# **BMJ Open** Investigating the impact of oral health on pregnancy and offspring outcomes: protocol for the Lifetime Impact of ORal heAlth (LIORA) cohort study

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# ABSTRACT

Introduction Oral health is a fundamental component of well-being, and is closely associated with overall health and quality of life. Oral health may also affect the next generation. The children of mothers with poor oral health are likely to also have poor oral health as they go through life. We aim to investigate associations between maternal oral health and general health, pregnancy outcomes, offspring oral health and offspring general health.

Methods and analysis The Lifetime Impact of Oral Health study is a prospective, observational cohort study being done at a single centre in Chongqing, China. A total of 1000 pregnant women will be recruited in their first trimester (11-14 weeks gestation). After obtaining informed consent, general and oral health assessments will be undertaken. Maternal lifestyle, demographic data and biospecimens (blood, hair, urine, nail clippings, saliva, dental plaque, buccal, vaginal and anal swabs) will be collected. Pregnancy outcomes will be recorded at the time of delivery. Cord blood and placenta samples will be collected. The offspring will be followed up for general and oral health examinations, neurodevelopmental assessments and biospecimen (dental plaque, saliva, buccal swabs, exfoliated primary dentition, urine, hair, nail clippings) collection until they are 15 years old. Biological samples will undergo comprehensive metabolomic, microbiome and epigenome analyses. Associations between maternal oral health and general health, pregnancy outcomes, offspring oral health and offspring general health will be investigated and the underlying mechanisms explored.

Ethics and dissemination This project has been approved by the Research Ethics Committee of the Affiliated Hospital of Stomatology of Chongging Medical University (CQHS-REC-2021 LSNo.23). Participants will be required to provide informed consent to participate in the study. Dissemination of findings will take the form of publications in peer-reviewed journals and presentations at national and international conferences. Trial registration number ChiCTR2100046898.

#### STRENGTHS AND LIMITATIONS OF THIS STUDY

- $\Rightarrow$  The Lifetime Impact of Oral Health study is a birth cohort study investigating the oral health of pregnant women and the health of their offspring in mainland China.
- $\Rightarrow$  Data collection will continue until the offspring are 15 years old.
- $\Rightarrow$  The longitudinal data and biospecimens collected will enable investigation of the factors contributing to early-life oral health and oral disease.
- $\Rightarrow$  The study location is limited to a single city in China (Chongqing).

#### INTRODUCTION

Oral health is a fundamental component of health,<sup>1</sup> and is among the top 10 standards of human health identified by the WHO. The oral cavity is considered the intersection of dentistry and medicine, and a window into uning, general health.<sup>2</sup> Thus, oral health is not only important on its own, but also closely associated with overall well-being and quality of life. For example, periodontitis, one of the most common oral diseases-characterised by microbially associated host-mediated inflammation that results in loss of periodontal attachment—is a progressive inflammatory disease.<sup>3</sup> It is associated with a number of **O** other chronic conditions such as cardiovascular disease, type 2 diabetes, pulmonary 8 disease, osteoporosis, stroke, hypertension, rheumatoid arthritis, dementia and kidney disease.<sup>2</sup> <sup>4–9</sup> It remains unclear whether those associations are causal. The major influences are thought to be environmental and lifestyle factors, as well as the host immune response to microorganisms. It has been shown that the relationship between periodontal disease and type 2 diabetes may be bidirectional, with

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the regulation of one condition positively affecting the other.

Pregnant women, as a special group of the population, are not exempt from the oral disease-systemic condition association. Rather, it may be amplified or present in other forms during gestation. Pregnancy is characterised by a series of structural and functional adjustments in the cardiovascular, immune, gastrointestinal and metabolic systems. The association between pregnancy complications and maternal periodontal disease has been shown in previous studies.<sup>4</sup> Changes in lifestyle, such as diet and physical activity, and certain changes during pregnancy, such as elevated hormone levels, may be risk factors for oral diseases such as periodontitis, gingivitis and dental caries-also known as tooth decay, which is a biofilmmediated, sugar-driven, multifactorial, dynamic disease resulting in the phasic demineralisation and remineralisation of dental hard tissues.<sup>10 11</sup> Morning sickness or oesophageal reflux, as well as changes in diet and oral hygiene practices during pregnancy, can promote tooth demineralisation and thus a higher risk of dental caries.<sup>12</sup> On the other hand, oral diseases may cause pregnancy complications. For instance, it has been proposed that oral microorganisms, their metabolites or the inflammatory mediators that are associated with periodontitis, may enter the circulation or the fetus to induce pregnancy complications or adverse outcomes, such as pre-eclampsia, gestational diabetes mellitis (GDM) or preterm birth.<sup>13 14</sup>

Birth cohort studies can help understand the causation of diseases in early life,<sup>15</sup> and such studies that include oral health can provide information on the association between maternal oral health and pregnancy outcomes. A review article has analysed 15 oral health birth cohort studies from 5 continents.<sup>15</sup> Of these, only four commenced during pregnancy and enable scrutiny of the association between maternal oral health and systemic health, as well as the association between maternal and child health. The four studies were conducted in Brazil (two), Thailand (one) and Uganda (one). One limitation of all four cohort studies was the limited number of biospecimen types and associated analytical methods.

With economic development, the general health of the mainland Chinese population has improved substantially in the past decade. However, a comparison of the findings of the Fourth National Oral Health Epidemiology Survey in China from 2015 to 2017 with those of the National Oral Health Epidemiology Survey conducted 10 years prior<sup>1617</sup> indicates that the dental caries experience of the Chinese population is worsening. The higher Decayed, missing, filled teeth (DMFT) scores may be due to changes in diet and difficulties in accessing dental care.<sup>17</sup> Efforts to improve oral health must be supported by ongoing research on the demographic, biological and sociobehavioural characteristics that affect susceptibility to oral diseases. Whether poor oral health affects pregnancy outcomes and what may be the underlying mechanism(s) are interesting and important questions. To our knowledge, there has been no birth cohort study investigating

the oral health of pregnant women and its impact on mothers and their offspring in mainland China.

#### **Objectives**

In order to improve both the long-term oral health and overall health of the Chinese population, and to provide valuable information on the relationship between maternal and child health, we aim to establish a pregnancy and birth cohort study in Chongqing, China. Our hypothesis is that maternal oral health has a detectable  $\neg$ and substantial impact on their general health and pregnancy outcomes, along with the child's health. The information collected on the health of the mothers and their offspring will include: (1) maternal oral health, such 2 as periodontal and dental health; (2) general maternal 8 health; (3) pregnancy outcomes (pregnancy complications, such as GDM and preterm birth); (4) fetal development; (5) child general health; (6) child oral health and (7) child neurodevelopment. The name of the proposed including for uses relat cohort study is the Lifetime Impact of Oral Health (LIORA) study.

#### **METHODS AND ANALYSIS** Study design

The LIORA study is a prospective, observational cohort study. A total of 1000 pregnant women and their offspring will be recruited, surveyed, examined and biospecimens will be collected from 11 to 14 weeks of pregnancy until the children reach 15 years of age.

#### Setting and participant recruitment

data Pregnant women who attend clinical visits at the two designated hospitals at 11-14 weeks of pregnancy between 2021 and 2024 will be invited to participate. The two study sites are the main hospital (located at Yuzhong District ≥ of Chongqing) and branch (Jinshan) hospital of the First Affiliated Hospital of Chongqing Medical University (CQMU). The obstetrics departments of both hospitals are operated by the same group of medical professionals, some of whom work in both hospitals. Staff at the Affiliated Stomatological Hospital of CQMU will undertake <u>0</u> the mothers' oral assessment and infant follow-up. E-advertising of the study will be placed in mass media, such as Weibo and WeChat, two popular social apps in China.

Pregnant women approached by research nurses will be provided with information related to the study. Special emphasis will be made about the longitudinal nature of the study and the need to provide multiple biospecimens **3** from themselves and their child, as well as to respond to a series of questionnaires throughout the study. They will also be informed that a paternal buccal swab is optional, requiring a separate consent from the father, and that the decision on whether to provide this sample will not affect their participation in the study. Following an opportunity to ask questions related to the study, the pregnant women will be given the option to consent to, or refuse, participation. The information and consent sheets will

be available in Mandarin, which is the main language spoken in the Chongqing region. Women who meet the inclusion criteria for the study and consent to participate will then receive an appointment for their first study visit. Participants will be welcome (though not obliged) to bring a support person with them when meeting the research team member to discuss the study and provide consent. They may also bring a support person to any study visit. After giving birth, each mother will be given contact information for the research team at the Affiliated Stomatological Hospital of CQMU, where the main follow-up assessments of the mothers and their children will be conducted.

#### Inclusion and exclusion criteria

Included in the study will be women who are: (1) aged 18-45 years, living in Chongqing, China; (2) at the 11-14 week stage of singleton pregnancy and (3) able to provide written informed consent. Excluded from the study will be those who have had a previous pregnancy with complications that resulted in delivery before 32 weeks.

#### **Withdrawal**

All participants will be free to withdraw from the study at any time for any reason without impacting on future medical care. If a participant withdraws before completing the study, the reason for the withdrawal will be recorded. If a participant does not return for a scheduled visit, every effort such as messages and phone calls will be made. Participants who refuse to attend any longer will be recorded as 'withdrawls' and those we are unable to contact will be recorded as 'drop-outs'. If a participant withdraws from the study, no further evaluation will be performed, nor any additional data will be collected. However, all previously collected data and biospecimens will remain in storage for analysis.

#### **Study timeline**

This cohort will run from 2021 until the final study visit is completed, when the youngest child in the cohort turns 15 years of age. It is anticipated that recruitment will take place over a 36-month period, starting once ethical and regulatory approvals are in place. Following up for a period of 15 years postnatally enables evaluation of the impact of the exposome in pregnancy on the long-term dental development and oral health of the children.

#### Visit schedule

The visit schedule for the participants is shown in tables 1 and 2. Each woman will need to visit the hospital at ten specific time points throughout the study. During each visit, different questionnaires, physical assessments and biospecimen collection will be conducted. Starting from birth, the children will be followed up for a period of 15 years.

 
 Table 1
 Visit schedule for maternal measures and samples
during pregnancy

	Visit			
	1	2	3	
Data and sample collected	11–14 weeks	22–28 weeks	32–34 weeks	
Lifestyle questionnaire (emotion, stress, physical exercise, smoking and drinking status)	$\checkmark$	V	$\checkmark$	
Nutritional questionnaire (food frequency and 24-hour food and supplement intake)	$\checkmark$	$\checkmark$	$\checkmark$	
Oral health questionnaire	$\checkmark$	$\checkmark$	$\checkmark$	
Abdominal ultrasound	$\checkmark$	$\checkmark$	$\checkmark$	
Maternal anthropometry	$\checkmark$	$\checkmark$	$\checkmark$	
Oral glucose tolerance test		$\checkmark$		
Oral health examination	$\checkmark$	$\checkmark$	$\checkmark$	
Saliva, dental plaque and buccal swabs	$\checkmark$	$\checkmark$	$\checkmark$	
Blood	$\checkmark$		$\checkmark$	
Urine	$\checkmark$	$\checkmark$	$\checkmark$	
Hair	$\checkmark$		$\checkmark$	
Finger nail clippings	$\checkmark$	$\checkmark$	$\checkmark$	
Vaginal swab		$\checkmark$	$\checkmark$	
Anal swab		$\checkmark$	$\checkmark$	
Paternal buccal swab (optional)	$\checkmark$	$\checkmark$	$\checkmark$	

#### Sample size

Protected by copyright, including for uses related to text and data mining A sample size of 1000 is considered sufficient to allow studies of children and common oral conditions such ≥ as dental caries. For example, the prevalence of Early l trair Childhood Caries (ECC), the dental caries affecting children up to 6 years of age,<sup>18</sup> was 63.5% in 3–5 years old in China.<sup>19</sup> A maternal sample size was also calculated based on the prevalence of GDM in women with periodontitis (ie, the prevalence of periodontitis in women with GDM S was 44.8% and 13.2% in women without GDM).<sup>20</sup> For a type I error of 0.05, a type II error of 0.2 and a power of 0.8, the sample size required is 32. To take into account ē a 20% loss rate in follow up,<sup>21 22</sup> the required sample size increases to 40. With the prevalence of GDM in China estimated to be 17%,<sup>23 24</sup> we expect our sample size of 1000 pregnant women will include 170 women with GDM, and, of these, there will be approximately 76 with periodontitis. Therefore, a recruitment sample of 1000 women will have sufficient power for the study.

### **Data collection**

#### Maternal data

Data collection will include information from the women on environmental, health and lifestyle factors at study visits 1, 2, 3 and 5-10 (tables 1 and 2). Information to

Table 2	Schedule for maternal and offspring visits at and
after deliv	ery

	4	5	6–10
Data and samples collected	Delivery	1 year	3, 6, 9, 12, 15 years
Mother			
Delivery mode, gestational age, pregnancy outcome			
Lifestyle questionnaire (emotion, stress, physical exercise)			
Nutritional questionnaire (food frequency and 24- hour food)			
Oral health questionnaire		$\checkmark$	
Anthropometry			
Oral health examination			
Saliva, dental plaque and buccal swabs		$\checkmark$	
Urine		$\checkmark$	
Finger nail clippings		$\checkmark$	
Hair		$\checkmark$	
Offspring			
Cord blood and placenta sample			
Feeding and health			
Lifestyle questionnaire (emotion, stress, physical exercise)		$\checkmark$	$\checkmark$
Nutritional questionnaire (food frequency and 24- hour food)			$\checkmark$
Oral health questionnaire			
Neurodevelopment assessment (Gesell Developmental Schedules)			
Anthropometry		$\checkmark$	
Oral health examination			
Saliva and buccal swabs		$\checkmark$	
Exfoliated primary dentition			
Urine			$\checkmark$
Hair		$\checkmark$	
Finger nail clippings			

be collected from the women over the three prenatal visits will include past and present medical and health history, pregnancy and sociodemographic factors (age, education, occupation, family), along with lifestyle factors such as smoking and alcohol consumption.<sup>21 25</sup> Clinical assessments will be conducted at each visit, with a range of measurements (weight, height, waist circumference and blood pressure) recorded. Biological samples collected at these visits will include blood, urine, hair, saliva, dental

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plaque, buccal swabs and fingernail clippings. Liver and kidney function tests and fetal ultrasounds will also be conducted. Questionnaires seeking information about maternal stress, physical activity during pregnancy and dietary habits (including supplements) will also be completed using the WeChat applet Wenjuanxing (a platform providing functions equivalent to Amazon Mechanical Turk). Details of the questionnaires are provided below.

#### Birth data

The research nurses will attend the delivery. Detailed information will be collected from medical records related to the pregnancy, delivery and any medical complications. Pre-eclampsia is defined on the basis of pregnancyinduced hypertension, proteinuria and maternal systemic complications; small gestational age and large gestational age are defined with the 10th and 90th centile and the growth standards developed by Prof. Hua Zhang<sup>26</sup>; preterm birth is defined as deliveries before the threshold of 37 weeks<sup>27</sup>; GDM will be diagnosed using the criteria **Z** of the International Association of Diabetes & Pregnancy Study Groups Consensus Panel.<sup>28</sup> The sex,birth weight, birth length and head circumference of the infants, length of labour and whether admission to the neonatal unit were required, will be recorded along with Apgar scores. Placental tissue samples and cord blood will also be collected. texi

Should the birth occur at a hospital other than the two designated hospitals, the family will be excluded from the study at this point due to the lack of birth-related information and samples.

#### Offspring data

The mothers and their children will attend the Affiliated Stomatological Hospital of CQMU for study visits ⊳ 5–10, until the children reach 15 years of age. Lifestyle information about the children will be collected from their parents at each visit to evaluate their health status. Samples collected will include urine, hair, saliva, finger ھ nail clippings, dental plaque and buccal swabs. In addinan enppings, dentai plaque and buccal swabs. In addi-tion to questionnaires on infant feeding and food pref-erences, assessments related to temperament, sleep patterns, oral health, body measurements and neurode-velopment will be taken. Questionnaires All questionnaires, including Pregnancy Stress Scale (applet) Costational Nutrition Knowledge (cost of)

(applet), Gestational Nutrition Knowledge (paper), Attitudes and Behaviour (paper), Brief Infant Sleep Questionnaire (applet), Carey Toddler Temperament Questionnaire (paper), Food Frequency Questionnaire, 24-hour Food Recall Questionnaire (paper), Nutrition Questionnaire for Children (paper), and Medication use (paper) use validated scales and measures, according to a previously published protocol.<sup>29</sup> The Wenjuanxing applet or paper questionnaires will be used accordingly.

The responses in both forms will be entered in electronic databases by research staff.

#### Oral health

At enrolment and follow-up visits, the participants will complete a questionnaire regarding their age, socioeconomic status, sugar intake, oral hygiene practices, oral health knowledge and attitudes including information about past dental visits (recent dental treatment and dental fear), smoking status, alcohol consumption, and history of any systematic diseases. The oral-health-related quality of life will be evaluated with Locker's Global Oral Health Item<sup>30</sup> and the Oral Health Impact Profile-14 (Chinese version) questionnaire during study visit 1.<sup>31</sup> The Early Childhood Oral Health Impact Scale (ECOHIS, Chinese version) and short-form Parental-Caregiver Perceptions Questionnaire will be applied during study visits 5 and 6 to determine the impact of any dental conditions on children under 5 years of age and their families.<sup>32 33</sup> The Child Perceptions Questionnaire (Chinese version) will be used at study visit 9 to measure oral-health-related quality of life for children aged 11–14 years.<sup>34</sup>

#### Physical activity

The Pregnancy Physical Activity Questionnaire (Chinese version) will be used to assess the levels of physical activity during pregnancy.<sup>35</sup> For 6–15 years old children, the short form of International Physical Activity Questionnaire will be used to assess their level of physical activity.<sup>36</sup>

#### Gesell developmental schedules

Gesell Developmental Schedules (Chinese Revision) is a standardised assessment of motor (fine and gross), language (receptive and expressive) and cognitive development of infants, toddlers and preschool children aged from 2 months to 9 years. It will be administered by a trained professional and consists of a series of play tasks.

#### **Assessments**

All maternal body measurements-including anthropometry, liver and kidney function, ultrasound scans and oral glucose tolerance test, and also all child body measurements-will be conducted according to a previously published protocol.<sup>29</sup>

#### Oral health examination

Oral health of the mothers will be evaluated at visits 1, 2, 3 and 5-10, while the oral health assessments for the children will be conducted once a year during visits 5–10 by trained and callibrated dentists. Dental caries, malocclusion and other oral conditions will be recorded according to a Chinese standard used in national surveys.<sup>37</sup> Clinical definitions of periodontitis and gingivitis will use the staging and grading classification proposed by Tonetti et al.<sup>3</sup> Periodontal data including bleeding on probing, probing depth (PD), clinical attachment loss at six sites (mesiobuccal, midbuccal, distobuccal, distolongual, midlingual and mesiolingual) per tooth (except for the third molars) will be collected. Enamel defects on the

children's teeth will be recorded using the DDE index at age 9 or 10 for teeth 16, 26, 36, 46, 14, 13, 12, 11, 21, 22, 23 and 24. Extraoral facial scans at rest position and intraoral scans will be taken for mothers and children.

#### **Biospecimens**

All maternal and offspring biospecimens including peripheral blood, cord blood, placenta, urine, hair, finger nail clippings, saliva and buccal swabs will be collected according to a previously published protocol<sup>29</sup> and stored as described in the following section.

#### Blood

Protected by copy A total of 12mL of venous blood will be collected at each visit: 6 mL in a 10 mL coagulant tube (for serum) and 6mL in a 10mL EDTA anticoagulant tube (plasma, leucocytes and red cells). Fasting blood samples will be preferred, and postprandial samples will be noted by the researchers. The samples will be temporarily placed in a thermal bag (containing two frozen 200 mL ice packs) immediately following collection, and transported to the Bu laboratory within 30 min where they will be centrifuged to obtain serum, plasma, red cells and leucocytes. Ten Q 200 µL serum aliquots, 10×200 µL plasma aliquots and uses related 2×200 µL red cell aliquots will be stored.

#### Cord blood and placental tissues

Umbilical cord blood will be collected from the umbilical vein into a 10 mL EDTA anticoagulant tube and a 10 mL procoagulant tube. Twenty pieces (2 mm × 2 mm × 4 mm) of placental tissue from each of the fetal and maternal a surfaces will be randomly collected using a 4mm skin data mining, biopsy punch. The samples will be immediately placed in precooled (4°C) saline solution for transportation to the laboratory within 30 min of collection, for storage.

#### Urine

Al trair A midstream urine sample will be collected at each maternal prenatal visit. A 15mL sterile centrifuge tube will be used to collect 10 mL of urine. The urine samples will be placed in a thermal bag (containing two frozen 200 mL ice packs) and transported to the laboratory nd within 30 min where they will be centrifuged at  $845 \times g$ similar for 10min and then 10×200 µL supernatant aliquots will be stored.

### Hair

technolog Approximately 30 hairs will be cut from near the scalp of the mothers and their children. For newborn babies, three tufts of infant hair will be collected instead. Aluminium foil will be wrapped around hairs at the time of collection and labelled to identify the scalp proximal end. The samples will be transported in a thermal bag (containing two frozen 200 mL ice packs) to the laboratory within 30 min of collection, and stored.

#### Nails

Maternal fingernail samples will be collected at each prenatal visit. Following collection, the clippings will be immediately wrapped in aluminium foil and then transported to the laboratory within 30 min for storage.

#### **Oral samples**

Study participants will be advised to avoid eating, drinking, smoking or chewing gum 30 min prior to providing oral samples. A clean water mouth rinse will be provided 10 min prior to sample collection. Saliva will be be collected using a passive drool method. To do this, Participants will be asked to allow saliva to pool in their mouth, then lower their head and drool 2-3 mL of saliva into a specimen tube. The saliva will be immediately put into a thermal bag (containing two frozen 200 mL ice packs) and transported to the laboratory within 2 hours. On arrival, the sample will be centrigufed at  $9391 \times g$ ,  $4^{\circ}C$ for 15 min. The supernatant will be transferred into a new tube for metabolomics analysis, while the pellet will be resuspended in bacterial preserving solution provided by Puroton Technology (Chongging, China). The prepared samples will be stored at -80°C. Following saliva sample collection, the coverage of teeth by dental plaque will be recorded. Dental plaque/oral biofilm samples will be collected from the buccal surface of the upper anterior teeth, the mid-dorsal surface of the tongue and the glossal supragingival surface of a lower molar using disposable dental microbrush applicators. A periodontal probe will be used to collect plaque from the deepest periodontal pockets on the left-hand and right-hand sides, or interproximally between posterior molars in the absence of deep periodontal pockets. Buccal swabs will be collected from the mothers at visits 1, 2 and 3. A sterile swab will be used to rub the entire area of both left and right buccal mucosa for 15 times each. The swabs will then be immediately stored in sterile tubes. These will be used to measure DNA methylation profiles throughout pregnancy to identify potential epigenetic biomarkers.

#### Exfoliated primary dentition

Parents will be asked to collect all of their child's primary teeth that have either been shed naturally or extracted manually, and to rinse them in plain tap water, gently pat them dry with absorbent paper, store them in a sealed container in their home freezer and bring them to the researchers on the following visit. Once received, the soft tissue and contaminants on the teeth will be removed and the teeth will be washed with distilled water in our laboratory. The teeth will then be dried, sealed in a labled bag and stored.

#### Vaginal swab

Vaginal swabs will be collected from the mothers at visits 1, 2 and 3 to analyse the microbiome. Prior to collection, participants will be asked to refrain from sexual intercourse for 24 hours. A sterile swab will be used to rub the inside of vagina by the gynaecologist. The swabs will then be immediately stored in sterile tubes with stabilising buffer (Puroton, Chongqing, China).

## Anal swab

Anal swabs will be collected from the mothers at visits 1, 2 and 3 to analyse the microbiome. A sterile swab will be used to rub the inside of anus by the gynaecologist. The swabs will then be immediately stored in sterile tubes with stabilising buffer (Puroton, Chongqing, China).

#### Sample storage and biobank

All samples except hair and nail clippings will be stored in 0.5 or 1 mL cyrovials with preprinted barcodes (Nest, China). The hair and nail samples, wrapped in aluminium foil, will be stored in resealable barcoded bags. Prior to storage in a biobank facility, each sample will be scanned by a Tracxer code reader (Micronics) enabling the sample and its location to be tracked in the database (Yiducloud, China). Hair and nail samples will be stored in -20°C freezers (Zhongke MeiLing, China). Placental tissues will be preserved in liquid nitrogen tanks (Locator 4 Plus). All other samples will be stored in -80°C freezers (907, Thermo Fisher Scientific, American). The -80°C freezers (907, Thermo Fisher Scientific, American) have Bu temperature sensors (PT100) and a monitoring system ō (Smart-Vue, Thermo Fisher Scientific, France) as well as a backup liquid nitrogen cooling system (DPL452, Taylor uses related Wharton, USA).

#### Sample analysis

Based on the purpose, the samples will be subjected to to text different types of analyses, including genome sequencing and metabolomics.

#### Metabolomics analysis

Samples will be first extracted using organic solvents and then analysed using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Both targeted and untargeted approaches will be used to discover and identify potential biomarkers.

#### Microbiome analysis

, Al train The V1-V3 regions of bacterial 16S rRNA and the fungal Bul ITS2 region of rRNA will be amplified from microbial genomic DNA using universal primers. Demultiplexed ھ sequencing data will be analysed using QIIME software. Alpha diversity will be measured by R software, using the phyloseq package. Beta diversity will be represented using weighted UniFrac distance measure, and contributions to technologies. the differences in the beta diversity will be presented as principlal coordinate analysis using OIIME.

#### **Epigenetic analysis**

DNA methylation will be analysed using targeted bisulfite sequencingfor specific loci and the Infinium MethylationEPIC BeadChip for genome-wide assessment. Both approaches require bisulfite conversion of DNA, followed by multiplexed PCR and library preparation for targeted bisulfite sequencing.

#### **Data storage**

The data will be deidentified (the names of participants will be replaced with unique identity numbers) by the

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research team and then stored in a database run by Taimei Medical Technology, (China). To protect privacy and confidentiality, access and use of the data will be under strict conditions with approval from all principal investigators required.

#### **Data analysis**

The data will be analysed by team members who are trained in multivariate and longitudinal data analysis, and are experienced in the organisation and analysis of cohort study data. The team keeps current by taking courses and workshops in epidemiology and statistics, and by working collaboratively with scientists who are recognised as leaders in their field. Statistical analyses will be conducted using SPSS V.24.0 software (IBM), Stata V.16.1 (StataCorp) and R V.4.1.2.

The first analytical task will to 'distill' the very large number of site-level and surface-level maternal oral health variables to a set of meaningful person-level variables. Those derived variables will represent static/cumulative (prevalence, severity) measures of disease experience for the mothers but, as the prospective child oral health data collection continues, dynamic (incidence, increment) child-level measures of disease experience will become available. The team has expertise and considerable experience in reducing both cross-sectional and longitudinal dental data-sets in this way. The primary analytical principle is collection of the oral health data at site/surface level, but analysis of those data at the person level. Once the person-level oral health and disease variables have been created, univariable and multivariable analyses will be used to examine and test the study hypotheses, with appropriate regression approaches used to test hypotheses about the emergence, persistence and developmental antecedents of child oral diseases and conditions such as developmental defects of enamel.

Continuous variables will be reported as numbers of observed and missing values, mean, SD of the mean, median and range. Categorical variables will be described as frequencies and percentages. Univariable and multivariable analyses will be conducted using various methods to examine the study hypothesis. Cross-tabulations (and  $\chi^2$  tests), t-tests and analysis of variance will be used to compare two or more groups. Regression modelling will be used to reveal the emergence, persistence and developmental antecedents of child oral diseases and conditions. Principal components analysis, orthogonal partial least squares discriminant analysis and correlation analysis will be used for the metabolomics and microbiomics data.

### Patient and public involvement

None.

#### ETHICS AND DISSEMINATION

The LIORA Study was approved by the Chongqing Medical University Institutional Review Board in May 2021

(Ethical approval number: COHS-REC-2021(LSNo.23)), and registered in the Chinese Clinical Trial Registry (Registration number: ChiCTR2100046898). The Clinical Research Management Committee of the Affiliated Hospital of Stomatology of CQMU will be responsible for data and safety monitoring in this study. All members of the committee are independent of the study team. This committee will oversee all ethical and safety issues in accordance with the National Health Commission of the People's Republic of China regulations, policies and  $\neg$ guidance relating to clinical research. The study staff, or a designated member of the research team, will ensure the women receive a copy the Participant Information Š Sheet, that the details of the study have been explained to them and they have received responses to any questions concerning the study. The participants will be given sufficient time to decide whether they would like to to participate. If they agree, the research staff member and the participant must both sign and date the informed consent form before participation can begin. One copy of the consent will be kept by the participant, and one copy will be stored by the investigator in a locked cabinet.

The data will be analysed by professional researchers uses related and the findings will be broadly disseminated through conference presentations and peer-reviewed publications. Participants will be advised that they can access the publications and presentations through the project website (currently under construction).

#### DISCUSSION

China has a high prevalence of pregnancy complications such as GDM, as well as oral diseases such as periodontitis, ECC and malocclusion, requiring urgent government and academic attention.<sup>19 23 38 39</sup> Birth cohort studies provide insight into the earliest determinants and protective factors of oral health, and potential risk of oral disease, and the underlying mechanism of pregnancy complications.<sup>15</sup> To achieve our goal, we plan to recruit a large number of pregnant women and follow them throughout their pregnancy. We will continue to follow their children, once born, for more than a decade. At specified time points during the study, we will collect a variety of biospecimens and data. These will be analysed to better understand the prevalence and trajectory of common diseases in pregnancy and oral health.

To the best of our knowledge, this is one of only a few cohort studies on the oral and general health of mothers and their offspring in China. One such study was the Children of 1997, a Hong Kong Chinese birth cohort.<sup>40</sup> The strengths of our study include: (1) the comprehensive biospecimen collection, enabling metabolomic and microbiomic analysis rarely seen in previous studies; (2) the long-term follow-up and data collection beginning in early pregnancy and continuing during prenatal visits, birth, and the first 15 years of the offspring's life, which has not been done in many other birth cohort studies; (3) the focus on maternal oral health during pregnancy,

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in particular periodontitis, which is implicated in infection, inflammation and metabolic issues in mothers and in adverse birth outcomes; and (4) the investigation of a Chinese population in a developing Asian city that has not been extensively studied previously.

This cohort study will provide valuable information on maternal and offspring health in a typical Chinese community. It will require intensive effort and plenty of resources over an extended period of time.

To our knowledge, the LIORA study is the first birth cohort study investigating the oral health of pregnant women and its impact on mothers and their offspring in mainland China. For the offspring, data will be collected both prenatally and postnatally until they are 15 years old (eg, oral health, anthropometry, nutrition, cognitive outcomes) and we will also collect data relating to maternal lifestyle, oral health, living environment and nutrition predelivery and postdelivery. The wealth of longitudinal data and biospecimens collected as part of this study will enable an unparalleled investigation of the factors contributing to early-life oral health and oral disease. A wide range of women will be recruited to gain as representative sample as possible; however, a potential limitation is that the study location is limited to a single city in China (Chongqing).

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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.

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