

Neck Circumference to Height Ratio is a Reliable Predictor of Liver Stiffness and Nonalcoholic Fatty Liver Disease in Prediabetes

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Abstract

Background and Objectives: Nonalcoholic fatty liver disease (NAFLD) and dysglycemia are public health challenges. There is urgent need for anthropometric surrogates for NAFLD screening. This study evaluated role of neck circumference (NC) and neck-height ratio (NHtR) as predictors of liver stiffness measure (LSM) in individuals with prediabetes (IPD). **Methods:** In a cross-sectional study, 188 IPD from 1130 screened individuals underwent anthropometry, ultrasonography, Fibroscan[®] for LSM, dyslipidemia, insulin resistance (IR), and fetuin-A assessment. **Results:** Hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), metabolic syndrome (MetS), NAFLD, and significant liver stiffness (SLS) (LSM >8.5kPa) were observed in 53.7%, 31.4%, 71.3%, 73.9%, 24.5%, and 11.2% prediabetes individuals, respectively. Prediabetes with NAFLD had significantly higher body mass index (BMI), NC, NHtR, glycated hemoglobin, triglycerides, fatty liver index (FLI), and LSM. Prediabetes in highest NHtR quartile had significantly higher BMI, hypertension, MetS, fasting glucose, glycated hemoglobin, homeostatic model assessment-IR, NAFLD, LSM, SLS, and lower HDL-C. Stepwise forward linear regression revealed that NHtR, FLI, and LDL-C were best predictors of LSM, at baseline (Model-1), after adjusting for age and sex (Model-2), and adjusting model-2 plus systolic and diastolic blood pressure (Model-3). NHtR and NC (in females) and NHtR and BMI (in males) had largest area under the curves for predicting LSM, NAFLD, and MetS. NHtR ≥ 21.54 cm/m (sensitivity: 90%; specificity: 52.5%; females) and ≥ 21.62 cm/m (sensitivity: 80%; specificity: 49.4%; males) was best predictor of SLS. **Interpretation and Conclusion:** NHtR is a reliable tool for community screening of NAFLD and liver stiffness in prediabetes.

Keywords: Liver stiffness, metabolic syndrome, neck-height ratio, nonalcoholic fatty liver disease, prediabetes

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an example of ectopic fat deposition, representing the hepatic manifestation of metabolic syndrome (MetS).^[1] The prevalence of NAFLD in India ranges from 9% to 35% and is higher in obesity and type 2 diabetes (T2DM) (>50%).^[2] There is high prevalence of prediabetes (12%–14%) in India, another important manifestation of MetS.^[3,4] The younger onset of T2DM compounded by higher rates of prediabetes progression to T2DM (India, China, Finland, and USA being 14%–18%, 11%, 6%, and 2.5%, respectively) underpins a more aggressive disease explaining this increased burden of diabetes and

NAFLD.^[3-5] Since sonography is not easy to organize and cost-effective at community levels, there is an urgent need for anthropometric surrogates for population NAFLD screening. Individuals with increased measures of such anthropometric markers can then be taken up for confirmatory tests for NAFLD

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like ultrasonography (USG) or liver stiffness measure (LSM) assessment and for institution of appropriate therapeutic and preventive interventions.

Neck circumference (NC) is validated to be simple measure of upper body subcutaneous fat deposition, predictor of MetS, and promising predictor of NAFLD.^[6-8] Role of NC in predicting liver stiffness is not known. Neck-height ratio (NHtR) is considered to be superior to NC as a measure for upper body fat deposition, as it adjusts for difference in NC attributable to height. Hence, the aim of this study was to evaluate the role of NC and NHtR as predictors of liver stiffness, NAFLD, and MetS in Indians with prediabetes by comparing it with traditional anthropometric indices such as body mass index (BMI) and waist-hip ratio (WHR).

METHODS

In this cross-sectional study, consecutive prediabetes individuals 30–80 years age screened from diabetes awareness camps (conducted by the Department of Endocrinology and Metabolism, Institute of Postgraduate Medical Education and Research [IPGMER], Kolkata) with persistent impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) over 2 oral glucose tolerance tests (OGTT), fulfilling all

inclusion/exclusion criteria, and giving informed written consent were included [Figure 1].

Study subject recruitment protocol

Apparently healthy relatives of patients with T2DM, attending diabetes awareness camps in Calcutta, India were screened by finger prick capillary blood glucose estimation (Accu-Chek Active, Roche, India). Individuals with IFG (5.55–6.94 mmol/L) or postprandial blood glucose/random blood glucose 7.77–11.04 mmol/L were called on a separate day for 75 g OGTT. Individuals with IFG or IGT underwent a second OGTT within a week to confirm persistent prediabetes. Individuals with history of any oral antidiabetes medications, insulin use, any medication that can interfere with glucose metabolism (glucocorticoids, antiepileptics, hormone supplements), individuals with associated comorbid states such as chronic kidney disease, liver disease, any chronic illness, and malignancy were excluded. Individuals 30–80 years age, with persistent IFG or IGT over 2 OGTTs, fulfilling all inclusion/exclusion criteria, and giving informed written consent were included in the study.

The study duration was from August 2012 to September 2016. The institutional ethics committee approved the study. Selected prediabetes individuals attended outpatient services

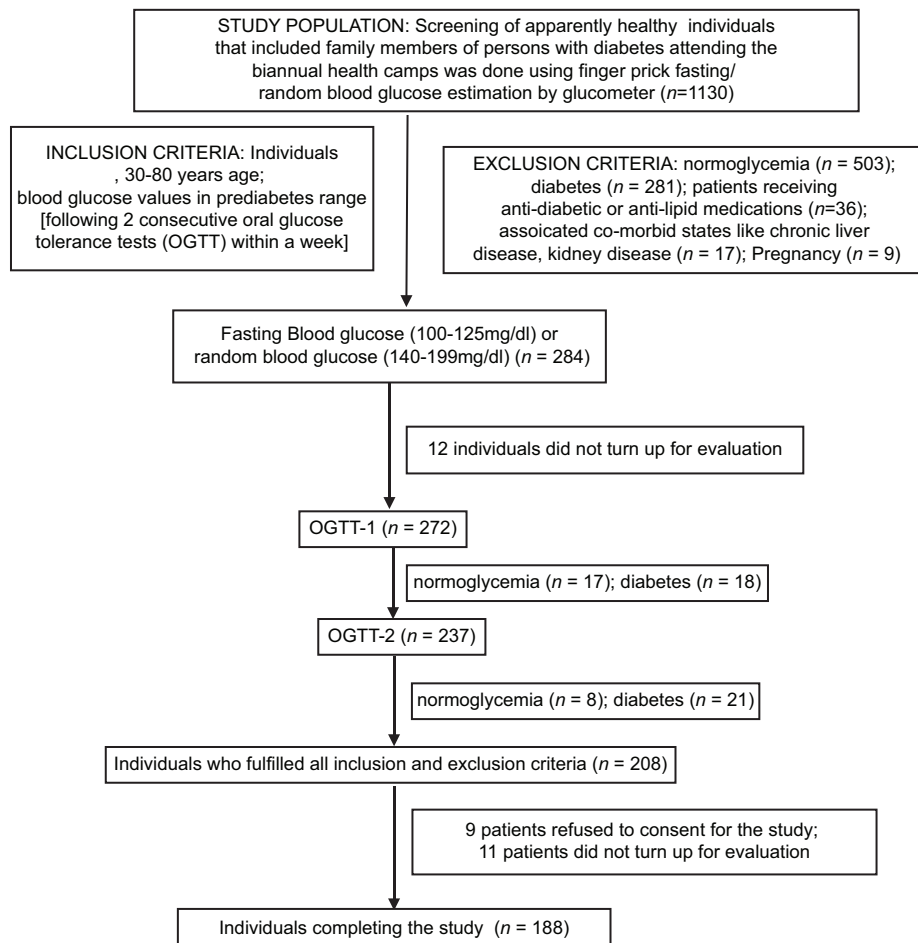


Figure 1: Flowchart elaborating the study protocol

of the Department of Endocrinology and Metabolism IPGMER Kolkata (through prior appointment) and after 12 h fast, they underwent anthropometric, biochemical, NAFLD, and liver stiffness assessment. Blood samples (10 ml) were collected, serum was separated and stored at -80°C for biochemical analysis.

Anthropometric assessment

Height (to ± 0.1 cm) was measured in all the individuals using a Charder HM200PW wall-mounted stadiometer (calibrated using a 36" calibration rod [Perspective Enterprise, Portage, Michigan, USA]), and body weight (to ± 100 g) measured using an electronic calibrated scale (Tanita, Japan, Model-HA521, Lot number-860525). NC was measured using a calibrated plastic tape, with the head positioned along the Frankfurt plane, at mid-neck height, between midcervical spine and midanterior neck, to within 1 mm.^[7] In men with, it was measured just below the Adam's apple.^[7] Patients with Grade 2 or bigger goiter were excluded. A single observer made all measurements in triplicate. The coefficient of variation of the NC measurement ranged from 3% to 6%. Waist circumference (WC) was measured at the end of a gentle expiration midway between the lower rib margin and iliac crest with the patient standing with feet 23–30 cm apart. WHR and NHtR were calculated.

Nonalcoholic fatty liver disease assessment using ultrasonography

NAFLD assessment was done using USG (Toshiba Xario-XG [Model: SSA-680A, Japan]) with 3.5 MHz transducer by an expert blinded to the study protocol. Grading was done comparing liver parenchyma echogenicity with right kidney.^[9,10] Liver echogenicity same as that of the right kidney was considered normal (no fatty liver, Grade-0), minimal diffuse increase in hepatic echogenicity, with normal diaphragm and intrahepatic vessels (mild fatty liver, Grade-1), increased hepatic echogenicity with mild deterioration in the image of diaphragm and intrahepatic vessels (moderate fatty liver, Grade-2), and highly echogenic liver along with difficult to display diaphragm, intrahepatic vessels, and posterior segment of right hepatic lobe was defined as severe fatty liver (Grade-3).

Fatty liver index assessment

Fatty liver index (FLI), an algorithm based on BMI, WC, triglycerides, and gamma-glutamyl-transferase, a validated noninvasive measure of fatty liver, was calculated. FLI score ranged from 0 to 100 with FLI <30 ruling out and ≥ 60 ruling in fatty liver.^[11] FLI has been demonstrated to be a good noninvasive predictor NAFLD.^[12-14]

Liver stiffness measure assessment using Fibroscan®

Transient elastographic examination (Fibroscan®, Echosense, Paris) was done for LSM assessment. A reliable result was defined as at least 10 valid shots, a success rate of at least 60%, and interquartile range $<30\%$ of the median LSM value.^[15-17] Results were considered unreliable if these

criteria were not met. Failure of the procedure was defined as no valid shot after at least 10 attempts.^[15-17] Previous studies have documented healthy range of LSM in our population to be 3.2–8.5 kPa.^[17] LSM >8.5 kPa was defined as a marker of significant liver fibrosis, which has also been used in this study.^[17]

Details of biochemical assays

Serum insulin was estimated using solid phase, enzyme-labeled chemiluminescent immunoassay (Immulite 1000, Siemens, Gwynedd, UK; analytical sensitivity: 2 $\mu\text{IU/ml}$; range: 2–300 $\mu\text{IU/ml}$; coefficient of variation [CV]: 5.9%–8%). Serum fetuin A was estimated using sandwich ELISA (Ray Biotech, Cat#: ELH-IL1fetuinA-001). The intra-assay CV was $<10\%$ and inter-assay CV was $<12\%$. The minimal detectable concentration was <0.2 ng/ml. Serum lipid profile, creatinine, calcium, phosphorus, and alkaline phosphate were estimated in all patients using clinical chemistry analyzer (Daytona, serial number-58260536, Furuno Electric, Nishomeya, Japan).

Statistical analysis

Normality of the distribution of variables was checked using the Kolmogorov–Smirnov test. Continuous normally distributed variables were expressed as mean \pm standard deviation. All nonnormally distributed variables were expressed as median (25th–75th percentile). For categorical data, frequencies and percentages were estimated. $P < 0.05$ was considered as statistically significant. ANOVA with *post hoc* analysis and Kruskal–Wallis nonparametric ANOVA with Dunn's postcorrection were performed for normally and nonnormally distributed variables, respectively. Chi-square tests were used for categorical variables. Pearson's or Spearman's correlation coefficient was calculated for normally and nonnormally distributed variables, respectively. The associations between metabolic risk factors and anthropometric parameters were assessed using partial correlation analysis. The receiver operating characteristic (ROC) curves were plotted, and areas under the curves with 95% confidence interval (CI) were calculated to explore the diagnostic efficacy of anthropometric parameters as diagnostic tests for detecting NAFLD and liver fibrosis and determine optimal sex-specific NC cutoffs in relation to NAFLD. The Youden index, defined as (sensitivity + specificity)–1, was used to determine the optimal cutoff points. SPSS version 20 (Chicago, IL, USA) was used for statistical analysis. The occurrence of NAFLD in quartile-1 ($n = 47$) and quartile-4 ($n = 44$) of NC was 14.89% and 43.18%, respectively. This evaluation achieved a power of 98%, keeping α (Type I error) at 0.05.

RESULTS

Out of 1130 consecutive individuals screened, data from 188 prediabetes individuals (age 46.96 ± 12.67 years; male:female = 119:69; BMI: 26.15 ± 5.23 kg/m²) who completed the study were analyzed [Figure 1 and Table 1]. Hypertension, MetS, NAFLD, and LSM >8.5 kPa were observed in 53.7%, 73.9%, 24.5%, and 11.2% individuals,

Table 1: Clinical, anthropometric, glycemic, lipid, insulin resistance, adipocytokine, and fatty liver disease parameters of individuals with prediabetes as per the quartiles of neck circumference to height ratio

Parameter	Neck circumference to height ratio (cm/m)				P
	Quartile-1 18.64-21.25 (n=47)	Quartile-2 21.25-22.05 (n=48)	Quartile-3 22.05-23.44 (n=49)	Quartile-4 23.44-27.44 (n=44)	
Age (years)	48.6±12.7	45.8±11.7	44.0±14.1	50.2±11.3	0.117
Sex (male:female) [#]	29:18	27:21	39:10	24:20	0.043
WC (cm)	87.6±7.5	90.6±4.9	92.5±7.8	97.4±6.7	<0.001
WHR	0.96±0.07	0.94±0.05	0.96±0.05	0.99±0.06	0.002
BMI (kg/m ²)	23.5±3.7	26.4±7.7	26.2±3.7	28.6±3.2	<0.001
Hypertension	15	28	29	29	0.006
MetS	26	38	34	41	<0.001
FBG (mmol/L) ^a	5.6 (5.3-6.2)	5.3 (5.1-6.1)	5.9 (5.4-6.4)	5.9 (5.4-6.4)	0.036
2hPGBG (mmol/L) ^a	8.4 (6.9-10.7)	9.2 (7.5-10.4)	10.2 (8.7-10.9)	9.5 (7.9-10.8)	0.128
HbA1c (mmol/mol)/% ^a	36 (33-38)/5.4 (5.2-5.7)	37 (33-41)/5.5 (5.2-5.9)	39 (37-43)/5.7 (5.5-6.1)	40 (36-45)/5.8 (5.5-6.3)	0.002
Triglyceride (mmol/L) ^a	1.6 (1.1-2.2)	1.5 (1.2-1.8)	1.5 (1.2-1.7)	1.7 (1.5-2.3)	0.061
LDL-C (mmol/L) ^a	2.6 (2.1-3.2)	2.8 (1.9-3.6)	3.1 (2.5-3.6)	3.1 (2.5-3.9)	0.105
HDL-C (mmol/L) ^a	1.1 (0.9-1.3)	0.9 (0.8-1.1)	0.9 (0.8-1.1)	1.0 (0.9-1.2)	0.001
QUICKI ^a	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.150
HOMA2-IR ^a	1.0 (0.5-1.3)	1.2 (0.9-1.3)	1.1 (0.7-1.5)	1.2 (0.9-2.8)	0.028
HOMA2β ^a	36.8 (24.1-55.0)	46.8 (34.6-74.8)	35.8 (25.5-49.7)	50.7 (29.8-78.1)	0.034
Fetuin-A (μg/ml) ^a	392.9 (339.2-438.0)	363.0 (197.8-467.4)	394.5 (294.6-548.2)	519.3 (289.4-684.3)	0.004
FLI	33.4 (20.2-58.5)	44.5 (29.3-57.5)	47.4 (37.1-65.9)	69.64 (62.18-76.93)	<0.001
USG NAFLD grades					
Absent	40	37	40	25	0.008
Grade-1	6	9	9	14	0.141
Grade-2	1	2	0	5	-
ALT (μkat/L) ^a	0.4 (0.3-0.6)	0.48 (0.3-0.7)	0.5 (0.3-0.7)	0.57 (0.4-1.1)	0.327
LSM (kPa) ^a	4.2 (3.4-5.6)	4.7 (3.3-6.4)	4.9 (4.6-5.6)	5.4 (4.0-9.9)	0.003
LSM >8.5 kPa ^b	2	5	2	12	0.001

All continuous variables expressed as mean±SD. ^aAll nonnormally distributed variable expressed as median (25th-75th percentile); ^bP value calculated using one-way ANOVA; ^aNot normally distributed, Kruskal-Wallis one-way ANOVA used for analysis; ^cP<0.05 considered statistically significant; [#]P-value calculated using Chi-square test, ^bLSM >8.5kPa is a marker of significant hepatic fibrosis. BMI: Body mass index, MetS: Metabolic syndrome, WC: Waist circumference, WHR: Waist-hip ratio, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, FBG: Fasting blood glucose, 2hPGBG: 2 h post 75-g anhydrous glucose blood glucose, MetS: Metabolic syndrome, HOMAβ: Homeostasis model assessment estimated beta cell function, HOMA-IR: Homeostasis model assessment of insulin resistance, QUICKI: Quantitative insulin sensitivity check index, FLI: Fatty liver index, USG: Ultrasonography, NAFLD: Nonalcoholic fatty liver disease, LSM: Liver stiffness measure (using Fibroscan®), SD: Standard deviation, ALT: Alanine transaminase

respectively. Triglycerides >1.69 mmol/L and high-density lipoprotein cholesterol (HDL-C) <1.04 mmol/L (males) and <1.29 mol/L (females) was observed in 31.4% and 71.3% individuals, respectively. Prediabetes individuals with NAFLD (n = 46) had significantly higher BMI, NC, NHtR, and WC compared to those without NAFLD (n = 142) [Table 2]. FLI, LSM, HbA1c triglycerides, and LDL-C were significantly higher, and HDL-C lower in individuals with prediabetes (IPD) with NAFLD [Table 2].

Of the 46 prediabetes individuals with NAFLD, 21 had significant liver stiffness (SLS) on fibroscan. Prediabetes individuals with NAFLD and SLS had higher serum triglycerides (1.83 [1.41–3.21] mmol/L vs. 1.60 [1.27–2.05] mmol/L; P=0.041), LDL-C (4.27 [3.26–6.89] mmol/L vs. 2.62 [1.92–3.26] mmol/L; P<0.001), fetuin-A (467.4 [301.43–721.39] μg/ml vs. 440.20 [298.72–517.18] μg/ml; P = 0.06), FLI (66.19 [50.55–83.32] vs. 58.49 [39.72–72.38]; P = 0.038), and ALT

(1.05 [0.70–1.77] μkat/L vs. 0.37 [0.30–0.67] μkat/L; P=0.004) as compared to those with NAFLD but normal liver stiffness.

The median (25th–75th percentile) for NC was 36.5 cm (36–38) in males and 34 cm (32–36.75) in females (P < 0.001); the corresponding NHtR was 22.09 (21.32–22.96) and 21.70 (21.03–23.62) cm/m, respectively (P = 0.272). Median WC to hip ratio (WHR) was 0.97 in males (0.93–1.00) and 0.95 in females (0.89–0.98) (P = 0.138). Prediabetes individuals in the highest NHtR quartile had significantly higher BMI, WC, WHR, hypertension, and MetS [Table 1]. They also had significantly higher FBG, HbA1c, homeostatic model assessment-insulin resistance (IR), and significantly lower HDL-C. Occurrence of NAFLD, FLI, LSM, and LSM >8.5 kPa were also significantly higher in prediabetes individuals in the highest quartile of NHtR [Table 1].

In males, all 4 anthropometric measurements had significant positive correlation with IR, fetuin-A, and FLI [Table 3].

Table 2: Clinical, anthropometric, glycemic, lipid, insulin resistance, adipocytokine, and fatty liver disease parameters of individuals with prediabetes having nonalcoholic fatty liver disease as compared to those without nonalcoholic fatty liver disease

Parameter	Prediabetes with NAFLD (n=46)	Prediabetes without NAFLD (n=142)	P
Age (years)	49.6±12.2	46.2±12.7	0.145
Sex (male:female) [#]	26:20	93:49	0.272
NC (cm) ^a	36.5 (35.2-38.0)	36.0 (34.0-37.1)	0.041
NHtR (cm/m) ^a	22.7 (21.4-23.9)	21.8 (21.2-22.8)	0.002
WC (cm)	93.8±7.6	91.3±7.6	0.047
WHR	0.97±0.07	0.96±0.06	0.440
BMI (kg/m ²)	27.5±6.2	25.7±4.8	0.046
Hypertension (%)	26 (56.5)	75 (52.8)	0.661
MetS (%)	40 (86.9)	99 (69.7)	0.020
FBG (mmol/L) ^a	5.7 (5.2-6.1)	5.8 (5.2-6.2)	0.369
2hPGBG (mmol/L) ^a	10.1 (7.9-10.8)	9.2 (7.5-10.8)	0.878
HbA1c (mmol/mol)/%	41 (34-44)/5.9 (5.3-6.2)	38 (34-40)/5.6 (5.3-5.8)	0.018
Triglyceride (mmol/L) ^a	1.8 (1.3-2.4)	1.5 (1.2-1.7)	0.003
LDL-C (mmol/L) ^a	3.2 (2.4-5.2)	2.8 (2.3-3.4)	<0.001
HDL-C (mmol/L) ^a	1.00 (0.9-1.2)	0.9 (0.8-1.2)	0.638
QUICKI ^a	0.37 (0.35-0.41)	0.38 (0.36-0.39)	0.670
HOMA-IR ^a	1.1 (0.7-1.6)	1.1 (0.8-1.4)	0.858
HOMAβ ^a	49.8 (24.6-67.6)	39.9 (27.9-61.1)	0.582
Fetuin-A (μg/ml) ^a	460.5 (301.4-589.5)	396.7 (278.7-519.3)	0.297
FLI ^a	61.6 (45.5-76.7)	44.8 (28.2-64.1)	0.001
ALT (μkat/L) ^a	0.6 (0.3-1.1)	0.5 (0.3-0.6)	0.003
LSM (kPa) ^a	7.9 (6.4-11.4)	4.3 (3.3-5.0)	<0.001
LSM >8.5 kPa ^b	21	0	-

All continuous variables expressed as mean±SD. ^aAll nonnormally distributed variable expressed as median (25th-75th percentile); *P* value calculated using one-way ANOVA; [#]Not normally distributed, Kruskal-Wallis one-way ANOVA used for analysis; *P*<0.05 considered statistically significant; [#]*P*-value calculated using Chi-square test, ^bLSM >8.5 kPa is a marker of significant hepatic fibrosis. BMI: Body mass index, MetS: Metabolic syndrome, WC: Waist circumference, WHR: Waist-hip ratio, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, FBG: Fasting blood glucose, 2hPGBG: 2 h post 75-g anhydrous glucose blood glucose, MetS: Metabolic syndrome, HOMAβ: Homeostasis model- assessment estimated beta cell function, HOMA-IR: Homeostasis model assessment of insulin resistance, QUICKI: Quantitative insulin sensitivity check index, FLI: Fatty liver index, USG: Ultrasonography, NAFLD: Nonalcoholic fatty liver disease, LSM: Liver stiffness measure (using Fibroscan[®]), SD: Standard deviation, ALT: Alanine transaminase, NC: Neck circumference

In females, anthropometric measurements had significant positive correlations with IR, triglycerides, HbA1c, fetuin-A, FLI, and LSM [Table 3]. Fetuin-A had significant positive correlation with triglycerides (σ : 0.192; *P*: 0.015), FLI (σ : 0.390; *P* < 0.001), and LSM (σ : 0.190; *P*: 0.017).

Stepwise forward linear regression analysis revealed that NHtR, FLI, and LDL-C were the most consistent predictors of LSM, without adjustment for any variables (Model-1), after adjusting

for age and sex (Model-2) and after adjusting for variables in model-2 plus systolic and diastolic blood (Model-3) [Table 4]. Serum fetuin-A was significant predictor of LSM after adjustment for age and sex only (Model-2) [Table 4]. Serum triglycerides were significant predictors of LSM without adjusting for any variables (Model-1). Serum HDL-C was a predictor of LSM after adjusting for variables in Model-2 and Model-3.

Logistic regression analysis, using presence of NAFLD as dependent variable, showed that relationship between NHtR and NAFLD after adjusting for sex was statistically significant (odds ratio 1.353 [95% CI 1.108–1.651]; *P* = 0.003). A similar logistic regression showed that the relationship between NC and NAFLD was statistically significant (odds ratio 1.127 [95% CI 1.003–1.266]; *P* = 0.044). Logistic regression, using presence of SLS (LSM >8.5 kPa) as the dependent variable, showed that the relationship between NHtR and liver stiffness after adjusting for sex was statistically significant (odds ratio 1.421 [95% CI 1.111–1.817]; *P* = 0.005). A similar logistic regression showed relation between NC and liver stiffness approached statistical significance (odds ratio 1.158 [95% CI 0.997–1.345]; *P* = 0.056).

The areas under the ROC curves area under the curves (AUCs) were constructed to evaluate the predictive values of anthropometric indices for NAFLD, liver fibrosis, and MetS [Table 5]. In females, NHtR (0.662; *P* = 0.036) and NC (0.667; *P* = 0.030) were the best predictors of NAFLD. NHtR (AUC: 0.816; *P* = 0.001) and NC (AUC: 0.744; *P* = 0.014) were best predictors of liver fibrosis in females. NHtR (AUC: 0.837; *P* = 0.007) followed by BMI (AUC: 0.745; *P* = 0.049) was best predictor of MetS in females [Table 5]. In males, NHtR (AUC: 0.626; *P* = 0.048) followed by BMI (AUC: 0.616; *P* = 0.050) was best predictor of NAFLD. BMI (AUC: 0.695; *P* = 0.025) and NHtR (AUC: 0.619; *P* = 0.061) were the best predictors of liver fibrosis in males. All 4 anthropometric parameters were significant predictors of MetS; AUC was highest for BMI (AUC: 0.811; *P* < 0.001) and NHtR (AUC: 0.796; *P* < 0.001).

In females, NHtR \geq 21.54 cm/m had a sensitivity of 90% and specificity of 52.5% in identifying prediabetes females with SLS. Nine out of the 10 females with SLS in this study had NHtR \geq 21.54 cm/m. In males, NHtR \geq 21.62 cm/m had sensitivity and specificity of 80% and 49.4% in identifying males with SLS. Nine out of 11 males with SLS in this study had NHtR of 21.62 cm/m.

A NC \geq 35.25 cm had a sensitivity of 80% and specificity of 62.7% in identifying females having SLS. NC \geq 36.25 cm had a sensitivity and specificity of 72.7% and 40.0%, respectively, in identifying SLS in males. NHtR \geq 21.54 cm/m had a sensitivity and specificity of 74.6% and 66.7% in identifying females with MetS. NHtR of \geq 21.62 cm/m had sensitivity and specificity of 80.3% and 69.8%, respectively, in identifying males with MetS. NC \geq 36.25 cm had sensitivity and specificity of 73.7% and 48.8% in identifying males with MetS.

Table 3: Correlation between anthropometric indices and lipid, glycemic insulin resistance, adipocytokine, and fatty liver disease parameters of individuals with prediabetes after adjusting for age

Parameter	NC ^a	NHtR ^a	WHR	BMI
Males (n=119)				
Triglycerides ^a	0.216 (0.061)	0.212 (0.065)	0.215 (0.062)	0.319 (0.005)
HDL-C	0.088 (0.450)	0.123 (0.212)	-0.031 (0.790)	-0.216 (0.060)
HbA1c% ^a	0.183 (0.113)	0.187 (0.105)	0.379 (0.001)	0.332 (0.003)
HOMA-IR ^a	0.345 (0.002)	0.230 (0.045)	0.273 (0.017)	0.471 (<0.001)
HOMA β ^a	0.228 (0.048)	0.210 (0.069)	0.230 (0.046)	0.298 (0.007)
QUICKI ^a	-0.365 (0.001)	-0.277 (0.016)	-0.248 (0.030)	-0.474 (<0.001)
Fetuin-A ^a	0.251 (0.024)	0.279 (0.012)	0.252 (0.023)	0.333 (0.002)
FLI ^a	0.631 (<0.001)	0.620 (<0.001)	0.617 (<0.001)	0.678 (<0.001)
LSM ^a	0.089 (0.445)	0.097 (0.406)	0.032 (0.781)	0.191 (0.098)
Females (n=69)				
Triglycerides ^a	0.414 (0.011)	0.385 (0.019)	0.425 (0.009)	0.021 (0.901)
HDL-C	-0.440 (0.006)	-0.487 (<0.001)	-0.478 (<0.001)	-0.424 (0.009)
HbA1c% ^a	0.387 (0.018)	0.328 (0.047)	0.101 (0.553)	0.008 (0.964)
HOMA-IR ^a	0.472 (0.003)	0.340 (0.039)	0.374 (0.016)	0.191 (0.258)
HOMA β ^a	0.034 (0.843)	-0.049 (0.772)	-0.005 (0.976)	0.006 (0.970)
QUICKI ^a	-0.240 (0.152)	-0.157 (0.354)	-0.308 (0.064)	-0.154 (0.364)
Fetuin-A ^a	0.099 (0.527)	0.100 (0.525)	0.205 (0.186)	0.329 (0.031)
FLI ^a	0.514 (<0.001)	0.532 (<0.001)	0.269 (0.108)	0.681 (<0.001)
LSM ^a	0.141 (0.406)	0.174 (0.304)	0.156 (0.357)	0.13 (0.432)

^aNot normally distributed; Spearman's correlation coefficient calculated; all values expressed as correlation coefficient (*P*-value); *P* <0.05 considered statistically significant. NC: Neck circumference, NHtR: Neck height ratio, WHR: Waist-hip ratio, BMI: Body mass index, HDL-C: High-density lipoprotein cholesterol, HbA1c: Glycated hemoglobin, HOMA-IR: Homeostatic model of assessment of insulin resistance, HOMA β : Homeostatic model of assessment of estimated beta cell function, QUICKI: Quantitative insulin sensitivity check index, FLI: Fatty liver index, LSM: Liver stiffness measure as measured using Fibroscan[®]

DISCUSSION

A quarter of prediabetes individuals in this study had USG evidence of NAFLD. In a study of 118 patients with NAFLD from Texas, prediabetes was observed in 85%.^[18] Occurrence of prediabetes in NAFLD was associated with more severe IR.^[18] We have previously documented increased prediabetes progression to diabetes in prediabetes individuals who had

higher FLI and NAFLD.^[3] Improvement in NAFLD was associated with decreased T2DM incidence over 12 years.^[19] In a Israeli study, NAFLD was a strong and independent risk factor for prediabetes over 7 years.^[20] Nearly half of prediabetes individuals with NAFLD (45.62%; 21/46) had SLS. In a Turkish study, prediabetes occurrence in NAFLD was associated with more severe portal inflammation and liver fibrosis.^[21] Hence, prediabetes and NAFLD are closely linked, and the exponential increase in the burden of prediabetes and diabetes in India would further accentuate the problem of NAFLD in the very near future.^[22,23]

We showed that prediabetes individuals in highest NHtR quartile had significantly worse glycemia, dyslipidemia, IR, elevated fetuin-A, and more severe NAFLD (FLI and liver stiffness). Two previous studies from China have observed NC to be a predictor of NAFLD.^[7,24] Huang *et al.* in a cross-sectional study of 4053 individuals from China demonstrated NC to be an independent predictor of NAFLD.^[7] In another population-based cross-sectional study of 2668 individuals from China, NC was an independent predictor of NAFLD in nonobese men but not women.^[24]

Fetuin-A is a hepatokine. Biologic roles attributed to fetuin-A has expanded exponentially in the last 2 decades. Fetuin-A has been linked to systemic inflammation, adverse glycemic outcomes in prediabetes, NAFLD, advanced hepatic fibrosis, microalbuminuria, carotid intima media thickness, arterial stiffness, coronary, and peripheral artery disease.^[6,25,26] Fetuin-A was evaluated in this study to explore its role as a potential future serologic marker of adverse clinical outcomes in prediabetes. Our study also demonstrated fetuin-A to be a predictor of liver stiffness in prediabetes. In a cohort of 967 IPD, FLI >60 was associated with increased progression to T2DM.^[7] In our study, fetuin-A had a positive correlation with FLI, in accordance with a Chinese study.^[27] Both FLI and NAFLD assessment by USG have been documented to be independent predictors of diabetes in the general population.^[13,14] FLI has been demonstrated to good surrogate marker of NAFLD and longterm predictor of diabetes, coronary artery disease, and increased all-cause mortality in IPD.^[28]

NHtR was consistently the best predictor of liver stiffness. Overall, NHtR had a better odds ratio than NC for predicting liver fibrosis. NHtR performed well with regard to WHR for predicting NAFLD, liver fibrosis, and MetS (higher AUCs). We identified NHtR ≥ 21.54 cm/m in females and ≥ 21.62 cm/m in males to be the best predictors of liver stiffness.

Optimal NC cutoff for detection of MetS was ≥ 32.5 cm and ≥ 36.25 cm in females and males, respectively, which was lower than observed in Turkey (35 cm and 39 cm in females and males)^[29] but similar to that observed in Chinese (33 cm and 37 cm in females and males).^[24] Different ethnicity and body composition of different populations may explain this difference. Limitations of this study include the small number of prediabetes individuals evaluated and the number of prediabetes individuals who had SLS. Furthermore,

Table 4: Linear regression analysis showing parameters that are independent predictors of liver stiffness measure in individuals with prediabetes

Variable	Model-1 ^a		Model-2 ^b		Model-3 ^c	
	Standardized coefficient (β)	P	Standardized coefficient (β)	P	Standardized coefficient (β)	P
NHtR	0.236	0.048	0.295	0.022	0.29260	0.024
QUICKI	0.037	0.606	0.051	0.503	0.052	0.497
Fetuin-A	0.161	0.101	0.182	0.047	0.175	0.065
FLI	0.595	0.001	0.656	<0.001	0.678	0.031
HbA1c	0.102	0.320	0.170	0.118	0.159	0.165
Triglycerides	0.466	0.029	0.436	0.052	0.430	0.057
VLDL-C	0.221	0.220	0.204	0.265	0.191	0.309
LDL-C	0.393	<0.001	0.382	<0.001	0.375	<0.001
HDL-C	0.194	0.052	0.226	0.030	0.226	0.031

Linear regression was initially performed with all parameters which are likely to influence LSM (age, BMI, WHR, NHtR, HbA1c, HOMA-IR, QUICKI, triglycerides, VLDL-C, HDL-C, LDL-C, fetuin-A and FLI). ^{a,b,c}Parameters with $P < 0.2$ were included into the step-wise forward linear regression analysis without adjustment for any variables (Model-1), after adjustment for age and sex (Model-2), and after adjustment for variables in Model-2 plus systolic and diastolic blood pressure (Model-3). Standardized coefficient (β): Change in odds ratio with 1 unit change in predictor variable. LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, NHtR: Neck height ratio, FLI: Fatty liver index, BMI: Body mass index, WHR: Waist-hip ratio, HOMA-IR: Homeostatic model of insulin resistance, QUICKI: Quantitative insulin sensitivity check index, LSM: Liver stiffness measure as measured using Fibroscan[®], HbA1c: Glycated hemoglobin

Table 5: Area under the receiver operating characteristic curve by different anthropometric indices as predictor of nonalcoholic fatty liver disease, liver fibrosis, and metabolic syndrome (n=188)

	NC		NHtR		WHR		BMI	
	AUC (95% CI)	P	AUC (95% CI)	P	AUC (95% CI)	P	AUC (95% CI)	P
Males (n=119)								
NAFLD (USG)	0.551 (0.424-0.678)	0.429	0.626 (0.494-0.738)	0.048	0.578 (0.455-0.702)	0.223	0.616 (0.506-0.746)	0.050
Liver fibrosis ^a	0.540 (0.366-0.652)	0.510	0.619 (0.401-0.720)	0.061	0.536 (0.391-0.680)	0.597	0.695 (0.565-0.845)	0.025
MetS	0.721 (0.628-0.815)	<0.001	0.796 (0.678-0.854)	<0.001	0.765 (0.673-0.856)	<0.001	0.811 (0.804-0.937)	<0.001
Females (n=69)								
NAFLD (USG)	0.662 (0.505-0.818)	0.036	0.667 (0.514-0.823)	0.030	0.545 (0.385-0.703)	0.556	0.552 (0.379-0.716)	0.500
Liver fibrosis	0.744 (0.563-0.925)	0.014	0.816 (0.673-0.959)	0.001	0.499 (0.291-0.708)	0.993	-0.583 (0.382-0.785)	0.404
MetS	0.610 (0.378-0.842)	0.377	0.837 (0.721-0.954)	0.007	0.525 (0.236-0.814)	0.840	0.745 (0.577-0.913)	0.049

^aLiver fibrosis: Significant liver fibrosis defined as liver stiffness measure (LSM; as measured using Fibroscan[®]) >8.5 kPa. NAFLD: Nonalcoholic fatty liver disease, MetS: Metabolic syndrome, NC: Neck circumference, NHtR: Neck height ratio, WHR: Waist-hip ratio, BMI: Body mass index, USG: Ultrasonography, ROC: Receiver operating characteristics, AUC: Areas under the ROC curves, CI: Confidence interval

ethnic people from Bengal only formed a part of this study. Hence, the results from this study need to be replicated in larger studies before its use can be recommended in routine clinical practice. Advantages of NHtR include its ease of measurement in the community, as compared to WHR, which some patients may not be comfortable with. Hepatic fat detection by USG has a threshold of 30% in contrast to only 5% for magnetic resonance spectroscopy^[10] and hence is a limitation of USG when used for NAFLD screening. Liver biopsy, though the gold standard for diagnosis for NAFLD, is limited by sampling error and is not a suitable option for NAFLD diagnosis in a large cohort of individuals as in our study.

CONCLUSION

To summarize, our study demonstrated that NHtR can be a good screening tool (good sensitivity [80%–90%] with a

relatively poor specificity [around 50%]) for detection of liver stiffness in prediabetes. The high sensitivity ensures that we will not miss cases in the community during screening. Patients thus detected can undergo further confirmatory tests to confirm the diagnosis of NAFLD. Hence, there is an urgent need for a large multicentric study involving different parts of India to ensure the replicability of this result and adjust for different ethnic variations in India, before this can be used in routine clinical practice.

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Conflicts of interest

There are no conflicts of interest.

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