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Stepwise evaluation for the risk of metabolic unhealthiness and significant non-alcoholic fatty liver disease in India

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Summary

Background Non-communicable diseases including metabolic health disorders are becoming area of concern for low/middle income countries with poor health-care resources. Present study was planned to assess the prevalence of metabolically unhealthy (MU) subjects in the community and proportion of the MU subjects having the risk of significant Non-alcoholic Fatty Liver Disease (NAFLD) using a step-wise evaluation strategy in a resource-poor setting.

Methods Study was performed in 19 community development blocks of Birbhum district, West Bengal, India. Every fifth member in the electoral list was included for the first step evaluation (n = 79,957/1,019,365, 7.8%) to detect any metabolic risk. Subjects with any metabolic risk in the first step (n = 9819/41,095, 24%) were taken for second step evaluation with Fasting blood glucose (FBG) and ALT. Subjects with elevated FBG and/or ALT in the second step (n = 1403/5283, 27%) were taken into third step evaluation.

Finding At least one risk factor was found in 51.4% (n = 41,095/79,957). 63% (n = 885/1403) of the subjects with metabolic abnormality (third step) had MU state making its overall prevalence of 1.1% (n = 885/79,957). 53% of MU subjects (n = 470/885) had 'persistently elevated ALT' suggesting the risk of having significant NAFLD.

Interpretation Step-wise evaluation strategy could detect the subjects at risk, actually having MU state and proportion of MU subjects at risk of having 'persistently elevated ALT' (surrogate of significant NAFLD) in the community with minimum utilization of scarce resources.

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Keywords: Community study; Fatty liver; Health resource; Health planning; Lean

Introduction

Transition of global disease burden from communicable diseases to non-communicable diseases (NCD) even in low and middle income countries (LMIC) has been already noted.[1] Much of the burden is attributed to disability, not to premature deaths.[1] Heterogeneity and changing pattern of demographic and epidemiological characteristics across the globe has been proposed to govern the differential changes in the burden. Specifically, population aging coupled with changing epidemiology has been proposed to play pivotal role making the NCDs in the LMICs comparable to high income countries in near future.[2] LMICs are expected to have more socio-economic impact of

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Research in context

Evidence before this study

Metabolic unhealthiness is a growing concern even for low and middle income countries. Effective screening protocol applicable to the resource poor setting of these countries is still not in vogue. We explored PubMed on 3rd August, 2022 for original research articles published in English language on the Metabolic unhealthiness in the community and its screening strategy using the term 'Metabolic unhealthiness or Metabolic Health' AND ("non-alcoholic fatty liver disease" OR "Community screening" OR "Community prevalence" OR "step-wise evaluation" OR "Community study") irrespective of date of publication. We excluded the studies not reporting evaluation strategy in the community. 'High risk' approach using different risk scores has been reported to be effective in screening for Diabetes in the community and less privileged section of the society. Also, lifestyle interventional strategy based on this 'high risk' approach was reported to be cost-effective. We could not find any study reporting minimum but rational use of simple anthropometric and metabolic variables in stepwise manner in a resource poor community setting.

Added value of this study

This study focused on overall metabolic health rather than Diabetes only and utilized simple and easily applicable markers in the place of any questionnaire or numerical risk score, avoiding inherent bias and minimizing the consumption of health resources and infrastructure. Additionally, this study considered the community screening for risk of significant non-alcoholic fatty liver disease (NAFLD), a metabolic ill-health related disease, which is emerging as a significant contributor to the liver disease burden of the countries like India. Given the urgent need for community screening policy for non-communicable disease, metabolic disorder in particular, in India and other resource-poor low-income countries, data from this study could help to plan cost-effective community programme.

Implications of all the available evidences

Altogether, these data on efficacy and feasibility of community screening for overall metabolic unhealthiness and related risk of significant non-alcoholic fatty liver disease (NAFLD) and their prevalences in community will help to develop and customize the community programme according to the resources available.

this shift as the people of relatively younger age groups, in the productive years of their lives, are anticipated to bear the burden, in contrast to older age groups in high income countries.[2] Disorders linked to metabolic health, like Diabetes, obesity, cardiovascular disease (CVD), hypertension and non-alcoholic fatty liver disease (NAFLD) are major contributors to the basket of NCD. People with normal body mass index (BMI) still predominates the population of LMICs and in south-east Asia in particular.[3] Metabolic unhealthiness and its consequences do not spare the non-obese subjects.[4] We previously reported the prevalence of Metabolic syndrome (MS) in the range of 20.3% and 10.7%, in females and males, respectively, in a rural Indian population.[5] Rural Indian population was also found to have cardio-metabolic risk and subclinical CVD at a lower BMI compared to multiethnic Americans.[6] Thus, metabolic unhealthiness in non-obese subjects along with rising trend of obesity put the metabolic health related disorders in the priority list of NCD in these countries.[3] Situation is made worse by the fact that these LMICs are least prepared to tackle this emerging disease burden.[2] In addition to this, 81% of the Asian population relies on out-of-pocket (OOP) payment for healthcare and about 70% of the OOP expenditure is for medicines in countries like Bangladesh, India and Vietnam.[7] Expected socio-economic impact of this scenario demands focused approach to NCDs and Metabolic Health disorders, particularly, in LMICs.

Present study, conducted in a rural community of the state of West Bengal in India, was designed to scale the magnitude of the metabolic unhealthiness utilizing minimum healthcare facilities in a resource-poor setting. In compliance with this purpose, objective of the present study was to assess the prevalence of metabolically unhealthy (MU) subjects in the community and proportion of the MU subjects having persistently elevated liver enzyme (ALT) as a surrogate of the 'risk of significant NAFLD' using a step-wise evaluation strategy.

Methods

Study design and setting

Stepwise evaluation strategy (Fig. 1) was adopted to maximize the utilization of limited resources available. Green, yellow and red colored cards were given to the subjects after each step of evaluation for visual appreciation of their metabolic risk status. Selected subjects (n = 79,957) were screened for the presence of metabolic risks i.e., overweight/obesity (BMI $\geq$ 23 kg/m^2), Hypertension and Random blood glucose (RBG) >200 mg/dL. Subjects without any risk factor were provided with a 'Green card' signifying 'no risk'. Subjects with at least one risk factor were provided with 'Yellow card' signifying 'potentially at risk' and were taken for second-step evaluation to detect dysglycemia in the form of impaired fasting glucose (IFG/Prediabetes) or Diabetes by measuring Fasting blood glucose (FBG) and to detect

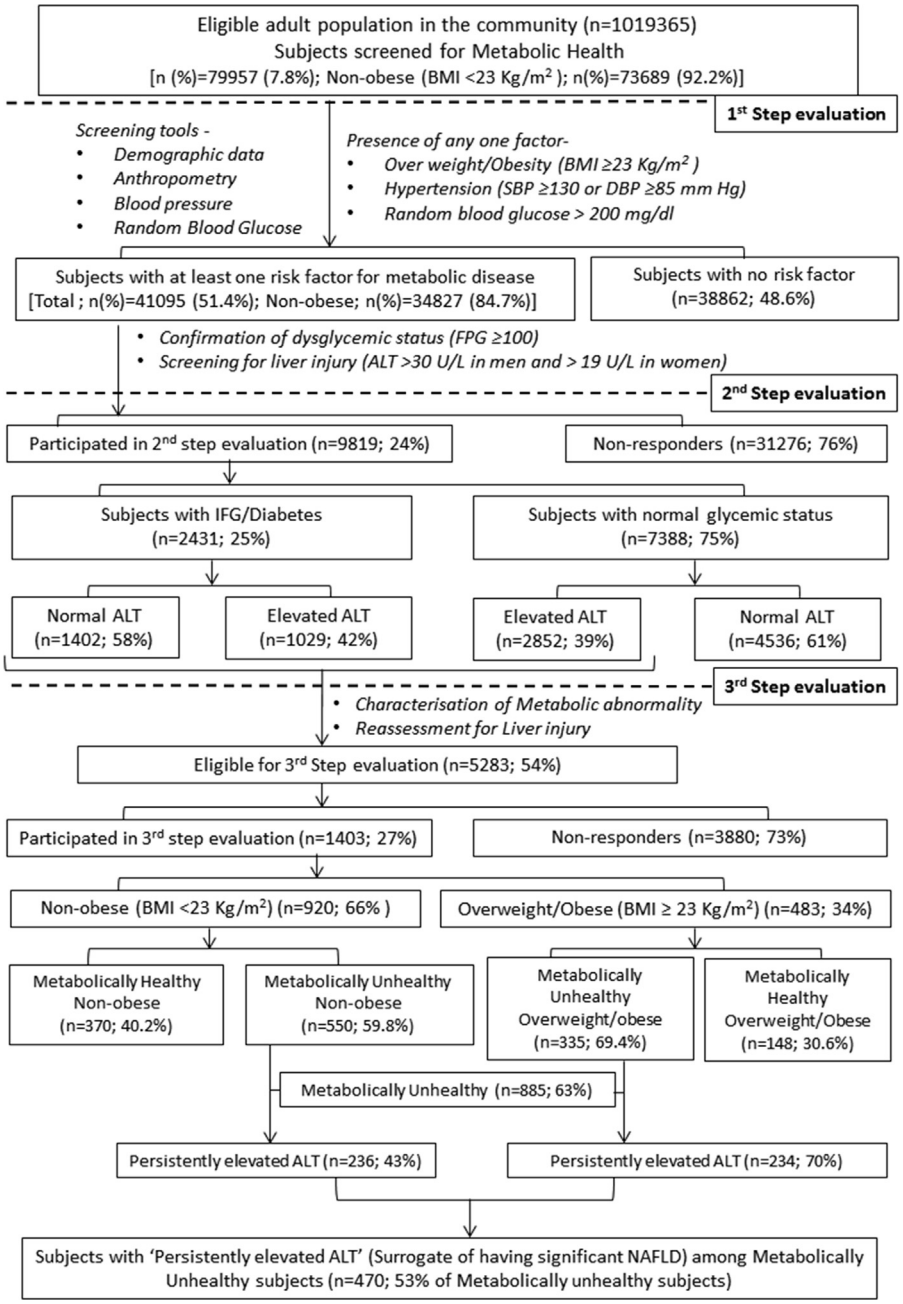

Fig. 1: **Stepwise approach to explore Metabolic Disorder and related risk of liver injury in the study population.** ALT, Alanine Amino-transferase; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; IFG, Impaired Fasting Glucose; NAFLD, Non-alcoholic Fatty Liver Disease; SBP, Systolic Blood Pressure.

evidence of liver injury by estimation of serum ALT. Subjects with IFG (Prediabetes)/Diabetes and those with elevated ALT without dysglycemia were provided with 'Red card' signifying 'at risk of being metabolically unhealthy (MU)' and were taken for third-step evaluation. In this step FBG was repeated to confirm the diagnosis of IFG (Prediabetes)/Diabetes, lipid profile was done and ALT was repeated to detect the subjects with persistently elevated ALT.

Peripheral and Central Metabolic ports were created as an organizational part of the study. 'Peripheral Metabolic Ports' (n = 50), housed in CD blocks (n = 19) in the community and were nodal point for the community field-work and socio-demographic and health

record keeping. Peripheral ports conducted door-to-door survey for initial interview using study-specific proforma and screening including anthropometric assessment (Height, body weight, BMI and abdominal circumference) recording blood pressure (BP) and measuring RBG using strip based glucometer (OneTouch® SelectSimple™ Blood Glucose monitoring system, LifeScan, Inc). All 'Peripheral Metabolic Ports' were connected with 'Central Metabolic Ports' (n = 4) which were equipped with technical facilities for performing detail blood biochemical tests for metabolic health assessment in second and third steps of the study. Thus each 'Central Metabolic Port' catered 12–13 'Peripheral Metabolic Ports'. 'Port monitors' were primarily responsible to maintain the Peripheral ports. They were trained to use the equipments for the first-step screening through a 2–week workshop. Designated 'Coordinators' supervised 'Port monitors' and maintained hierarchical liaison. All the activities in both peripheral and central ports were monitored and scrutinised by investigators. Ultrasonography for detection of fatty liver was not done as this facility and expertise is not available at community level healthcare. Participants were referred to 'Central Metabolic Ports' for blood biochemical tests in the second and third steps of the study.

Study population

Study was performed in 19 community development (CD) blocks of Birbhum district, West Bengal, India between the period March, 2015 and February, 2016. 'CD blocks' are rural administrative units defined as a collection of village councils (Panchayat) in a defined geographical area. Adults (age ≥ 18 years) enrolled in the independent electoral voter list (n = 1,019,365) published by Election Commission of India, 3–5 months before the start of the study, framed the sampling population. Every fifth member in the electoral list was included in the study making total of 79,957 participants i.e., 7.8% of the adult population, for the first-step evaluation (Fig. 1). Our community subjects, overall, did not have evidence of hazardous alcohol use according to DSM-IV criteria, which made it easier for our study to evaluate metabolic health without being influenced by alcohol use. A subject was marked as 'non-responder' if he did not turn up to participate after two telephonic communications and three home visits by the field workers. All participants provided informed consent. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by Institutional Ethics Committee for Human Research of the Institute of Post Graduate Medical Education & Research (IPGME&R Research Oversight Committee; Registration No. ECR/35/Inst/WB/2013, Government of India) (approval letter Memo No. Inst/IEC/2014/776 dated 03/07/2014).

Study tools

Study specific proforma was used to record individual identifiers and socio-demographic data in the household interviews by the field surveyors, to record anthropometric data, blood pressure and RBG data in the first step and blood biochemical test data done in the second and third steps. Anthropometric assessment included height, bodyweight and abdominal circumference measurements. Height was measured by using stature meter, bodyweight was measured by routinely calibrated Rossmax digital weighing machine (Rossmax Swiss GmbH, Switzerland) and abdominal circumference was measured as per the standard technique by using anthropometric tape (waist circumference).[8] Blood pressure was measured by routinely calibrated 'Rossmax Automatic Upper Arm Blood Pressure Monitor (Rossmax Swiss GmbH, Switzerland)'. RBG in the field was measured by using strip-based glucometer (OneTouch® SelectSimple™ Blood Glucose monitoring system, LifeScan, Inc). Blood biochemical parameters evaluated in the second step included FBG and ALT and in the third step included FBG, HbA1c, lipid profile (Triglyceride, HDL, LDL) and ALT and were measured in routinely calibrated standard automated analyser. Routine clinical assessment by clinicians was not a part of this stepwise community evaluation study protocol. Subjects with significant risks and condition requiring medical intervention were advised to attend concerned government health facilities.

Definitions used in the study

Hypertension was defined as Systolic BP (SBP) ≥130 mm Hg or Diastolic BP (DBP) ≥85 mm Hg as per the criteria proposed by International Diabetes Federation (IDF).[8]

IFG (Prediabetes) and Diabetes were diagnosed as FBG 100–125 and ≥126 mg/dL, respectively.[9]

Dyslipidemia was defined as triglyceride ≥150 mg/dL, low HDL level (<40 mg/dL in male and <50 mg/dL in female) or already on lipid-lowering medications.[8]

Upper limits of normal (ULN) for ALT were taken as 30 and 19 U/L for male and female, respectively.[10] 'Persistently elevated ALT' was defined as having elevated ALT in two occasions i.e., both in second and third step of the study.

Anthropometric classification: underweight, normal weight, overweight and obese were defined as BMI <18.5, 18.5–23, 23–27.5 and >27.5 kg/m^2, respectively, as per the criteria applicable to Asian population.[11] Waist circumference (WC) ≥90 cm in male and ≥80 cm in female, cut-off values for South-Asians, was used to define abdominal obesity.[8]

Metabolically unhealthy (MU) subject were defined as having two or more risk factors among – SBP/DBP ≥130/85 mm Hg or on antihypertensive medications, triglyceride ≥150 mg/dL, low HDL level (<40 mg/dL in

male and <50 mg/dL in female) or already on lipid-lowering medications, FBG ≥100 mg/dL or on anti-diabetic medication, HOMA-IR >90th percentile and hsCRP level >90th percentile.[12] All the risk factors except HOMA-IR and hsCRP were used in the present study.

Based on these criteria, metabolically healthy subjects with BMI <23 kg/m^2 were classified as 'Metabolically Healthy Non-obese (MHNO)', metabolically healthy subjects with BMI >23 kg/m^2 were classified as 'Metabolically Healthy Obese (MHO)', metabolically unhealthy subjects with BMI <23 kg/m^2 were classified as 'Metabolically Unhealthy Non-obese (MUNO)' and metabolically unhealthy subjects with BMI >23 kg/m^2 were classified as 'Metabolically Unhealthy Obese (MUO).'

Metabolic Syndrome (MS) was defined by using criteria proposed by IDF.[8]

Subjects at risk of having 'significant NAFLD' were defined as having MU state with persistently elevated ALT. We used the evidence of persistently elevated ALT as a surrogate of 'significant NAFLD' in absence of Ultrasonographic evidence of fatty liver, as unexplained elevation of ALT has been found to correlate most frequently with NAFLD, particularly in subjects with metabolic dysfunction and Insulin resistance.[13,14]

Statistical analysis

Participants of the study had complete data for all variables without any missing value. Data were anonymised and used for analysis. Continuous and Categorical data were presented as Median with interquartile range (IQR) expressed as 25th and 75th percentile values and number (n) with percentage (%), and respectively. Non-parametric tests, Mann–Whitney U and Chi-square tests (with yates correction for continuity) were done to compare the groups for continuous and categorical variables, respectively. Two-sided p-value <0.05 was considered statistically significant. Performance characteristics in the form of area under receiver operator characteristic curves (AUROC), sensitivity (Sn), Positive predictive value (PPV), negative predictive value (NPV) and accuracy (cases correctly classified) along with their respective 95% CI were calculated for the variables used in each step for detection of outcome in the next step (BMI ≥ 23 kg/m^2, Hypertension and RBG ≥ 200 mg/dL in the first-step for detection of IFG (Prediabetes)/Diabetes in the second-step; FBG ≥ 100 mg/dL and Elevated ALT in second-step for detection of MU subjects in third-step; MU overweight/obese subjects for detection of subjects with elevated ALT in the third-step). Binary logistic regressions (Backward method with Likelihood Ratio) were performed in the subjects evaluated in step 3 of the study (n = 1403) to find out demographic and anthropometric predictors (gender, age <30 vs ≥30 years and presence of abdominal obesity) of 'MU state' as well as 'elevated ALT in subjects with IFG (Prediabetes)/Diabetes'. Odds ratios (OR) with 95% confidence interval (CI) for the significant predictors are reported. Characteristics of the responders and non-responders in every step are provided in Supplementary Table S1 in descriptive fashion. SPSS software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for the data analysis.

Role of the funding source

Funding agency did not have any role in the study conceptualisation, designing, execution, data acquisition, data analysis, drafting of the manuscript and decision to publish.

Result

Study population consisted of 79,957 subjects [non-obese; n (%) 73,689 (92.16%)] from 19 CD blocks of the community (Fig. 1). Men contributed to 46.3% (n = 37,055) of the subjects. Median (IQR; 25th, 75th percentile) age was 41 (29, 51) years. At least one risk factor was found in 51.4% (n = 41,095). Non-obese subjects contributed to 84.74% (n = 34,827) of these at-risk subjects. Out of these 'at-risk' subjects (n = 41,095) 24% (n = 9819) participated in the second-step evaluation. Second-step evaluation revealed IFG (Prediabetes)/Diabetes in 25% of the subjects (n = 2431). Elevated ALT was detected in 42% (n = 1029) and 39% (n = 2852) of the subjects with (n = 2431) and without (n = 7388) IFG/Diabetes, respectively. Overall, 5283 subjects (n = 2431 and 2852 for subjects with 'IFG (Prediabetes)/Diabetes' and 'Normoglycemic with elevated ALT', respectively) were eligible for detail metabolic characterisation and reassessment of liver injury in the third-step. 27% of the eligible subjects (n = 1403) responded to participate in the third-step. Non-response rates were 76% (n = 31,276/41,095) and 73% (n = 3880/5283) in second and third steps, respectively. Overall, responders had higher proportion of self-employed individuals, were better educated, had higher FBG and were living closer to evaluation ports (Supplementary Table S1).

Performance of stepwise evaluation for detecting subjects at risk of metabolic disorder

As the study was designed to screen the subjects at metabolic risk at every step of evaluation, proportion of these subjects showed an increasing trend across the steps, though, test of significance for these differences are not logically acceptable and thus avoided. Proportion of Subjects with BMI <23 kg/m^2 decreased from 83.2% (n = 66,525) in the first-step to 74.8% (n = 7342) in second-step and 65.6% (n = 920) in the third-step (Table 1). Subjects with abdominal obesity increased from 14.1% (n = 11,279) in the first-step to 23.8% (n = 2336) and 28.1% (n = 394) in second and third steps, respectively (Table 1). Similarly, frequencies of hypertensive subjects increased from first to third step.

Subjects with IFG (Prediabetes)/Diabetes were more prevalent in third-step than second-step evaluation [n (%); 939 (66.9) and 2431 (24.8) in third and second step, respectively] (Table 1). Frequency of elevated ALT, also, increased in third-step [n (%); 958 (68.3) and 3881 (39.5) in third and second step, respectively] (Table 1).

AUROC, sensitivity, specificity, PPV, NPV and accuracy (cases correctly classified) along with 95% CI of different independent variables of first and second steps for prediction of outcomes in the second and third steps, respectively, are provided in Table 2. RBG ≥200 mg/dL in the first step had highest AUROC (95% CI) [0.64 (0.63–0.66)] for prediction of IFG (Prediabetes)/Diabetes in the second step with accuracy of 94.21% (95% CI, 93.64–94.56). FBG ≥100 mg/dL at second-step had highest AUROC (95% CI) [0.87 (0.86–0.88)] for prediction of 'MU (Metabolically Unhealthy) state in the third step with accuracy of 73.41% (95% CI, 71.02–75.71). Also in the third step evaluation, 'MU state with overweight/obesity' (BMI ≥ 23 kg/m^2) had AUROC (95% CI) of 0.66 (0.62–0.69) for prediction of 'persistently elevated ALT' among subjects with IFG (Prediabetes)/Diabetes with an accuracy of 61.92% (95% CI, 58.63–65.13) (Table 2).

Status of 'MU state' in the study population

MU state was detected in 63% (n = 885/1403) of the third-step participants (Table 1) making its' overall prevalence of 1.1% (n = 885/79,957). Whereas, MS was detected in 19.1% (n = 268/1403) (Table 1). MU subjects accounted for 59.8% and 69.4% of non-obese (n = 550) and overweight/obese subjects (n = 335), respectively (Fig. 1).

MU, both non-obese (MUNO) and overweight/obese (MUO) subjects, had higher WC and prevalence of abdominal obesity compared to their healthy counterparts (MHNO and MHO), despite having comparable BMI (Table 3). Median (IQR; 25th, 75th percentile) for WC and prevalence of abdominal obesity were 74 (67, 80) cm and 12.5% (n = 69/550) as well as 72 (65.5, 78) cm and 7.8% (n = 29/370) for metabolically unhealthy (MUNO) and healthy (MHNO) non-obese subjects, respectively (p = 0.01 and 0.02, respectively) (Table 3). Similarly, Median (IQR; 25th, 75th percentile) for WC and prevalence of abdominal obesity were 88 (82, 93) cm and 65.37% (n = 219/335) as well as 85.5 (81, 91) cm and 52% (n = 77/148) for metabolically unhealthy (MUO) and healthy (MHO) overweight/obese subjects, respectively (p = 0.004 and 0.01, respectively) (Table 3). MU non-obese (MUNO) subjects were comparable to overweight/obese (MUO) subjects in terms of having FBG

Steps of evaluation	Variables	All subjects (1st step evaluation) (n = 79,957)	Subjects at risk of Metabolic disorder (2nd step evaluation) (n = 9819)	Subjects with Metabolic disorder (3rd step evaluation) (n = 1403)
1st Step	Age (year); median (IQR)	41 (29, 51)	45 (34, 55)	44 (34, 54)
	Men; n (%)	37,055 (46.3)	3690 (37.6)	763 (54.4)
	Literate subjects; n (%)	8883 (11.1)	235 (2.4)	230 (16.4)
	BMI (kg/m^2); median (IQR)	19.4 (17.5, 21.8)	20.3 (18, 23)	21.23 (5.3)
	Subjects with BMI <23 kg/m^2; n (%)	66,525 (83.2)	7342 (74.8)	920 (65.6)
	Waist circumference (cm); median (IQR)	71 (65, 78)	75 (68, 82)	78 (70, 86)
	Subjects with abdominal obesity; n (%)	11,279 (14.1)	2336 (23.8)	394 (28.1)
	Hypertensive; n (%)	33,016 (41.3)	6060 (61.7)	929 (66.2)
	RBG (mg/dL) >200 mg/dL; n (%)	1332 (1.7)	460 (4.7)	175 (12.5)
2nd Step	FBG (mg/dL); Median (IQR)	NA	87 (79, 99)	107 (87, 132)
	Subjects with elevated FBG; n (%)	NA		
	(a) All subjects with FBG ≥100 mg/dL		2431 (24.8)	939 (66.9)
	(b) IFG/Prediabetes		1656 (16.9)	461 (32.9)
	(c) Diabetes		775 (7.9)	478 (34.0)
	ALT (IU/L); Median (IQR)	NA	20 (14, 29)	35 (20, 52)
	Subjects with elevated ALT; n (%)	NA	3881 (39.5)	958 (68.3)
3rd Step	TG (mg/dl); Median (IQR)	NA	NA	142 (122, 167)
	MU subjects; n (%)	NA	NA	885 (63.1)
	Subjects with MS; n (%)	NA	NA	268 (19.1)
	MU Subjects with persistently elevated ALT; n (%)	NA	NA	470/885 (53.0)

ALT, Alanine Aminotransferase; DM, Diabetes Mellitus; FBG, Fasting Blood Glucose; IFG, Impaired fasting Glucose (Prediabetes); IQR, interquartile range expressed as 25th and 75th percentile values, respectively; MS, Metabolic Syndrome (defined by the guideline of International Diabetes Federation (IDF)); MU Metabolically Unhealthy; NA, Not Applicable; RBG, Random Blood Glucose; TG, Triglyceride. Abdominal obesity was defined as waist circumference ≥90 cm in male and ≥80 cm in female, specific for South-Asian ethnicity; MU subjects were defined as having metabolic risk factors ≥2.

Table 1: **Characteristics of study population at each step of the study.**

Steps of evaluation	Outcome	Predictor variables (risk factors)	AUROC (95% CI); p Value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (cases correctly classified) (95% CI)
From 1st to 2nd step (n = 9819)	Detection of IFG/Diabetes (n = 2425)	BMI ≥23 kg/m^2 (n = 2477)	0.56 (0.55–0.56); <0.0001	31.96 (30.10–33.86)	76.99 (76.00–77.94)	80.64 (79.50–81.73)	27.39 (26.79–27.99)	43.21 (42.23–44.52)
		Presence of Hypertension (n = 6060)	0.57 (0.55–0.58); <0.0001	67.71 (65.81–69.57)	40.25 (39.13–41.38)	64.63 (63.85–65.38)	43.61 (42.05–45.19)	57.20 (56.21–58.18)
		RBG ≥200 mg/dL (n = 460)	0.64 (0.63–0.66); <0.0001	13.23 (11.91–14.65)	98.10 (97.76–98.40)	25.54 (22.04–29.38)	95.83 (95.77–95.99)	94.21 (93.64–94.56)
From 2nd to 3rd step (n = 1403)	Detection of MU subjects (n = 885)	Presence of FBG ≥100 mg/dL (n = 900)	0.87 (0.86–0.88); <0.0001	78.44 (75.61–81.09)	64.41 (60.05–68.60)	79.77 (77.73–81.68)	62.55 (59.20–65.78)	73.41 (71.02–75.71)
		Elevated ALT (n = 930)	0.67 (0.65–0.69); <0.0001	58.31 (54.98–61.58)	20.08 (16.71–23.79)	55.48 (35.74–57.22)	21.99 (18.92–25.39)	44.19 (41.57–46.84)
3rd step (n = 1403)	Detection of persistently elevated ALT among MU subjects (n = 470)	MU overweight/obese subject (n = 335)	0.66 (0.62–0.69); <0.0001	46.79 (45.17–54.40)	75.66 (71.24–79.72)	69.85 (65.65–73.74)	57.09 (54.56–59.65)	61.92 (58.63–65.13)

ALT, Alanine Aminotransferase; AUROC, Area under Receiver operating characteristics (ROC) curve; BMI, Body Mass Index; CI, Confidence Interval; FBG, Fasting Blood Glucose; IFG, Impaired fasting Glucose; MU, Metabolically Unhealthy; NPV, Negative Predictive value; PPV, Positive Predictive Value; RBG, Random Blood Glucose. Calculations were done by using 2 × 2 contingency table (occurrence/non-occurrence of Outcome vs presence/absence of risk factor). ROCs were constructed taking the continuous variable forms (BMI, Systolic Blood pressure, RBG, FBG and ALT) of the categorical risk variables as test variables.

Table 2: **Performance of variables at different steps of evaluation to predict outcome in the next step.**

≥100 mg/dL [n (%); 434 (78.9) and 236 (70.4) in MU non-obese (MUNO) and overweight/obese (MUO) subjects, respectively, p = 0.59] and dyslipidemia [n (%); 313 (56.9) and 196 (58.5) in MU non-obese (MUNO) and overweight/obese (MUO) subjects, respectively, p = 0.52] (Table 3). But MS prevalence was

Variables	Metabolically healthy		Metabolically unhealthy		p value[e]
	Non-obese subjects (MHNO)[a] (n = 370)	Overweight/Obese subjects (MHO)[b] (n = 148)	Non-obese subjects (MUNO)[c] (n = 550)	Overweight/Obese subjects (MUO)[d] (n = 335)	
Age (year); median (IQR)	39 (28, 49)	39 (27, 45)	49 (40, 58)	44 (36, 53)	a vs b 0.20 c vs d <0.0001
Men; n (%)	214 (57.8)	88 (59.5)	296 (53.8)	165 (49.2)	a vs b 0.75 c vs d 0.19
BMI (kg/m^2); median (IQR)	19.5 (17.9, 20.9)	25.2 (23.7, 26.6)	19.8 (17.7, 21.4)	25.4 (24.1, 27.1)	a vs c 0.17 b vs d 0.12
WC (cm); median (IQR)	72 (65.5, 78)	85.5 (81, 91)	74 (67, 80)	88 (82, 93)	a vs c 0.017 b vs d 0.0041
Subjects with abdominal obesity; n (%)	29 (7.8)	77 (52.0)	69 (12.5)	219 (65.4)	a vs c 0.028 b vs d 0.011
Hypertensive; n (%)	113 (30.5)	54 (36.5)	460 (83.6)	302 (90.1)	a vs b 0.19 c vs d 0.014
Subjects with FBG ≥100 mg/dL; n (%)	106 (28.6)	30 (20.3)	434 (78.9)	236 (70.4)	a vs b 0.22 c vs d 0.59
Subjects with dyslipidemia; n (%)	42 (11.3)	20 (13.5)	313 (56.9)	196 (58.5)	a vs b 0.49 c vs d 0.52
Subjects with MS; n (%)	0 (0)	0 (0)	67 (12.2)	201 (60.0)	c vs d <0.0001
Subjects with persistently elevated ALT; n (%)	267 (72.2)	122 (82.4)	236 (42.9)	234 (69.8)	a vs b 0.015 c vs d <0.0001

ALT, Alanine Aminotransferase; BMI, Body Mass Index; FBG, Fasting Blood Glucose; IQR, interquartile range expressed as 25th and 75th percentile values, respectively; MHNO, Metabolically Healthy Non-obese; MHO, Metabolically Healthy Overweight/obese; MS, Metabolic Syndrome; MUNO, Metabolically unhealthy Non-obese; MUO, Metabolically Unhealthy Obese; WC, Waist Circumference. Abdominal obesity was defined as waist circumference ≥90 cm in male and ≥80 cm in female, specific for South-Asian ethnicity. [e]p values were derived from Mann–Whitney U test and Chi square test (with yates correction for continuity) for continuous and categorical variables, respectively.

Table 3: **Comparative description of the subjects with different metabolic health status.**

Subjects included in the regression model	Subjects with the outcome	Independent variables (n)	Coefficient of regression	SE	Odds ratio (95% CI)	p value
Predictors of Metabolically Unhealthy state						
Evaluated in Step 3 (n = 1403)	MU subjects (n = 885)	Age ≥30 years (n = 1148)	1.40	0.15	4.06 (3.04–5.42)	<0.0001
		Presence of abdominal obesity (n = 394)	0.51	0.13	1.66 (1.28–2.17)	<0.0001
Predictors of persistently elevated ALT in subjects with IFG (Prediabetes) and Diabetes						
Subjects having IFG/DM (n = 900)	Subjects with persistently elevated ALT (n = 376)	Age ≥30 years (n = 814)	−0.76	0.23	0.47 (0.29–0.74)	0.0017
		Presence of abdominal obesity (n = 268)	0.88	0.15	2.42 (1.78–3.24)	<0.0001

ALT, Alanine Aminotransferase; CI, Confidence Interval; DM, Diabetes Mellitus; IFG, Impaired Fasting Glucose; MU, Metabolically Unhealthy; SE, Standard Error. Predictors were derived from Binary logistic regressions (Backward method with Likelihood Ratio); Abdominal obesity was defined as waist circumference ≥90 cm in male and ≥80 cm in female, specific for South-Asian ethnicity.

Table 4: **Predictors of Metabolically Unhealthy state and elevated ALT.**

significantly higher in MU overweight/obese (MUO) subjects [n (%); 201 (60) and 67 (12.2) in MU overweight/obese (MUO) and non-obese (MUNO) subjects, respectively, $p < 0.0001$].

Binary logistic regression model revealed increased age (≥30 years) [OR (95% CI); 4.06 (3.04–5.42), $p < 0.0001$] and presence of abdominal obesity [OR (95% CI); 1.66 (1.28–2.17), $p < 0.0001$] as significant predictors of Metabolically Unhealthy (MU) state (Table 4).

Risk of significant NAFLD

Proportion of the subjects with elevated ALT increased significantly in those with metabolic disorder (third-step) from those at risk of metabolic disorder (second-step) [n (%) 958 (68.3) and 3881 (39.5) in third and second steps, respectively, $p < 0.0001$] (Table 1). 53% of MU subjects (n = 470/885) were at risk of having 'significant NAFLD' reflected by having 'persistently elevated ALT' (Table 1 and Fig. 1).

Degree of ALT elevation increased significantly in overweight/obese persons (BMI ≥ 23 kg/m^2) compared to those with normal BMI (≤23 kg/m^2), in absence of any other metabolic risk factor [ALT, xULN; Median (IQR; 25th, 75th percentile) 2.2 (1.6, 2.7) vs 1.3 (0.9, 1.9) in BMI ≥23 kg/m^2 and ≤23 kg/m^2, without any metabolic risk factors, respectively, $p < 0.0001$] (Fig. 2). Gradual accumulation of metabolic risk factors in those overweight/obese subjects did not add to the severity of elevation in ALT (Fig. 2). However, among MU subjects, overweight/obese (MUO) had higher proportion of subjects with elevated ALT compared to their non-obese (MUNO) counterpart [n (%) 234/335 (69.8) vs 236/550 (42.9) in overweight/obese (MUO) and non-obese (MUNO) MU subjects, respectively, $p < 0.0001$] (Table 3).

As the subjects with IFG (Prediabetes)/Diabetes had higher risk of having significant NAFLD, binary logistic regression analysis was performed to find out predictors of elevated ALT in them. 'Presence of abdominal obesity' was a significant predictor for 'persistently elevated ALT' in IFG (Prediabetes)/Diabetes subjects [OR (95% CI); 2.42 (1.78–3.24), $p < 0.0001$] (Table 4). Increasing age (≥30 years) was a negative predictor for 'persistently elevated ALT' in them [OR (95% CI); 0.47 (0.29–0.74), $p < 0.0001$] (Table 4).

Discussion

Our present study was focused to pick up the subjects at highest risk of having metabolic unhealthiness in the community, by stepwise risk evaluation, to minimize the utilization of healthcare facilities. First-step screened the subjects with any metabolic risk and subsequent two steps identified the subject at highest risk of metabolic unhealthiness. 'High risk' approach using different risk scores has been reported to be effective in screening for Diabetes in the community and less privileged section of the society.[15,16] We used simple and easily available parameters in place of any risk score based assessment. Unlike other cross-sectional studies, our study explored the spectrum of metabolic ill-health from minimum or no risk to specifically defined 'MU' state and 'MS' in a comparative frame. Thus, our present study could help to devise a plan to screen for metabolic ill-health in the community, implementable in resource-poor LMICs. India, like other LMICs, is facing the epidemiological transition of disease burden towards NCDs with simultaneous presence of communicable diseases.[17,18] Future burden of NCDs is expected to be much higher in these countries that are least prepared to handle the situation.[2] Particularly in India, healthcare infrastructure is not robust enough to deal with the combined burden of communicable and non-communicable diseases.[19] So, prudent utilization of both formal and informal healthcare resources is regarded as a major necessity towards addressing this issue.[19]

As most of the burden of NCD is related to obesity and metabolic ill-health, our study provides timely information in this issue from a real life scenario of the community at large. India houses a large portion of underweight/non-obese people of the world along with increasing trend in obesity.[3] Thus, we had the

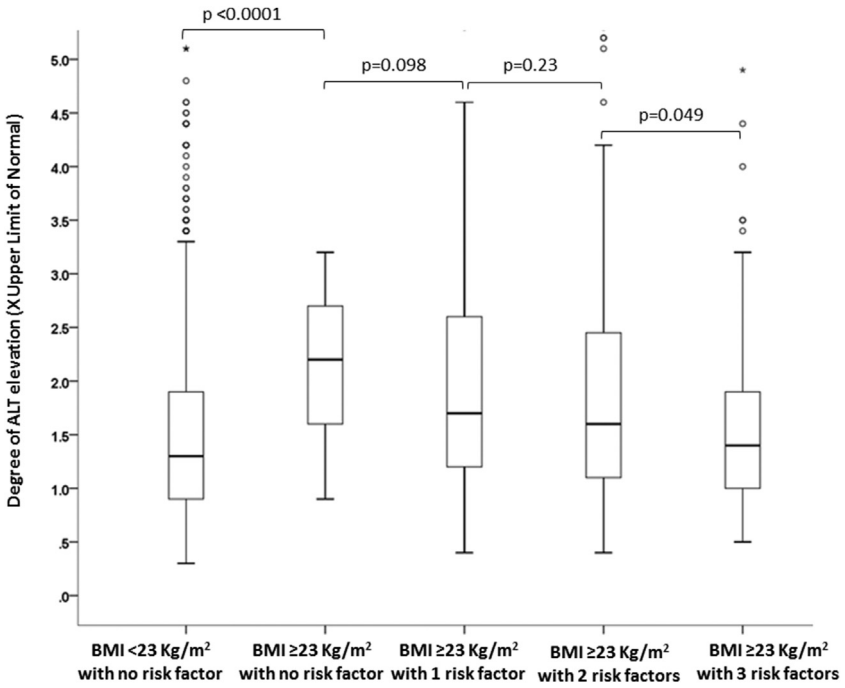

***Fig. 2:* Boxplot showing relationship between Obesity, number of metabolic risk factors and degree of ALT elevation.** ALT, Alanine Aminotransferase; BMI, Body Mass Index.

opportunity to explore the wider spectrum of adiposity in an ethnically homogeneous population. We observed that about half of community people had at least one metabolic risk factor. Metabolic unhealthiness and NAFLD goes hand-in-hand to contribute to a significant share of liver disease burden. Segregation of the subjects with highest degree of metabolic abnormality and those with at-risk of significant NAFLD will provide important information to the healthcare policy makers for future preparedness. We found 'MU' state and MS in 63% and 19.1% of the metabolically high risk subjects, respectively. Thus, we could reach these subjects with highest risk status without exploring a large portion of the community, which minimized resource utilization.

We dealt with a population where non-obese subjects predominated (92.2%). Metabolic ill-health and risk of significant NAFLD in non-obese population is a growing concern and area of research worldwide. Non-obese MU subjects are found to have insulin resistance and increased liver fat in comparison to their healthy counterpart.[4] In the midst of scarce data, our observation of 59.8% of metabolically high-risk non-obese subjects having MU state extends the spectrum of metabolic unhealthiness and related NAFLD beyond obesity. It also re-emphasizes that abdominal obesity plays key role toward MU state even in normal BMI subject. As a result, non-obese individuals acquire metabolic abnormalities comparable to those with obesity. Definitely, overweight/obese subjects developed severe metabolic dysfunction more frequently, reflected by significantly higher proportion of subjects with MS in them. Apart from anthropometric risks, demographic changes are also expected to influence the risk and burden of NCDs. Burden of disability related to NCDs is expected to increase in future along with increase in life expectancy in majority of the countries.[20] Gradual accumulation of risk factors with the age could contribute to the increasing burden of metabolic ill-health in the community. Our observation of increasing age as a strong risk factor for MU state points towards this possibility.

NAFLD is a known accompaniment of MS because of shared risk factors and common pathogenetic role of insulin resistant state.[21,22] Thus, increasing prevalence of metabolic ill-health is going to increase the burden of NAFLD and related chronic liver disease in the community. Our study did not avail the facility of ultrasound to detect NAFLD. Instead, persistently elevated liver enzyme was regarded as a surrogate of possible significant NAFLD, despite knowing the fact that not all the subjects with metabolic abnormality and elevated liver enzymes harbor Non-alcoholic steatohepatitis (NASH) or significant NAFLD.[21] However, 24% of symptomatic and 35% of asymptomatic subjects with elevated ALT were found to have significant fatty liver disease in the form of steatohepatitis.[23] Considering the frequency of ALT elevation in advanced NAFLD, it could be utilized

as a tool to screen those subjects with possibility of significant NAFLD.[24] Still we consider this as one of the limitations of our study and had to go with that for the sake of simplicity and wider community applicability of the screening model. We observed that 53% of the MU subjects were at risk of having significant NAFLD reflected by having its surrogate i.e., persistently elevated ALT. Overweight/obese MU subjects had higher proportion of at-risk subjects for significant NAFLD. This observation is in concordance with other studies showing higher risk of NASH in obese subjects.[25]

In the present study, degree of ALT elevation increased above BMI 23 kg/m^2. But, gradual accumulation of metabolic risk factors over and above increased BMI did not affect the degree of ALT elevation suggesting the predominant role of adiposity affecting the severity of NAFLD. Particularly, in subjects with severe metabolic ill-health i.e., IFG (Prediabetes)/Diabetes, 'presence of abdominal obesity' was associated with high risk of having 'persistently elevated ALT as a surrogate of the risk of 'significant NAFLD'. Well conducted community studies reported that metabolic abnormalities like IFG, Diabetes, Insulin resistance and MS were associated with NAFLD.[21,22] But community studies showing predictors of severity of NAFLD are limited.[21] Based on our observation and current state of knowledge, it can be summarised that metabolic abnormalities along with adiposity could increase the risk of NAFLD, whereas, severity of NAFLD could be sometimes influenced by the degree of adiposity, independent of other metabolic derangements. Further pathophysiological studies are required to clarify this issue.

Higher proportion of non-response in each step of the study was possibly related to the unwillingness to undergo laboratory tests and visit to healthcare facilities on the part of otherwise healthy and physically active population. Overall, responders were better educated, had higher proportion of self-employed individuals and belonged to the households closer to the evaluation ports which could have influenced the awareness, commitment and practical feasibility to adhere to the study protocol. However, response rates in each step were almost similar (24% and 27% in second and third steps, respectively). In a Dutch community study, which focused on nonresponse and its determinants, reported participation rate of 28.9% with variation across occupations, educational level and household income.[26] Unlike community studies, higher response rates are observed mostly in only interview based studies, telephonic surveys and state sponsored census surveys. Our study had large number of participants even after excluding non-responders, which is expected to nullify non-response bias out of non-response rate.[27] Even then, we consider this as one of the limitations of our study and accept as a real life scenario, as nonresponse is not always responsible for introduction of non-response bias and affect the data representativeness in the community studies.[27]

Our stepwise evaluation study performed satisfactorily to screen subjects with metabolic unhealthiness in the community. MU state and MS were detected in 63% and 19.1% of the subjects at high-risk of metabolic abnormality, respectively. MU non-obese subjects had higher abdominal obesity than their healthy counterparts and were comparable to MU overweight/obese subjects in terms of metabolic derangements. Overall 53% of MU subjects had persistently elevated ALT as a surrogate of risk of having significant NAFLD.

Contributors

All the authors had access to the data and all of them share the responsibility for the submission for publication. Data were verified by *Kausik Das*, *Partha Sarathi Mukherjee*, *Dipesh Kumar Das* and *Pabak Sarkar*. *Partha Sarathi Mukherjee* was involved in onceptualization, methodology, project administration, supervision, data review, draft review and editing. *Sujoy Ghosh* was involved in conceptualization, methodology, data review and draft review. *Pradip Mukhopadhyay* was involved in conceptualization, methodology, data verification and draft review. *Dipesh Kumar Das* was involved in supervision of community work as Project Supervisor, data acquisition, data verification and draft review. *Pabak Sarkar* was involved in supervision of community work as Programme Manager, data acquisition, data verification and draft review. *Saibal Majumdar* was involved in methodology, project administration, training of field workers, field work monitoring and draft review. *Kajal Chatterjee* was involved in methodology, project administration, training of field workers, field work monitoring and draft review. *Kausik Das* was involved in methodology, data review, data analysis, writing original draft and editing. *Abhijit Chowdhury* was involved in conceptualization, methodology, project administration, training of field workers, draft review.

Data sharing statement

Original data (deidentified participant data) in the excel spreadsheet format may be provided on personal communication with the corresponding author or with the office of the 'Liver Foundation, West Bengal; Website: https://www.liverfoundation.in/' (Office address: Liver Foundation, West Bengal, Room No. 12, 16th Floor). Chatterjee International Center, 33A, Jawaharlal Nehru Rd Kolkata, 700071, India; Email: liv.fwb@gmail.com after publication of the article.

Declaration of interests

We were not paid by any pharmaceutical company or any other agency to write this article. Authors were not precluded from accessing data in the study and all of them accept the responsibility to submit for publication. All the authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lansea.2023.100142.

References

1 Murray CJ, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a

systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2197–2223.
2 Bollyky TJ, Templin T, Cohen M, Dieleman JL. Lower-income countries that face the most rapid shift in noncommunicable disease burden are also the least prepared. *Health Aff (Millwood)*. 2017;36(11):1866–1875.
3 NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet*. 2016;387(10026):1377–1396.
4 Stefan N, Schick F, Häring HU. Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. *Cell Metab*. 2017;26(2):292–300.
5 Barik A, Das K, Chowdhury A, Rai RK. Metabolic syndrome among rural Indian adults. *Clin Nutr ESPEN*. 2018;23:129–135.
6 Barik A, Shah RV, Spahillari A, et al. Hepatic steatosis is associated with cardiometabolic risk in a rural Indian population: a prospective cohort study. *Int J Cardiol*. 2016;225:161–166.
7 van Doorslaer E, O'Donnell O, Rannan-Eliya RP, et al. Catastrophic payments for health care in Asia. *Health Econ*. 2007;16(11):1159–1184.
8 Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabet Med*. 2006;23(5):469–480.
9 American Diabetes Association. Standards of medical care in diabetes. *Diabetes Care*. 2004;27 Suppl 1:S15–S35.
10 Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med*. 2002;137(1):1–10.
11 WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*. 2004;363(9403):157–163.
12 Wildman RP, Muntner P, Reynolds K, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med*. 2008;168(15):1617–1624.
13 Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol*. 2003;98(5):960–967.
14 Suzuki A, Angulo P, Lymp J, et al. Chronological development of elevated aminotransferases in a nonalcoholic population. *Hepatology*. 2005;41(1):64–71.
15 Sathish T, Shaw JE, Tapp RJ, et al. Targeted screening for prediabetes and undiagnosed diabetes in a community setting in India. *Diabetes Metab Syndr*. 2019;13(3):1785–1790.
16 Timm L, Harcke K, Karlsson I, et al. Early detection of type 2 diabetes in socioeconomically disadvantaged areas in Stockholm - comparing reach of community and facility-based screening. *Glob Health Action*. 2020;13(1):1795439.
17 Samb B, Desai N, Nishtar S, et al. Prevention and management of chronic disease: a litmus test for health-systems strengthening in low-income and middle-income countries. *Lancet*. 2010;376(9754):1785–1797.
18 India State-Level Disease Burden Initiative Collaborators. Nations within a nation: variations in epidemiological transition across the states of India, 1990-2016 in the Global Burden of Disease Study. *Lancet*. 2017;390(10111):2437–2460.
19 Mohanan M, Hay K, Mor N. Quality of health care in India: challenges, priorities, and the road ahead. *Health Aff (Millwood)*. 2016;35(10):1753–1758.
20 Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006;3(11):e442.
21 Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2018;15(1):11–20.
22 Speliotes EK, Massaro JM, Hoffmann U, et al. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology*. 2010;51(6):1979–1987.
23 Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. *Am J Gastroenterol*. 1999;94(10):3010–3014.
24 Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC, Public Policy Committee of the American Association for the Study of Liver Disease. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology*. 2008;47(4):1363–1370.
25 de Alwis NM, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol*. 2008;48 Suppl 1:S104–S112.
26 Boshuizen HC, Viet AL, Picavet HS, Botterweck A, van Loon AJ. Non-response in a survey of cardiovascular risk factors in the Dutch population: determinants and resulting biases. *Public Health*. 2006;120(4):297–308.
27 Lee S, Brown ER, Grant D, Belin TR, Brick JM. Exploring nonresponse bias in a health survey using neighborhood characteristics. *Am J Public Health*. 2009;99(10):1811–1817.