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Assessment of dietary, genetic and metabolic factors in South Indian adolescents with metabolic dysfunction-associated steatotic liver disease: a case–control study protocol

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ABSTRACT

Introduction Metabolic dysfunction-associated steatotic liver disease (MASLD) is a leading cause of liver disease among adolescents. The objectives of this study are to investigate the associations of dietary, genetic and metabolic factors with MASLD in South Indian adolescents.

Methods and analysis The study will employ a case–control study design. We will recruit 280 adolescents (140 cases and 140 controls) from hospital and school settings. The hospital setting will be the paediatric gastroenterology outpatient department (OPD) at the study institution and the school setting will be selected urban schools from Ernakulam, Kerala. At the hospital, cases and controls will be selected from the patients who are attending the paediatric gastroenterology OPD with complaints of generalised abdominal pain or constipation with no other significant medical complaints or use of medications. A sensitisation programme on MASLD for parents of adolescents will be conducted in schools. All consenting parents along with their adolescent wards will be invited for study participation. Cases will be defined as adolescents having evidence of hepatic steatosis in ultrasound and meeting any one of the paediatric cardiometabolic criteria for MASLD. Those who fail to satisfy this criteria will be defined as controls. All participants will undergo nutritional and physical activity assessments using validated questionnaires along with blood sampling for biochemical analysis and genetic testing. We will examine the associations between MASLD and dietary parameters using Pearson's χ^2 tests after stratifying dietary variables into categorical groups. Logistic regression will be used to assess the impact of dietary parameters and single-nucleotide polymorphisms (SNPs) on the risk of MASLD.

Ethics and dissemination Ethics approval was obtained from the Ethics Committee of Amrita School of Medicine, Kochi. Informed consent will be obtained from participants and their legal guardians before enrolment. The study findings will provide valuable insights into the evolution of MASLD among adolescents in South India.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Metabolic dysfunction-associated steatotic liver disease (MASLD) is more common in adolescents who are overweight or obese, have high blood sugar, high blood pressure or high cholesterol.
⇒ The first-line treatment for MASLD includes lifestyle changes such as eating a healthy diet, exercising and losing weight.
⇒ Several single nucleotide polymorphisms have been identified as conferring increased risk for MASLD, *PNPLA3* being the most common and consistently associated with the disease.

WHAT THIS STUDY ADDS

⇒ This study will probably identify specific dietary patterns and/or nutritional factors that may contribute to MASLD in the school-going adolescent population from south India.
⇒ This study will explore the role of specific genetic variations in adolescents with MASLD.
⇒ This study will help in understanding whether these genetic variations influence individual responses to different macronutrients, thereby affecting the development of MASLD in adolescents.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study may provide insights into how genetic variations interact with dietary factors to influence the risk of MASLD.
⇒ The study may contribute to the development of personalised dietary recommendations based on genetic profiles for adolescents at risk of MASLD, which could allow for early intervention and prevention strategies.
⇒ The study may contribute to the development of public health initiatives aimed at promoting healthy eating habits and physical activity among adolescents in a school setting.

INTRODUCTION

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously referred

to as non-alcoholic fatty liver disease (NAFLD), is now a leading cause of liver disease among children and adolescents.[1 2] The diagnostic pathway for paediatric MASLD differs from that of adult MASLD.[2] MASLD encompasses a spectrum of severity, ranging from steatosis (accumulation of liver fat without inflammation) to metabolic dysfunction-associated steatohepatitis (MASH). MASH, previously known as non-alcoholic steatohepatitis (NASH), is charaterised by histological features of lobular inflammation and hepatocellular ballooning.[1] MASH can advance to stages that include fibrosis, cirrhosis and even hepatocellular carcinoma (HCC).[3] This paper uses both MASLD and NAFLD, as a significant portion of the literature uses the term NAFLD.

The global pooled prevalence of NAFLD rose from 25% in 2016 to 30.05% in 2019 (covering the period from 1990 to 2019) as per a recent meta-analysis.[4] A recent meta-analysis of studies conducted in India revealed that NAFLD affects 38.6% of adults and 35.4% of children.[5] It has been estimated that the prevalence of NAFLD in obese children is five times greater than that reported in non-obese.[6]

The current evidence suggests an element of heritability in MASLD. Previous studies have identified several SNPs that are associated with NAFLD. The major SNPs include patatin-like phospholipase domain-containing 3 (*PNPLA3*) rs738409, transmembrane 6 superfamily member 2 (*TM6SF2*) rs58542926, glucokinase regulator (*GCKR*) rs1260326 and membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*) rs641738 among other SNPs.[7] The *PNPLA3* rs738409 variant has been identified as one of the significant determinants of inter-individual and ethnicity-related differences in hepatic fat content.[8] These differences appear to be independent of insulin resistance and serum lipid concentration.[9 10]

Valenti *et al* investigated the correlation between the *PNPLA3* genotype and histological characteristics of NASH in a group of 149 Italian children (aged 6–13 years).[11] They evaluated liver tissue using the NASH Clinical Research Network scoring system and found the risk allele (*PNPLA3*-I148M) was significantly associated with hepatic steatosis, with an OR of 18.9 (95% CI 7.1 to 47; p≤0.0001) for moderate or severe steatosis.[11]

In a multiethnic study done in obese children and adolescents, Goffredo *et al* found that individuals carrying the minor allele for *TM6SF2* rs58542926 had a higher risk of hepatic steatosis and marked liver injury.[12] A multiethnic study, done in Caucasian, African American and Hispanic obese children and adolescents, showed an association between *GCKR* rs1260326 and hepatic fat content.[13] A study done in Finland showed an association in increased plasma alanine transferase (ALT) levels with increasing number of T alleles of the *MBOAT7* rs641738 polymorphism in children.[14] A similar association was observed in another study done in Italian children with carriers of the T allele.[15]

Ultra-processed foods (UPFs) are industrial formulations which have five or more ingredients.[16] Besides salt, sugar, oils and fats, these contain ingredients other than substances that are not generally used as culinary ingredients.[16] UPFs represent a significant component of modern diets, and understanding their influence on gene–diet interactions is becoming increasingly crucial in the pursuit of personalised nutrition strategies. A recent meta-analysis investigating the relationship between UPF consumption and NAFLD suggested that the risk of developing NAFLD increases as the quantity of UPF consumption rises, indicating a positive association between UPFs and NAFLD.[17]

The current study aims to investigate the genetic factors predisposing adolescents in South India to MASLD, focusing on the association of selected SNPs in genes like *PNPLA3*, *TM6SF2*, *MBOAT7* and *GCKR*. It also aims to study the association of dietary habits, including UPFs, dietary diversity and physical activity with the prevalence and severity of MASLD. Lastly, we will explore the impact of interaction between the genetic variation and dietary factors, mainly UPFs, on the susceptibility of MASLD in adolescents.

METHODS AND ANALYSIS

Case–control study

Protocol design

Primary objective

- To examine the effect of macronutrient intake on the risk of MASLD in school-going adolescents.

Secondary objectives

- To investigate the effect of percentage energy intake from UPFs on the risk of MASLD.
- To investigate the effect of selected SNPs of the genes *PNPLA3*, *TM6SF2*, *MBOAT7* and *GCKR* on the risk of MASLD during adolescence.
- To investigate the effect of dietary diversity as measured by Individual Dietary Diversity Score (IDDS) on the risk of MASLD.
- To investigate the effect of physical activity levels as measured by the Physical Activity Questionnaire for children and adolescents (PAQ-C and PAQ-A) on the risk of MASLD during adolescence.
- To examine the interactions between UPFs and genetic variations on the risk of MASLD in adolescents.

This approach aims to provide insights into the multifaceted factors influencing MASLD in this population, including dietary habits, genetic predispositions and lifestyle factors such as physical activity.

Selection criteria

This is a case–control study currently ongoing at Amrita Institute of Medical Sciences, Kerala, India. The inclusion and exclusion criteria are described in figure 1. Informed consent will be obtained from adolescents and their legal guardians before study enrolment.

Significance of the study

This study explores the link between genetics and dietary factors in MASLD among South Indian adolescents. It is

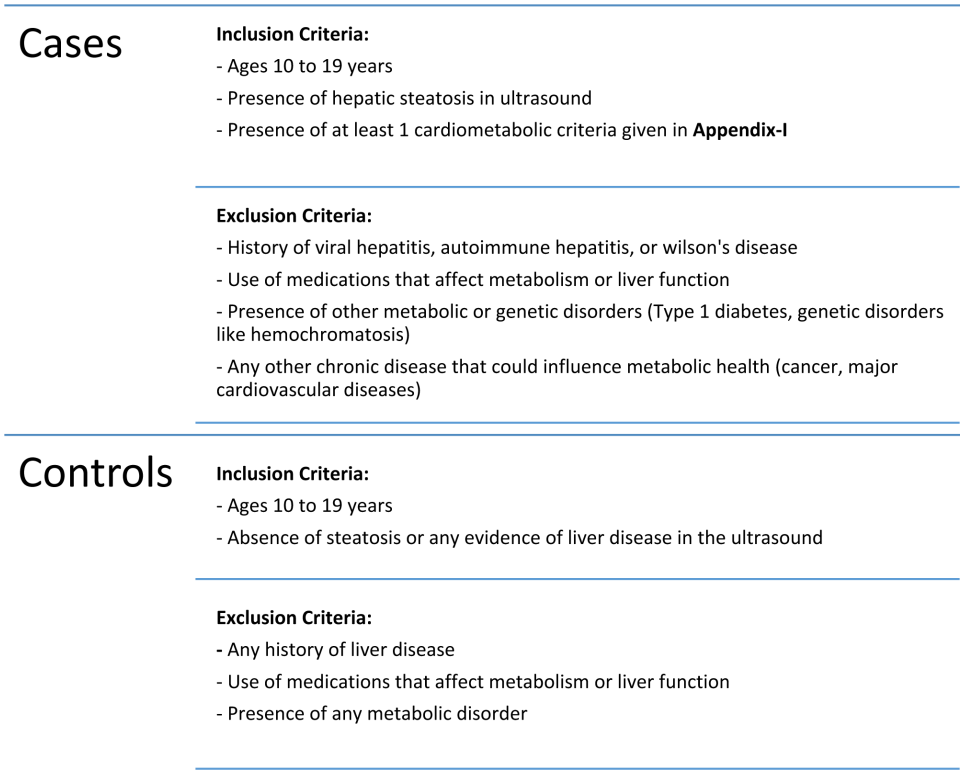

Figure 1 Selection criteria for the study.

crucial to comprehend the potential impact of certain genes on susceptibility to MASLD and how dietary choices can modify these genetic effects. These examinations are currently relevant considering the increasing occurrence of MASLD and the substantial genetic variation among the Indian population. Early detection, risk factor identification and effective management would help to prevent or reduce adverse outcomes associated with MASLD, such as MASH, cirrhosis and HCC. Reductions in these adverse outcomes by proper diagnosis and appropriate management may ultimately reduce the need for liver transplantation in subjects with MASLD. Knowledge from this study may also assist in developing personalised dietary interventions targeting MASLD.

Sample size

We used the following equation to calculate the sample size for this unmatched case–control study design. We computed the sample size using a conservative estimate of 50% prevalence of exposure among controls and a minimally important OR of 2.0 with equal ratio of cases to controls.

$$n = \frac{(Z_{\alpha_2}+Z_{\beta})^2}{(p_1-p_0)^2} \times \frac{p_0(1-p_0)+p_1(1-p_1)}{r}$$

Where:

- P_0 is the proportion of exposure in controls (0.5).
- P_1 is the proportion of exposure in cases (0.667).
- OR is the minimally important odds ratio (2.0).
- $Z_{\alpha 2}$ is the standard normal value for significance level (1.96).
- Z_{β} is the standard normal value for power (0.84 for 80% power).
- r is the case–control ratio (1).

The sample size estimation provided us with a requirement of 138 cases (with MASLD) and 138 controls totalling 276 participants. We will be recruiting a total of 280 participants for this study.

Participant recruitment

Cases and controls would be recruited from both hospital and school settings subject to the inclusion and exclusion criteria mentioned earlier (figure 1). The majority of cases will be recruited from the paediatric gastroenterology outpatient department (OPD) of the study centre (hospital). Patients presenting with generalised abdominal pain, constipation, gastritis and similar complaints, lacking significant medical history and prolonged medication use, will be recruited and subjected to a structured workup for MASLD that includes an abdominal ultrasound (figure 2). Participants identified to have steatosis will be classified as cases, provided they satisfy the cardiometabolic criteria for MASLD (online supplemental appendix 1) and are tested negative for hepatitis B and C viruses. Those with steatosis but who fail to satisfy the cardiometabolic criteria for MASLD will be excluded from the study. All participants without steatosis will be designated as controls (figure 2).

Participants will also be recruited from five selected schools in the Ernakulam District, Kerala. School health awareness classes will be conducted in these schools for parents of adolescents. Those parents expressing interest

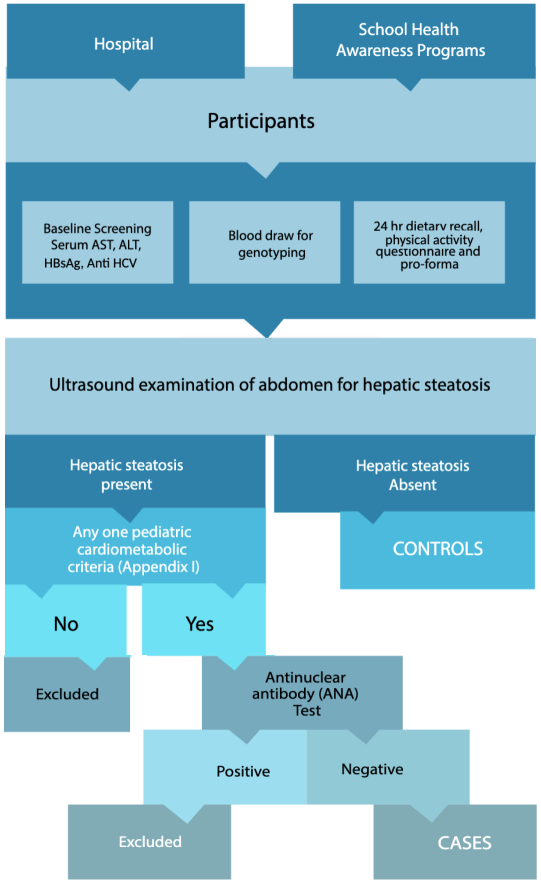

Figure 2 Flow chart illustrating participant recruitment process. ALT, alanine transferase; AST, aspartate aminotransferase; HBsAg, Hepatatis B surface Antigen; HCV, Hepatitis C virus.

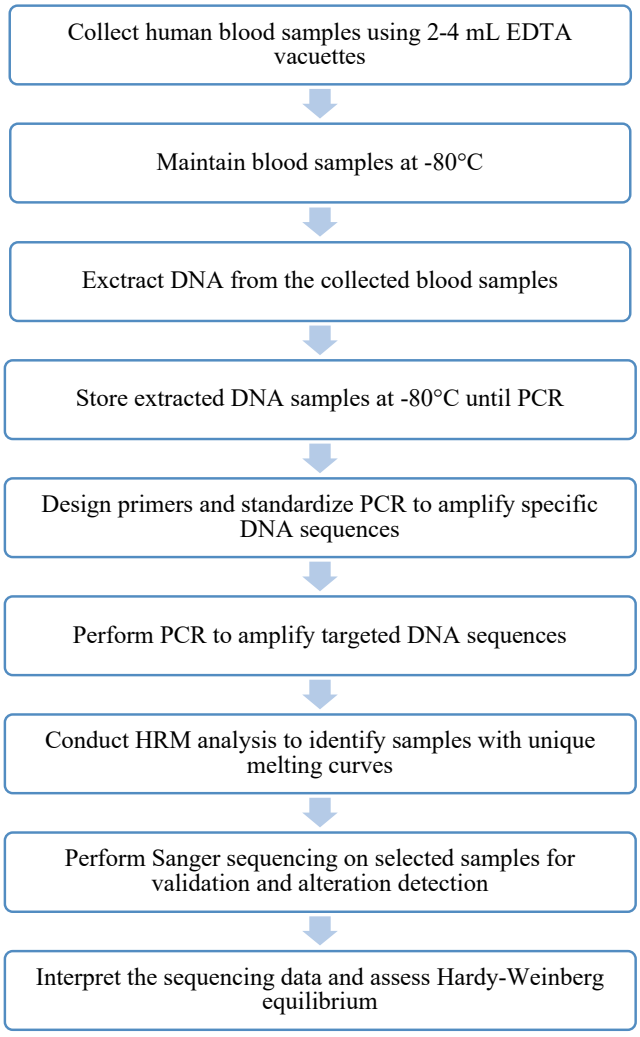

Figure 3 Process of genotyping the samples.

will be invited to the hospital for further screening for MASLD in the same pattern mentioned above. The detailed methodology is described as a flow chart in figure 2.

All cases and controls recruited as mentioned above will be subjected to nutritional assessment as well as physical activity assessment. The nutritional assessments will be done using a 3-day 24-hour dietary recall. Physical activity levels will be assessed using the validated PAQ-A, established by the College of Kinesiology, Canada.[18] The 24-hour dietary recall will yield comprehensive data on the participants' food patterns, while the PAQ-A will offer an understanding of their levels of physical activity. These assessments will help in identifying any possible correlations between food, physical activity and MASLD. Blood samples will be obtained for biochemical analysis and genetic testing. The biochemical analysis will include serum aspartate aminotransferase, alanine aminotransferase, fasting glucose, fasting lipid profile, fasting insulin, serum ceruloplasmin, anti-HBsAg and anti-HCV. The process of genotyping is presented as figure 3. A detailed description of the selected SNPs is presented under measurements.

Informed consent will be obtained from all the participants aged 18 years and above, and assent from individuals below 18 years of age. In addition, we will be documenting parental consent before enrolling the adolescent participants.

Measurements

Anthropometrics

We will do anthropometric measurements in accordance with the revised guidelines provided by the Indian Academy of Paediatrics.[19] The measurements will include height (in centimetres), weight (in kilograms), waist circumference (in centimetres) and BMI (in kilograms per square metre). Obesity is defined as having a BMI that is equal to or greater than the 95th percentile for age and sex in children aged 5 years and above. Overweight is defined as a BMI equal to or greater than the 85th percentile for age and sex in children aged 5 years and above.[19] Central adiposity is described as having a waist circumference that is larger than the 70th percentile for age and sex in children aged 2 years and above.[20]

DietCal—A Tool of Dietary Assessment and Planning' V.13.0 (Profound Tech Solution; http://dietcal.in/)

DietCal is a standardised tool for diet and nutrient analysis. The newer version of the DietCal software has been updated with the new Database from the Indian Food Composition Tables (IFCT) given by the National Institute of Nutrition in 2017.[21] (Commonly referred to as IFCT 2017). The software enables us to calculate the values of 177 nutrients for food items and also their sum total. The software also has a pictorial display of 431 food items to increase the accuracy of dietary data capture.

Liver ultrasound

A radiologist will perform sonography with a Philips Affiniti 70G ultrasound machine using a 2.5 MHz convex transducer to image the liver and to determine and grade liver steatosis for both the cases and controls. The grading will be based on a visual analysis of the intensity of echogenicity. The typical liver has a uniform appearance on ultrasound, with a level of brightness that is either the same as or slightly higher than that of the renal cortex and spleen. In the event of fatty infiltration, the liver exhibits increased echogenicity compared with the renal cortex and spleen. The radiologist will be visually assessing the intensity of echogenicity, assuming optimal gain setting to determine the grading of steatosis (0–3).[22]

Study proforma

A comprehensive study proforma was developed to collect the demographic and baseline details of the subjects for the study. This proforma is included as online supplemental appendix 2.

Nutrition and Physical Activity Questionnaires

A 3-day 24-hour recall

A 24-hour dietary recall is a structured interview intended to capture detailed information about all foods and beverages, along with portion sizes, consumed by the respondent in the past 24 hours. We will collect this information through structured interviews to accurately assess their dietary and nutrient intake patterns for three days (two weekdays and one weekend).

Individual Dietary Diversity Score

The Food and Agriculture Organization's IDDS measures an individual's dietary diversity by examining the variety of food types eaten over a given time frame.[23] Individuals provide information about their food intake by selecting from a predetermined list of nine food categories, and this information will be used to assess IDDS. The common food groups include starchy staples, dark green leafy vegetables, vitamin A-rich fruits and vegetables, other fruits and vegetables, meat and fish, eggs, legumes, nuts, seeds, and milk and milk products. The diversity score increases with food group usage. A diet with a high IDDS will have more nutrients and more food groups. This test helps identify an individual's areas for improvement in respect to the diversity of their diet. IDDS will be assessed using 24-hour dietary recalls.

PAQ-C and PAQ-A

PAQ-C and PAQ-A are validated questionnaires to assess the physical activity of school-going adolescents and are developed by the College of Kinesiology, Canada.[18] We will be using PAQ-C for children between the ages of 10 and 14 years and PAQ-A for children above the age of 14 years (online supplemental appendix 3).

PAQ-C and PAQ-A are also designed to assess physical activity of children aged 8–18 years. The PAQ-C is a self-administered tool using a recall of activities over a period of 7 days whose summary score is derived from nine items rated on a 5-point scale. The score computation is based on the assessment of spare time activity and incidence of the activity a week later, through five steps involved in scoring. The PAQ-A designed for grade levels 9–12 is identical to this except that the recess item is not included and eight items are summarised. Both instruments provide ordinal scales from 1 to 5 as in activity levels; the scores thus obtained reflect the general physical activity levels of children.[18 24 25]

SNP selection

There is a documented interaction between *PNPLA3* rs738409 and omega-6/omega-3 PUFA on hepatic fat content and ALT levels in children and adolescents of Caucasian, African American and Hispanic populations.[26] Individuals with *PNPLA3* rs738409 have a higher risk of fibrosis severity if they have a higher intake of carbohydrates (%) (29). This risk is particularly higher in the G-allele carriers.[27] *TM6SF2* rs58542926 variant predisposes to an increase in hepatic fat content.[28] This is thought to be associated with the impairment of very low-density lipoprotein release by the liver and accumulation of lipids.[28] This has shown a strong association with NASH in several studies.[29 30] *MBOAT7* rs641738 has also shown a strong association with NASH in several populations, including European and Asian populations.[31] The *GCKR* rs1260326 variant disrupts *GCKR* function, increasing hepatic glucose uptake and glycolysis, leading to acetyl-CoA overload and de novo lipogenesis.[32 33] The T allele of this variant reduces *GCKR's* ability to inhibit glucokinase, lowering blood glucose levels and promoting liver fat accumulation.[32 33]

Statistical analysis

We will use SPSS V.27 for the statistical analysis of this study. All study data will be captured on paper and will be converted to Excel for further analysis. We will report the association between MASLD and listed dietary parameters by using Pearson's χ^2 tests. All dietary variables (exposures) that are continuous in nature will be stratified into categorical groups after examining their distribution for conducting χ^2 tests. We will report the association between MASLD and listed SNPs using Pearson's χ^2 test. We will report the risk of MASLD across various levels of dietary parameters by using logistic regression. Similarly, the risk of MASLD based on the presence of selected SNPs will also be explored using logistic regression.

Dissemination

We will ensure that the participants' anonymity and privacy are maintained during the study by deidentifying data before sharing and statistical analysis. We will be securely storing all study data after encryption to maintain confidentiality. The study findings will be shared at various research conferences and published in peer-reviewed journals.

Patient or public participation

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

CONCLUSIONS

MASLD develops early in adolescence due to poor lifestyle habits, and this evolution may be influenced by genetic susceptibility. Modern research emphasises the role of nutrition, lifestyle changes and weight control in MASLD treatment. This study seeks to discover adolescent MASLD risk factors. Additionally, it will examine the genetic impact of key SNPs like *GCKR, PNPLA3, TM6SF2* and *MBOAT7* in MASLD. Exploring gene–nutrient interactions would help us build more effective MASLD management choices and early interventions. Nutrigenetic research in adolescents with MASLD is scarce in the Indian subcontinent. The current study seeks to fill these gaps.

Current study status

The study has started recruiting participants after receiving ethical clearance from the institutional review board of the study institution. We have initiated the genotyping process of these samples.

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Contributors RG, MR and SV designed the research question and prepared the initial draft of the protocol. RG, MR and LB contributed significantly to the development of the methodology. This was shared with LB, BVP and RP who provided their feedback. RG, MR and SV were responsible for obtaining ethics committee approval and recruiting participants for the study. MR was responsible for planning the statistical part of this study. The revised protocol was read and accepted by all the authors. MR will act as the guarantor of this study protocol.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Ethics Committee of Amrita School of Medicine, Amrita Institute of Medical Sciences, Kochi, Kerala, India (ID: ECASM-AIMS-2024-209). Participants gave informed consent to participate in the study before taking part.

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