


ORIGINAL ARTICLE

High prevalence of non-alcoholic fatty liver disease among healthy male blood donors of urban India

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Key words

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Abstract

Background: There is limited data on the community prevalence of non-alcoholic fatty liver disease (NAFLD). The present study evaluated the prevalence of NAFLD in a large number of healthy male blood donors of urban north India.

Methodology: In a prospective study performed over 18 months, voluntary blood donors fulfilling the requisite blood donation criteria and consenting to participate in the study were evaluated. The study received the approval of the institute's ethics committee. Diagnosis of NAFLD was made by excluding significant alcohol intake, ultrasound showing hepatic steatosis, and exclusion of transfusion associated infections. Subjects were also evaluated for various metabolic risk factors and the presence of metabolic syndrome.

Results: Of 1388 subjects who consented for participation, 386 did not come for evaluation. Three females, nine (0.9%) HBsAg-positive, and four (0.4%) anti-HCV positive subjects were excluded. Of the 986 males evaluated with hepatobiliary ultrasound, 543(55.1%) had fatty liver on ultrasonography [15 (1.5%) alcoholic fatty liver and 528 (53.5%) NAFLD]. Among those with NAFLD, 469 (88.8%), 54 (10.2%), and 5 (0.9%) had mild, moderate, and severe hepatic steatosis, respectively. Subjects with NAFLD, when compared to those without NAFLD, had significantly higher age, BMI, waist circumference, blood pressure, total cholesterol and triglycerides, low-density lipoprotein, and fasting plasma glucose. Multivariate regression analysis demonstrated age, BMI, waist circumference, systolic blood pressure, total cholesterol, and number of metabolic syndrome criteria as independent predictors of NAFLD.

Conclusions: Urban Indian healthy male blood donors have a high prevalence of NAFLD.

Introduction

Non-alcoholic liver disease (NAFLD) is a spectrum ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH), which has the propensity to progress to liver cirrhosis and hepatocellular carcinoma (HCC).¹ Rather than affecting only the liver, NAFLD is now recognized as a multisystem disease and has been associated with not only increased liver-related morbidity and mortality but also with increased morbidity- and mortality-related cardiovascular disease, chronic kidney disease, osteoporosis, and extrahepatic malignancy.² NAFLD has now become the most common liver disease the world over, affecting up to one fourth of the global population; the prevalence, however, may differ from country to country and region to region.³ The general population prevalence in the Western world may be slightly higher than the Asia Pacific.⁴ Within the Asia Pacific, there are countries with higher and lower prevalence.⁵ Within the

Asia Pacific, Asian-Indians are somehow more susceptible to insulin resistance (even at lower BMI) and thus to NAFLD. This fact has been established in various multiracial studies and may have a genetic basis.^{6–8} Studies from India have shown variable prevalence, with major difference between rural and urban parts of the country.^{9–12} Even within urban India, the highest prevalence reported is close to 30%, but the data are limited to east, west, and southern India.^{10–12} In the present study, we assessed the prevalence of NAFLD in a large number of healthy blood donors of urban north India.

Methodology

Study design. In a prospective cross-sectional study performed over a period of 18 months, all blood donors coming to the blood donation center of the institute and fulfilling the

requisite blood donation criteria were included in a voluntary, nonrandom manner after giving informed consent. The study received approval from the institute's ethics committee. In order to capture the healthier group, only voluntary blood donors were included, and replacement/family-directed donors were excluded from the study.

The blood collection of the department of transfusion medicine of the institute is predominantly from an outdoor volunteer program (85%), leaving only 15% of collection at the blood donation center of the institute. Because of the controlled working environment, this study was carried out at the blood donation center of the institute and not on the donors at outdoor camps. Even the blood donation center at the institute collects approximately 8000 blood donors per year (12 000 over 18 months of study period), but of these, a majority are replacement and family-directed donations who were excluded from the study. Finally, only volunteer donor population constituting about 3000 volunteers was initially screened, and those consenting ($n = 1388$, 46%) were included in the study.

Personal and family medical history, including history liver disease, hypertension, diabetes mellitus and cardiovascular disease, medication use, or substance abuse, was taken from all the participants. Subjects were assessed for history of smoking and alcohol consumption.

Definition of non-alcoholic and exclusion of other diseases.

Subjects were divided into complete abstainers and alcohol consumers. Alcohol consumers were inquired in detail about the type of alcohol consumed, duration of consumption, and average quantity consumed per day to quantify the amount of absolute alcohol consumed per day. Subjects were further divided into those who were consuming <20 g/day (defined as non-alcoholic along with abstainers) or > 20 g/day.¹³

All blood donors were screened for hepatitis B virus surface antigen (HBsAg, ELISA, InTec Products Inc., Hialang, Xiamen), antibodies for hepatitis C virus (anti HCV, SD HCV ELISA 3.0, Standard Diagnostics, Inc., Kyonggi-do, Korea), human immunodeficiency virus antibody (anti -HIV-1/HIV-2, SD HIV1/2 ELISA 3.0, Standard Diagnostics, Inc., Kyonggi-do, Korea), syphilis, and malaria. Donors testing positive for any of the above infections were excluded from further analysis and referred to the relevant clinics for further work-up.

Anthropometry. Body weight and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated by dividing the weight (in kg) by the squared height (in meters). Asia Pacific Criteria were used to define overweight (BMI ≥ 23 and < 25 kg/m²) or obese (BMI > 25 kg/m²). Obese subjects with BMI > 25 and < 30 kg/m² were considered obesity class I and BMI > 30 kg/m² considered obesity class II.¹⁴ Waist circumference was measured by placing a measuring tape in a horizontal plane around abdomen at level of iliac crest at the end of normal expiration. Waist circumference ≥ 90 cm for males and ≥ 80 cm for females were used to define central obesity.¹⁴ Any subject with known hypertension on antihypertensive medication or having either systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg was labeled as hypertensive.

Biochemistry. An overnight fasting blood sample was collected and processed immediately for the estimation of liver function tests (LFT) and lipid profile. The sera were separated and used for estimation of different parameters (fasting serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and liver function tests – serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total protein). Another 2 mL of blood was taken in a sodium fluoride vial for estimation of fasting plasma glucose (FPG). Subjects with a history of taking oral hypoglycemic agents were defined as having type 2 diabetes mellitus (DM). Lipid profile cut-off values were based on criteria laid down by the National Cholesterol Education Programme (NCEP)/Adult Treatment Panel (ATP) III guidelines.¹⁵ Abnormal values were defined as total cholesterol (TC) ≥ 200 mg/dL; serum triglycerides ≥ 150 mg/dL; serum HDL < 40 mg/dL for males, < 50 mg/dL for females; and serum LDL ≥ 130 mg/dL.¹⁵

Metabolic syndrome. Subjects were evaluated for the presence of metabolic syndrome based on American Heart Association/Updated NCEP ATP III criteria, as shown below, with modified waist for Asians. Subjects with \geq three of the parameters were diagnosed with metabolic syndrome.¹⁵

Updated NCEP/ATP III criteria

1. *Elevated waist circumference:* men ≥ 90 cm, women ≥ 80 cm
2. *Elevated triglycerides:* ≥ 150 mg/dL
3. *Reduced HDL cholesterol:* men < 40 mg/dL, women < 50 mg/dL
4. *Elevated blood pressure:* SBP ≥ 130 or DBP ≥ 85 mm Hg or use of medication for HTN
5. *Elevated fasting glucose:* ≥ 100 mg/dL or use of medication for hyperglycemia

Diagnosis of hepatic steatosis. All the study subjects underwent ultrasonography (USG) of the abdomen to assess the hepatic parenchyma and biliary tree, carried out by a hepatologist experienced in abdominal USG. B-mode examination for grading of fatty liver was performed using the same system (Philips, HDI-3500 Diagnostic Ultrasound System with a Philips C5-2 curved array transducer 2.0–5.0 MHz, Bothell, WA 98041–3003, USA). The presence or absence and grading of fatty infiltration of the liver were recorded as grades 0, 1, 2, or 3. In grade 1 (mild) fatty infiltration, echogenicity was slightly increased, with normal visualization of the diaphragm and the intrahepatic vessel borders.¹⁶ In grade 2 (moderate) fatty infiltration, echogenicity was moderately increased, with slightly impaired visualization of the diaphragm or intrahepatic vessels. Grade 3 (severe) fatty infiltration was considered to be markedly increased echogenicity with deep attenuation causing poor or nonvisualization of the diaphragm, the intrahepatic vessels, and the posterior portion of the right lobe.¹⁶ Fatty liver subjects were further divided into alcoholic fatty liver (alcohol consumption > 20 g/day and ultrasound evidence of hepatic steatosis) and non-alcoholic fatty liver disease (NAFLD - alcohol abstinence or consumption < 20 g/day with ultrasound evidence of hepatic steatosis).

Statistical analysis. For quantitative data, the data were presented as mean \pm SD or median and interquartile range (IQR), as appropriate. Normality of data was checked by measures of Kolmogorov Smirnov tests of normality. For categorical variables, number and percentages were calculated. Correlation between various anthropometric and biochemical parameters was evaluated using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for data that was not normally distributed. Multivariate regression analysis was performed to remove the confounding effect of different variables. All calculations were two sided and performed using SPSS version 17 (Statistical Packages for the Social Sciences, Chicago, IL).

Results

Study population and prevalence of NAFLD. As detailed in the methodology, even though a larger number of blood donations occurred during the study period, only voluntary blood donors fulfilling the requisite criteria and providing consent were evaluated. Of the 1388 healthy blood donors who consented for the study, 386 did not come for the evaluation. Mean age of the study population was 30.7 ± 8.2 years for males and 44.7 ± 8.1 years for females. As we had only three females, further analysis was performed only in 999 males. Nine male subjects (0.9%) were excluded on account of HBsAg positivity and four (0.4%) on account of anti-HCV positivity. None of the

participants tested positive for HIV. A total of 986 males were further evaluated for history of smoking and alcohol consumption, body mass index (BMI), features of metabolic syndrome, liver function tests, and hepatobiliary ultrasound (Fig. 1).

Of the 986 males in the whole study, 93 subjects (9.4%) were smokers, 22 (2.2%) smoked bidis, while 71 (7.2%) smoked cigarettes, with mean duration of 8.4 ± 7.1 years of smoking. A total of 747 subjects (74.6%) did not consume alcohol. Among the 255 subjects (25.4%) who consumed alcohol, 240 (94.1%) consumed <20 g alcohol per day, while 15 subjects (5.9%) consumed >20 g alcohol per day. Even though alcohol is a common cause of liver disease in this part of the country, as per the experience of our blood bank, most of the volunteer blood donors are non-alcoholic. This is because the volunteer blood donors are regular repeat blood donors and are more conscious of the blood donor selection criteria compared to first-time and replacement blood donors. Of the 986 males evaluated with hepatobiliary ultrasound, 543 (55.1%) had evidence of fatty liver, while 443 (44.9%) had normal liver ultrasonography. With further stratification, 15 subjects (1.5%) had alcoholic fatty liver (alcohol consumption >20 g/day), while 528 (53.5%) had NAFLD (alcohol abstinence or consumption <20 g/day). Among the NAFLD group, 469 (88.8%), 54 (10.2%), and 5 (0.9%) had mild, moderate, and severe hepatic steatosis, respectively. The median age among the NAFLD subjects was 32 (IQR 27–39) years. The maximum number of subjects [220 (41.7%)] with NAFLD were in the age group 18–30 years (Table 1).

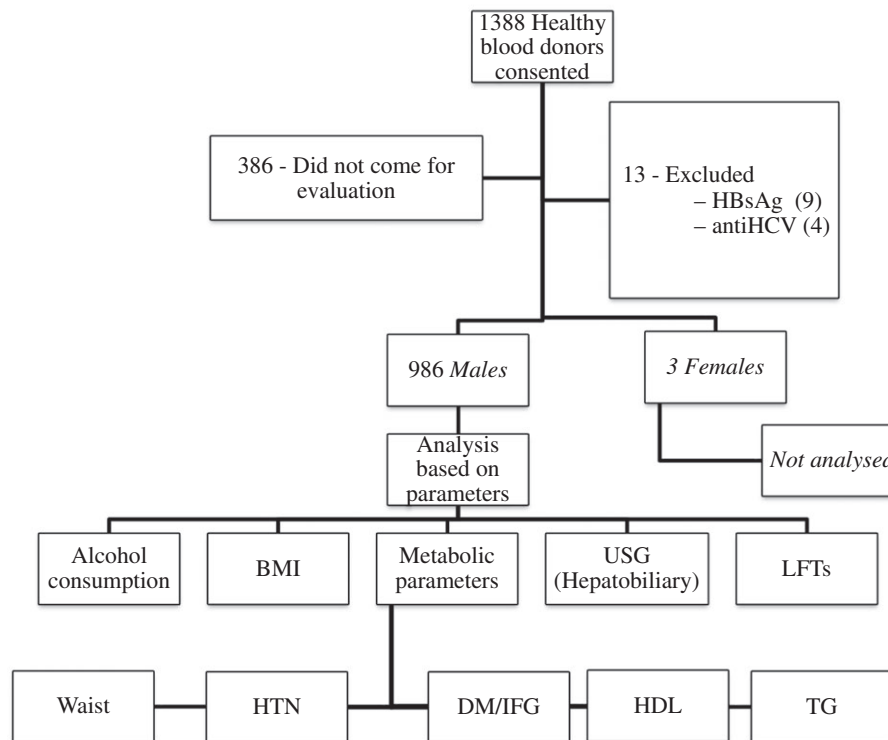


Figure 1 Showing the flow of inclusion and the work-up performed in the subjects. Anti HCV, hepatitis C virus antibody; BMI, body mass index; DM, diabetes mellitus; HBsAg, hepatitis B surface antigen; HDL, serum high density lipoprotein; HTN, hypertension; IFG, impaired fasting glucose; LFT, liver function test; NAFLD, nonalcoholic fatty liver disease; TG, serum triglycerides; USG, ultrasound.

Table 1 Showing the baseline characteristics of NAFLD subjects ($n = 528$)

Parameters	NAFLD (number/percentage)
Age (years)	
18–30	220 (41.7)
30–40	203 (38.4)
40–50	91 (17.2)
50–65	14 (2.65)
Alcohol	
Abstinent	341 (64.6)
<20 gm	187 (35.4)
BMI (kg/m ²)	
<23	36 (6.8)
23–24.99	85 (16.1)
25–29.99	332 (62.9)
≥30	75 (14.2)
Waist ≥ 90 cm	357 (67.6)
HTN	215 (40.7)
DM/IFG	145 (27.5)
TG ≥ 150 mg/dL	342 (64.8)
HDL < 40 mg/dL	315 (69.7)
Metabolic syndrome criteria	
0	22(4.17)
1	81 (15.3)
2	139 (26.3)
3	166 (31.4)
4	83 (15.7)
5	37 (7.0)
Metabolic syndrome	286 (54.2)

BMI, body mass index; DM, diabetes mellitus; HDL, serum high-density lipoprotein; HTN, hypertension; IFG, impaired fasting glucose; NAFLD, non-alcoholic fatty liver disease; TG, serum triglycerides.

Metabolic syndrome and NAFLD. The median BMI among NAFLD subjects was 26.79 (IQR 25.11–28.67) kgm⁻². The prevalence of obesity among the NAFLD population was 77.1%, while the prevalence of NAFLD among obese subjects was 88.7% (407 of the 459). Thirty-six NAFLD subjects (6.8%) had normal BMI (<23 kg/m²), whereas 492 subjects (93.2%) were either overweight or obese. Among the obese group, 332 subjects (62.9%) had class I obesity, while 75 subjects (14.2%) had class II obesity. The median waist circumference was 93 cm (IQR 87–100), with 357 NAFLD subjects (67.7%) having a waist circumference ≥ 90 cm (Table 1). A total of 215 subjects (40.7%) with NAFLD had hypertension. The median FPG among the NAFLD subjects was 94.6 mg/dL. Of the subjects, 145 (27.5%) had diabetes or impaired fasting glucose. The median TG among the study population was 174.50 (IQR 130.29–221.91) mg/dL, with 342 subjects (64.8%) having TG ≥ 150 mg/dL. The median HDL was 38.47 (IQR 33.29–42.87) mg/dL, with 315 subjects (69.7%) having HDL < 40 mg/dL. Of the NAFLD participants, 280 (54.2%) had presence of metabolic syndrome (ATP III criteria ≥ 3 components). Among 528 subjects with NAFLD, one component of metabolic syndrome was present in 81 subjects (15.3%), two components in 139 subjects (26.3%), three components in 166 subjects (31.4%), four components in 83 subjects (15.7%), and five components in 37 subjects (7.0%). At least one

component of metabolic syndrome was present in 82.0% (809 of 986) of the blood donors and 506 of 528 (95.8%) subjects with NAFLD, and only 22 subjects (4.17%) not fulfilling any criteria for metabolic syndrome had NAFLD (Table 1).

Comparison of NAFLD and non-NAFLD cohort. A total of 528 subjects with NAFLD were compared with 443 subjects without fatty liver on ultrasonography. Subjects with NAFLD had significantly higher age, BMI, waist circumference, blood pressure, TC, TG, LDL and FPG, as shown in Table 2 [all p -values <0.001], when compared with subjects with normal liver on ultrasonography.

Correlation of various parameters with NAFLD. Age, weight, BMI, waist circumference, blood pressure (SBP/DBP), FPG, TG, TC, LDL, number of metabolic syndrome parameters, AST, and ALT had a significant positive correlation with the prevalence of the fatty liver. Serum HDL had a significant negative correlation with the prevalence of NAFLD (Table 3). To remove the confounding effect of variables, multivariate regression analysis was performed, which revealed age, BMI, waist circumference, SBP, total cholesterol, and number of metabolic syndrome criteria to be the independent predictors of NAFLD (Table 3).

Discussion

NAFLD prevalence. Prevalence rates of NAFLD in the general population are variable depending on the modality used to detect fatty liver and the regional location within the country. Studies from the United States and Europe have shown that 25–46% of the general population has NAFLD as studied by ultrasound and magnetic resonance spectroscopy (MRS).^{3,17,18} Ultrasound studies from the Asia Pacific suggest that a quarter of the general population in Asia Pacific has NAFLD.¹⁹ Within

Table 2 Showing the comparison between various parameters among subjects with and without NAFLD

	Subjects without fatty liver ($n = 443$)	Subjects with NAFLD ($n = 528$)	P -value
	Median (IQR)	Median (IQR)	
Age (years)	25 (22–30)	32 (27–39)	0.000
BMI (kg/m ²)	22.2 (20.4–23.6)	26.8 (25.1–28.7)	0.000
Waist (cm)	79 (73–84)	93 (87–100)	0.000
SBP (mm Hg)	120 (110–120)	122 (120–130)	0.000
FPG (mg/dL)	87 (81–93)	95 (86–101)	0.000
TG (mg/dL)	131 (94–180)	175 (130–222)	0.000
HDL (mg/dL)	42 (36–46)	38 (33–43)	0.000
TC (mg/dL)	158 (139–178)	183 (161–210)	0.000
LDL (mg/dL)	93 (78–108)	114 (91–133)	0.000
AST (IU/L)	23 (20–27)	36 (27–47)	0.000
ALT (IU/L)	24 (20–29)	51 (38–77)	0.000

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; FPG, fasting plasma glucose; HDL, serum high-density lipoprotein; HTN, hypertension; IFG, impaired fasting glucose; LDL, serum low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, serum triglycerides.

Table 3 Showing correlation of various parameters with NAFLD

Factor	Correlation		Multivariate regression analysis			
	Correlation coefficient	P value	Regression coefficient	SE	T value	Significance
Age	-0.396(**)	0.000	-0.092	0.002	3.550	0.000
BMI	0.682(**)	0.000	0.384	0.005	10.833	0.000
Waist	0.656(**)	0.000	0.179	0.002	4.853	0.000
SBP	0.490(**)	0.000	0.092	0.002	3.328	0.001
DBP	0.348(**)	0.000	0.384	-0.871	-0.028	0.561
FPG	0.339(**)	0.000	0.729	0.346	0.011	0.747
HDL	-0.092(**)	0.004	0.282	-1.076	-0.034	0.917
TG	0.174(**)	0.000	0.301	-1.035	-0.033	0.845
TC	0.360(**)	0.000	0.093	0.000	4.02	0.000
LDL	0.327(**)	0.000	0.937	0.079	0.003	0.256
Number of metabolic syndrome parameters	0.401(**)	0.000	0.110	0.031	4.598	0.000

BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL, serum high-density lipoprotein; LDL, serum low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; TC, total cholesterol; TG, serum triglycerides.

Asia Pacific, Asian-Indian men are probably more prone to insulin resistance and NAFLD even at a lower BMI. In a large study from the United States including young, lean, healthy, sedentary, nonsmoking Eastern Asians, Asian-Indians, Blacks, Caucasians, and Hispanics, the prevalence of insulin resistance was approximately two- to threefold higher in the Asian-Indians compared with all other ethnic groups.⁶ This was further associated with an approximately twofold increase in hepatic triglyceride content and plasma IL-6 concentrations in Asian-Indian men compared with Caucasian men.⁶ Two multiethnic studies from Malaysia also found higher prevalence of NAFLD in Indian and Malay males in comparison with Chinese males.^{7,8} Differences in the prevalence of NAFLD among different races may be dependent on environmental and genetic factors. There is paucity of published literature regarding the prevalence of NAFLD in India, with most studies describing a small number of patients. Epidemiological studies suggest prevalence of NAFLD in around 9–32% of general population with maximum prevalence in those between 40 and 50 years of age.^{9–12} The lowest prevalence of 9% was reported from the rural part of West Bengal (East India), whereas the highest prevalence of 32% was reported from the urban part of southern India.^{9,12} The prevalence of NAFLD in our study was 53.5%, with the highest prevalence in the 18–30 years age group (median age 32 years) (Table 1). Even though we did not have a comparative group, the high prevalence of NAFLD in our only male population study substantiates the proneness of young Indian males for NAFLD as described earlier in multiracial studies.^{6–8} The higher prevalence of NAFLD in our study could also be related to the high prevalence of metabolic risk factors in the blood donors, with at least one component of metabolic syndrome present in 82.0% (809 of 986) of the blood donors. In addition to metabolic risk factors, whether our population is also genetically predisposed to NAFLD has not been evaluated in the present study.

Most patients with noncirrhotic NAFLD are initially asymptomatic and are diagnosed due to an incidental detection of raised liver enzymes or fatty liver on USG. In some patients, these findings are detected during work-up for dyspeptic symptoms, malaise, or fatigability or during work-up for other illnesses.²⁰ Our study was performed on voluntary blood donors

who were asymptomatic for NAFLD. The higher prevalence of NAFLD/hypertension and diabetes/IFG in healthy blood donors may represent the spread of a modern lifestyle epidemic in the general population and would require adequate preventive steps at various levels to check this menace. Even though not evaluated in our study, many patients with fatty liver on ultrasound may have severe liver disease, that is, non-alcoholic steatohepatitis (NASH), NASH with significant fibrosis, and even compensated NASH-related cirrhosis, adding significantly to the present and future disease burden of chronic liver disease in Asia Pacific in general and in India in particular.

NAFLD and metabolic syndrome. As metabolic risk factors are so common in patients with NAFLD, there is evidence now to show that NAFLD may actually be a hepatic manifestation of metabolic syndrome.²¹ Majority of the patients with NAFLD are obese and, consequently, are at a nearly fivefold higher risk of developing hepatic steatosis. The risk of NAFLD is also high in those with impaired fasting glucose, type 2 diabetes mellitus, dyslipidemia, and hypertension.²² Majority of the subjects (93%) in our study had overweight (16.1%) or obesity (77.1%), and only 36 (6.8%) NAFLD subjects had normal weight. Our data and some of the earlier data challenge the concept of so-called 'Lean NASH' in India.^{20,22} The high prevalence of overweight and obesity is attributed to the use of Asia Pacific criteria in our study, and the lower prevalence of obesity in some of the earlier studies was related to the use of international criteria to define overweight and obesity.^{23,24} Central obesity with waist circumference ≥ 90 cm was seen in 67.6%. Central or abdominal obesity, which is more commonly associated with insulin resistance and NAFLD, has been observed in 72–90% of Indian patients with NAFLD.²² The prevalence of hypertension (HTN) among NAFLD subjects was 40.7%, which was significantly higher than the prevalence of 10% in previous studies from India.^{20,22,25} The reason for higher prevalence of HTN in our subjects could be related to the 'white coat' effect as only 13 subjects were previously diagnosed as being hypertensive.

Type 2 diabetes mellitus (DM) is an important determinant of both the presence and severity of NAFLD. We found that 145 (27.5%) of the NAFLD subjects had either diabetes mellitus

or impaired fasting glucose, slightly higher than our previous experience in hospital-based patients with NAFLD.^{20,22,25} Hypertriglyceridemia (TG \geq 150 mg/dL) was observed in 342 subjects (64.8%) with NAFLD, while abnormal HDL (<40 mg/dL) was seen in 315 subjects (69.7%) similar to the earlier reported high prevalence of abnormal TG and HDL in patients with NAFLD.^{22,26}

Among 528 subjects with NAFLD in our study, at least one component of metabolic syndrome was present in 506 (95.8%) subjects, and only 22 subjects (4.17%) not fulfilling any criteria for metabolic syndrome had NAFLD. However, among the 528 NAFLD subjects, only 242 (54.2%) had metabolic syndrome, which is consistent with previous reports.^{20,22,25}

Correlation of various parameters with NAFLD.

Multivariate regression analysis demonstrated age, BMI, waist circumference, SBP, total cholesterol, and number of metabolic syndrome criteria to be the independent predictors of NAFLD. Our observations are similar to earlier studies reporting female gender, BMI, waist, hypercholesterolemia, LDL levels, elevated fasting blood sugar, and raised AST and ALT as independent predictors associated with presence and severity of NAFLD.^{10,27}

Limitations. One of the limitations of our study is that it is a male-centric study. Of 1002 donors who participated in the present study, 999 (99.7%) were males, with only three females (0.3%). The paucity of the female gender in our study could be a reflection of the differential gender distribution among the donors in our institute. Because of this limitation, it may not be possible to generalize the results for the whole country, and a high prevalence of NAFLD may be limited to young males of urban north India. The study was also limited by the fact that, of 1388 healthy blood donors who initially consented for the study, 386 did not come for the evaluation. This is because the volunteer blood donors have no symptoms, and it is beyond the control of the blood bank to force the follow up of such volunteers. Another limitation was that the diagnosis of NAFLD in our subjects was based on the history of alcohol intake and the hepatobiliary ultrasound with exclusion of secondary causes by history and investigations. Even though the sensitivity of the ultrasound for the diagnosis of moderate to severe fatty liver is around 85%, it still may remain the preferred and practical modality for the detection of hepatic steatosis in epidemiological settings or while screening a large number of patients where other modalities like CT/MRS and liver biopsy may be impractical. Our study was also limited by the lack of availability of socioeconomic and dietary practice data in blood donors, which may have a bearing on the high prevalence of metabolic risk factors and NAFLD in them.

In conclusion, our study highlights a high prevalence of NAFLD in the young urban north Indian healthy male blood donors. A study to look into the environmental and genetic reasons would be required to check the spread of this lifestyle disease.

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