. The prevalence of NAFL, NAFL with elevated alanine aminotransferase, and cryptogenic cirrhosis was 8.7%, 2.3%, and 0.2%, respectively. Seventy-five percent of NAFL subjects had a body mass index (BMI) <25 kg/m^2, and 54% were neither overweight nor had abdominal obesity. The subjects with the highest risk of having NAFL were those with a BMI >25 kg/m^2 (odds ratio 4.3, 95% confidence interval 1.6-11.5). Abdominal obesity, dysglycemia (fasting plasma glucose >100 mg/dL or elevated homeostatic model assessment of insulin resistance), and higher income were the other risk factors. Even having a normal BMI (18.5-24.9 kg/m^2) was associated with a 2-fold increased risk of NAFL versus those with a BMI <18.5 kg/m^2. *Conclusion:* There is a significant prevalence of NAFL and potentially significant liver disease, including cryptogenic cirrhosis, in this predominantly nonobese, nonaffluent population in a developing country. NAFL will be a major determinant of future liver disease burden in countries of the developing world. (HEPATOLOGY 2010;51:1593-1602)

See Editorial on Page 1491.

Cirrhosis of the liver ranked as the thirteenth most common cause of mortality worldwide toward the end of the last decade.[1] Chronic viral hepatitis due to hepatitis B virus (HBV) and hepatitis C virus (HCV) is the most common cause of cirrhosis and hepatocellular carcinoma in developing countries.[2] Epidemiological studies on liver disease in developing countries have focused mostly on viral hepatitis.[3,4] This pattern is different from that seen in the developed nations of the West, where nonalcoholic fatty liver disease (NAFLD) ranks next only to HCV infection and alcoholism as the third most commonly diagnosed liver disease at United

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CI, confidence interval; CT, computed tomography; FPG, fasting plasma glucose; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostatic model assessment of insulin resistance; IR, insulin resistance; LSM, liver stiffness measure; MS, metabolic syndrome; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; OR, odds ratio; SD, standard deviation.

From the Divisions of [1]Hepatology; [2]Gastroenterology, and [3]Pathology and [4]Center for Liver Research, School of Digestive and Liver Diseases, Institute of Postgraduate Medical Education and Research, Kolkata, India; [5]Liver Foundation, West Bengal, India; the [6]Department of Radiodiagnosis, Bangur Institute of Neurosciences and Psychiatry, Kolkata, India; and [7]National Institute of Cholera and Enteric Diseases, Kolkata, India.

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States gastroenterology practices.[5] The scenario is changing in developing countries, where an upward trend in the prevalence of noncommunicable diseases is evident as a result of economic prosperity and changes in sociodemographics and lifestyle.[6,7] Characterstically, many of these emerging public health priorities are clinical expressions of the metabolic syndrome (MS) and insulin resistance (IR).[6-8]

NAFLD, which is considered to be the hepatic manifestation of MS,[8] is a distinct clinico-pathologic entity characterized histologically by a spectrum ranging from bland steatosis to steatohepatitis and cirrhosis and even hepatocellular carcinoma.[9] Recent studies have indicated that the prevalence of NAFLD is fairly significant in Asian countries.[10] However, these studies have focused largely on economically developed segments of populations in these regions.[11,12] The prevalence of obesity and diabetes is rising in developing countries,[13] further underscoring the need for in-depth assessment of NAFLD epidemiology in these countries.

In general, there is limited epidemiological data on the prevalence of nonalcoholic fatty liver (NAFL) in the general population, even from the West.[14,15] Inconsistency in methodological designs to detect NAFL, heterogeneity of the population analyzed, and exclusion of liver biopsy in the assessment of a disease that is primarily defined histologically are some of the drawbacks of available studies.[11,12,14,15]

To define the prevalence and identify the risk factors of NAFL and significant liver disease in a developing country, we undertook a community-based study in a defined rural population from the Birbhum District, West Bengal, India.

Subjects and Methods

Population Sample. Adult (>18 years) inhabitants of Nagari Gram Panchayat in the Birbhum District of West Bengal, India, were included. A Gram Panchayat is the most peripheral rural administrative unit in India and comprises several villages in the vicinity. We purposely chose the village unit in the present study based on the framework of our previous population-based epidemiological work on HBV and HCV infection.[16,17] The voters list, an independent list of all adult eligible voters prepared by the Election Commission of India, updated 9 months prior to the study initiation, was used as the sampling frame for the present study. A 1:3 subsample was selected by including every third person registered in the voters list of 7,218 individuals (3,863 men, 3,355 women). A total of 2,406 individuals (1,266 men, 1,140 women) were invited to participate in the study. Fifty-nine of these individuals were unavailable to participate because they were migrant laborers, and another 172 did not give consent (overall population participation rate, ≈90%). An additional 219 individuals were excluded because of any amount of alcohol intake (n = 168) or other comorbidities (n = 51) that were deemed exclusionary in view of their possible influence on the study implementation and outcome (Fig. 1). Another 45 individuals who tested positive for chronic hepatitis viral infections (HBV and HCV) were also excluded. This yielded a final population sample of 1,911 individuals (Fig. 1).

Alcohol and drug intake were excluded and laboratory studies were performed (see Supporting Information for details).

Study Design. A multistage staggered approach starting in the community and then detailed analysis in the Institute was used (Fig. 1).

In the first phase, which was performed in the community, all subjects who consented to participate were interviewed by a trained public health nurse in the form a structured questionnaire to derive socio-economic and demographic data as well as details of drug intake and alcohol use. Subsequently, anthropometric measurements (height, weight, body mass index, and waist circumference) were performed (see Supporting Information). Obesity was defined by body mass index (BMI) according to World Health Organization standards,[18] and abdominal obesity (waist circumference >90 cm for men, >80 cm for women) was defined according to the International Diabetes Federation's cutoff for South Asians.[19] Components of MS were defined according to International Diabetes Federation criteria.

At the end of the interview, blood samples were collected for estimation of liver enzymes, fasting plasma glucose (FPG), and viral markers. Finally, hepatic ultrasonographic examination was performed by a single radiologist (D. B.) using a portable ultrasound

Address reprint requests to: Abhijit Chowdhury, Professor and Head, School of Digestive and Liver Diseases, Institute of Postgraduate Medical Education and Research, Kolkata 700020, India. E-mail: achowdhury2002@yahoo.co.in; Fax: (91)-3322235435.

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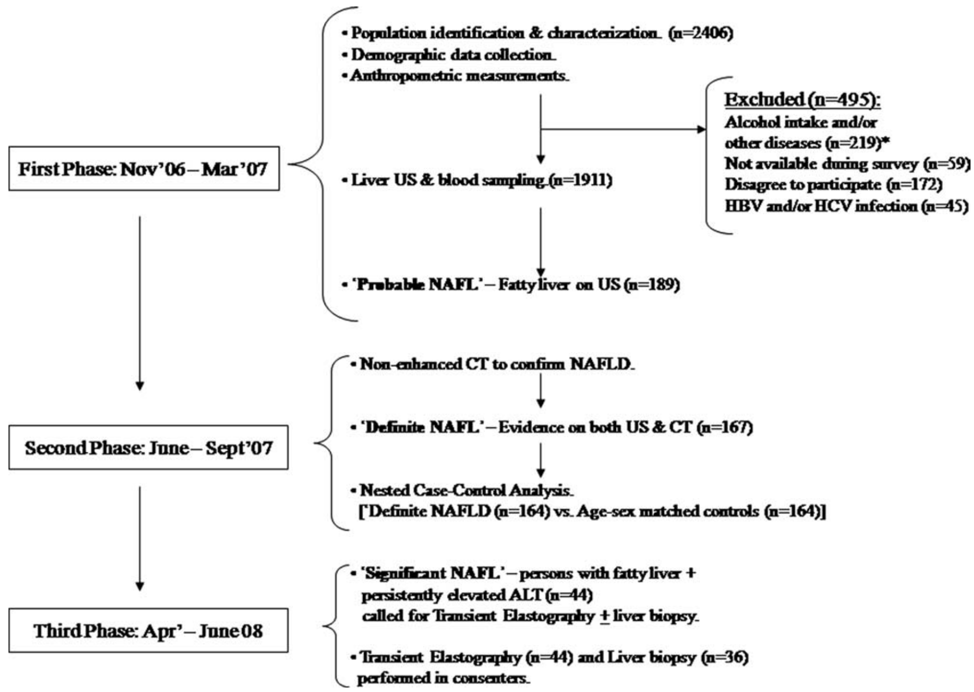

Fig. 1. Study algorithm. *Of the 219 subjects excluded, 168 had a history of alcohol intake, 28 had tuberculosis and were receiving directly observed therapy for tuberculosis, nine had obstructive airway disease, six were on antileprosy drugs, five had general geriatric debility, and three had advanced cardiovascular disease.

device (Sonosite 180 Plus®, SonoSite Inc., USA) after a 6-hour fast.

Subjects with fatty liver on ultrasonographic examination, diagnosed by standard criteria,[20] were designated as having probable NAFL (n = 189).

In the second phase, which was performed in an institutional setting, subjects with probable NAFL underwent nonenhanced computed tomography (CT) examination (Asteon, Toshiba, Japan) for confirmation of fatty liver, defined by a liver attenuation index of ≤ -14 HU on CT.[21]

Subjects with fatty liver on both imaging modalities used (ultrasonography and CT) were designated as having definite NAFL (n = 167).

To identify the role of MS and IR in NAFL, a nested case-control study was performed between subjects with definite NAFL versus age- and sex-matched controls drawn from the same background population. Three individuals did not agree to participate, leaving a final cohort of 164 individuals (cases). Besides the anthropometric data already recorded, skin-fold thicknesses at various sites were recorded using Lange calipers. Blood pressure was recorded in the left arm with the subject in a supine position after 5 minutes of rest using a mercury spyghmomanometer. Total body fat mass and percentage were assessed using leg-to-leg bioelectrical impedance analysis equipment (Tanita Corporation, Tokyo, Japan) in a fasting state by standard methodology.[22] Laboratory parameters analyzed included serum fasting lipid profile, C-reactive protein and iron levels, repeat FPG, alanine aminotransferase (ALT), and aspartate aminotransferase.

In the final phase, subjects with definite NAFL who had persistently elevated ALT levels (>40 U/L in both sexes at both the first and second phase of the study) were defined as having potentially significant NAFL. These subjects underwent transient elastographic examination (Fibroscan, Echosense, Paris) performed by a single operator (K. D.) for liver stiffness measure (LSM). An LSM value >8 kPa was considered abnormal, reflecting significant liver fibrosis.[23] Liver biopsy was performed using an 18-gauge Menghini needle as described[24] in subjects with potentially significant NAFL who consented. Liver tissue was stained with hematoxylin-eosin, reticulin, and Masson's trichrome stains. Histological assessment included determination of relevant objective parameters, NAFLD activity score, and NAFLD fibrosis score[25] by a single pathologist (A. R. M.) blinded to the clinical data.

Consent and Ethical Clearance. Informed consent was obtained from all participants prior to interviews and investigations at each phase. The study was approved by the Institutional Review Board of the Institute of Postgraduate Medical Education and Research, Kolkata, India.

Statistical Analysis. Means, medians, standard deviations (SDs), ranges, and proportions were calculated as appropriate. For parametric data, categorical variables were compared using Pearson's chi-square test or Fisher's exact test, and continuous variables were

Table 1. Characteristics of Study Population and NAFL Subjects

Parameters (Normal Range)	Total (n = 1,911)	Subjects With NAFL (n = 164)	Subjects Without NAFL (n = 1,747)
Age, years (mean ± SD)*	35.5 ± 12.4	39.0 ± 12.7	35.2 ± 12.3
Male sex (%)	53	54	53
Occupation (%)†			
Manual laborer	59	46	60
Other occupations	41	54	40
Economic status (%)*,‡			
<$1.00/day	48	31	50
$1.00-$2.00/day	42	48	41
>$2.00/day	10	21	9
Educational status (%)			
Up to primary level	61	51	62
Secondary level	19	23	19
Higher secondary level and above	20	26	19
Presence of smoking habit (%)	29	31	28
BMI, kg/m^2 (mean ± SD)*	19.6 ± 6.6	23.0 ± 4.2	19.3 ± 6.7
Persons having BMI (%)*			
<18.5	47	12	49
18.5-22.9	40	40	40
23.0-24.9	6	23	6
≥25.0	7	25	5
Presence of abdominal obesity§ (%)*	11	39	7
Fasting blood glucose, mg/dL (mean ± SD)*	80.0 ± 23.8	93.5 ± 38.1	78.7 ± 21.4
FBG >100 mg/dL (%)*	13	26	12
ALT, IU/L (mean ± SD)†	29.9 ± 26.2	35.3 ± 23.4	29.4 ± 26.4

*$P < 0.001$ between subjects with and without NAFLD.
†$P < 0.01$ between subjects with and without NAFLD.
‡Expressed in US dollars ($).
§Abdominal obesity was defined as waist circumference ≥90 cm in men and ≥80 cm in women.

compared using the Student *t* test or analysis of variance. For nonparametric data, we used Fisher's exact test for categorical data and the Mann-Whitney U or Kruksall-Wallis H test for continuous variables. Spearman's rho was calculated as appropriate. Binary logistic regression was performed using presence of NAFL, a qualitative dichotomous outcome, as a dependent variable to calculate the odds ratio and for multivariate analysis. The α level adopted for significance was $P < 0.05$ (two-tailed). Statistical analysis was performed using SPSS version 13 software (SPSS, Chicago, IL).

Results

Population Profile. The mean (±SD) age of the 1,911 subjects in the study was 35.5 ± 12.4 years (men, 35.9 ± 13.0 years; women, 35.1 ± 11.6 years), with a male:female ratio of 1.1:1. The majority were agricultural workers and/or manual laborers (59% [men, 70%; women, 50%]) and were economically poor (household income <$2.00/day in 90%). The mean (±SD) BMI was in the lower normal range (19.6 ± 6.6 kg/m^2 [men, 19.5 ± 8.2 kg/m^2, women, 19.7 ± 3.9 kg/m^2]). Only 7% (men, 6%; women, 8%) of the study population were overweight (BMI >25 kg/m^2). Eleven percent of the entire population had abdominal obesity. The majority of the individuals in the population were undernourished, with 47% having a BMI <18.5 kg/m^2. Twenty-nine percent were smokers and were predominantly men (36% versus women, 3%). The prevalence of dysglycemia (FPG >100 mg/dL) was 13% (men, 12.5%; women, 13%). Overall, the population was young, poor, physically active, and nonobese, typical of rural settings in developing countries. The charachteristics of the study population are given in Table 1.

Prevalence of NAFL, Potentially Significant NAFL, and Cirrhosis. Overall, 8.7% (167/1,911) of the study population had definite NAFL. Potentially significant NAFL (NAFL with elevated liver enzymes) was present in 44 (2.3%).

Transient elastographic examination (Fibroscan, Echosense, Paris) to measure LSM was performed in all 44 subjects with potentially significant NAFL. The median (range) LSM was 8.1 (3.7-46.4) kPa, and 26/44 (58%) subjects had significant liver fibrosis (LSM >8 kPa). The mean (±SD) interquartile range was 0.9 (±0.9) kPa, and the median success rate of acquisition was 100% (range, 60%-100%). LSM did not correlate with NAFLD activity score, age, and sex (data not shown), but showed a positive correlation with increasing stages of fibrosis (Spearman's rho, 0.55).

Table 2. Characteristics of 36 Patients Who Underwent Liver Biopsy

Parameters	Values
Median age, years (range)	40 (22-70)
Sex (male:female), n	23:13
Median BMI, kg/m^2 (range)	25.6 (18.7-37.3)
Median LSM, kPa (range)	8.1 (2.6-46.4)
Median length of liver tissue, cm (range)	2.0 (1.5-3.5)
Steatosis grade, n:	
Grade 1	19
Grade 2	10
Grade 3	7
NAFLD fibrosis stage, n:	
Fibrosis stage 0	17
Fibrosis stage 1A	9
Fibrosis stage 2	6
Fibrosis stage 4 (cirrhosis)	4
NASH (NAFLD activity score ≥5), n (%)	11 (31)
Median NAFLD activity score (range)	4 (0-7)

Of the 44 subjects with potentially significant NAFL, 36 (82%) agreed to undergo liver biopsy (Table 2). Steatosis was seen in all subjects. Histologic nonalcoholic steatohepatitis (NASH) (NAFLD activity score of ≥5) was seen in 11/36 (31%) subjects, four of whom had cirrhosis. Thus, 2.4% (4/167) of subjects with NAFL had cirrhosis. The prevalence of cirrhosis in the entire population was at least 4/1,911 (0.2%).

Mean (±SD) LSM values were significantly higher in subjects with stage 2 and stage 4 fibrosis/cirrhosis than those with stage 1A fibrosis (Fig. 2). The four patients with cirrhosis were recalled for re-evaluation after liver biopsy and had a negative autoimmune and Wilson's work-up.

Subjects with NAFL. The prevalence of NAFL was similar in both males (88/1018 [8.6%]) and females (76/893 [8.5%]). Subjects with NAFL were older than those without NAFL. The prevalence of fatty liver increased with age in both sexes with the peak prevalence attained by the fourth decade in men and women (data not shown).

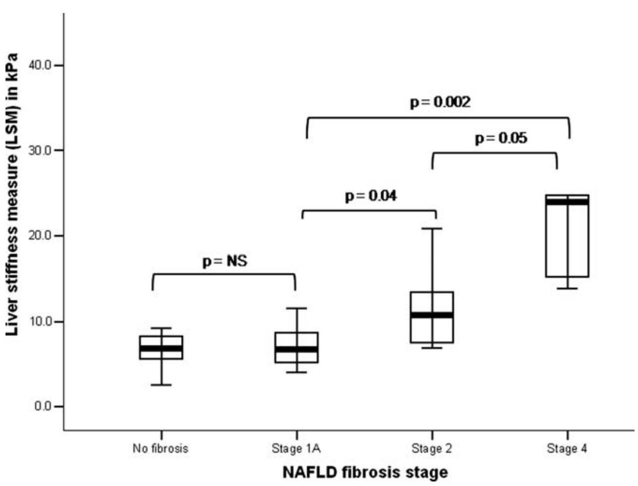

Fig. 2. Distribution of LSM in different stages of fibrosis. Mean (±SD) LSM values were: stage 0/no fibrosis, 8.4 ± 2.3 kPa; stage 1A, 7.2 ± 2.4 kPa; stage 2, 11.7 ± 5.2 kPa; stage 4/cirrhosis, 24.9 ± 13 kPa.

Although the average income of the population was low, subjects with NAFL were more likely to be economically better off than those who did not have fatty liver (income >$2.00/day; 21% versus 9%, respectively [$P < 0.001$]). There was no significant difference in the educational status of the two groups. Persons with NAFL were, however, less likely to be as physically active as manual laborers (46% versus 60% [$P < 0.01$]). They had a higher BMI, a higher prevalence of abdominal obesity, a higher mean fasting plasma glucose, and a higher prevalence of dysglycemia (FPG >100 mg/dL). Their mean ALT levels were also higher (Table 1).

In multivariate analysis (Table 3), after adjusting for age and sex, the odds ratio (OR) of having NAFL independently rose with increasing income (income $1.00-$2.00/day, OR 1.8 [$P = 0.05$]; income >$2.00/day, OR 2.4 [$P = 0.01$]) and with the presence of dysglycemia (OR 2.6 [$P = 0.001$]) and abdominal obesity (OR 3.6 [$P < 0.001$]). Having a normal BMI (18.5-24.9 kg/m^2) was associated with a 2-fold increased risk of having fatty liver (OR 2.0 [$P = 0.03$]). The highest risk was in those with a BMI > 25 kg/m^2 (OR 4.3 [$P = 0.001$]).

However, despite having a higher mean BMI, most of the NAFL subjects (75%) were not overweight; 103/164 (63%) had normal BMI (18.5-24.9 kg/m^2), and 20/164 (12%) were underweight (BMI <18.5 kg/m^2). Abdominal obesity was present in only 39% of NAFL subjects. Ninety subjects (54%) with NAFL

Table 3. Multiple Logistic Regression for Risk Factors for NAFL in the Whole Population

Variables	OR (95% CI)	*P* Value
Presence of abdominal obesity*	3.6 (1.7-7.2)	<0.001
Presence of dysglycemia (FBG >100 mg/dL)	2.6 (1.5-4.6)	0.001
Family income†		
<$1.00/day	1.0	
$1.00-$2.00/day	1.8 (1.0-3.2)	0.05
>$2.00/day	2.4 (1.2-5.0)	0.01
BMI		
<18.5 kg/m^2	1.0	
18.5-24.9 kg/m^2	2.0 (1.1-3.8)	0.03
≥25 kg/m^2	4.3 (1.6-11.5)	0.001

Age, sex, and profession did not achieve statistical significance.

*Abdominal obesity was defined as a waist circumference ≥90 cm in men and ≥80 cm in women.

†Expressed in US dollars ($).

Table 4. Comparison of Subjects With and Without NAFL (Nested Case-Control)

Parameters	Cases with NAFL (n = 164)	Controls Without NAFL (n = 164)	*P* Value
Age, years (mean ± SD)	39 ± 13	39 ± 13	NS
Sex (male:female), n	88:76	88:76	NS
Anthropometric values			
BMI, kg/m^2 (mean ± SD)	22.70 ± 3.90	20.60 ± 5.10	NS
BMI categories, n (%)			
<18.5 kg/m^2	20 (12)	55 (33)	
18.5-24.9 kg/m^2	103 (63)	107 (65)	
≥25 kg/m^2	41 (25)	2 (1)	
Waist circumference, cm (mean ± SD)	80.01 ± 12.10	75.00 ± 9.01	0.03
Presence of abdominal obesity (%)	39	18	<0.0001
Triceps skin-fold thickness, mm (mean ± SD)	10.00 ± 5.70	9.00 ± 5.20	0.05
Subscapular skin-fold thickness, mm (mean ± SD)	17.02 ± 8.30	14.40 ± 7.30	0.05
Fat percentage (mean ± SD)	20.9 ± 6.9	14 ± 7.4	0.001
FBG, mg/dL (mean ± SD)	94 ± 37	80 ± 21	NS
FBG >100 mg/dL, n (%)	43 (26)	21 (13)	<0.001
Serum insulin, mIU/mL (mean ± SD)	7.90 ± 4.81	6.7 ± 2.28	NS
HOMA-IR (mean ± SD)	2.24 ± 3.16	1.44 ± 0.96	0.02
Presence of diabetes,* n (%)	12 (7)	7 (4)	<0.001
Triglyceride, mg/dL (mean ± SD)	125.9 ± 65	98 ± 36	0.05
HDL, mg/dL (mean ± SD)	44 ± 12.3	41.6 ± 7.4	0.04
Total cholesterol, mg/dL (mean ± SD)	179.3 ± 63.6	177 ± 48	0.001
Hypertension, n (%)	10 (6)	7 (4)	<0.001
ALT >40 IU/L, n (%)	44 (27)	25 (15)	<0.01
CRP, mg/dL (mean ± SD)	2.3 ± 1.3	2.0 ± 1.0	NS

*The presence of diabetes was based on FBG only, which may underestimate the fact.
Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; NS, not significant.

were neither overweight nor had abdominal obesity. NAFL subjects with BMI <18.5 kg/m^2 were significantly younger and had significantly lower mean total serum cholesterol, body fat content (as fat percentage assessed by leg-to-leg bioelectrical impedance), and prevalence of abdominal obesity than NAFL subjects who had normal BMI or who were overweight (Supporting Table 1).

Nested Case-Control Analysis. In this subanalysis of 164 cases with NAFL and 164 controls without NAFL (Table 4), NAFL subjects were significantly more likely to be overweight (BMI >25 kg/m^2, 25% versus 1%; [$P < 0.0001$]), have abdominal obesity, and a significantly higher body fat content (mean ± SD fat percentage, 20.9 ± 6.9% versus 14 ± 7.4% [$P < 0.001$]). They had higher triceps and subscapular skin-fold thicknesses, although the difference achieved borderline significance. Subjects with NAFL were more likely to have dysglycemia and had a higher mean value for homeostatic model assessment of insulin resistance (HOMA-IR). Although they were more likely to be hyperinsulinemic, the difference did not achieve statistical significance. They also had significantly higher mean serum levels of total cholesterol, high-density lipoprotein cholesterol, and triglycerides and had a higher frequency of hypertension. Thus, subjects with NAFL were more likely to have abnormalities in various components of MS than those without NAFL.

On multivariate analysis (data not shown), using BMI, abdominal obesity, HOMA-IR (marker for dysglycemia), and high-density lipoprotein levels (marker for dyslipidemia), having a BMI in the normal range (18.5-24.9 kg/m^2 [OR 2.5, 95% confidence interval (CI) 1.4-4.6 ($P < 0.01$)] or >25 kg/m^2 [OR 53, 95% CI 11.5-240 ($P < 0.001$)]) and a rising HOMA-IR (OR 1.2, 95% CI 1.0-1.4 [$P = 0.05$]) were independently associated with an increased risk of NAFL.

Nonobese NAFL. As both the NAFL subjects and general population had a low prevalence of overweight or abdominal obesity, we conducted a subgroup comparative analysis among the cases and controls who had BMI <25 kg/m^2 as well as a normal waist circumference (Table 5). Even within this subgroup, the mean values for BMI; skin-fold thicknesses from various sites; body fat percentage; and serum FPG, triglyceride, and total cholesterol levels were significantly elevated in the NAFL subjects. On multivariate analysis, even in this subgroup with no obesity, the only two independent predictors of fatty liver were increased BMI (OR 1.2, 95% CI 1.1-1.4 [$P < 0.01$]) and biceps skin-fold thickness (OR 1.2, 95% CI 1.1-1.3 [$P < 0.01$]).

In order to clarify the role of MS in the subgroup of NAFL subjects with BMI <18.5 kg/m^2, a comparative case-control analysis was performed (Supporting Table 2). Compared with controls with BMI <18.5

Table 5. Comparison of Nonobese NAFL Cases With Nonobese Controls

Parameters	Nonobese NAFL Cases (n = 90)	Nonobese Controls (n = 134)	*P* Value
Age, years (mean ± SD)	36 ± 13	39 ± 13	NS
Sex (male:female), n	63:27	81:53	NS
Anthropometric values			
BMI, kg/m^2 (mean ± SD)	20.7 ± 2.7	19.5 ± 2.7	0.002
Waist circumference, cm (mean ± SD)	73.01 ± 9.12	72.23 ± 8.32	NS
Biceps skin-fold thickness, mm (mean ± SD)	7.0 ± 4.0	4.9 ± 2.2	<0.0001
Triceps skin-fold thickness, mm (mean ± SD)	10.6 ± 6.0	8.0 ± 4.2	0.002
Subscapular skin-fold thickness, mm (mean ± SD)	17.6 ± 7.8	13.1 ± 6.7	<0.0001
Suprailiac skin-fold thickness, mm (mean ± SD)	13.7 ± 8.4	8.3 ± 4.6	<0.0001
Fat percentage (mean ± SD)	18.5 ± 5.9	12.3± 6.0	<0.0001
FPG, mg/dL (mean ± SD)	86 ± 25	80 ± 20	0.03
Serum insulin, mIU/mL (mean ± SD)	6.83 ± 3.24	6.66 ± 2.19	NS
HOMA-IR (mean ± SD)	1.63 ± 1.65	1.41 ± 0.89	NS
Triglyceride, mg/dL (mean ± SD)	118.2 ± 66.3	93.4 ± 34.6	0.001
HDL, mg/dL (mean ± SD)	43.6 ± 12.7	41.8 ± 7.3	NS
Total cholesterol, mg/dL (mean ± SD)	159.4 ± 60.4	177.7 ± 50.3	0.02
ALT >40 IU/L n (%)	21 (23%)	21 (15.8%)	NS
CRP, mg/dL (mean ± SD)	1.9 ± 1.0	2.0 ± 0.9	NS
Ferritin, mg/dL (mean ± SD)	39 ± 14	38 ± 15	NS

A nonobese person was defined as having a BMI <25 kg/m^2 and a waist circumference <90 cm in men and <80 cm in women.
Abbreviations: CRP, C-reactive protein; HDL, high-density lipoprotein; NS, not significant.

kg/m^2, NAFL subjects had significantly higher skin-fold thicknesses, body fat percentage, and mean serum triglyceride levels but lower mean serum total cholesterol levels. They also had a higher prevalence of hypertension and higher mean serum FPG, although there was no statistically significant difference in the markers of dysglycemia.

Discussion

In this prospective multistaged community-based epidemiological study performed in a rural Indian population, we found an 8.7% prevalence of NAFL, including ≈0.2% prevalence of cryptogenic cirrhosis. The major methodological strengths of our study are its population-based prospective design, adoption of stringent imaging criteria for diagnosis of fatty liver, strict exclusion of alcohol consumption and viral hepatitis to derive a true metabolic fatty liver and, most importantly, performance of liver biopsy in a significant subset of NAFL subjects for the first time in an epidemiological study of NAFL.

We found a high prevalence of NAFL (8.7%), potentially significant NAFL (2.3%), and silent cirrhosis (≈0.2%). This is intriguing considering that 47% of our study population had a BMI <18.5 kg/m^2 and 87% had normal BMI. Remarkably, three fourths of those with NAFL were not even overweight, and half of them had neither generalized nor abdominal obesity. This was a reflection of the very low (7%) overall prevalence of overweight individuals in the population. Despite this, the association of NAFL with MS and adiposity was preserved, albeit in a modified manner. The linear association of increasing obesity with increasing prevalence of NAFL in the population, is established in developed countries as well as the socioeconomically upward moving segment of the low to middle income nations,[10-12,14,15] although the vast majority of the world population live beyond the boundaries of such social order. The present study is unique in that it expands the NAFL ambit beyond its classical overweight-obesity paradigm. It also provides evidence, for the first time, that fatty liver will be an important determinant of liver disease burden even in the poor and emerging economies, where a disease burden transition is already occurring.[2,26]

Previous epidemiological studies have mostly been in a preselected population[12,14] or did not completely exclude alcohol consumption[11,12,14,15] or viral hepatitis[15] in calculating prevalence data of NAFL. Two previous well-designed community studies on NAFL are the Dionosys study from Italy[14] and the Minnesota study from the United States.[27] Whereas the former study was an epidemiological one, the latter was a clinically defined cohort providing useful information on the epidemiology of NAFL in developed countries as well as liver disease behavior. However, the NAFL prevalence reported here is lower than other imaging-based epidemiological studies (15%-29%).[9,28] The stepwise dual-screening using both ultrasonography and CT, rigid exclusion of alcohol intake, younger age, and low background prevalence of obesity and MS may explain the lower prevalence figures reported here. What is more concerning is the fact that, as the population ages, the prevalence of fatty liver will rise with its consequent health burden.

Another remarkable feature of this study is the provision of histological evaluation of the liver in a community sample of NAFL subjects. Absence of histology has been a persistent lacunae of epidemiological studies of NAFL.[9,28] Elevated ALT in NAFL is indicative of the presence of NASH and fibrosis.[28] Therefore, using this as our guide, we selected a subset of potentially significant NAFL for biopsy, because our Institutional Review Board allowed us to perform liver biopsy, with its attendant potential complications,[29] in only those NAFL subjects who had abnormal liver enzymes. Histologically, NASH and silent cirrhosis was found in 31% and 2.4%, respectively, of those subjects with NAFL and elevated ALT who consented to liver biopsy. This is lower than the 76% and 2% baseline prevalence of NASH and cirrhosis, respectively, in a community-based cohort study from Minnesota.[27] This can be attributed to the higher prevalence of obesity in the Minnesota cohort compared with our population (71% versus 25%, respectively). Moreover, the Minnesota cohort was made up of subjects who had visited the physician and had a diagnosis of fatty liver and therefore was clinically assembled with a referral bias. Our NAFL subjects were detected on population screening, hence our data are more representative of the general population as seen in our country. It should be stressed that NAFL subjects with normal ALT may also harbor advanced liver disease,[28] indicating that our figures may have underestimated the true prevalence of NASH or cirrhosis. On the other hand, we found histological NASH in only one third of our patients with NAFL and elevated enzymes underscoring the fact that elevated ALT may be a poor surrogate marker of underlying NASH in subjects with fatty liver.

IR has been implicated in the pathogenesis of NAFL.[8,9,28] The fact that elevated BMI, abdominal obesity, and dysglycemia (represented by either a FPG $>$100 mg/dL or increasing HOMA-IR), all markers of IR, were independent risk factors of NAFL, even within this predominantly nonobese population, upholds the strong biological relationship of NAFL with MS or IR across socio-economic and anthropometric phenotypes. According to the thrifty-genotype hypothesis,[30] IR has evolved as an energy-conserving mechanism in humans in the face of historical relative lack of abundance of food in pre-agricultural society. This has become maladaptive in situations of energy excess and sedentarism, usually associated with economic prosperity. In our population comprised predominantly of manual laborers, NAFL subjects were more likely to have a higher income and less likely to be manual laborers than subjects without NAFL. Moreover, on multivariate analysis, increasing family income and even a normal BMI were independent risk factors for NAFL. These suggest the subtle unfolding of maladaptive potential of IR in the form of NAFL in our population.

Asians have increased body fat compared with Europeans, even at the same BMI.[19] This may also explain why a normal BMI was an independent risk factor for NAFL in our study. This is partly supported by the fact that, in the case-control analysis, the body fat content, measured as body fat percentage by bioelectric impedance analysis, was significantly higher in subjects with NAFL. The increased risk of NAFL with abdominal obesity, the higher subcutaneous skin-fold thicknesses in the NAFL subjects, and the increased risk of NAFL with increasing biceps skin-fold thickness in the subgroup of nonobese NAFL are important highlights of our study. Moreover, the fact that even NAFL subjects with BMI $<$18.5 kg/m^2, compared with controls with BMI $<$18.5 kg/m^2, had increased markers of adiposity in the form of higher subcutaneous skin-fold thicknesses and higher body fat percentage on bioelectric impedance analysis should provoke the need for more studies on the complex relationship of body fat patterning with liver fat deposition in different ethnic groups. The recent discovery of an ethnicity-specific association between the genes encoding a fat-metabolizing protein with NAFL further augments the relationship between genes, ethnicity-specific body-fat distribution, and NAFL.[31]

Another noteworthy feature is that 12% of NAFL subjects had undernutrition (BMI $<$18.5 kg/m^2). Could undernutrition also be responsible for NAFL in our population? Studies in humans have demonstrated that liver accumulates fat during starvation in adults[32] or during protein-energy malnutrition in children.[33] Molecular pathogenesis of obesity-associated liver disease and undernutrition-related liver damage are quite similar.[34,35] To answer this question, we conducted a case-control study of subjects with and without NAFL with a BMI $<$18.5 kg/m^2 and looked for markers of adiposity and MS. Interestingly, even in this subgroup, subjects with NAFL had higher indices of adiposity and higher prevalence of markers of MS versus those without NAFL. This highlights for the first time a "*third-world NAFL*" phenotype in which, instead of overt obesity, subtle measures of increased adiposity predispose to NAFL.

Transient elastography, though not well validated for measuring fibrosis in NAFLD, has been used in liver disease of different etiologies.[36-38] Despite being used in NAFLD, the issue of whether steatosis and inflammation influence the stiffness value is not yet settled.[27] However, studies that included liver diseases of various etiologies report nonuniformity of cutoff values for exclusion of cirrhosis among different etiologies. Higher cutoff value for LSM was reported to achieve best diagnostic accuracy and acceptable sensitivity and specificity for NAFLD.[36] LSM values increase with increasing stages of fibrosis in NAFLD.[37] Significant correlation was also demonstrated between LSM and stages of fibrosis,[38] which was also reproduced in our study.

One weakness of our study was our inability to do the full component of all MS parameters in all subjects and the fact that our population sample is not representative of all the diverse socio-demographic groups in our country. Thus, previous studies in urban Indians involving smaller sample sizes and having higher baseline obesity have reported a higher prevalence of fatty liver on ultrasound.[39,40] While acknowledging these limitations, the data presented herein mandate the need for larger study sample and inclusion of a wider battery of metabolic parameters at baseline.

In conclusion, our study found a significant prevalence of NAFL and cryptogenic cirrhosis in a predominantly poor, nonobese, nonsedentary population. Abdominal obesity, overweight, dysglycemia, rising income, and even a normal BMI were found to be independent risk factors of NAFL in our population.

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