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## PAKistan Study of prEmature coronary atHerosclerosis in young AdultTs (PAK-SEHAT): A prospective longitudinal study protocol investigating the prevalence, severity and determinants of atherosclerotic cardiovascular disease in the young adult Pakistani population

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### Recommended Citation

Hanif, B., Sheikh, S., Peerwani, G., Cainzos-Achirica, M., Javed, W., Baqar, J. B., Samad, Z., Bashir, F., Virani, S. S., Nasir, K. (2023). PAKistan Study of prEmature coronary atHerosclerosis in young AdultTs (PAK-SEHAT): A prospective longitudinal study protocol investigating the prevalence, severity and determinants of atherosclerotic cardiovascular disease in the young adult Pakistani population. *BMJ Open*, 13(11), e076045.




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# BMJ Open PAKistan Study of prEmature coronary atHerosclerosis in young AdulTs (PAK-SEHAT): a prospective longitudinal study protocol investigating the prevalence, severity and determinants of atherosclerotic cardiovascular disease in the young adult Pakistani population

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**To cite:** Hanif B, Sheikh S, Peerwani G, *et al.* PAKistan Study of prEmature coronary atHerosclerosis in young AdulTs (PAK-SEHAT): a prospective longitudinal study protocol investigating the prevalence, severity and determinants of atherosclerotic cardiovascular disease in the young adult Pakistani population. *BMJ Open* 2023;**13**:e076045. doi:10.1136/bmjopen-2023-076045

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2023-076045>).

Received 26 May 2023  
Accepted 17 August 2023



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## ABSTRACT

**Introduction** Atherosclerotic cardiovascular disease (ASCVD) is a major cause of morbidity, mortality and health expenditures worldwide. Despite having higher ASCVD in the Pakistani population, data on subclinical coronary atherosclerosis in young Pakistanis remain scarce. The PAKistan Study of prEmature coronary atHerosclerosis in young AdulTs (PAK-SEHAT) aims to assess the prevalence, severity and determinants of subclinical coronary atherosclerosis among Pakistani men (35–60 years) and women (35–65 years) free of clinically symptomatic ASCVD and will assess 5-year rates of ASCVD events.

**Methods and analysis** PAK-SEHAT is an ongoing prospective cohort study with 2000 participants from all provinces of Pakistan who will be interviewed at the baseline along with phlebotomy, measurement of carotid intima-media thickness (CIMT) and coronary CT angiography (CCTA). Phlebotomy will be repeated at 2.5 years, whereas CIMT and CCTA will be repeated at 5 years. We will report the frequency of maximal coronary stenosis  $\geq 50\%$  and  $\geq 70\%$ , number of coronary vessels with plaque and the number of coronary segments affected per participant on CCTA. We will use Cox proportional hazards regression models to evaluate the association between baseline characteristics and incident ASCVD events during follow-up. These associations will be presented as HRs with 95% CIs.

**Ethics and dissemination** The study protocol was approved by the Tabba Heart Institute Institutional Review Board (THI/IRB/FQ/22-09-2021/016). All study procedures are consistent with the principles of the Declaration of Helsinki. Findings of the study will be disseminated via peer-reviewed publications and conference presentations.

**Trial registration number** NCT05156736.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is a prospective longitudinal nationwide study with a comprehensive follow-up of 5 years.
- ⇒ Adjudication of plaque burden and morphology via coronary CT angiography (CCTA).
- ⇒ Biobanking and genome sequencing on the sample DNA of participants to understand the genetics of the South Asian population vulnerable to atherosclerotic cardiovascular disease (ASCVD).
- ⇒ Sufficiently large and adequately powered sample size.
- ⇒ Participants with body mass index higher than 40 might be at a higher risk of ASCVD but due to the limitation of CCTA assessment, we will not be able to enrol such participants.

## INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) remains one of the leading causes of morbidity, mortality and health expenditures worldwide,<sup>1</sup> and is now a major public health issue in many densely populated low-income and middle-income countries undergoing rapid urbanisation.<sup>2</sup> Among them, multinational studies such as INTERHEART and PURE, as well as immigrant studies in Western countries, have shown that South Asian (SA) populations harbour a higher burden of ASCVD than most other geographical and ethnic groups.<sup>3–10</sup> For instance, in a recent analysis in the UK Biobank, SA participants had a 2.03 age and sex-adjusted HR for ASCVD compared with participants of European ancestry.<sup>5</sup> Moreover, ASCVD

typically presents at a younger age in SAs,<sup>3 11–13</sup> and when compared with patients of other ethnicities with a premature event, SA patients tend to have more severe coronary atherosclerosis.<sup>12 14 15</sup> Studies also suggest that the burden of ASCVD is heterogeneous across SA populations, with research in countries such as the UK or Spain suggesting that Pakistanis may have a higher risk of ASCVD than Indians.<sup>5 16</sup> Despite these concerning trends, studies of the burden and determinants of premature ASCVD in Pakistan have so far been scarce.<sup>17–19</sup>

Subclinical coronary atherosclerosis is a powerful driver of clinically overt ASCVD events later in time.<sup>20</sup> In recent years, coronary CT angiography (CCTA) has become increasingly available, and the associated radiation dose has been significantly reduced, and studies such as SCAPIS in Sweden and the Miami Heart Study in the USA have used CCTA to assess coronary plaque in large asymptomatic populations.<sup>21 22</sup> Characterising the prevalence and determinants of subclinical plaque in a population at increased risk of premature ASCVD can help inform primary prevention strategies and potential plaque-screening approaches in Pakistan. These initiatives, informed by high-quality, contemporary data, can ultimately help reduce the burden of ASCVD in the country.

The PAKistan Study of prEmature coronary atHerosclerosis in young AdulTs (PAK-SEHAT) aims (1) to determine the prevalence and severity of subclinical coronary atherosclerosis among young Pakistani men and women free of clinically symptomatic ASCVD, and (2) to assess the 5-year rates of ASCVD clinical events and progression using repeat CCTA and carotid intima-media thickness (CIMT) among Pakistanis with varying degrees of subclinical coronary plaque burden. The secondary objectives of the study are to (1) determine the prevalence of diabetes, hypertension, dyslipidaemia and other cardiovascular risk factors; (2) identify the key determinants of subclinical coronary atherosclerosis and ASCVD in this population; (3) estimate the incidence of diabetes, hypertension, dyslipidaemia and other cardiovascular risk factors at 5 years of follow-up.

## METHODS AND ANALYSIS

We will recruit 2000 young Pakistani men and women with no known clinical ASCVD (ratio of 1:1) through a prospective cohort study with a follow-up of 5 years. The age range of male participants at enrolment is 35–60 years and 35–65 years for female participants. **Box 1** summarises the study's inclusion and exclusion criteria. To ensure a representative sampling across the entire age spectrum, age is divided into five age categories in men (intervals of 5) and six age categories in women (intervals of 5), and almost 200 participants will be recruited in each age category. First-degree relatives of index participants will not be recruited to avoid clustering of genetic effects. The baseline data collection of this study started in March 2023 and is expected to end in January 2024.

### Box 1 Inclusion and exclusion criteria in PAK-SEHAT

#### Inclusion criteria

- ⇒ Men 35–60 years of age and women 35–65 years of age.
- ⇒ Asymptomatic individuals without known cardiovascular disease.
- ⇒ Able to comprehend and sign an informed consent form.

#### Exclusion criteria

- ⇒ Participants assessed to be unavailable for study follow-up, for example, migrating out of the country, migrating to another city where PAK-SEHAT Programme is not available.
- ⇒ History of major cardiovascular events (angina, myocardial infarction, prior coronary revascularisation), cerebrovascular disease including stroke and transient ischaemic attack, peripheral arterial disease, either diagnosis or surgery for abdominal aortic aneurysm, or heart failure.
- ⇒ Serum creatinine >1.5 mg/dL.
- ⇒ BMI >40; the CT scan machine specification does not allow to screen patients with higher BMI and morbid obesity.
- ⇒ Any contraindication for CT scanning or non-iodinated contrast.
- ⇒ Active malignancy.
- ⇒ Current pregnancy (assessed by dipstick in women who have amenorrhoea in the last 1 month).
- ⇒ Failure to provide informed consent.

BMI, body mass index; PAK-SEHAT, PAKistan Study of prEmature coronary atHerosclerosis in young AdulTs.

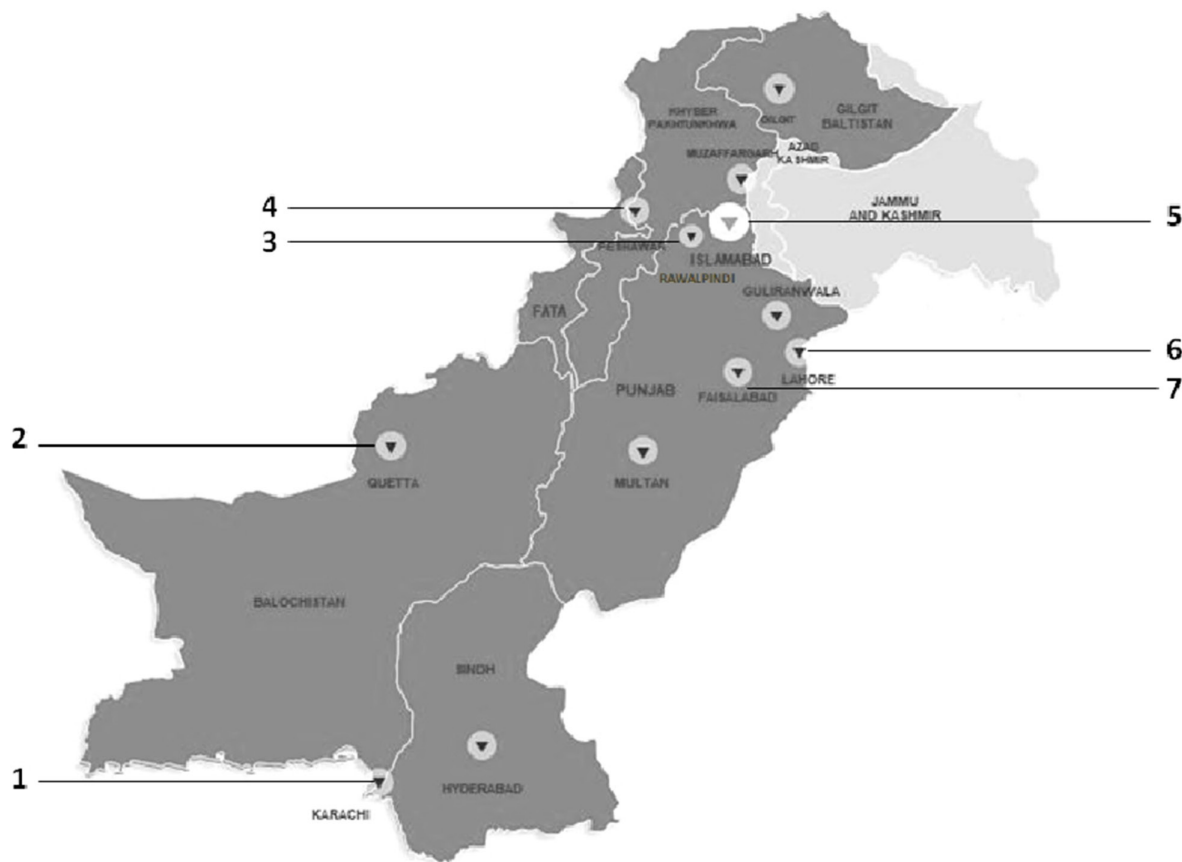
The follow-up for each enrolment is 5 years; hence, the study will end by January 2029.

### Sample size

The sample size of 2000 participants was estimated based on an expected 30% positivity of plaque on CCTA, the margin of error of 2.1% and alpha of 5% using OpenEpi sample size calculator.<sup>23 24</sup> The sample size was estimated to be 1826 individuals. To account for the potential poor quality of some CCTA images and loss to follow-up, we plan to recruit a total of 2000 participants (online supplemental table 1: distribution of sample across provinces).

### Participant enrolment

PAK-SEHAT will recruit participants from all four provinces of Pakistan and one federal territory; Sindh, Punjab, Baluchistan, Khyber Pakhtunkhwa and Islamabad. A multistage cluster sampling will be employed to identify and recruit potential study participants. The first stage of sampling will occur at the province level, which is the largest administrative division in the country. The distribution of the sample across the provinces is done proportionate to the size sampling method (see online supplemental table 1). It is followed by cities and then clusters. We define a cluster as the selected tertiary care hospital that has the provision of cardiac services. A line listing of all the potential hospitals was curated and screened for eligibility. We have collaborated with eight hospitals in provinces across Pakistan: four from Punjab (two from Lahore, one each from Faisalabad and Rawalpindi), and one each from Sindh (Karachi), Baluchistan (Quetta) and Khyber Pakhtunkhwa (Peshawar), and from federal territory, that is, Islamabad (figure 1).



No	Province	City	Hospital(s)
1.	Sindh	Karachi	Tabba Heart Hospital, Karachi
2.	Baluchistan	Quetta	Tabba Heart Institute, Quetta
3.	Punjab	Rawalpindi	Safari Hospital
4.	Khyber Pakhtunkhwa	Peshawar	Peshawar Institute of Cardiology
5.	Federal Territory of Islamabad	-	Medikay Cardiac center
6.	Punjab	Lahore	Bahria International Hospital Doctors hospital & medical Center
7.	Punjab	Faisalabad	Faisalabad Institute of Cardiology

**Figure 1** Map of Pakistan with selected provinces, cities and site hospitals.

To enrol the participants, we are implementing active and passive strategies.

#### Active strategy

The attendants of patients with any condition visiting the study hospitals will be offered to participate in the study. After taking the consent, their eligibility for enrolment in the study will be assessed. Through this strategy, we will recruit 15% of the sample to avoid clustering of genetic and lifestyle factors related to ASCVD.

#### Passive strategy

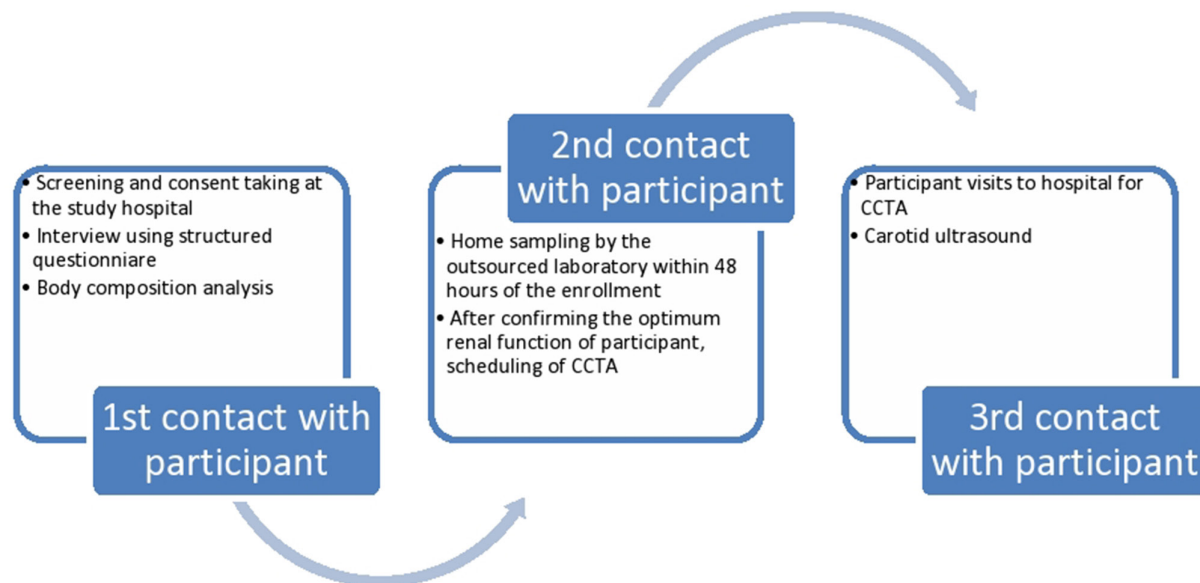
The study will be advertised using pamphlets, posters and banners within the study hospitals, and general physician clinics. Electronic media such as FM radio and digital platforms such as Facebook, Twitter and Instagram will also be used to invite volunteers to participate in the study. The volunteers willing to participate in the study

will contact the research staff via a hotline number available in the advertisement. They will then be scheduled to visit the hospital for consent and screening of eligibility.

#### Training of research and hospital staff

The 2-day training of research associates and coordinators has been performed to acquaint them with study protocols. They were trained in assessing the eligibility and administration of consent and the questionnaire. Mock consent exercises and interview training were also conducted. Hands-on training for using Tanita body composition monitors (Tanita Corporation of America, which uses advanced bioelectric impedance analysis (BIA) technology) for body composition analysis,<sup>25 26</sup> a stadiometer for height measurement and Omron (Omron Healthcare USA) digital blood pressure device for measuring blood pressure was also done.





**Figure 2** Procedural flow chart of baseline investigations of participants. CCTA, coronary CT angiography.

To ensure uniformity and standardisation of CCTA and CIMT across hospitals, live CCTA of a volunteer was undertaken to demonstrate the standard of procedure for the study. In CIMT training, the process of measuring the common carotids was demonstrated on a volunteer.

#### Baseline testing sequence

The baseline testing sequence is the same for participants recruited through active or passive strategy. In both cases, the first in-person contact for the participants will be the research team at the site hospital. At the study sites, after confirming the eligibility and consent signing, participants will be interviewed, and their body composition analysis will be performed. After that, the phlebotomy at a convenient time for the participants at their homes will be performed. If the participant's renal function is optimised, they will be scheduled to visit the study hospital for CCTA and CIMT. [Figure 2](#) depicts the flow of the baseline study investigations. [Table 1](#) depicts scheduled procedures and investigations at the study baseline and follow-up.

#### Study interviews and questionnaires

Participants will be interviewed for sociodemographic data and information on medical history, family history and lifestyle (diet, physical activity, tobacco product use) using structured questionnaires via electronic tablet devices. The medical history questions will comprise of whether the participants have a history of hypertension, dyslipidaemia or diabetes along with the time duration for these comorbidities (time from date of diagnosis) and treatment. Physical activity and mental health will be evaluated using a short form of the International Physical Activity Questionnaire and Patient Health Questionnaire-9 that is already validated in Pakistan.<sup>27</sup> Dietary patterns will be assessed by a structured shortened food frequency questionnaire which has been used

previously in the Asian population.<sup>28</sup> [Table 2](#) summarises the domains that will be evaluated and the questionnaires used in PAK-SEHAT.

#### Blood pressure measurement

Systolic and diastolic blood pressure will be measured in each participant using Omron digital blood pressure device, with participants sitting in a comfortable position for 5 min. Three readings are taken: the first reading will be discarded, and the average of the last two readings will be captured.

**Table 1** Scheduled procedures, investigations and data collection at study baseline and during the first 5 years of follow-up in PAK-SEHAT

Procedure	Baseline visit	30 months	60 months
Enrolment and informed consent	X		
Lifestyle, diet, physical activity questionnaires	X		
Vitals	X		
Anthropometrics and body composition	X		
Laboratory analyses	X	X	X
Cardiac CT for CAC scoring and CCTA	X		X
Carotid ultrasound for IMT measurement	X		X
Follow-up for clinical events		X	X

CAC, coronary artery calcium; CCTA, coronary CT angiography; IMT, intima-media thickness; PAK-SEHAT, PAKistan Study of prEmature coronary atHerosclerosis in young Adults.

**Table 2** PAK-SEHAT Study questionnaires

Focus	Description
Demographics	Age, sex, ethnicity, religion, educational attainment, employment status, marital status, household assets to assess socioeconomic status
Diet	Food habits and frequency
Family history	History of cardiovascular diseases in first-degree relatives, mortality in first-degree relatives, age and cause of death in first-degree relatives
History of consanguinity	Consanguine marriage of parents
Medical history	History, duration and treatment of hypertension, diabetes and dyslipidaemia; history of chronic lung disease, chronic liver disease, chronic kidney disease, cancer, venous thromboembolism, congenital and valvular heart disease
Use of tobacco products	Frequency and duration of smoking tobacco such as cigarettes, sheesha, huqqa, type and form of tobacco used, duration and frequency of usage of chewing tobacco such as gutka, mawa, menpuri and others, exposure and duration of passive smoking
Mental health	Patient Health Questionnaire-9
Physical health	International Physical Activity Questionnaire

PAK-SEHAT, PAKistan Study of prEmature coronary atherosclerosis in young Adults.

### Anthropometry and body composition

Height (cm) will be measured using a stadiometer. Body composition will be analysed using the Tanita-DC-240 body composition analyser (Tanita Corporation of America).<sup>26</sup> Participants will be asked to stand barefoot on the body composition analyser for less than a minute. A very low, safe electrical signal will be sent from four metal electrodes to the upper body through the feet using BIA, body mass, visceral fat, muscle and bone mass, and total body water.

### Blood measurements

Approximately 30 mL of venous blood will be collected from each participant. Blood (15 mL) will be used for laboratory tests, including high-sensitivity C reactive protein, non-fasting lipids, glycosylated haemoglobin A1c (HbA1c), serum creatinine, complete blood count, lipoprotein a (Lp(a)) and liver function tests. Lp(a) will be measured by an immunoturbidometric assay using the Alinity c Lp(a) reagent. The reagent is a suspension of polystyrene latex particles of uniform size coated with IgG anti-human Lp(a). [Table 3](#) summarises the planned blood measurements in PAK-SEHAT.

### Biobanking and genetic testing

Blood sample (15 mL of 30 mL) will be used for biobanking and genetic testing. The blood samples will be tagged by the study identification number (study ID), and only the principal investigator will be able to access the participant's identifier via study ID. The blood samples will be transported at 4°C for DNA extraction. Inorganic and organic methods will be employed to extract DNA from the blood sample using the following steps:

1. Cell lysis.
2. Protein separation.
3. DNA isolation and purification.
4. DNA quantification by Qubit.

Once DNA extraction is done, 15 aliquots (each of 1 mL) will be made and biobanked at -80°C for further genetic testing. The entire genome sequencing will be done on the sample DNA to test for their association with CVDs, arrhythmias, structural CVDs, metabolic syndrome and other non-communicable diseases. The aliquots will be stored for a maximum period of 10 years and will be destroyed once the genomic sequencing is done.

Consent will be taken from each participant specifically for biobanking and genetic testing; if a participant will not consent, then their samples will not be biobanked.

### Cardiac CT scans

Participants will first undergo a non-contrast-enhanced cardiac CT for coronary artery calcium (CAC) scoring, followed by a contrast-enhanced CCTA. Multislice CT scanners and retrospective ECG triggering will be used. Participants will be evaluated before the CT scan by study personnel for creatinine of less than 1.5 mg/dL, heart rate and blood pressure. If the heart rate is above 65 beats per minute (bpm), they will be pretreated with a beta-blocker (or ivabradine if beta-blocker contraindicated) to achieve a target heart rate of ~60 bpm. Sublingual nitroglycerin will be administered as per standard protocols approximately 3 min before the CCTA scan, and intravenous contrast will be injected at a rate of 5.5–7 mL/s depending on the body habitus of the participant; for an average patient, the dose of contrast is expected to be approximately 60–80 mL. The non-contrast-enhanced images will be transferred to the cardiology CT core laboratory and will be evaluated by trained radiologists, who will quantify CAC according to Agatston's method.<sup>29</sup> Contrast-enhanced tomographic images will be evaluated in dedicated workstations for description of plaque subtypes (calcified, predominantly calcified, predominantly non-calcified and non-calcified), maximal stenosis,



**Table 3** Baseline and follow-up blood measurements in PAK-SEHAT

Group of measurements	Specific measurements
Blood count	<ul style="list-style-type: none"> <li>▶ Haemoglobin</li> <li>▶ White cell count</li> <li>▶ Neutrophils</li> <li>▶ Basophils</li> <li>▶ Eosinophils</li> <li>▶ Monocytes</li> <li>▶ Leucocytes</li> <li>▶ Platelets</li> <li>▶ Haematocrit</li> </ul>
Coagulation	<ul style="list-style-type: none"> <li>▶ Prothrombin time</li> </ul>
Glucose metabolism	<ul style="list-style-type: none"> <li>▶ Glycosylated haemoglobin A1c</li> </ul>
Inflammation	<ul style="list-style-type: none"> <li>▶ High-sensitivity C reactive protein</li> </ul>
Kidney function	<ul style="list-style-type: none"> <li>▶ Creatinine</li> </ul>
Non-fasting lipid levels	<ul style="list-style-type: none"> <li>▶ Total cholesterol</li> <li>▶ Low-density lipoprotein cholesterol</li> <li>▶ High-density lipoprotein cholesterol</li> <li>▶ Triglycerides</li> </ul>
Lipoprotein a	<ul style="list-style-type: none"> <li>▶ Lp(a)</li> </ul>
Liver function	<ul style="list-style-type: none"> <li>▶ Serum glutamic pyruvic transaminase</li> <li>▶ Serum glutamic oxaloacetic transaminase</li> <li>▶ Alkaline phosphatase</li> <li>▶ Direct bilirubin</li> <li>▶ Indirect bilirubin</li> </ul>
<p>The same laboratory measurements will be conducted at baseline, 30 months of follow-up and 60 months of follow-up (see <a href="#">table 1</a> for more details). PAK-SEHAT, PAKistan Study of prEmature coronary atHerosclerosis in young Adults.</p>	

number of coronary segments affected and presence of high-risk plaque features. Coronary arteries will be evaluated on a per-segment basis according to a modified American Heart Association 18-segment classification, and a segment involvement score will be calculated in each participant as the number of coronary segments with plaque (ranging from 0 to 18).<sup>22</sup> Each coronary segment will be assessed to semiquantify plaque burden (0: no plaque, 1: minimal or mild, 2: moderate, 3: severe) and the degree of maximal luminal stenosis (0: none, 1: <30%, 2: 30–49%, 3: 50–69%, 4: 70–99% and 5: 100%). The location of plaque in the main coronary vessels (left main, left anterior descending coronary artery, left circumflex coronary artery or right coronary artery) and the number of main vessels affected will be reported as well. The total plaque severity score will be calculated as the sum of the individual segment plaque severity scores, and the total segment stenosis score will be calculated by adding the individual segment stenosis scores. The following plaque

vulnerability features will be evaluated: positive remodeling (defined as a remodelling index >1.1), low CT attenuation (mean CT number <30 Hounsfield units), spotty calcifications (defined as calcification with length <3 mm and occupying  $\leq 90^\circ$  of the vessel arc) and napkin-ring sign (defined by the presence of a ring-like peripheral higher attenuation with central low CT attenuation).<sup>22</sup> The kappa statistics will be estimated to determine the inter-reader agreement for CT reporting. Also, a random sample of 10% will be shared for quality control with the study's international advisory team.

#### Carotid ultrasound imaging

All participants will undergo carotid ultrasound imaging for assessment of the presence of carotid plaque and CIMT evaluation. This will be assessed with automated measurement over bilateral carotid arteries using a Toshiba Aplio 500 ultrasound system (Otawara, Japan), using a B-mode ultrasound examination with a 10 MHz multifrequency linear array transducer. Participants will be examined in the supine position, and standard measurements are performed.<sup>30</sup> Trained personnel evaluate ultrasound images, and the de-identified images of randomly selected study participants will be shared with the study's international advisory team for quality control.

CT scans and carotid ultrasounds will be reported at the core laboratory, that is, at Tabba Heart Institute (THI) by two independent reporters. The images will be saved in compact discs and then will be transferred from the regional centres to the central site on a regular basis. For quality check, 5% of the sampled images will be reviewed by international experts, and any discrepancy will be resolved after discussion.

Once the CCTA and CIMT are done, participants will be reimbursed for their time and transport expenses via a bank transfer.

#### Baseline data management and quality

Study data will be collected on tablets and will be transferred regularly (typically on a weekly basis) to a central server. The centralised study database will be stored using a secure server, and data privacy and confidentiality will be secured at all times. For quality control purposes, the data collection application in the study tablets has in-built features to limit erroneous data entry and inconsistency in recording participants' responses. Also, a field coordinator will monitor data collection and spot checks 5–10% of the interviews. Spot checks include observing live interviews, study procedures and data entry. At the centralised study database, data error reports will be generated monthly to monitor completeness, invalid values and inconsistencies in responses. In addition, a live dashboard has been developed for pre-identified key variables to monitor and evaluate the project's progress.

Participants will be provided the results of all their laboratory and radiological investigations within the due time frame of the hospitals' standard reporting.



### Follow-up and event ascertainment

As part of the study follow-up, participants will be contacted at 30 and 60 months (5 years) after baseline for repeat investigations. Also, the participants will be contacted every 6 months via phone call to determine the study outcomes. They will be interviewed for the occurrence of cardiac events or the secondary outcomes of diabetes, hypertension and dyslipidaemia. In case of an affirmative answer during follow-up, participants will be interviewed in detail about the specific event through another questionnaire. For hypertension, dyslipidaemia and diabetes, the date of diagnosis will be assessed. If the participant does not know, then the approximate duration since the time of diagnosis will be assessed from the participant. The follow-up questionnaire will collect information on the timing of the event and treatment, and participants will also be asked to provide hospital records to confirm the event. ASCVD events will include myocardial infarction (MI), stroke, coronary revascularisation and cardiovascular death. The reported events will be adjudicated by the two physicians using the information collected through follow-up questionnaires and hospital records. If there is disagreement in event adjudication between the two assigned experts, a third independent physician adjudicator will review the evidence to break the tie. The confirmed ASCVD events will be recorded and participants' follow-up will be continued until the end of the study period.

Two cardiologists from the centralised PAK-SEHAT adjudication committee will classify events as definite, probable or absent, based primarily on available hospital documentation. The ASCVD events include definite and probable MI, definite ASCVD death, stroke and revascularisation. To ascertain the cause of death as CVD or non-CVD, death certificates or hospital records will be asked from the families of the participants. If documents are not provided, then a verbal autopsy will be performed. The information provided in verbal autopsy will be reviewed by the two physicians to categorise death into CVD or non-CVD death. If there is a discrepancy about the cause of death between the two physicians, then the information will be reviewed by the third physician and the cause of death will be assigned.

Definite CVD death will be labelled if the deceased had an MI within 28 days of death, chest pain within the 72 hours before death, or had a history of coronary heart disease and there was no known non-atherosclerotic or non-cardiac cause of death. Stroke will require if there was a focal neurological deficit for at least 24 hours or until death, along with evidence of stroke from medical records and no non-vascular cause.

At the 30th month of the baseline assessment, the participants will be contacted again for laboratory investigations. Then, at the 60th month from the baseline examination (table 1), participants will be contacted for the second time to have repeat CT and laboratory investigations.

Other than the information provided by the study participants during follow-up interviews, the information generated by the blood tests will be used to also identify incident cases of diabetes and dyslipidaemia. The repeat CCTA at 60 months of follow-up will be used to evaluate the progression/regression of coronary plaque burden.

### Cardiovascular risk management of study participants

This is an observational study, and no cardiovascular risk management intervention will be implemented as part of the study. However, participants will be informed about the results of their study tests and encouraged to discuss those with their treating physician. Best efforts are made to create linkages to care for participants. Participants with high-risk findings, such as blood pressure of  $\geq 180/100$  mm Hg indicating advanced stages of hypertension, HbA1c of 9% indicating advanced uncontrolled diabetes or low-density lipoprotein cholesterol of  $\geq 190$  mg/dL, are referred to a relevant health professional. Participants with  $\geq 50\%$  stenosis in the left main coronary artery and  $\geq 70\%$  stenosis in major epicardial vessels are also referred to cardiology hospitals for further assessment as it indicates obstructive coronary artery disease.

### Statistical analyses

For analyses using baseline data, we will describe the frequency and distribution of cardiovascular risk factors (known, unknown), coronary plaque burden, and other key baseline variables using number and frequency (%) for categorical variables and mean (SD) or median (IQR) for normally and not normally distributed continuous variables, respectively. Specifically, for coronary plaque characteristics, the CAC score will be categorised as 0,  $>0$ – $<100$ ,  $\geq 100$ , and the distribution of CAC scores and proportion of each stratum will be reported. Regarding CCTA findings, the proportion of participants with any coronary plaque and the distribution of plaque subtypes will be reported with 95% CI. We will also report the frequency of having maximal coronary stenosis  $\geq 50\%$  and  $\geq 70\%$ , respectively, and the proportion of participants with high-risk plaque features. The distributions of the number of coronary vessels with plaque, the number of coronary segments affected per participant and the location of coronary plaques (left main, anterior descending, circumflex and right coronary artery) will also be described.

In analyses of incident events during follow-up, we will use standard survival analysis and time-to-event techniques. Cumulative incidence at 5 years (in %) and incidence rates per 1000 person-years (with 95% CI) will be computed for the primary and secondary study outcomes.

We will use Cox proportional hazards regression models to evaluate the association between important baseline characteristics (demographics, lifestyle, metabolic factors, coronary plaque burden and characteristics, genetics and other features) and incident ASCVD events

during follow-up. These associations will be presented as HRs with 95% CIs.

All analyses will be conducted overall and stratified by age, sex and province. Additional analyses will be considered to evaluate additional research questions relevant to premature coronary atherosclerosis and ASCVD in Pakistanis. Statistical analyses will be conducted using the latest versions available of R and Stata software, and a p value of 0.05 will be used as a threshold of statistical significance.

### Research ethics and dissemination

The study protocol was reviewed and approved by the THI Institutional Review Board (IRB) (THI/IRB/FQ/22-09-2021/016) and National Bioethics Committee, Pakistan. Prior to inclusion in the study, written informed consent is requested from each participant, and candidates for study enrolment who fail to consent for participation are excluded. All study procedures are consistent with the principles of the Declaration of Helsinki.<sup>31</sup> Findings of the study will be disseminated via peer-reviewed publications, conference presentations and clinical communications.

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**Funding** This study is funded by our study partner Getz Pharma Private Limited (study registration number: GPPL-PH-002).

**Map disclaimer** The inclusion of any map (including the depiction of any boundaries therein), or of any geographic or locational reference, does not imply the expression of any opinion whatsoever on the part of BMJ concerning the legal status of any country, territory, jurisdiction or area or of its authorities. Any such expression remains solely that of the relevant source and is not endorsed by BMJ. Maps are provided without any warranty of any kind, either express or implied.

**Competing interests** WJ is the head of public health and JBB is a senior manager at Getz Pharma Private Limited.

**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 required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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