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Research Brief

Screening for non-alcoholic fatty liver disease (NAFLD) among adults in Puducherry, India: A community-based cross-sectional study

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***Background & objectives*: Non-alcoholic fatty liver disease (NAFLD) is an emerging public health issue and affects 20–30 per cent of the general population globally and 9–53 per cent in India. We aimed to estimate the prevalence of NAFLD and associated risk factors among adults at high risk for NAFLD (≥30 yr) in Puducherry.**

***Methods*: A community-based cross-sectional study was conducted among 350 adults at high risk for NAFLD (simple random sampling) in a rural area of Puducherry. Clinical assessment, and blood investigations (liver function tests, lipid profile) were performed. NAFLD was diagnosed using the fatty liver index and risk stratification for advanced liver fibrosis by using the FIB-4 score. The indeterminate/ high-risk participants underwent vibration controlled transient elastography (VCTE). Prevalence of NAFLD [proportions with 95 per cent confidence interval (CI)] and risk factors associated with NAFLD (multivariable regression) were reported.**

***Results*: Prevalence of NAFLD among adults at high risk was 22.8 per cent (95% CI:18.7% –27.5%); and higher among men (35.2%), those with diabetes mellitus (33.3%) and obesity (40.2%). Male sex, diabetes mellitus and elevated alanine transferase were significantly associated with high risk of NAFLD.**

***Interpretation & conclusions*: One in four screened adults had NAFLD, increasing to one in two among those with comorbidities.**

Keywords Advanced liver fibrosis - FIB-4 score - non-alcoholic fatty liver disease - risk assessment - vibration controlled transient elastography

Fatty liver is a condition where at least five per cent of the weight of the liver is contributed by fat[1]. Non-alcoholic fatty liver disease (NAFLD) refers to a state of fatty liver that can result in the absence of a significant alcohol intake (≥ 30g/day for men and ≥ 20g/day for women) and other secondary causes[2]. NAFLD includes multiple entities, from simple fat accumulation to liver failure requiring transplantation. Individuals with Type 2 diabetes mellitus (T2DM), overweight or obese individuals, and those with

metabolic syndrome are identified as the high-risk group for NAFLD[3].

Globally, NAFLD is now frequently cited as the cause of liver disease, affecting 20-30 per cent of the world's general population and 42-70 per cent of individuals with T2DM[4,5]. Overall 29.6 per cent of Asians have NAFLD and 10 per cent of deaths in the Asia Pacific region are attributable to liver cancer and cirrhosis in people with NAFLD[6]. In India, about 9-53 per cent of the general population and 65-75 per cent of the high-risk population (overweight, obese, T2DM) are affected by NAFLD[7–9]. Ultrasonography of the abdomen is the recommended modality for diagnosing NAFLD[10,11]. Fatty liver index and FIB-4 (Fibrosis-4) score are the proxy indicators for fatty liver and advanced liver fibrosis respectively, and are used in low-resource settings.[12,13] Vibration controlled transient elastography (VCTE) is used to identify liver fibrosis with higher sensitivity and specificity[14]. There is an increased risk of cardiovascular disease (CVD) with NAFLD[15,16]. Population-based screening using a community-based assessment checklist under the National Programme for Prevention and Control of Non communicable Diseases (NP-NCD) has facilitated early identification, prevention and control of NCDs, including those at high risk for NAFLD. We aimed to estimate the prevalence of NAFLD and the clinical factors associated with high risk of NAFLD among adults of more than 30 yr in Puducherry.

Materials & Methods

This cross-sectional study was undertaken by the department of Preventive and Social Medicine, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India.

Study design and participants: A community-based cross-sectional study was carried out from July 2021 to July 2023 in the rural field practice area of Rural Health and Wellness Centre (HWC)-Primary Health Centre (PHC), which caters to a population of 11,200 (four villages) in Puducherry, India[17]. All eligible adults above 30 yr residing in Ramanathapuram village who were willing to participate were included in the study. Non-random selection of study site was done. Simple random sampling of participants was done by using random number assignment applying Excel function. The individuals were screened using community-based assessment checklist and those with a score of more than four were included. Those who tested positive for hepatitis B and C (*i.e.*, hepatitis B surface antigen or active hepatitis C) or taking drugs that cause fatty liver and those with alcohol intake more than recommended (men >30g/day and women >20g/day) were excluded. Assuming the prevalence of NAFLD among the high-risk individuals to be 40 per cent,10 per cent relative precision, 5 per cent alpha error, the estimated sample size was 373 using the OpenEpi online version 3.0.

Study tools: Sociodemographic and clinical details were collected using data collection proforma. Screening for hepatitis B & C was done using a rapid diagnostic kit. The high-performance liquid chromatography method was used for HbA1C testing. Serum albumin, liver enzymes (AST, ALT, GGT) triglycerides, HDL-cholesterol (HDL-C), LDL-cholesterol and total cholesterol were estimated based on spectrophotometry using autoanalyzer (Beckman Coulter Inc, Brea, California, USA) in a quality assured laboratory. Fatty liver index and FIB-4 score online calculators were used to diagnose fatty liver and risk of advanced liver fibrosis (reported as low, indeterminate, high). VCTE (FibroScan®, EchoSens, Paris, France) is a point-of-care non-invasive test using M probe to diagnose ALF. The M probe is designed for the general adult population and it operates at a central frequency of 3.5 MHz. It measures liver stiffness at a depth from 2.5 to 6.5 cm from the skin with a shear wave frequency of 50Hz. It provides an objective report on fatty liver (S0-S3) and liver fibrosis (F1-F4) expressed as controlled attenuation parameter (CAP) score (in dB/m) and liver stiffness measurement (LSM) score (in kPa), respectively at the same time.

Study procedure: According to the stepwise screening cascade under NP-NCD guidelines[18], Population-based screening was carried out using community-based assessment checklist. The line list of individuals with score > 4 was obtained, and participants were recruited using a simple random sampling. They were interviewed for sociodemographic and clinical history. Height, weight, and waist circumference were recorded following standard protocols. Fasting venous blood samples (6–8 mL) were collected for liver function tests, lipid profile, HbA1c, and platelet count on the same day. After calculating fatty liver index and FIB-4 scores, referral to the tertiary care centre and further management of indeterminate and high-risk individuals for advanced liver fibrosis were carried out as per the operational guidelines for integration of NAFLD into NP-NCD. The participants with indeterminate and high risk of liver fibrosis were

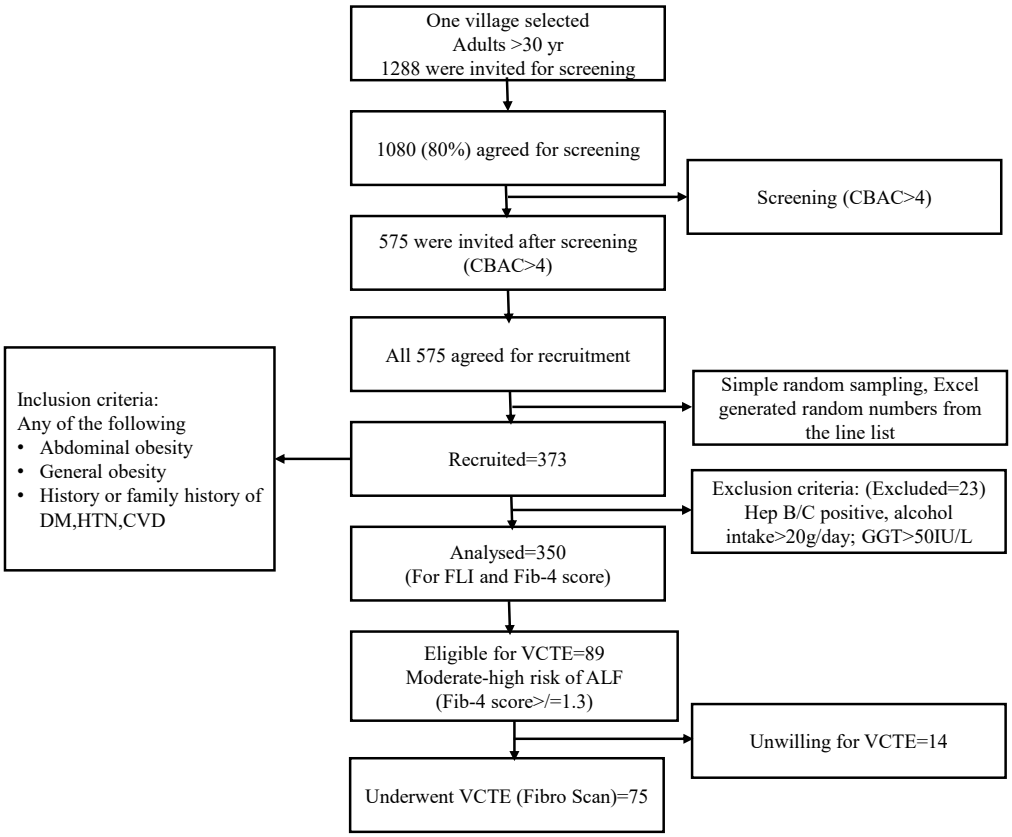

Figure. Schema of participant flow in the screening cascade in the present study. The eligible adults of Ramanathapuram village, more than 30 yr (n=1288), were invited for screening of which 1080 agreed for screening using CBAC (Community based assessment checklist). Following the screening cascade, 575 had CBAC>4. However, using simple random sampling, 373 were recruited. Out of them, 23 were excluded. 350 adults at high risk for NAFLD were considered for analysis. CBAC, community-Based Assessment Checklist; DM, diabetes mellitus; FLI, fatty liver index; GGT, gamma glutamyl transferase; Hep B/C, hepatitis B/C; HTN, hypertension; VCTE, vibration controlled transient elastography.

mobilized to the primary health centre by camp basis on five different days for VCTE (about 5–7 min per participant). For each participant, liver stiffness score was recorded when ten valid measurements were done, and the result was reported as the median of these valid measurements in kilopascals (kPa). VCTE provides information on hepatic steatosis (fat in the liver) and hepatic fibrosis (scarring in the liver) objectively. We used a liver fibrosis scale comprising four grades: F0 (≤ 7 kPa), F1 (7.1–10.0 kPa), F2 (10.1–13.0 kPa), F3 (13.1–16.0 kPa) and F4 (≥ 16.1kPa).

Statistical analysis: Data were entered using Epicollect5 mobile app and analysed using STATA v14 software[19,20]. All the categorical variables were summarized in the form of frequency with percentage. Based on the normality of the distribution of the data, the continuous variables were summarized using mean with standard deviation (SD) or median with interquartile range (IQR). The prevalence of NAFLD (fatty liver index> 60) was calculated, and risk stratification (FIB-4 score) was presented as a proportion with 95 per cent CI. Association between socio demographic details, clinical details with high risk of NAFLD was assessed using Chi-square test or Fisher's exact test. Univariate regression was performed to explore associations of demographic and clinical variables with NAFLD. Variables with *P* value less than 0.2 in univariate analysis and those of biological relevance were included in multivariable logistic regression to calculate adjusted prevalence ratios (aPR) with 95 per cent CI. $P<0.05$ was considered statistically significant.

Results

Participant recruitment and study flow details are depicted in figure. The mean (SD) age of the participants was 49 (12) yr and majority (60.3%) of

Table. Association between clinical and biochemical factors with high risk of NAFLD among the study participants

Variable	Total (N=350)	NAFLDs present, (n=80) [£] n, (%)	Univariate analysis		Multivariable analysis	
			PR[$] (95% CI)	*P* value	aPR[$] (95% CI)	*P* value
Sex						
Male	139	49 (35.2)	2.3 (1.6 - 3.5)	<0.01	1.9 (1.3-2.9)	<0.01*
Female	211	31 (14.6)	Ref		Ref	
Physical activity						
Yes	30	11 (36.6)	Ref	0.04	-	-
No	320	69 (21.5)	0.5 (0.3-0.9)		-	-
Diabetes mellitus						
Yes	108	36 (33.3)	1.8(1.3 -2.7)	<0.01	1.6 (1.1-2.3)	<0.01*
No	242	46 (19)	Ref		Ref	
HDL cholesterol						
HDL ≤40 mg/dL	117	34 (29)	1.5 (1.0-2.2)	0.04	-	-
HDL >40 mg/dL	233	46 (19.7)	Ref		-	-
Elevated LDL Cholesterol (>150mg/dL)						
Yes	76	18 (23.6)	1.0 (0.7-1.7)	0.85	-	-
No	274	62 (22.6)	Ref		-	-
Elevated total cholesterol (>200 mg/dL)						
Yes	140	34 (24.2)	1.1 (0.8-1.6)	0.60	-	-
No	210	46 (21.9)	Ref		-	-
Elevated ALT (>40 IU/L)						
Yes	32	17 (53.1)	2.7 (1.8-3.9)	<0.01	1.8 (1.1-3.0)	<0.01*
No	318	63 (19.8)	Ref		Ref	

[£] Chi-square test, *P**<0.05 is statistically significant. $Prevalence ratio (PR); adjusted prevalence ratio (aPR) after adjusting for confounders – using logistic regression analysis

Only those variables with *P* value <0.2 was considered significant among univariate model and carried on for multivariable analysis. Regression model with least BIC (Bayesian information criterion) was considered robust model. We have used step wise selection model for including variables in regression model. HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine transaminase

them were women. The mean (SD) BMI was 25.1 (4.9) kg/m^2 and one in two (49.1%) were found to be obese. Majority (91.4%) were physically inactive. The mean (SD) HbA1C per cent was found to be 6.5 (1.7) per cent. About one-third (30.8%) of the participants were found to have T2DM. The median (IQR) ALT was 18 (13.5-25) and 9.1 per cent (n=32) had elevated ALT (>40 IU/L). The median (IQR) GGT was 21 (16-31) IU/L and 8 per cent (n=28) had elevated GGT (>50 IU/L). Out of 350 adults at high risk for NAFLD, 42 per cent of the participants had low, 35.1 per cent had indeterminate and 22.8 per cent had high fatty liver index. The median (IQR) fatty liver index was 38 (19-59). The prevalence of NAFLD among adults at high risk for NAFLD more than 30 years was found to be 22.8 per cent (95% CI: 18.7-27.5%).

The median (IQR) FIB-4 score was 0.89 (0.63-1.31). On risk stratification, 23 per cent (n=80) had indeterminate FIB-4 score (1.3-2.6), 2 per cent (n=9) had high FIB-4 score (>2.6); These 89 participants (indeterminate and high-risk) were eligible for VCTE imaging. Of these, 14 were unable to undergo VCTE. Finally, 75 participants underwent VCTE. The mean CAP value for fatty liver was 246.7 dB/m. Out of 75 participants, 37(49%) had no fatty liver, 11(15%) had Grade-I, 9(12%) had Grade-II, and 18 (24%) had Grade-III fatty liver. The mean LSM value was 9.2 kPa. Majority (n=57, 76%) had no liver fibrosis, and 14 (19%) had mild advanced liver fibrosis, two had cirrhosis and one each had moderate and severe fibrosis. Out of nine participants with high FIB-4 score, only 2 (22.2%) had VCTE-confirmed fibrosis, out of 80 with indeterminate FIB-4 score, 16 (20%) had VCTE-confirmed fibrosis. Multivariate analysis results are shown in the table. The prevalence of NAFLD was 1.9 times higher among men and 1.6 times higher among

people with T2DM. Those with elevated ALT levels (>40 mg/dL) were at 1.8 times more risk of developing NAFLD compared to those with normal ALT levels after adjusting for sex, T2DM, HDL-C and AST levels.

Discussion

In our study setting, the population eligible for screening was 43 per cent (> 30 yr), unlike the recommended 37 per cent as per the population-based screening guidelines; due to geographical variation, a higher proportion of the ageing population. The prevalence (22.8%) is lower compared to a few studies (rural), which reported 30.7 per cent and 28.1 per cent (by ultrasound) in Haryana and Maharashtra, respectively[21,22]. The prevalence of NAFLD among men and women is consistent with the previous studies based on fatty liver index in the general population (18.2%)[23]. In urban areas like Delhi and Patna reported NAFLD was 57.6 per cent and 57.2 per cent respectively, owing to the sedentary lifestyle and dietary habits contributing to NAFLD in urban than in rural areas[24,25]. This high burden warrants urgent multisectoral, health promotion activities in rural and urban areas.

Our results on multivariate analysis are similar to other studies showing higher prevalence of NAFLD in men and those with T2DM[22,26–28]. Though total cholesterol is a known risk factor for NAFLD, our study did not show any significant association with NAFLD[28]. Those with elevated ALT levels (>40 mg/dL) were at 1.8 times more risk of developing NAFLD compared to those with normal ALT levels after adjusting for sex, T2DM, HDL-C and AST levels. Earlier study has found 53 per cent have elevated ALT, considered as surrogate marker for significant NAFLD[29]. NAFLD is associated with metabolic risk factor derangements (T2DM, hypercholesteremia, *etc*). A multidisciplinary approach linking high-risk individuals and primary care physicians with specialists through referral pathways and teleconsultation is the need of the hour.

The study's main strengths include the use of validated non-invasive tools with good sensitivity and specificity and robust study design. Provision of VCTE at the community level for risk stratification prevented unnecessary referral to higher centres. One of the limitations of the study is that the conclusions drawn pertaining to physical activity and tobacco use were self-reported, unlike using a questionnaire, though the status of alcohol consumption was confirmed using GGT. Many studies reported a significant association between BMI, high waist circumference, elevated GGT and hypertriglyceridemia with NAFLD. But those variables result in collinearity due to use of fatty liver index and FIB-4 score; hence, these variables could not be used in univariate and multivariable analysis.

Use of validated non-invasive tools at the primary care level and stepwise screening cascade offers a pragmatic pathway for early NAFLD detection, risk stratification, and referral, with direct relevance for India's population-based NCD control initiatives.

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Conflicts of Interest: None.

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