BMJ Open

Association of iron deficiency anemia with dental caries in the permanent first molars of children aged 7–12 years in Karachi, Sindh, Pakistan: protocol for an analytical cross-sectional study

Sadaf Syed ,[1] Iqbal Azam,[1] Waseem Ahmed Jamalvi,[2] Farhan Raza Khan,[3] Ali Faisal Saleem ,[4] Shiyam S Tikmani[5]

To cite: Syed S, Azam I, Jamalvi WA, *et al*. Association of iron deficiency anemia with dental caries in the permanent first molars of children aged 7–12 years in Karachi, Sindh, Pakistan: protocol for an analytical cross-sectional study. *BMJ Open* 2025;**15**:e092312. doi:10.1136/bmjopen-2024-092312

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2024-092312).

Received 11 August 2024
Accepted 16 December 2024

Check for updates

© Author(s) (or their employer(s)) 2025. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ Group.

For numbered affiliations see end of article.

Correspondence to
Dr Shiyam S Tikmani;
shiyam.sunder@aku.edu

ABSTRACT

Introduction Iron deficiency anaemia (IDA) and dental caries are prevalent diseases among Pakistani children. Limited research has been done to explore their association with permanent teeth. Given the caries susceptibility of permanent first molars and their role in the development of ideal occlusion, this study aimed to estimate caries frequency in these molars and assess its association with IDA in 7–12 year-old children.

Methods and analysis This analytical cross-sectional study will include 141 children aged 7–12 years visiting physicians in the paediatric OPD of Dr. Ruth K.M. Pfau, Civil Hospital Karachi. Using consecutive sampling, children who met initial screening criteria were further evaluated to determine eligibility for the study. Data collection will involve physical examinations (including weight and height), oral examinations (including the relevant oral hygiene and caries assessments) and laboratory examinations (including the prescribed tests). In addition, questions will be asked about sociodemographic characteristics, history of IDA, oral hygiene habits, smokeless tobacco use and the frequency of cariogenic dietary consumption. Exposure variable will include the presence of IDA, assessed using complete blood count, C-reactive protein and ferritin tests and treated as a dichotomous variable. Outcome variable will include dental caries in at least one permanent first molar, assessed using the Decayed, Missing, and Filled Teeth index and also treated as a dichotomous variable. Analysis will include Poisson regression with robust variance, reporting prevalence ratios with 95% CIs for the association of IDA and dental caries in the permanent first molars. Frequency of children with carious permanent first molars with 95% CIs will also be reported.

Ethics and dissemination This research has been approved by ethical review committee of Aga Khan University (Reference number: 2024-9692-30593) and the institutional review board of Dow University of Health Sciences (IRB Reference: IRB-3556/DUHS/Approval/2024/196) before participant recruitment. Results will be disseminated through seminars and peer-reviewed publications.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will use objective laboratory tests for the diagnosis of iron deficiency anaemia and the validated Decayed, Missing, and Filled Teeth index, recommended by WHO, for caries diagnosis in children.
⇒ It will control for the confounding associated with the mixed dentition stage, by specifically focusing on the permanent first molar which has the greatest caries risk.
⇒ In addition, this study will be conducted among children belonging to the lower socioeconomic background, who are more likely to experience both the exposure and outcome; thereby controlling confounding associated with the socioeconomic status.
⇒ Even though this focus on permanent first molars of children belonging solely to low socioeconomic status will improve the internal validity of the study, it may compromise its external validity.
⇒ Furthermore, because this is a cross-sectional study and the exposure and outcome are measured at one point in time, it is inherently susceptible to temporal bias.

INTRODUCTION

Dental caries is the most prevalent global disease,[1] causing significant pain and impacting oral health-related quality of life[2] and nutritional intake[3] of children. In 2017, untreated dental caries affected approximately 2.3 billion people.[4] Caries prevalence in permanent teeth is highest in Africa (58.9%), followed by Asia (58.8%) and Australia (54.9%).[5] Regionally, its prevalence in the Eastern Mediterranean Region is 61% among 12 year olds and 70% among 15 year olds.[6] Although locally, its overall prevalence in Pakistan is 56.6%, with 61.2% in mixed dentition and 57.2% in permanent dentition.[7]

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Anaemia affects over 1.9 billion people globally[8] and had impacted 24.3% of the global population in 2021.[8] The highest prevalence of anaemia is found in Western and Central sub-Saharan Africa (47.4% and 35.7%, respectively) and South Asia (43%),[8] which overlaps with the regions heavily affected by dental caries. Dietary iron deficiency has been reported to be its leading cause among most demographic groups, including children.[8]

Despite its significant impact, particularly in children, research on iron deficiency anaemia (IDA) in Pakistani children aged 5–12 is limited, with varying prevalence rates reported across regions: 19% for 5–16 year-olds in rural Swat,[9] 62.2% for children under 15 in Lahore,[10] and 15.5% for 11–13 year-olds in Shaheed Benazirabad.[11]

Studies worldwide have found a significant association of IDA with caries in children,[12–14] although, most have focused on younger age groups, with limited representation of school-age children. Some studies have proposed mechanisms supporting this association such as impaired saliva buffering,[15] reduced enamel protection against demineralization,[16] and antibacterial effects of iron.[17 18]

Given the prevalence of both conditions and their significant adverse effects on children's health, establishing a link between IDA and caries in Pakistani school-age children is a crucial research gap. Both conditions may strain the healthcare system and socioeconomic conditions despite being preventable. This study aimed to address this gap by estimating the frequency of carious permanent first molars among children aged 7–12 and assessing its association with IDA. The focus on first permanent molars is significant due to their high caries risk[19] and their role in developing ideal occlusion within this age group.[20] By providing insights into this association, the study's findings could inform future longitudinal research and enhance the integrated management of dental and haematological care for affected children.

Objectives

1. To investigate the association between IDA and dental caries in the permanent first molars among children aged 7–12 years visiting a public tertiary care hospital in Karachi, Pakistan.
2. To estimate the frequency of children aged 7–12 years with carious permanent first molars visiting a public tertiary care hospital in Karachi, Pakistan.

Hypothesis

The prevalence of dental caries in the permanent first molars of 7–12 year-old children who have IDA is more than two times as compared with children who do not have IDA after adjusting for a priori confounders and other independent variables.

METHODS AND ANALYSIS

Study design

An analytical cross-sectional study will be used. This type of design measures the burden of exposure and outcome in a specific population simultaneously and, in doing so, assesses their association in comparative terms.

To ensure that the study design and reporting are transparent and comprehensive in presenting the findings of the study, the Strengthening the Reporting of Observational Studies in Epidemiology checklist will be followed.

Study site and setting

To overcome time constraints and for ethical reasons, we will conduct a hospital-based study to ensure an adequate number of children are recruited. Furthermore, to obtain a homogenous sample, Dr. Ruth K.M. Pfau, Civil Hospital Karachi located in Karachi, Sindh, Pakistan will be used in this study.

Civil Hospital Karachi

CHK is a 1900-bed public tertiary care and teaching hospital located on Mission Road in Karachi, Pakistan. Affiliated with Dow Medical College, it is government-funded and provides free treatment to all patients, mainly including patients from diverse backgrounds, predominantly from lower socioeconomic groups. The paediatric outpatient department (OPD) at CHK, operational Monday through Saturday during daytime hours, sees approximately 130 children aged 7–12 years daily. It has been selected as the study site due to its diversity and practicality in recruitment. This setting offers a balanced sample of healthier children, including those who may not attend school. This selection helps enhance the precision and internal validity of the study while acknowledging potential limitations in external validity.

Data collection will commence after receiving ethical approval from the institutional review board (IRB) and ethical review committee (ERC) of Dow University of Health Sciences (DUHS) and Aga Khan University (AKU), respectively. A pre-testing phase will take place from 2 to 7 December 2024, followed by recruitment and data collection from 9 December 2024 to mid-February 2025. The recruitment process aims to enroll between 1 and 4 participants per day. Despite the limited operational hours and detailed selection criteria, the recruitment process remains feasible. The high patient volume ensures a steady pace of enrollment. Data cleaning and analysis will take approximately 1 month, and manuscript preparation is expected to be completed by mid-April 2025.

Sampling technique and study population

In this study, the target population includes children aged 7–12 years visiting physicians at CHK, who have been prescribed C-reactive protein (CRP), ferritin and complete blood count (CBC) tests. Consecutive sampling technique will be used as participants will be recruited in the order they present at the paediatric OPD. This approach will also allow for efficient identification of eligible participants through an initial screening process based on key criteria that can be quickly assessed. The initial screening will determine whether the child is

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

accompanied by a family member from the same household, whether both individuals can understand Urdu, whether the child has been prescribed the relevant blood tests, and if the child has at least one fully erupted permanent central incisor in both arches, along with all fully erupted permanent first molars.

After this preliminary screening, a more thorough evaluation will be conducted to ensure that participants meet the full set of inclusion and exclusion criteria. This sampling approach facilitates the rapid identification of potential participants while ensuring rigorous verification of eligibility. It allows for a targeted and efficient selection process for the study.

Eligibility criteria

Inclusion criteria

The study will include children aged 7–12 years who reside with an adult family member in the same household and are able to communicate effectively in Urdu. Both the child and the family member must understand Urdu. This requirement is necessary because the first author, who is the sole data collector, is proficient only in this language. Financial constraints make hiring translators or additional data collectors infeasible. This requirement ensures clear communication and improves the accuracy and reliability of the data collected.

A key criterion for inclusion is that the child must be prescribed CRP, ferritin and CBC tests by a physician at the study site. This requirement ensures that the blood tests are medically justified. It also reassures the parents that the tests serve both clinical and research purposes, increasing their comfort with participation.

In addition, the child must be accompanied by an adult family member who resides in the same household. This is crucial for obtaining accurate socio-demographic information, details about the child's health behaviours and relevant household data, such as monthly expenditure. Children visiting the paediatric OPD are sometimes accompanied by relatives or non-family members who may not have sufficient knowledge of the child's health and household circumstances. Therefore, the inclusion of a household-residing family member helps ensure the accuracy of the information collected.

Furthermore, informed assent from the child and informed consent from the accompanying adult family member are mandatory for participation, in line with ethical guidelines to protect participant autonomy.

Exclusion criteria

However, children will be excluded from the study if they have been recently diagnosed with anaemia (eg, thalassemia, sickle cell anaemia or hereditary spherocytosis) by a healthcare professional. Other exclusion criteria include a history of chronic diseases such as diabetes, or chronic obstructive pulmonary disease as diagnosed by a healthcare professional, repeated blood transfusions; or long-term drug use (more than 3 months). Drugs such as antihypertensives, antihistamines, antiepileptics and non-steroidal anti-inflammatory drugs, are included, as they may adversely affect salivary flow.[21 22] Children will also be excluded if they are taking iron supplements, have a known history of cleft lip and/or palate, are undergoing orthodontic intervention, or have a known mental or physical disability. Exclusion criteria also include partially erupted or unerupted permanent first molar or both permanent central incisors in either jaw. In addition, children with overt fillings or crowns in the first permanent molars (placed for reasons other than caries in these teeth) or with overt developmental enamel defects such as enamel hypoplasia, hypomineralization and fluorosis in these teeth will be excluded.

Recruitment of participants

Potentially eligible children, along with their accompanying family members, will be respectfully approached in the paediatric OPD of CHK, for screening purposes. The child will be assessed for eligibility in the study through a screening questionnaire and a brief oral examination. If deemed eligible, these children and the adult family members will be invited to participate in the study.

The data collection process will include a pre-tested questionnaire, as well as physical, oral and laboratory examinations. The adult family member will provide information on the child's sociodemographic background and medical history. The child will answer questions about oral hygiene habits, other relevant behaviours and their cariogenic diet. Once the questionnaire is completed, the child will undergo a physical examination to assess height and weight. This will be followed by an oral examination, to evaluate oral hygiene using the Simplified Oral Hygiene Index (OHI-S) and carious status through the Decayed, Missing, and Filled Teeth (DMFT/dmft) index. This examination will focus on the permanent first molars and adjacent teeth but will also assess the entire dentition for carious teeth. Any findings from the oral examination will be communicated to the family member, and a referral slip for free treatment at the hospital's dental OPD will be provided if needed. In addition, oral hygiene instructions will be offered to the family through an educational flyer.

All data will be collected via the REDCap software. The first author will be responsible for administering the questionnaires, conducting the physical and oral examinations, and overseeing the study procedures. Blood samples, however, will be collected by trained personnel. The costs for laboratory tests, including CBC, CRP and ferritin, will be covered by the first author. These test results, typically available within 12–48 hours, will be accessible directly to both the first author and the participants. All study procedures will be completed within a single visit, ensuring convenience for participants while maintaining ethical standards through pre-obtained informed consent. Follow-up visits will not be required.

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Study variables

The study outcome variable is dental caries in the permanent first molars, assessed using the DMFT index.[23] Each permanent first molar will be examined and coded as 'D' for decay, 'M' for missing, 'F' for filled or 0 for sound. If the DMFT index score>0 of at least one permanent first molar, the child will be considered carious and will be treated as such in analysis (carious/non-carious).

The primary exposure in this study is IDA, defined by anaemia with haemoglobin level below 11.5 g/dL[24] and absolute iron deficiency. Iron deficiency will be indicated by ferritin levels below 15 µg/L,[25] when CRP is under 10 mg/L or by ferritin level below 70 µg/L when CRP is 10 mg/L or higher. These thresholds will account for the effect of infection or inflammation on ferritin.[26 27]

A priori confounders will include the usual monthly household expenditure, recorded as the total monthly household spending on food, utilities (gas, water and electricity), medical costs, clothing, social activities and miscellaneous items. Body mass index (BMI) will be calculated and categorised using the WHO growth charts for boys and girls aged 5–19 years.[28] Smokeless tobacco use will also be included as an a priori confounder, given its prevalence in Pakistan, where around 7.7% of adults use smokeless tobacco,[29] suggesting its use in children, particularly among the lower socioeconomic groups.[30] Other a priori confounders include parental and child education levels, the child's gender and age.

Additional independent variables that will be assessed as potential confounders, include previous diagnoses of IDA (since age 6), time of last dental visit, reasons for the visit, tooth brushing frequency and use of fluoridated toothpaste. Oral hygiene practices such as regular use (defined as at least once a day) of toothpowder, mouthwash, miswak and dandasa, will also be considered. The number of carious adjacent teeth (to the permanent first molar) will be assessed using the DMFT/dmft index.[23] A composite score of the frequency of cariogenic dietary consumption over the past 6 months will also be included as another independent variable. Lastly, OHI-S[31] will reflect plaque and calculus levels in the mouth. Scores will be categorised as follows: 0–1.2 as 'good', 1.3–3 as 'fair' and 3.1–6 as 'poor'.[32]

Data collection method and tools

Data collection method will include:

- Filling the screening questionnaire to assess the eligibility of the participant.
- After obtaining appropriate consent, filling the structured questionnaire that asks for information on the outcome variable, primary exposure variable, a priori confounders and the independent variables.

The questionnaires will be administered to the study population in Urdu. They are expected to take around 35–50 min to complete.

Screening questionnaire

The screening questionnaire will be administered after obtaining appropriate verbal consent. It will include questions and oral examinations consistent with the eligibility criteria of the study. After the relevant questions are asked and the child is considered eligible based on them, oral examination will take place.

The oral cavity examination will be conducted under adequate illumination. Occlusal assessment will involve retracting the cheek and asking the child to close their mouth to determine whether the relevant teeth of the opposing arches meet. This will verify if the permanent first molars and permanent central incisors have fully erupted. Unerupted or partially erupted status will only be noted for the molars if no prior extraction due to caries is confirmed.

Following this procedure, the permanent first molars will be visually inspected for overt fillings or crowns. The accompanying adult will be asked whether these restorations were placed for reasons other than decay. If both permanent central incisors in either arch or at least one permanent first molar is not fully erupted, and there are any fillings or crowns not related to decay in the permanent first molars, the child will be considered ineligible.

The oral examination will also assess each permanent first molar for enamel developmental defects using the modified developmental defects of enamel index (mDDE).[33] This index was originally introduced by the Federation Dentaire International (FDI) Commission on Oral Health in 1982[34] and modified by Clarkson et al.[33] It has been widely used in various studies, including one in Islamabad, Pakistan.[35] It will serve as a reference for detecting enamel defects predisposing the child to caries.[36 37] Demarcated opacity of ≤1 mm will be considered sound.[38] Defects will also be considered sound, if uncertainties arise, except for demarcated brown opacities, in which case a history will be taken.

Under proper illumination, the data collector will retract the child's cheek with a mouth mirror and use a dental explorer to enhance examination sensitivity. Assessment of the buccal, lingual/palatal and occlusal surfaces of the permanent first molar will be conducted to detect any white/cream/yellow or brown demarcated opacity. Its presence will result in exclusion. However, if there is any uncertainty regarding the brown opacities, history of frequent betel leaf and/or betel nut (with or without tobacco), gutkha, tea or coffee consumption will be taken. The absence of these habits will result in exclusion. In addition, inspection will identify diffuse white opacity with linear, patchy or confluent (combining into a chalky white area) distributions. It will also assess for hypoplasia including single or multiple pits and missing enamel (grooves, or larger areas of missing enamel). The presence of any of these conditions will render the child ineligible for the study.

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Study questionnaire

This questionnaire will have the following seven sections (for access to study questionnaire, refer to online supplemental material).

Sociodemographic information

This section will include the child's age, gender and education level. It will also include questions on the education level of both parents and the usual monthly household expenditure on food items, utilities including gas, water and electricity, medical-related, clothing items, social activities and miscellaneous items.

Medical history

This section will include questions on if the child has ever been diagnosed with IDA from the age of 6 by a healthcare professional, and how many times have they been diagnosed with it since then.

Oral hygiene-related behaviour

This section will include questions on when the child last visited the dentist, the reason for that visit, how frequently the child brushes their teeth, whether the child uses toothpaste, what toothpaste they use and whether they regularly use any of the listed oral hygiene practices at least once a day. These questions were obtained from the WHO Oral Health Assessment Form for Children[23] and were somewhat adapted to the objectives of this study.

Therefore, due to this adaptation, content validation was done by a team of eight dental experts. These experts rated each question for clarity and relevance on a scale of 1 (not relevant/clear) to 4 (very relevant/clear) and provided feedback for further improvement. Results indicated item-level content validity index (I-CVI) scores ranging from 0.875 to 1. The scale-level content validity index score, based on the average (S-CVI/Ave) and universal agreement method (S-CVI/UA), were 0.975 and 0.8, respectively, for both relevance and clarity. These scores indicate that this section of the study questionnaire is appropriately relevant and clear.[39]

Other habits

This section will include one question on how often the child uses smokeless tobacco (such as sinus, snuff and chewable tobacco like gutkha, betel leaf and/or areca nut with tobacco). This question was also obtained from the WHO Oral Health Assessment Form for Children[23] and adapted appropriately.

Dietary information

This section will include questions on how often, on average, in the last 6 months the child had consumed cariogenic food items. Recognising that processed starch and extrinsic sugars enhance dietary cariogenicity, the questionnaire focuses on food items that contain either or both and are commonly found in Pakistan.[40]

Physical and oral examination

Physical examination

The child's height will be measured using an inch tape, recorded in centimetres. It will be later converted to metres for calculating BMI. Weight will be measured in kilograms using the Tanita HA621 Manual Weight Scale, manufactured by Tanita Corporation, Japan.

Oral examination will include the OHI-S and the DMFT/dmft index of the permanent first molars and their adjacent teeth.

Simplified Oral Hygiene Index

The OHI-S, developed by Greene and Vermilion in 1964,[41] provides a quick, quantitative assessment of an individual's oral hygiene[41] by combining the simplified debris and calculus indices, which measure the presence of plaque and calculus, respectively.[31]

Before the oral examination begins, the child will be comfortably seated. The permanent first molars and central incisors will be visually inspected under sufficient lighting, using an explorer to enhance detection. A mouth mirror will be used to retract the cheek as needed. If the upper right or lower left permanent central incisor is missing, the adjacent permanent central incisor will be inspected instead. For the OHI-S, the buccal surfaces of the upper permanent first molars, upper right and lower left permanent central incisors, and the lingual surfaces of the lower permanent first molars will be inspected.

Debris Index Simplified (DI-S)

DI-S will assess the amount of debris present on each tooth surface. Scores will range from 0 to 3: 0 indicates no debris or extrinsic stain, 1 indicates debris covering no more than one-third of the tooth surface or the presence of extrinsic stain, 2 indicates debris covering between one-third and two-thirds and 3 indicates debris covering more than two-thirds of the tooth surface. The overall debris index for each child will be calculated by summing the scores of all surfaces examined and dividing by the number of surfaces assessed.

Calculus Index Simplified

Calculus Index Simplified (CI-S) will similarly assess the presence of supragingival or subgingival calculus. A score of 0 indicates no calculus. A score of 1 indicates supragingival calculus covering no more than one-third of the surface. A score of 2 indicates coverage between one-third and two-thirds, or the presence of subgingival calculus flecks. A score of 3 indicates supragingival calculus covering more than two-thirds, and/or a continuous band of subgingival calculus. The overall calculus index will be calculated similarly to the debris index, by summing the scores of all surfaces and dividing by the number of surfaces examined.

DMFT/dmft index of the permanent first molar and its adjacent teeth

The DMFT index, widely used for caries diagnosis, is an aggregate of the total number of decayed, missing (caused by caries) and filled teeth, reflecting the carious

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

status of the dentition. Although it is typically used for both primary and permanent dentitions, it can also be applied specifically to certain teeth, such as the permanent first molars,[42] as is the case in this study.

Oral examination will be conducted under adequate illumination. The cheek will be retracted with a mouth mirror as necessary, and the teeth will be dried with gauze to enhance the visual inspection. Furthermore, an explorer will also be used to aid caries detection. Each permanent first molar and its adjacent permanent tooth will be assessed. The teeth will be classified as 'D' (decayed), 'M' (missing due to caries), 'F' (filled) or '0' (sound). A tooth will be coded as 'D' if an overt cavity, undermined enamel or softened walls/floor are detected on probing, with or without a permanent filling. If a temporary filling is present, the tooth will still be recorded as decayed ('D'). If a permanent filling is present due to previous decay (as confirmed by questioning the child and the accompanying adult), and no signs of active decay are observed, the tooth will be coded as 'F'. In cases where a tooth is missing and confirmed to be missing due to caries (through questioning the child and the accompanying adult), it will be coded as 'M'. If no such condition is present, the tooth will be considered sound and coded as '0'.

The DMFT score for each child will be calculated separately for the permanent first molars and their adjacent permanent teeth by adding their respective scores, with equal weight given to the 'D', 'M' and 'F' codes. This process will also be applied to primary second molars, using the dmft index, with results recorded accordingly.[23]

Laboratory-related information

Data will be collected on ferritin, haemoglobin and CRP to diagnose IDA. Ferritin will be measured in micrograms/L, haemoglobin in g/L and CRP in mg/L. All blood tests will be conducted at the collection centre of Dow Diagnostic Research and Reference Laboratory (DDRRL), located adjacent to CHK. Established in 2007, DDRRL adheres to international standards, providing high-quality diagnostic services, emphasising on accuracy and reliability.[43] The laboratory maintains internal quality assurance through daily controls.[43] Its medical directorate is also trained in compliance with ISO 15189:2022 standards for medical laboratories.[44]

For each participant, 2 mL of venous blood will be drawn into an ethylenediaminetetraacetic acid (EDTA) tube for haemoglobin analysis. EDTA tubes prevent coagulation and preserve cellular components.[45] In addition, 6 mL of blood will be collected in two 3 mL gel tubes for CRP and ferritin analyses. Gel tubes are necessary for serum separation, which is required for these tests.[45] The collected samples will be transported to DDRRL on the same day, using ice packs to maintain appropriate temperatures during transit. The ice packs will be monitored and replaced as needed to preserve sample integrity.

On arrival at the laboratory, the samples will be promptly analysed. CBC will be analysed using the Abbott Alinity hq Haematology Analyzer, CRP using the Atellica CH CRP_2 by Siemens Healthineers and ferritin using the Atellica IM Ferritin system, also by Siemens Healthineers.

Data management

Data will be collected using a web-based questionnaire through REDCap software. The first author will perform daily checks to ensure completeness, accuracy and logical consistency. Any inconsistent or missing information (marked as 'I don't know') will be verified by contacting the accompanying adult family member. REDCap's conditional logic and range checks will help maintain data accuracy. All study questions will be set as required fields to minimise missing values.

Data cleaning and detection of missing values will be managed by the first author by sorting each variable in ascending and descending order. To further ensure data quality, the first author, a qualified and trained dentist, will be actively involved in conducting data collection. This involvement will ensure ethical and sensitive handling of information, minimise bias and promote uniformity throughout the process.

To ensure cultural appropriateness, the questionnaires were translated from English to Urdu by a translator, and reviewed by an expert. Necessary adjustments were made during the review, and a back translation was performed to preserve the original intent and meaning of the questions. Pre-testing of the study tools will also be conducted on 5% of the study sample (n=7 children) at the study site to identify any questions requiring paraphrasing or re-translation.

Validity

Several potential biases could affect the validity of this hospital-based study. First, confounding bias may arise due to unequal distribution of undiagnosed or unreported medical conditions, medication histories and unaccounted-for cariogenic items in the child's diet, which could influence caries risk.

Second, with bias might be introduced through study questions on sociodemographic factors, diet, smokeless tobacco consumption and oral hygiene behaviours. This can be mitigated by refining the questions to be more neutral and less judgmental during pre-testing. In addition, a more objective assessment of oral hygiene will be performed using the OHI-S to complement self-reported data.

Third, recall bias could occur when collecting information on the child's cariogenic diet. This will be mitigated by focusing on the child's diet over the past 6 months and verifying responses with the accompanying family member.

Lastly, non-differential misclassification could result from using a manual weight scale. This will be minimised by calibrating the machine daily and verifying its accuracy with a 1 kg weight. Each child will be weighed twice; if discrepancies arise between the measurements, the scale will be recalibrated and the weight reassessed.

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

Sample size

The sample size is calculated for both objectives as follows.

Frequency of children aged 7–12 with carious first permanent molars

The sample size is calculated using the WHO Sample Size Determination in Health Studies Software, Version 2.0.21. A previous study conducted in Pakistan reported a frequency of 38.5%[46] for carious permanent first molars. Using this figure, with an absolute precision of 9% and a 95% confidence level (chosen to maintain study feasibility), a minimum sample size of 113 children aged 7–12 years is calculated.

Association of IDA and dental caries in first permanent molars in children aged 7–12

The sample size for this objective was calculated using STATA Software, Version 15.0, with a power of 80% and a significance level of 5%. The ratio of exposed to unexposed children is derived from a study conducted in the Shaheed Benazir district of Pakistan among 11–13 year-olds, which reported an IDA prevalence of 15.5%.[11] This yields a ratio of 1:5. The proportion of unexposed children with the outcome (dental caries) is obtained from a study conducted in India,[47] which reported that 37.8% of children without IDA have dental caries. Using these parameters, a minimum sample size of 76 children aged 7–12 years is calculated to assess the association between IDA and dental caries in first permanent molars. This sample size will detect a one-sided prevalence ratio of 2.

Since the sample size calculated for the first objective is larger, it will be used for this study. After accounting for a 20% non-response and ineligibility rate, a final minimum sample size of approximately 141 children aged 7–12 years is obtained.

Statistical analysis

All analyses will be conducted using STATA Software, Version 15.0. The frequency of children with carious permanent first molars will be reported along with 95% CIs.

Descriptive statistics will be presented for all independent variables (such as age, gender, monthly household expenditure, parental and child education levels and BMI, etc.) stratified by IDA status. Categorical variables (eg, gender, child's education status) will be reported as frequencies and percentages. The distribution of quantitative variables will be assessed for normality using box plots, histograms and the Kolmogorov-Smirnov test. For normally distributed variables (eg, age, number of adjacent carious teeth), means and SD will be provided. For skewed distributions, medians and interquartile ranges (IQRs) will be reported.

Differences between exposure groups for categorical variables will be assessed using the chi-square test, provided the expected frequency per cell is at least 5; otherwise, Fisher's exact test will be used. For quantitative predictors, independent sample t-tests will be conducted, and p values will be reported.

To analyse the cariogenic dietary construct, Principal Component Analysis will reduce this variable into a single composite score, categorising the frequency of cariogenic food consumption into low, medium and high levels.

To assess the association between IDA and dental caries in permanent first molars, Poisson regression with robust variance will be used. Initially, univariate analyses will assess IDA, along with a priori confounders and other independent variables (eg, age, gender, education level, tooth brushing frequency, etc.), for their association with caries in permanent first molars. Crude prevalence ratios with 95% CIs will be reported. Variables with a p value of <0.25 at the univariate level will be checked for multicollinearity along with IDA (r>0.8).

Subsequently, confounding and multivariable analyses will be conducted. A stepwise model-building approach will be used to construct the multivariable model, starting with IDA and sequentially adding variables. Likelihood ratio tests will be used to determine whether each added variable significantly improves the model fit ($p < 0.05$). Biologically plausible interactions will also be assessed ($p < 0.1$). The final, parsimonious model will be determined when no additional variables can be included, and none can be removed without affecting the model's fit. Adjusted prevalence ratios with 95% CIs for caries in permanent first molars will be reported.

Patient and public involvement statement

The patients and the public are and will not be involved in the design, conduct, reporting, or dissemination plans of this research.

ETHICS AND DISSEMINATION

All study activities will be conducted in accordance with the Declaration of Helsinki and the International Council for Harmonisation (ICH) guidelines for Good Clinical Practice (GCP). Ethical approval has been obtained from the ERC of AKU (Reference number: 2024-9692-30593) and the IRB of DUHS (IRB Reference: IRB-3556/DUHS/Approval/2024/196).

Before enrollment, informed assent and consent will be obtained from the participating children and their accompanying adult family members, respectively. Data collection will take place in a private space to protect their confidentiality. Sensitive questions, including those related to tobacco use, will be posed in a neutral and non-judgmental manner. This approach will foster a comfortable environment for participants, thereby minimising any potential discomfort associated with discussing sensitive topics and upholding the highest ethical standards in our research.

To ensure participant safety, required blood tests will be performed by trained personnel. Any child needing dental treatment will be referred to dentists at CHK for free dental care. The 'tell-show-do' technique will be used

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

to help ease anxiety during the oral examination, and instructions on paediatric oral hygiene will be provided via flyers.

Confidentiality will be maintained throughout the study. Data will be anonymized by assigning each participant a unique eight-character alphanumeric identification (ID), with no direct link to their personal information in documents used for analysis or dissemination. The link between participant contact details and ID will be kept in a separate file under the custody of the first author. Data will be stored in a password-protected database, with backup files, and will be securely deleted 7 years after study completion using erasure software.

The study results will be disseminated through seminars and published in a peer-reviewed academic journal.

Author affiliations
[1]Epidemiology and Biostatistics Section, Department of Community Health Sciences, Aga Khan University, Karachi, Pakistan
[2]Department of Pediatrics, Dr. Ruth KM PFau Civil Hospital, Dow University of Health Sciences, Karachi, Pakistan
[3]Dental Surgery Section, Department of Surgery, Aga Khan University, Karachi, Pakistan
[4]Paediatrics and Child Health, Aga Khan University, Karachi, Pakistan
[5]Population & Reproductive Health Section, Department of Community Health Sciences, Aga Khan University, Karachi, Pakistan

Acknowledgements The authors acknowledge the use of ChatGPT, a Large Language Model developed by OpenAI, for paraphrasing the text in an attempt to enhance its readability. However, the authors are solely responsible for the content and accuracy of this protocol.

Contributors SS and SST conceived and designed the study. SS will be responsible for data collection and data management. SS, SST and IA will be involved in data analysis and interpretation. SST, IA, WAJ, AFS and FRK have revised the work and have provided their intellectual input. SS wrote the first draft of the article. SS is the guarantor. The authors acknowledge the use of ChatGPT, a Large Language Model developed by OpenAI, for paraphrasing the text in an attempt to enhance its readability. However, the authors are solely responsible for the content and accuracy of this protocol.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

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.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Sadaf Syed http://orcid.org/0009-0008-8735-0199
Ali Faisal Saleem http://orcid.org/0000-0003-1804-9868

REFERENCES

1 Wen PYF, Chen MX, Zhong YJ, *et al*. Global Burden and Inequality of Dental Caries, 1990 to 2019. *J Dent Res* 2022;101:392–9.
2 Lawal FB, Oke GA. Satisfaction with dental condition and oral health-related quality of life of school-age children with dental pain in Ibadan, Nigeria. *SAGE Open Med* 2021;9:20503121211025944.
3 Shim S-H, Han D-H, Khang Y-H. Association between Dental Caries and Delayed Growth in Korean Children. *Caries Res* 2018;52:71–7.
4 Bernabe E, Marcenes W, Hernandez CR, *et al*. Global, Regional, and National Levels and Trends in Burden of Oral Conditions from 1990 to 2017: A Systematic Analysis for the Global Burden of Disease 2017 Study. *J Dent Res* 2020;99:362–73.
5 Kazeminia M, Abdi A, Shohaimi S, *et al*. Dental caries in primary and permanent teeth in children's worldwide, 1995 to 2019: a systematic review and meta-analysis. *Head Face Med* 2020;16:22.
6 Kale S, Kakodkar P, Shetiya S, *et al*. Prevalence of dental caries among children aged 5–15 years from 9 countries in the Eastern Mediterranean Region: a meta-analysis. *East Mediterr Health J* 2020;26:726–35.
7 Siddiqui AA, Alshammary F, Mulla M, *et al*. Prevalence of dental caries in Pakistan: a systematic review and meta-analysis. *BMC Oral Health* 2021;21:450.
8 GBD 2021 Anaemia Collaborators. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990-2021: findings from the Global Burden of Disease Study 2021. *Lancet Haematol* 2023;10:e713–34.
9 Akhtar N, Zeb J, Ali H, *et al*. Anemia and iron deficiency anemia in school children of district Swat Khyber Pakhtunkhwa Pakistan. *J Entomol Zool Stud* 2016;4:366–8.
10 Kishwar F, Ashraf T, Hanif I, *et al*. IRON DEFICIENCY ANEMIA: PREVALENCE IN CHILDREN OF LAHORE. *The Prof Med J* 2015;22:1122–5.
11 Jamali NH, Mahesar H, Bhutto MA. Prevalence of Iron Deficiency Anaemia in School and College Going Students of District Shaheed Benazirabad Sindh Province, Pakistan. *OJBD* 2016;06:67–78.
12 Younus MZ, Shah SM, Mushtaque U, *et al*. Deficiency of Vitamin D and Anemia among Pakistani Children with Early Childhood Caries: A Case-Control Study. *PJMHS* 2022;16:941–3.
13 Schroth RJ, Levi J, Kliewer E, *et al*. Association between iron status, iron deficiency anaemia, and severe early childhood caries: a case-control study. *BMC Pediatr* 2013;13:22.
14 Ji S, Guan X, Ma L, *et al*. Iron deficiency anemia associated factors and early childhood caries in Qingdao. *BMC Oral Health* 2022;22:104.
15 Mahantesha T, Reddy K, Ellore V, *et al*. Evaluation and association of iron deficiency anemia with salivary pH and buffering capacity in children aged 6-12 years. *Natl J Physiol Pharm Pharmacol* 2014;4:229.
16 Xu L, Wang J, Han R, *et al*. Iron level participates in the pathological damages of dental caries in infant rats by affecting enamel mineralization. *J Clin Pediatr Dent* 2023;47:86–94.
17 Ribeiro CCC, Ccahuana-Vásquez RA, Carmo C do, *et al*. The effect of iron on Streptococcus mutans biofilm and on enamel demineralization. *Braz Oral Res* 2012;26:300–5.
18 Pecharki GD, Cury JA, Paes Leme AF, *et al*. Effect of sucrose containing iron (II) on dental biofilm and enamel demineralization in situ. *Caries Res* 2005;39:123–9.
19 Nelson SJ. Wheeler's Dental Anatomy, Physiology And Occlusion. Eleventh Ed. S.L: Elsevier - Health Science, 2019.
20 Proffit WR. Early Stages of Development. Contemporary Orthodontics. Fifth Ed. St. Louis, MO, USA: Elsevier Mosby, 2013:66–91.
21 Glick A, Sista V, Johnson C. Oral Manifestations of Commonly Prescribed Drugs. *Am Fam Physician* 2020;102:613–21.
22 Zhou C, Shen J, Wen H-Y, *et al*. Reported oral side effects of antiepileptic medications. *Aust N Z J Dent Oral Health Ther* 2019;7.
23 Poul Erik BRJ, Petersen. Oral Health Surveys: Basic Methods. Fifth Ed. Geneva: World Health Organization, 2013:125.
24 Ahmad MS, Farooq H, Maham SN, *et al*. Frequency of Anemia and Iron Deficiency among Children Starting First Year of School Life and Their Association with Weight and Height. *Anemia* 2018;2018:8906258.
25 Unicef UNU, World Health Organization. Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers. Geneva, 2001.
26 Pagana KD, Pagana TJ, Pagana TN. Mosby's®diagnostic and laboratory test reference. Fifteenth ed. Elsevier, 2020.

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

27 WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. Geneva World Health Organization2020; 2020.82.
28 Organization WH. Growth reference 5-19 years - bmi-for-age (5-19 years). 2007. Available: https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age
29 Zhao L, Mbulo L, Twentyman E, *et al*. Disparities in smokeless tobacco use in Bangladesh, India, and Pakistan: Findings from the Global Adult Tobacco Survey, 2014-2017. *PLoS One* 2021;16:e0250144.
30 Abbasi HQ, Oduoye MO, Valentina Sih CM. The hidden menace of smokeless tobacco in Pakistan: understanding the risks and possible solutions; a letter to the editor. *Int J Surg* 2023;6:e0169.
31 Wei SH, Lang NP. Periodontal epidemiological indices for children and adolescents: II. Evaluation of oral hygiene; III. Clinical applications. *Pediatr Dent* 1982;4:64–73.
32 Pudentiana Rr RE, Purnama T, Tauchid SN, *et al*. Knowledge of Oral and Dental Health Impacts the Oral Hygiene Index Simplified (OHI-S) of Primary School Children. *Ind J Forensic Med Toxicol* 2021;15:2179–83.
33 Clarkson J, O'Mullane D. A modified DDE Index for use in epidemiological studies of enamel defects. *J Dent Res* 1989;68:445–50.
34 An epidemiological index of developmental defects of dental enamel (DDE Index). Commission on Oral Health, Research and Epidemiology. *Int Dent J* 1982;32:159–67.
35 Maxood SZA, Abdullah S. Enamel defects frequency in deciduous teeth. *Pak Oral Dent J* 2014;34.
36 Seow WK. Developmental defects of enamel and dentine: challenges for basic science research and clinical management. *Aust Dent J* 2014;59 Suppl 1:143–54.
37 Abanto Alvarez J, Rezende KMPC, Marocho SMS, *et al*. Dental fluorosis: exposure, prevention and management. *Med Oral Patol Oral Cir Bucal* 2009;14:E103–7.
38 Lygidakis NA, Wong F, Jälevik B, *et al*. Best Clinical Practice Guidance for clinicians dealing with children presenting with Molar-Incisor-Hypomineralisation (MIH): An EAPD Policy Document. *Eur Arch Paediatr Dent* 2010;11:75–81.
39 Yusoff MSB, Department of Medical Education, School of Medical Sciences, Universiti Sains Malaysia, MALAYSIA. ABC of Content Validation and Content Validity Index Calculation. *EIMJ* 2019;11:49–54.
40 Amezdroz E, Carpenter L, Johnson S, *et al*. Feasibility and development of a cariogenic diet scale for epidemiological research. *Int J Paediatr Dent* 2019;29:310–24.
41 Greene JC, Vermillion JR. THE SIMPLIFIED ORAL HYGIENE INDEX. *J Am Dent Assoc* 1964;68:7–13.
42 Gudipaneni RK, Alkuwaykibi AS, Ganji KK, *et al*. Assessment of caries diagnostic thresholds of DMFT, ICDAS II and CAST in the estimation of caries prevalence rate in first permanent molars in early permanent dentition-a cross-sectional study. *BMC Oral Health* 2022;22:133.
43 Sciences DUoH. DOW diagnostic research and reference laboratory.
44 Sciences DUoH. Dow Diagnostic Research & Reference Laboratory (DDRRL) team achieves milestone in medical diagnostics.
45 Buck DB, Buck GA. Chapter 8 - utilizing diagnostic studies. In: Ballweg R, Sullivan EM, Brown D, et al, eds. *Physician assistant*. Philadelphia: W.B. Saunders, 2008: 120–45.
46 Asghar S, Butt RA, Jafari SIA, *et al*. Frequency Of Dental Caries On Individual Tooth Surfaces Of First Permanent Molars Between 6-13 Years Old Children. *J Bahria Univ Med Dent Coll* 2015;5:16–9.
47 Bansal K, Goyal M, Dhingra R. Association of severe early childhood caries with iron deficiency anemia. *J Indian Soc Pedod Prev Dent* 2016;34:36–42.

BMJ Open: first published as 10.1136/bmjopen-2024-092312 on 7 January 2025. Downloaded from http://bmjopen.bmj.com/ on June 14, 2025 at Agence Bibliographique de l Enseignement Superieur (ABES) . Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.