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Prevalence, risk factors and metabolic profile of the non-obese and obese non-alcoholic fatty liver disease in a rural community of South Asia

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ABSTRACT

Introduction Since there is a paucity of data on the epidemiology of the non-alcoholic fatty liver disease (NAFLD), particularly in rural areas in Asia, we undertook such a study among the population of a rural community in Bangladesh with the aims to (1) determine the prevalence of non-obese and obese NAFLD, (2) compare the sociodemographic clinical and metabolic characteristics between non-obese and obese NAFLD subjects, and (3) determine the risk factors of NAFLD and no-nobese NAFLD.

Methods In this door-to-door survey, clinical examination, anthropometric measurements, biochemical tests and ultrasonography were performed on the adult population (≥18 years) of three villages in Bangladesh.

Results Of 1682, 1353 (80.44%) responded. After the exclusion of 48 subjects for alcohol consumption, HBsAg or anti-hepatitis C virus positivity, 1305 ((mean age 41.28±15.10 years, female 908 (69.6%)) were included in the final analysis. On ultrasonography, among the study population, 57 (4.4%) non-obese, 185 (14.2%) obese and, overall, 242 (18.5 %, (male 23.40%, female 16.4%, p=0. 003)) participants had NAFLD. NAFLD was detected in 57/804 (7.1%) of non-obese and 185/501 (36.93%) obese participants. Among the lean subjects, 24/592 (4.1%) had NAFLD. Among NAFLD subjects, 57 (23.55%) were non-obese, and 53 (22%) had raised alanine aminotransferase. On multivariate analysis, age >40 years, male gender, metabolic syndrome (MS), diabetes mellitus (DM), abdominal obesity, hypertension, dyslipidaemia and obesity were found as the risk factors for NAFLD. There were no differences in sociodemographic characteristics, DM, MS, abdominal obesity, hypertension and dyslipidaemia between non-obese and obese NAFLD (all p>0.05).

Conclusion In this community study in Bangladesh, NAFLD was present in 18.5% participants, one-quarter of whom were non-obese. Apart from body mass index, the metabolic profile was comparable between obese and non-obese NAFLD. Public health measures are needed to control and prevent NAFLD and MS and their adverse health consequences.

Summary box

What is already known about this subject?

- Non-alcoholic fatty liver disease (NAFLD) is common in the urban community of the developed country.
- Although more common in obese subjects, non-obese individuals may develop NAFLD.

What are the new findings?

- This is the first door-to-door survey on the prevalence of NAFLD in a rural community of Bangladesh.
- About one-quarter of the NAFLD subjects are non-obese in the rural community.
- The metabolic profile of non-obese NAFLD is different from the non-obese healthy counterpart.
- The metabolic profile of obese and non-obese NAFLD is comparable in a rural community of Bangladesh.

How might it impact on clinical practice in the foreseeable future?

- NAFLD should be considered an important cause of abnormal liver function tests and chronic liver disease among non-obese persons.
- Metabolic syndrome is also common among non-obese subjects like obese subjects.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition affecting one-quarter of the world's population.[1] NAFLD is a spectrum of diseases ranging from steatosis, non-alcoholic-steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC).[2 3] Globally, it is the leading cause of chronic liver disease.[2 3] It is also one of the leading cause of liver transplantation and HCC.[4 5] The most common cause of death among patients with NAFLD is cardiovascular disease,[6] which carries twice the mortality risk than liver disease.[7]

The pathogenesis and progression of NAFLD are complex and multifactorial that develops through the concerted actions of

multiple environmental and genetic factors.[8–11] Dietary factors and gut microbiota play an important role in the pathogenesis of NAFLD.[9 12] The effect of genetic risk factors is strongly influenced by modifiable environmental risk factors, such as obesity and insulin resistance. Genetic studies have found that variants in PNPLA3, TM6SF2, GCKR, MBOAT7 and HSD17B13 are associated with the full spectrum of NAFLD.[11] Pathological stimuli like lipid accumulation induce hepatic cells to produce inflammatory cytokines. Cytokines and chemokines like tumour necrosis factor (TNF)-α, transforming growth factor (TGF) -β1, interleukins (IL)-6, IL-10, might play an active role in the development and the potential progression of NAFLD through stimulation of hepatic inflammation, cell necrosis and apoptosis and induction of fibrosis.[13] Dysregulated production or secretion of anti-inflammatory and proinflammatory adipokines such as adiponectin, leptin, resistin, visfatin, TNF-α, TGF-β, IL1, IL-6, IL-10, IL-18 caused by excess adipose tissue and adipose tissue dysfunction can contribute to the development of insulin resistance and obesity-related metabolic diseases including NAFLD.[14]

Traditionally, NAFLD is considered as a disease of affluent society since its association with metabolic syndrome (MS), obesity, diabetes mellitus (DM), dyslipidaemia and hypertension.[7 15] Recent studies suggest that the prevalence of NAFLD in Asia is comparable to that of Western countries.[1 16] Moreover, the prevalence of NAFLD is rising faster in Asian countries.[16–20] Rapid industrialisation, socioeconomic development and urbanisation leading to a sedentary lifestyle and western diet may be the cause of such high prevalence and faster rise of the prevalence of NAFLD in Asia.[17 19] Moreover, genetic factors and body composition differences in fat and muscle may contribute to such a high burden in Asia.[16 19] Although more common in obese subjects, non-obese individuals may develop NAFLD, a distinct phenotype, known as lean or non-obese NAFLD. Lean NAFLD has been found more frequently among Asian subjects.[16] Asia is a vast area with multiple ethnicities and wide differences in socioeconomic status and dietary habit. Hence, the prevalence of NAFLD may vary in different countries, even in the different regions of the same country in Asia. However, most of the studies on NAFLD are from the urban community, and there are only a handful of studies outside the urban community in Asia where most of the people live in the villages.

Bangladesh achieved considerable economic development progress with a concomitant increase in calorie-rich diet intake and a sedentary lifestyle in the last few decades.[21] In Bangladesh, the prevalence of overweight and obesity, defined by body mass index (BMI) ≥25 and 30 kg/m^2, respectively, increased from 4.9% in 1975 to 23.6% in 2016.[22] An ultrasonographic survey among the adult volunteers found the prevalence of NAFLD to be 33.8%.[23] Despite the increasing prevalence of obesity, the prevalence and risk factors of NAFLD, the metabolic profile, and the risk factors for non-obese subjects to develop NAFLD are mostly unknown, particularly in the rural community of Bangladesh. Hence, we undertook such a community-based study in the rural population of Bangladesh with the following aims: (1) to find out the prevalence of NAFLD, including non-obese and obese NAFLD (2) to compare the sociodemographic, clinical and metabolic characteristics between the subjects with non-obese NAFLD and obese NAFLD and (3) to determine the risk factors of NAFLD and non-obese NAFLD.

METHODS

Study population

This cross-sectional study was undertaken among the adult population aged 18 years or more in a defined area of three villages (Charcharia and Churain of Nawabganj upazila of Dhaka district and Kharrah of Srinagar upazila of Munshiganj district of Bangladesh) during a period between April to August 2014 and July to September 2016. A manual census was done to identify the adult population. All the adult males and non-pregnant adult females who gave informed consent were included in the study. Persons with any amount of alcohol consumption or with established chronic liver disease with known aetiology, including hepatitis B virus, hepatitis C virus (HCV) infection, autoimmune hepatitis, primary biliary cholangitis were excluded from the study.

Study design

Three trained research assistants took the interview using a structured questionnaire during the door-to-door survey. Figure 1 shows the study protocol. The trained research assistants undertook the anthropometric measurements that included height (cm), weight (kg) and waist circumference (cm). Physical examinations, including pulse and systolic (mm Hg) and diastolic (mm Hg) blood pressure (BP), were measured in resting position by the investigators (MMR, MGK, NS and MA). Ultrasonography of the liver and biochemical tests were done after overnight fasting. Written informed consent was obtained from the study participants before the interview and all other examinations.

Questionnaire

The structured questionnaire, applied during the interviews, collected data on sociodemographic characteristics that included age, sex, occupation, monthly family income, education, marital status, religion, smoking and alcohol intake. The questionnaire also collected data on the history of chronic disease that included DM, hypertension, dyslipidaemia, chronic hepatitis B and C, autoimmune hepatitis, primary biliary cholangitis and drug-induced liver injury. The medication history for DM, hypertension and dyslipidaemia was also included in the questionnaire.

Biochemical tests

Blood samples of 5 mL were collected from the antecubital vein of fasting subjects by sterile disposable syringe

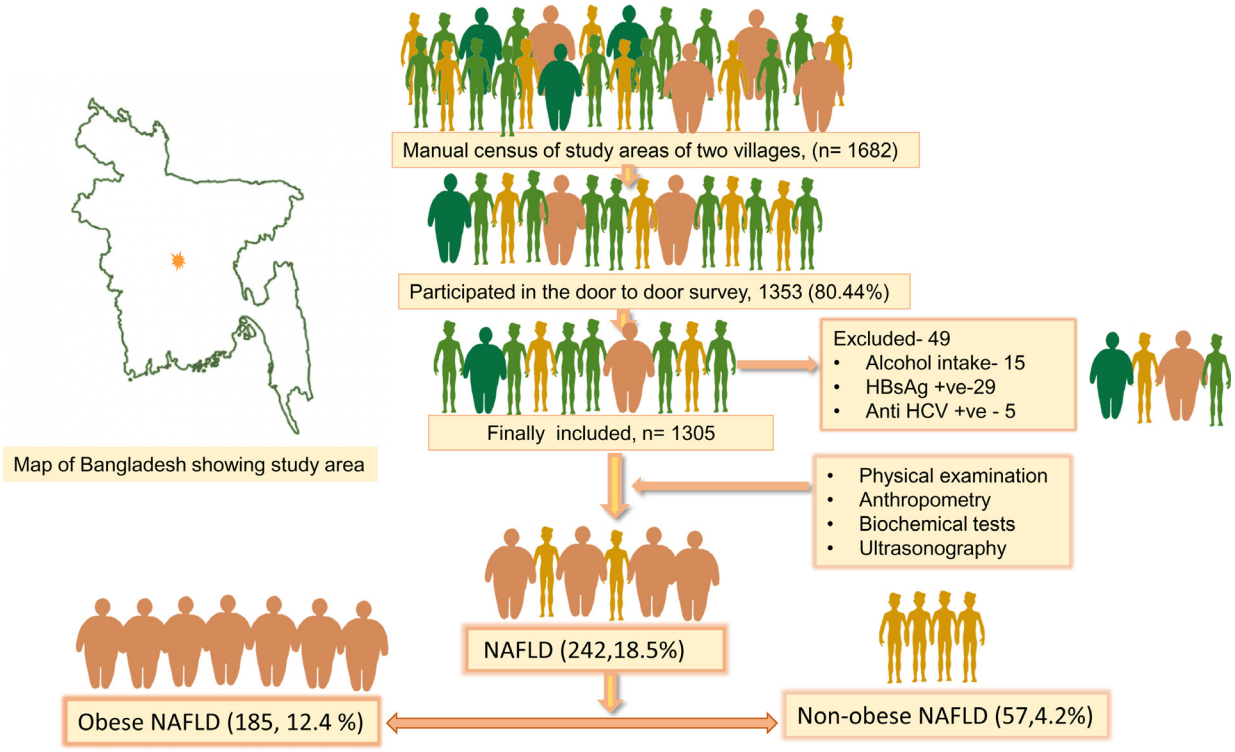

Figure 1 Study outline. HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease.

in an aseptic manner. Sera were separated and stored at-20°C for future testing. Serum glucose, alanine aminotransferase (ALT) triglyceride (TG), total cholesterol (TCh), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were determined by automatic biochemistry analyzer (Roche, Rotkreuz, Switzerland). Serum HBsAg and anti HCV were detected by ELISA.

Abdominal ultrasonography

Ultrasonography of the liver (Model 280C, Wuxi Haiying International Trade, Wuxi, China) was done in a local healthcare centre on fasting patients to detect NAFLD by a senior radiologist trained in ultrasonography (HB). Fatty liver was diagnosed in the presence of two of the three following criteria: (1) increased hepatic echogenicity compared with the spleen or the kidney, (2) blurring of liver vasculature and (3) deep attenuation of the ultrasonographic signal.[24]

Definitions

Obesity was categorised by BMI criteria for Asians by the regional office for the Western Pacific Region of the WHO.[25] BMI ≥25, 23.0–24.9, 18.5–22.9 and <18.5 kg/m^2 were defined as obese, overweight, normal and underweight, respectively.[25] Lean NAFLD and non-obese NAFLD were defined by BMI <23 kg/m^2 and <25 kg/m^2, respectively. DM was diagnosed if the fasting blood glucose (FBG) value ≥7.0 mmol/L or already on DM medications. Impaired fasting glucose was diagnosed if the FBG is between ≥6.1 and <7.0 mmol/L.[26] Revised National Cholesterol Education Programme, Adult treatment panel III clinical definition of the MS was used to define MS that requires the presence of three or more of the following features: (1) waist circumference ≥90 cm in men or ≥80 cm in women; (2) TG level 150 mg/dL or higher; (3) HDL-C level less than 40 mg/dL in men and less than 50 mg/dL in women; (4) systolic blood pressure 130 mm Hg or higher or diastolic pressure 85 mm Hg or greater and (5) fasting plasma glucose level 6.1 mmol/L or higher.[27] Dyslipidaemia was diagnosed in the presence of one or more of the followings: (1) TCh ≥200 mg/dL, (2) LDL ≥130 mg/dL, (3) HDL <40 mg/dL or (4) TG ≥150 mg/dL.[28]

Statistical analysis

Statistical analysis was done by IBM SPSS Statistics for Windows, V.25 (IBM). One investigator (NS) entered the data, 10% cross-checked by another investigator (MMR.). Categorical data were presented as proportion. Continuous data with normal distribution were presented as mean and SD and without normal distribution as median and IQR. Categorical data were analysed using the χ^2 test. Normally distributed continuous data were analysed using unpaired t-test. Non-parametric continuous data were analysed using Mann-Whitney U tests. Binary logistic regression analysis was used for the adjusted OR and 95% CI. All the factors considered to be associated with the dependent variable in univariate analysis were entered into the logistic regression analysis. P values less than 0.05 were considered significant.

RESULTS

Of the 1682 subjects approached, 1353 (80.44%) responded in the survey. After excluding 29 for HBsAg, 5

Table 1 Sociodemographic characteristics and metabolic profile of the study subjects with and without NAFLD

Variables	Study population (n=1305)	NAFLD absent (n=1063)	NAFLD present (n=242)	*P value
Age less than 40 Age more than 40	656 (50.3%) 649 (49.7%)	577 (54.3%) 486 (45.7%)	79 (32.6%) 163 (67.4%)	<0.001
Sex				0.002
► Male ► Female	397 (30.4%) 908 (69.6%)	304 (28.6%) 759 (71.4%)	93 (38.4%) 149 (61.6%)	
Marital status				0.001
► Married ► Single	1083 (83%) 222 (17%)	866 (81.5%) 197 (18.5%)	217 (89.7%) 25 (10.3%)	
Occupation				0.06
► Housewife ► Cultivator and day labour ► Service holders and others	854 (65.4%) 125 (9.6%) 326 (25%)	711 (66.9%) 97 (9.1%) 254 (23.9%)	142 (58.9%) 28 (11.6%) 71 (29.5%)	
Family income†				<0.001
► Lower ► Higher	799 (61.3%) 505 (38.7%)	672 (63.8%) 381 (36.2%)	118 (49.6%) 120 (50.4%)	
Education				0.175
► Up to class V ► More than class V	641 (49.1%) 663 (50.8%)	528 (49.9%) 530 (50.1%)	108 (44.8%) 133 (55.2%)	
History of smoking (current or past)	177 (13.6%)	140 (13.2%)	37 (15.3%)	0.405
Religion				0.581
► Muslim ► Hindu	934 (71.6%) 371 (28.4%)	757 (71.2%) 306 (28.8%)	177 (73.1%) 65 (26.9%)	
Presence of MS	463 (35.5%)	286 (26.9%)	177 (73.1%)	<0.001
Weight status				<0.001
► Underweight ► Normal weight ► Overweight ► Obese	161 (12.3%) 431 (33%) 212 (16.2%) 501 (38.4%)	161 (15.1%) 407 (38.3%) 179 (16.8%) 316 (29.7%)	0 (0%) 24 (9.9%) 33 (13.6%) 185 (76.4%)	
BMI kg/m^2 (mean±SD)	23.81±4.50	22.87±4.06	27.93±4.04	<0.001
Presence of abdominal obesity	398 (30.5%)	254 (23.9%)	144 (59.5%)	<0.001
Presence of DM	168 (12.9%)	86 (8.1%)	82 (33.9%)	<0.001
ALT >40 U/L	117 (9%)	64 (6%)	53 (22%)	<0.001
Presence of hypertension	309 (23.7%)	199 (18.7%)	110 (45.5%)	<0.001
Presence of dyslipidaemia	1108 (84.9%)	879 (82.7%)	220 (94.6%)	<0.001

*P value between subjects with and without NAFLD.
†Lower family income <৳15000/month, higher family income >৳15000/month.
ALT, alanine transaminase; BMI, body mass index; DM, Diabetes Mellitus; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease.

for anti-HCV positivity, and 15 for any amount of alcohol consumption, 1305 were included in the final analysis (figure 1). Overall, the mean age of the study participants was 41.28±15.10 years. Among them, 397 (30.4%) and 908 (69.4%) were male and female.

Prevalence and factors associated with NAFLD

On ultrasonography, overall, 242 (18.5 %) participants had NAFLD, as shown in figure 1. The prevalences of MS, DM, dyslipidaema, hypertenson and abdominal obesity were 35.5%, 12.9%, 84.9%, 23.7% and 30.5% participants, respectively (table 1). The mean age of the subjects with and without NAFLD was 46.10±12.3 and 40.18±15.5 years, respectively (p<0.001). Table 1 shows the sociodemographic characteristics, MS and parameters among the subjects with and without NAFLD. NAFLD prevalence was higher among the subjects aged 40 years or older (25.1%) than younger than 40 years (12%). The prevalence of NAFLD among males was higher (23.4%) than females (16.4%). NAFLD was more common among married persons, large family-income groups, cultivators and service holders, and other professions than homemakers, as shown in table 1. Among the subjects with NAFLD, 73.1% had MS, and 33.9% had DM, which were significantly higher than their counterparts without

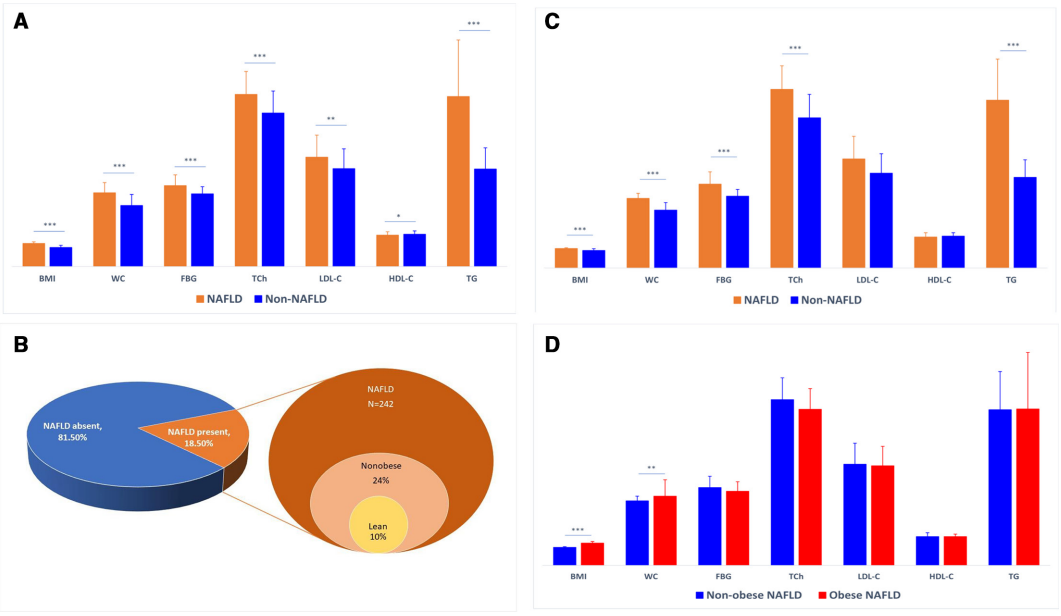

Figure 2 (A) Comparison of BMI, waist circumference (WC), fasting blood glucose (FBG), TCh, LDL-C, HDL-C and TG among subjects with and without NAFLD. (B) Prevalence of NAFLD, non-obese NAFLD and lean NAFLD. (C) Comparison of BMI, WC, FBG, TCh, LDL-C, HDL-C and TG among non-obese subjects with and without NAFLD. (D) Comparison of BMI, WC, FBG, TCh, LDL-C, HDL-C and TG among subjects with non-obese NAFLD and obese NAFLD. *P<0.05, **p<0.01, ***p<0.001. BMI, body mass index; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; TCh, total cholesterol; TG, triglyceride; WC, waist circumference.

NAFLD. The prevalence of NAFLD among the subjects with MS and DM was 38% and 48.8%, respectively. The BMI of the subjects with NAFLD was significantly higher than the subjects without NAFLD. Among the subjects with NAFLD, 76.4% were obese, 13.6% overweight and 9.9% were normal weight (table 1). Abdominal obesity was present in 59.5% of the subjects with NAFLD and 23.9% of the subjects without NAFLD. Overall, 53 (4.1%) participants had NAFLD with raised ALT. The elevated ALT level was found in 22% and 6% of the subjects with and without NAFLD. Among the NAFLD subjects with and without DM, 20 of 82 (24.4%) and 33 of 159 (20.8%) had raised ALT, respectively(p=0.313). Figure 2A shows the comparisons of BMI, waist circumference, FBG, TCh, TG, HDL-C and LDL-C among subjects with and without NAFLD.

Multivariate analysis for risk factors of NAFLD

In the multivariate analysis, age more than 40 years, male gender, MS, DM, abdominal obesity, hypertension, dyslipidaemia and overweight and obesity were independent risk factors for NAFLD (table 2). Among the subjects with NAFLD, the odds of having MS, DM, abdominal obesity, overweight, and obesity were 2.3 and 2.2, 2.2, 3.4 and 8.4, respectively.

Prevalence and factors associated with non-obese NAFLD

Of all, 804 (61.6%) participants were non-obese and 501 (38.4%) were obese. Among the study population, 57 non-obese and 185 obese subjects had NAFLD with a prevalence of non-obese and obese NAFLD 4.4% and 14.2%, respectively (figure 1). Among the 592 lean subjects, 24 had NAFLD, with a prevalence of 4.1% among the lean population. The prevalence of lean NAFLD in the whole study population was 1.8%. NAFLD was detected in 57 of 804 (7.1%) non-obese and 185 of 501 (36.93%) obese participants. Overall, about 76% of the participants with NAFLD were obese, 24% non-obese, and 10% lean, as shown in figure 2B.

The mean age of the non-obese subjects with and without NAFLD were 48.98±13.77 and 39.98±16.12 years, respectively(p<0.001). (online supplemental table 1) shows the sociodemographic characteristics, MS, and parameters among the non-obese population with or without NAFLD. Non-obese NAFLD was more common among participants older than 40 years. Among the non-obese NAFLD, 77% of subjects had MS, which was higher than their counterpart without NAFLD. The mean BMI of the subjects with non-obese NAFLD was significantly higher than that of non-obese participants without NAFLD. Abdominal obesity and DM were present among 52.6% and 31.6% of the subjects with non-obese NAFLD, respectively, and were significantly higher than their counterpart without NAFLD. Elevated ALT was present among about 16% of the subjects with non-obese NAFLD. The mean serum TG, TCh and FBG levels were higher among non-obese NAFLD than non-obese participants without NAFLD. HDL-C and LDL-C were comparable among the subjects with and without NAFLD across the non-obese population, as shown figure 2C.

Multivariate analysis for risk factors of non-obese NAFLD

On multivariate analysis, increased BMI ((adjusted OR, AOR 1.46 (95% CI 1.2 to 1.77)), presence of DM (AOR

Table 2 Multivariate analysis for risk factors of NAFLD

Variables	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age				
Less than 40 years	Reference		Reference	
More than 40 years	2.45 (1.83 to 3.29)	<0.001	1.54 (1.04 to 2.28)	0.03
Sex				
Female	Reference		Reference	
Male	1.56 (1.17 to 2.08)	0.003	2.86 (1.6 to 6.03)	0.006
BMI				
Under-normal weight	Reference		Reference	
Overweight	4.36 (2.51 to 7.58)	<0.001	3.32 (1.81 to 6.08)	<0.001
Obese	13.85 (8.86 to 21.66)	<0.001	7.90 (4.77 to 13.05)	<0.001
DM				
Absent	Reference		Reference	
Present	5.82 (4.12 to 8.23)	<0.001	2.21 (1.43 to 3.41)	<0.001
Metabolic syndrome				
Absent	Reference		Reference	
Present	7.40 (5.40 to 1014)	<0.001	1.72 (1.11 to 2.67)	0.015
Abdominal obesity				
Absent	Reference		Reference	
Present	4.68 (3.49 to 6.27)	<0.001	2.26 (1.72 to 3.99)	<0.001
Hypertension				
Absent	Reference		Reference	
Present	3.62 (2.69 to 4.87)	<0.001	1.89 (1.28 to 2.79)	0.001
Dyslipidaemia				
Absent	Reference		Reference	
Present	3.69 (2.06 to 6.59)	<0.001	2.31 (1.18 to 4.51)	0.014

Marital status, smoking habits, occupation and religions and education status did not achieve statistical significance.
BMI, body mass index; DM, diabetes mellitus; NAFLD, non-alcoholic fatty liver disease.

2.44 (95% CI 1.08 to 5.53)), MS (AOR 3.4 (95% CI 1.46 to 7.86)) and abdominal obesity (AOR 3.43 (95% CI 1.43 to 819)) were found as the risk factors for non-obese NAFLD.

Comparison of obese and non-obese NAFLD

Table 3 shows the comparisons of socio-demographic factors, MS, and metabolic parameters among the subjects with non-obese and obese NAFLD. There were no differences in age, sex, marital status, education level, occupation, smoking habits, religion and family income between non-obese and obese NAFLD subjects. The frequency of DM, MS, abdominal obesity was also comparable between the two groups. The fasting serum TG, TCh, LDL-C, and HDL-C, and blood glucose levels were also comparable between the two groups, as shown in figure 2D. However, there was a significant difference in BMI between the two groups.

DISCUSSION

This cross-sectional study was conducted in a rural community of Bangladesh where about one-third of the study population had normal body weight; about half had either obesity or overweight and the rest had undernutrition. This study demonstrated that 18.5% of the adult population had NAFLD, as detected by ultrasonography. NAFLD prevalence among the lean, non-obese and obese population was about 4%, 7% and 37%, respectively. About 24% of the NAFLD subjects were non-obese. Age more than 40 years, male gender, MS, DM, abdominal obesity, overweight and obesity were the risk factors for NAFLD. Similarly, increased BMI, MS, DM and abdominal obesity were found to be the risk factors for non-obese NAFLD. Apart from BMI, there were no differences in socio-demographic, clinical, or biochemical characteristics between the subjects with non-obese and obese NAFLD.

A previous ultrasonographic survey in Bangladesh among 2782 adult populations reported a 34% prevalence of NAFLD, which is higher than the 18.5% prevalence in the present study. This difference in prevalence may result from the differences in survey methods with the previous study's possible selection bias since it included

Table 3 Sociodemographic characteristics and metabolic profile among subjects with non-obese NAFLD and obese NAFLD

Characteristics	Non-obese NAFLD (n=57)	Obese NAFLD (n=185)	P value
Age (mean±SD)	48.98±13.78	45.22±11.71	0.043
Age less than 40	14 (24.6%)	65 (35.1%)	0.137
Age of more than 40	43 (75.4%)	120 (64.9%)	
Sex-			0.733
► Male	23 (40.4%)	70 (37.8%)	
► Female	34 (59.6%)	115 (62.2%)	
Marital status			0.580
► Married	50 (87.7%)	167 (90.3%)	
► Single	7 (12.3%)	18 (9.7%)	
Occupation			0.06
► Housewife	28 (49.1%)	114 (62%)	
► Cultivator and day labour	9 (15.8%)	19 (10.3%)	
► Service holders and others	20 (35.1%)	51 (27.7%)	
Family income (taka/month)			0.289
► Lower	32 (56.1%)	89 (48.1%)	
► Higher	25 (43.9%)	96 (51.9%)	
Education			0.686
► Up to class V	27 (47.4%)	82 (44.3%)	
► More than class V	30 (52.6%)	103 (55.7%)	
Smoker current or past smoker	8 (14%)	29 (15.7%)	0.763
Religion			0.654
► Muslim	43 (75.4%)	134 (72.4%)	
► Hindu	14 (24.6%)	51 (27.6%)	
Presence of MS	44 (77.2%)	133 (71.9%)	0.430
Presence of MS components			0.300
► Absent	1 (1.8%)	1 (0.50)	
► One	6 (10.5%)	13 (7%)	
► Two	6 (10.5)	38 (20.5%)	
► Three	19 (33.3%)	44 (23.8%)	
► Four	17 (29.8%)	67 (36.2%)	
► Five	8 (14%)	22 (11.9%)	
BMI kg/m^2 (mean±SD)	23.07±1.38	29.42±3.36	<0.001
Presence of abdominal obesity	30 (52.6%)	114 (61.6%)	0.227
Presence of DM	18 (31.6%)	64 (34.6%)	0.750
Presence of hypertension	26 (45.6%)	84 (45.4%)	0.978
Presence of dyslipidaemia	55 (96.5%)	174 (94.1%)	0.476
ALT>40 U/L	9 (15.8%)	44 (23.9%)	0.196

*P value between subjects with and without NAFLD.
†Lower family income <৳15 000/month, higher family income >৳15 000/month.
ALT, Alanine transaminase; BMI, body mass index; DM, diabetes mellitus; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease.

adult volunteers, and the study participants included mixed urban and rural populations. Additionally, the population's metabolic profile, which the previous study did not report, may differ from the present study population.[23]

The prevalence of NAFLD varies in Asia, from 8.7% to 57.37%.[29 30] In India, the prevalence of NAFLD varies from 16.6% to 32% in the urban population.[31 32] A community-based study among the rural population in West-Bengal, India, a neighbouring state of Bangladesh, found the prevalence of NAFLD to be 8.7%.[30] This lower prevalence of NAFLD may be related to the differences in the obesity status of West Bengal's rural population compared with our study population. The study population in the West-Bengal was mostly non-obese, and only 7% population were obese.

In contrast, 38% of the population in the present study were obese. The difference in obesity may be attributed

to dietary habits between the two population groups. Of note, the majority of the population of West Bengal are vegetarians. The prevalence of NAFLD among the non-obese population in the present study was 7.1%, similar to that of the mostly non-obese population in the West Bengal. These data suggest that even rural population of low-income and middle-income countries are affected by non-obese NAFLD.

A current systematic review and meta-analysis on the global prevalence of non-obese or lean NAFLD found that the overall prevalence of non-obese NAFLD was 12.1% among the general population and 8.6% in population-based studies. The prevalence of non-obese NAFLD in Southern Asia was 10%.[33] In a subanalysis of another meta-analysis of population-based studies of the Eastern population with BMI level <25 kg/m^2, the estimated prevalence of NAFLD was 11%, which was lower than the Western population.[34] The prevalence of non-obese NAFLD in different studies may be due to differences in study settings like population-based and healthcare centres, the cut-off value used to define the non-obese population, and the geographical regions and ethnicity.

The present study suggests that one-quarter of NAFLD subjects in the community are non-obese. A previous hospital-based study in Bangladesh also found that 26% of NAFLD patients were non-obese.[35] Two recent meta-analyses found that the pooled prevalence of non-obese participants among the NAFLD subjects were 25.3% and 40.8%.[33 36] There are controversies about the pathophysiology and outcome of lean or non-obese NAFLD.[17] However, a recent study demonstrated that lean patients have distinct metabolic, genetic, histological, bile acid profile, 7-alpha-hydroxy-4-cholesten-3-one (C4) levels, farnesoid X receptor activity and gut microbiota compared with non-lean NAFLD and lean healing controls.[37] Generally, lean/non-obese NAFLD is considered to have a more favourable biochemical profile, less severe histological findings.[34 38] However, a recent meta-analysis found that almost 40% of non-obese NAFLD have NASH, and almost 30% have significant fibrosis.[33] The hospital-based study in Bangladesh found no differences in metabolic parameters, insulin resistance, NASH and advanced fibrosis between non-obese and obese NAFLD.[35] In the present study, we observed that non-obese NAFLD subjects had increased odds for central obesity, DM, and MS. These findings corroborate well with a meta-analysis of 20 studies, including 5515 subjects with lean/non-obese NAFLD and 54 652 healthy controls.[38]

NAFLD is associated with gut dysbiosis.[12 33 38–40] Moreover, there is evidence for the association between gut bacteria and obesity.[9 39] The gut microbiome composition of lean and obese NAFLD was found to be different. Moreover, lean NAFLD patients had a differential microbiota profile compared with healthy lean controls.[37] A multistrain probiotic was found to reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients.[40] Microbiota-directed interventions, including diet and probiotics, probably guided by baseline microbial composition, is a potential therapeutic target and need to be explored in high-quality clinical trials.

There were no differences in abdominal obesity frequency despite the significant differences in BMI between obese and non-obese NAFLD. Abdominal obesity was an independent predictor of NAFLD among the non-obese population and the population as a whole in this study. Asians are more likely to have abdominal fat deposition at a lower BMI and are more prone to develop obesity-related complications due to excess visceral fat even with a similar BMI to the Western population.[41 42] This abdominal obesity also predisposes Asians to DM and cardiovascular risk factors despite lower BMI.[43] High prevalence of NAFLD despite lower BMI in Asians may result from excess viscera fat accumulation.[17 41]

In Bangladesh, the prevalence of obesity has increased significantly over the last few decades.[22] The prevalence of MS in one-third, dyslipidaemia in the majority and DM in about 13% of the population in the present study suggests a high prevalence of cardiovascular disease risk. These data also suggest that the non-communicable disease is a considerable burden in addition to the existing communicable diseases in Bangladesh. In fact, cardiovascular disease, diabetes, cancer and chronic respiratory disease are responsible for 67% of all death in Bangladesh currently.[44] The burden of non-communicable disease is likely to increase in Bangladesh like other Asian countries with continued socioeconomic development in the coming years.

The finding of the present study has important clinical implications. The NAFLD should be considered as an important differential diagnosis of chronic liver disease or raised ALT in Bangladesh, where chronic viral hepatitis, particularly hepatitis B virus is common.[45] Such consideration is important not only for obese but also the lean or non-obese individual with abnormal liver function tests or chronic liver disease. The present study also highlights the need for evaluation for metabolic profile both the obese and non-obese NAFLD considering the high rate of MS among such participants. The current study findings in the rural community of Bangladesh suggest increasing awareness and urgent need of targeted public health strategies to prevent NAFLD determinants. The findings may influence healthcare resources allocation and clinical trials substantially. This data supports the need for further research and resource allocation for NAFLD and metabolic disorders which are often viewed as non-priority, particularly in countries like Bangladesh, where non-communicable diseases are common.

To the best of our knowledge, this is the first household survey documenting the prevalence of NAFLD and MS in the rural community of Bangladesh. We acknowledge some limitations of our study. One of the limitations of the study is that it was done in a selected area. Bangladesh is a small country with an area of 146 480 km^2 and a homogenous population. Most of the people live in the villages in Bangladesh. Hence, the present study is likely

to reflect the current prevalence of NAFLD in rural areas of Bangladesh. The subjectivity of ultrasonographic interpretation in the diagnosis of NAFLD may be a limitation. Moreover, ultrasonography has a low sensitivity for mild steatosis. Another limitation of the study was that the proportion of progressive liver disease and cirrhosis were not estimated due to difficulties in performing a liver biopsy in this community survey. Non-invasive estimation of fibrosis status was not obtained either. Dietary, genetic factors and gut microbiota which were not assessed in the present study, might play an important role in the pathogenesis of obese and non-obese NAFLD.

In conclusion, although, traditionally regarded as a disease of the affluent society, NAFLD was a significant burden in a less affluent rural community of Bangladesh. The MS and its parameters like obesity, DM, hypertension and dyslipidaemia are also a considerable burden. About a quarter of NAFLD subjects are non-obese. Apart from BMI, the metabolic profile was comparable between obese and non-obese NAFLD. Further studies are needed to ascertain fibrosis and cirrhosis prevalence in the population. Public health measures are also needed to control and prevent the NAFLD and MS and their adverse health consequences.

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