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Cohort profile: Bushehr Elderly Health (BEH) Program, Phase III

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Abstract

The Bushehr Elderly Health (BEH) Program is a population-based prospective cohort study, designed to address the increasing health challenges associated with an aging population. The third phase of the BEH program was conducted to repeat measurements from previous phases of the study, assessing the state of non-communicable diseases (NCDs)—particularly cardiovascular and musculoskeletal diseases and cognitive function—as well as their determinants and consequences among older adults. This article presents the study profile for this phase, detailing the methodology, recruitment process, and baseline participant characteristics. The resulting longitudinal data will be used in future analytical studies. Phase III of the BEH Program was conducted six years after the second phase (stage 2), involving repeated measurements among participants aged 60 and above who were residing in Bushehr city and had participated in the previous phases of the first recruitment of the study. Previous phase participants were invited to take part in this phase. This phase repeated the measurements conducted in the previous phases included comprehensive questionnaires, physical and anthropometric examinations, whole body and bone density scan, diagnostic laboratory tests, and biobanking of biological samples including serum, EDP, stool, and saliva. The successful assessment has been completed for participants invited from the earlier phases of the BEH program, yielding extensive data for future analysis. In this phase of the BEH program, a total of 1,345 participants were enrolled in the study, a response rate of 64.7%. The mean (standard deviation) age was 74.3 (4.6) years and 50.4% of the participants were males. The third phase of the BEH program successfully repeated comprehensive measurements and expanded the biobank to include microbiota samples. These repeated measures will provide valuable longitudinal insights once analyzed, enabling researchers to examine changes in health-related variables over time and better understand the needs of older Iranian adults. Future analyses will address the specific objectives related to cardiovascular, musculoskeletal, and cognitive health.

Introduction

Alongside the rising life expectancy, the world's population is aging rapidly. It is anticipated that the global population of individuals aged 65 years and older will rise from 9.3% in 2020 to approximately 16% in 2050 (1). The elderly population will increase from 0.7 billion in 2019 to 1.5 billion in 2050 (2) and 2.2 billion by the 2070s (3). This increase in the older adult population is expected to continue, especially in lower and middle-income countries (4).

In Iran, the population of older adults aged 65 and older stands at 9.2% in 2025 and is predicted to rise to approximately 22% (19 million) by 2050. Also, the population aged 80 and older is projected to reach 3.8% by 2050 (5–7). This increase in the proportion and the number of older adults highlights the growing need to understand the impacts and outcomes of aging on health, social structures, and economies.

The health and economic burden of aging are significant both globally and in Iran. (8). According to a report between 2004 and 2030, there will be a 55% increase in global disability-adjusted life years

(DALYs) among those aged 60 and older due to population aging. Also, health conditions among older adults aged 60 and above account for 23% of the total global burden of disease. (9).

Considering the demographic shifts, the necessity for comprehensive research aimed at developing effective prevention and management strategies for non-communicable diseases (NCDs), which are associated with older adults, becomes more important (10). Among NCDs, cardiovascular, musculoskeletal, and cognitive diseases are highly important with the leading contributors to disease burden of 30.3%, 7·5%, and 6·6%, respectively (9). In Iran, between 1990 and 2015, CVDs were the first leading cause of DALYs, and there was an increase in age-standardized DALY due to musculoskeletal, mental, and neurological disorders (11). These conditions directly impact the quality of life, independence, and daily functioning of older adults, and require special attention in health and treatment programs. Furthermore, as the proportion of elderly people increases, so does the potential burden on healthcare systems, social services, and economic resources. Therefore, elderly health has become one of the main priorities of public health systems (4).

In response to these serious concerns, Bushehr Elderly Health (BEH) program, as a population-based prospective cohort study in southern Iran, was designed and executed with the aim of determining the factors affecting the health of older adults, and providing the information needed for decision-making by clinicians, policy makers, and health program managers. BEH program is a cohort that fills a critical geographical and methodological gap, allowing for unique insights into the aging process across distinct genetic backgrounds, lifestyle factors, and environmental exposures in an understudied region of Iran.

The baseline data gathering of the first recruitment of the BEH program was carried out in two phases (12, 13). This article presents the profile of the third phase of the study's first recruitment, which is a cohort profile paper detailing the methodology, measurements, and baseline participants characteristics for this phase (see Fig. 1). This phase was designed and implemented to repeat the measurements of the previous phases and investigated the prevalence, risk factors, and consequences of cardiovascular diseases, musculoskeletal diseases, and cognitive function in the participants of the BEH program's previous recruitments. In the design of the BEH program phase III, the primary application of its repeated measurements and longitudinal data is to monitor and track changes in health variables and their influencing factors over time. This phase also included the development of a microbiota biobank.

Methods

Study design

Specific objectives

The BEH program is a population-based prospective cohort study with the purpose of investigating the prevalence of NCDs and their risk factors, as well as the incidence of outcomes associated with NCDs in the older adult population (12, 13). This study is a collaborative effort between Tehran University of Medical Sciences and Bushehr University of Medical Sciences. The third phase (repeated

measurements) was conducted six years after the successful completion of the first and second phases (stage 2) of the first recruitment (the baseline measurements) of this study (Fig. 1).

This phase of the study was carried out with the following specific objectives, which will be addressed in future analytical studies using the longitudinal data collected in this phase:

- Determining the state of cardiovascular diseases and their determinants and consequences in the older adults
- Determining the state of musculoskeletal diseases and their determinants and consequences in the older adults
- Determining the state of cognitive function and its determinants and consequences among the older adults

1.2. Study population and inclusion and exclusion criteria

The first recruitment of BEH program population included people aged 60 years and older living in Bushehr city. In the first phase, conducted from March 2013 to October 2014, 3000 eligible people living in Bushehr were selected and enrolled in the study. In the second phase (stage 2) of this recruitment of the study at 2015–2016, 2,426 individuals participated. In the present phase, all individuals who had participated in the previous phase of the first recruitment were invited to take part in the study (Fig. 1). The inclusion criteria were as follows: men and women aged 60 years and older who had participated in the previous phases of the first recruitment of the study; residing in Bushehr with no plans to leave the city for at least the next five years; and having sufficient physical and mental capability to participate in the study. Participants are required to sign a written informed consent form (ICF). Older adults living in nursing homes were excluded from the study.

1.3. Sampling method

To ensure a representative sample of the people aged 60 and over in Bushehr city, 3000 eligible individuals were selected through a multi-stage cluster random sampling method in 2013, which has been described in detail in previous article (12).

1.4. Invitation

Participants from the second phase (stage II) of the first recruitment were invited to the third phase of the study via phone call. Up to 10 phone calls were made to both landline and mobile numbers, or to the contact numbers of their companions. The inviter, after introducing themselves and explaining the reason for the call, invited individuals to return for the new phase. After each phone call, the status and outcome of the call, as well as the reason for any disagreement, were recorded. If the participant accepted the invitation, the study nurse reminded participants to bring all their medications and insurance cards, wear light clothing, fast for 12 hours before the test, arrive on time at the study site, and be assured of the confidentiality of their information.

2. Ethics and dissemination

The third phase of the BEH program was approved by the ethical committee of the Endocrinology and Metabolism Research Institute of Tehran University of Medical Sciences (IR.TUMS.EMRI.REC.1396.00223). Study participants were provided with an information sheet containing all necessary details about the study's purpose, procedures, benefits, and participants' rights, and they signed ICFs in duplicate. These forms covered all components of the study, including data and biological sample collection, utilization, secondary data analysis, and potential publication of findings. One copy was archived at the Persian Gulf Biomedical Sciences Research Institute of Bushehr University of Medical Sciences, while the other was retained by the participant. Participants' right to withdraw from the study at any time is respected without any impact on their healthcare management plan. Additionally, after withdrawing, they were free to allow researchers to use the data collected from them in the study or to request their deletion. If people were diagnosed with diseases requiring treatment during the study, the necessary treatments were initiated, or they were referred to relevant specialists. This study was conducted in accordance with the Declaration of Helsinki and adhered to Iranian national guidelines for ethics in research.

3. Measurements

Data were collected using questionnaires, physical examinations, body composition and Bone Mineral Density (BMD) assessments through Dual-energy X-ray absorptiometry (DXA (, and diagnostic tests. All collected data were entered into an electronic case report form (eCRF).

3.1. Questionnaires

The list of standardized questionnaires for this study is presented in Table 1. The questionnaires were completed through face-to-face interviews with study participants.

Table 1
The list of questionnaires of phase III of the BEH Program

1	**The questionnaire name**	**Domain/component/description**
	Participants' Demographic Characteristics	**Personal information** **Age** **Sex** **Marital status** **Contact information**
2	Socio-Economic Status	Employment status Basic Insurance Supplementary Insurance Income Assets Residence status
3	Cardiovascular Diseases Risk Factors	Smoking (Life-time, Current, History, Frequency, Quantity, Quit attempts, Ex-smoking) Alcohol Consumption (Current, History, amount) Drug abuse (Life-time, Type, Age of onset, Duration)
4	International Physical Activity Questionnaire (IPAQ) (14)	Work-Related Activities Transport Activities Domestic Activities Leisure Activities Sitting
5	Geriatric Locomotive Function Scale-5 (GLFS-5)	Difficulty in climbing up and down stairs Difficulty in fast walking Difficulty in carrying a 2 kg weight Difficulty in carrying tasks Overall assessment of locomotion
6	Fertility History Questions (for women only)	Menstrual cycle start Sex hormone replacement therapy Contraceptive History

1	**The questionnaire name**	**Domain/component/description**
	Participants' Demographic Characteristics	**Personal information** **Age** **Sex** **Marital status** **Contact information**
		Hysterectomy /Oophorectomy
7	Cardiovascular Diseases and its Family History Questionnaire	Familial Diabetes, Hypertension, and Stroke Stroke
8	Rose Questionnaire (15, 16)	Awareness of Cardiovascular Symptoms
9	Heart Failure-Specific Health Literacy Scale (17)	Physical Functioning and Cardiovascular Related Events
10	Bone and Muscle Health Questionnaire (13)	Sunlight exposure Diagnosis, treatment, and adherence to treatment of Osteoporosis History of long corticosteroid use Self and parental history of fragility fractures Fall history Muscle health Height loss Screening for secondary osteoporosis Medication history affecting osteoporosis
11	Health-Related Quality of Life Questionnaire (EQ-5D) (18)	Mobility Self-Care Usual activities Pain/Discomfort Anxiety/Depression
12	Quality of Life Questionnaire 12-item Short Form	Physical functioning Limited Physical Role Function

1	**The questionnaire name**	**Domain/component/description**
	Participants' Demographic Characteristics	**Personal information** **Age** **Sex** **Marital status** **Contact information**
	Health Survey (SF-12) (19)	Physical pain Vitality (energy) Perceived mental health Limited emotional role function Social functioning General health
13	Social Frailty (20)	Living Situation Social Relations Social Support Social Activities Emotional Well-being
14	Medical History	- History of Hypertension, Dyslipidemia, Myocardial Infarction, Stroke, Heart Failure, Diabetes, Hyperthyroidism, Hypothyroidism, Parkinson's Disease, Alzheimer's Disease, Rheumatism, Osteoarthritis, Kidney Stones, Chronic Kidney Failure, Epilepsy, Depression, Lung Disease, Liver Disease, Cancer, HIV infection, Viral Hepatitis, Gastrointestinal Surgery (excluding Appendectomy and Cholecystectomy), and Inflammatory Bowel Disease - History of Hospitalization (Cause, Duration, and Location) -Unintentional Weight Loss of More than 5 kg in the Last Year -COVID-19 and history of diagnosis and Vaccination
15	Medication Registration Form	Drug Name Type of Drug Dose of drug Duration of Use

1	**The questionnaire name**	**Domain/component/description**
	Participants' Demographic Characteristics	**Personal information**
		Age
		Sex
		Marital status
		Contact information
16	Assessment of Activities of Daily Living (ADL)(21)	Basic Activities of Daily Living (BADL):
		Ambulating
		Feeding
		Dressing
		Personal Hygiene
		Toileting
		Continence
		Instrumental Activities of Daily Living (IADL):
		Use of telephone
		Shopping
		Meal preparation
		Housekeeping, and laundry
		Mode of transportation
		Medication management
		Money management
17	Cognitive Impairment Test (6CIT) (22)	What year is it?
		What month is it?
		Remember an address phrase with 5 components
		About what time is it?
		Count backwards from 20 to 1
		Say the months of the year in reverse order
		Repeat the 5-component address phrase to assess delayed memory
18	Patient Health Questionnaire-9	Loss of Interest or Pleasure

1	The questionnaire name	Domain/component/description
	Participants' Demographic Characteristics	**Personal information**
		Age
		Sex
		Marital status
		Contact information
	(PHQ-9) (23)	Depressed Mood or Hopelessness
		Sleep Disturbances
		Fatigue or Low Energy
		Appetite Changes
		Negative Self-Perception
		Difficulty Concentrating
		Psychomotor Changes (Restlessness or Slowness)
		Suicidal Thoughts or Self-Harm
19	24-hour Dietary Recall Assessment	Details of all foods and beverages consumed in seven meals over the past day
20	Mini Nutritional Assessment (MNA) in the elderly (24)	Changes in food intake over the past three months
		Recent weight loss
		Mobility status
		Psychological stress or acute disease history
		Neuropsychological problems
		Body Mass Index (BMI)
21	Pittsburgh Sleep Quality Questionnaire (PSQI) (25)	Subjective Sleep Quality
		Sleep Latency
		Sleep Duration
		Habitual Sleep Efficiency
		Sleep Disturbances
		Use of Sleeping Medication
		Daytime Dysfunction

1	The questionnaire name	Domain/component/description
	Participants' Demographic Characteristics	**Personal information** **Age** **Sex** **Marital status** **Contact information**
22	Epworth Sleepiness Scale (ESS) (26)	When: Reading Watching TV Inactivity in Public (e.g., theater) Car Passenger for an Hour Afternoon Resting Talking to Someone Sitting Quietly After Lunch Stopped in Traffic

3.2. Physical examinations

The physical examinations included various anthropometric measurements such as height, weight, waist circumference (WC), hip circumference (HC), neck circumference, upper arm circumference, forearm circumference, wrist circumference, thigh circumference, calf circumference, upper arm length, and lower leg length. Additional tests included the hand grip strength measurement, single leg stance test, short physical performance battery (SPPB), which includes five times sit to stand test, balance test, and gait speed test. Blood pressure measurement, an electrocardiogram (ECG), a visual acuity test, and a whispering test were also performed.

3.2.1. Anthropometric measurements

Anthropometric measurements were conducted with participants wearing light clothing and no shoes. Height and weight were measured using a fixed stadiometer and calibrated digital scale. Other anthropometric indices were measured using a flexible tape. WC was measured at the horizontal plane midway between the lowest ribs and the iliac crest. HC was measured at the maximum extension of the buttocks. Neck circumference was measured in the midway of the neck, between mid-cervical spine and mid anterior neck. Upper arm circumference was measured at the midpoint between the olecranon process and the acromion while the right arm was hanging relaxed. Right forearm circumference was

measured around the widest part, just below the elbow. Wrist circumference was measured around the right wrist, just above the wrist bone. Thigh circumference was measured at the midway between the trochanterion (top of the thigh bone, femur) and lateral tibia (top of the tibia bone) of right thigh. Calf circumference was measured at the widest point of the calf. The upper arm length was measured while the individual was standing upright with their right arm flexed at a 90º angle, from the acromial landmark (shoulder tip) to the posterior surface of the olecranon (the bony prominence of the elbow). The lower leg length was measured while the individual was sitting with their back straight and knees at a 90-degree angle, from the top of the patella (kneecap) to the underside of the foot. All measurements were read to the nearest 0.1 cm while the participant was standing upright.

3.2.2. Performance-based tests and muscle strength

Handgrip strength was measured bilaterally using a digital calibrated dynamometer. For each hand, three measurements were taken for each hand. The dominant hand was evaluated first, followed by a 15-second rest period, after which the non-dominant hand was assessed.

The Single Leg Stance Test for both the right and left legs was performed twice with the participant's eyes open and arms placed on their hips. The participant was asked to stand unassisted on one leg, and the time was recorded in seconds from the moment one foot was lifted off the floor until it either touches the ground, contacts the standing leg, or an arm moves away from the hips.

The five times sit to stand test was done to assess the lower limb strength, balance, postural control and functional mobility. This test involves the individual standing up from a chair and sitting back down five times with the arms crossed over the chest. The time taken to complete all five stands was measured, test was stopped if the individual could not complete the stands or showed signs of discomfort or was not suitable for participation.

The balance tests, including Side-by-Side Stance, Semi-Tandem Stance and Full Tandem Stance were conducted with the support of the interviewer. In the Side-by-Side Stance, participants stood with their feet together, side by side. For the Semi-Tandem Stance, the heel of one foot was placed next to the big toe of the other foot. In the Full Tandem Stance, one foot was positioned directly in front of the other, with the heel touching the toe. The timer for these tests was stopped when 10 seconds had elapsed or when participants moved their feet. To assess '4.57-m and 2.44-m gait speed,' the usual walking speed was measured over a distance of 4.57 and 2.44 meters. While assessing gait speed, the participant's usual walking style and use of assistive devices like canes or walkers were also considered.

3.2.3. Blood pressure measurements and electrocardiogram

Blood was measured using a mercury sphygmomanometer to determine systolic and diastolic pressures by assessing blood flow in the arm. Participants were seated comfortably for five minutes with their arm supported at heart level. The first and fifth Korotkoff sounds indicated the systolic and diastolic pressures, respectively, and each measurement was recorded separately after two readings.

Electrocardiography was performed by a trained nurse using a 12-lead system to screen for ischemia, arrhythmias, and other cardiac conditions. The procedure required the participant to lie comfortably on the bed, with electrodes attached to the upper limbs (above the wrists) and the lower limbs (above the ankles) and thoracic leads fixed in six specific precordial positions (V1-V6) to accurately record the heart's electrical activity, which was then displayed as an electrocardiogram (ECG).

3.2.4 Visual acuity test

The Tumbling E chart was used to assess visual acuity. Participants were asked to identify the direction of the "E" letters from a distance of 3 meters. The level of visual acuity was defined as the smallest row in which the patient can identify at least half of the letters. If the participants wore glasses, they were asked to wear them during the test.

3.2.5 Whisper test

The **whisper test** was performed to assess participants' hearing ability. The interviewer stood 60 cm behind the seated person, whispering a sequence of three words consisting of numbers and letters, and asked the patient to repeat them. A total of six words were used for each ear. If the participants had a hearing aid, the test was conducted with the hearing aid on.

3.3. Expanded Bone Mineral Density Assessment

Bone Mineral Density (BMD) was measured for all participants in the study, using Dual-energy X-ray Absorptiometry (DXA) (Horizon® DXA System, Hologic, USA). Scans were performed at the lumbar spine (L1 to L4), the total hip, and the femoral neck, according to standardized protocols.

To perform BMD measurements, participants were positioned as follows: For lumbar vertebrae scans, participants were placed supine with their hips positioned over a foam block, when necessary, to eliminate lumbar lordosis, ensuring the spine was centered and aligned with the scanner's longitudinal axis.

For femur measurements, legs were internally rotated using a triangular positioning device to optimize femoral neck alignment. The dual-hip scanning protocol was used, allowing simultaneous bilateral acquisition; individual positioning adjustments may still be necessary to ensure consistent abduction angles and minimize inter-scan variability.

According to the world health organization (WHO) recommendations on the NHANES III study (27), Caucasian women aged 20–29 were used as the reference population to calculate T-scores. A T-score of -2.5 or less indicates osteoporosis. This standardized protocol minimized postural variability across measurements.

In addition to BMD body composition analysis (BCA) by whole body DXA, vertebral fracture assessment (VFA), and trabecular bone score (TBS) analysis were also integrated. Whole-body scans required participants to lie supine with their arms parallel to their torso (palms flat), their legs secured at the

ankles with Velcro straps while toes pointing vertically. A radiolucent pillow was used to support the head when needed to maintain a neutral position. Whole-body scans provided body composition metrics, including fat mass (%), lean mass (g), bone mineral content (BMC), and appendicular skeletal muscle mass (ASM) calculated as the sum of muscle mass in all four limbs.

VFA was performed using lateral spine images acquired in dual-energy mode, with vertebral deformities identified using the scanner's integrated morphometric software. For trabecular bone microstructure evaluation, TBS iNsight® software (v4.1, Med-Imaps) analyzed lumbar spine DXA images, with T-scores referenced to NHANES-based European normative data on Caucasian women aged 20–29 (validated for BMI 15–37 kg/m²).

These measurements were conducted at the study site by a trained technician. The DXA device was calibrated prior to the start of data collection process began and regularly throughout the study period, to ensure accuracy, consistency, and robust quality control of all measurements.

Individuals weighing over 150 kg and those with bilateral hip replacement were excluded from this measurement. Participants were instructed to remove radiopaque objects and to avoid calcium supplements for 24 hours prior to the measurement.

3.4. Biological samples collection and biobanking

Biological samples (blood, feces, and saliva) were collected from participants for testing and biobank storage. Approximately 8 cc of venous blood was collected in two sampling tubes containing clot activator, and 2 cc was collected in two separate tubes containing K2EDTA. These samples were processed immediately to separate serum and plasma, which then were used for biochemical measurements and stored in the biobank. Additionally, with the aim of developing a microbiota biobank, fecal and saliva samples were collected from a sub-sample of the participants. This work was conducted according to the specific sample collection, storage, and transportation protocols established for the operator and participants to ensure the integrity and viability of microbiota.

3.5. Diagnostic Laboratory Tests

Complete Blood Count (CBC), Fasting Blood Sugar (FBS), Hemoglobin A1C (HbA1c), Cholesterol, Triglycerides (TG), High-density lipoprotein cholesterol (HDL-C), Low-density lipoprotein cholesterol (LDL-C), Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Parathyroid Hormone (PTH), Thyroid-stimulating hormone (TSH), Free Thyroxine (T4), Vitamin D (25OH), Iron, Total iron-binding capacity (TIBC), Phosphate, Calcium, Urea, Uric Acid, Creatinine, and Albumin are biochemical markers that were measured in this phase of the study. CBC was measured using the automated hematology analyzer KX-21 (Sysmex Corporation, Kobe, Japan). The biochemical measurements were conducted using a BS-200 chemical analyzer (Shenzhen Mindray Bio-Medical Electronics Co, Ltd, Shenzhen, China), except for the PTH test, which was performed using the Cobas e411 autoanalyzer (Roche Diagnostics, Basel, Switzerland) and TSH, T4, and Vitamin D (25OH), which were measured using Dynex automated ELISA DS2® processing system (Dynex technologies, Chantilly,

VA, USA). The biochemical kits used are presented in Table 2. All laboratory procedures were conducted in strict accordance with the intended Standard Operating Procedures (OSFs) and the manufacturers' guidelines ensuring essential quality system requirements (28, 29). Internal quality control checks were regularly performed to maintain data accuracy. The analytical reliability and inter-laboratory consistency of key biochemical assays used in the BEH program were confirmed through external quality assessment (EQA) when available. In addition, laboratory equipment calibration and maintenance were performed in compliance with standards and the manufacturers' instructions to ensure consistent analytical performance (30).

Table 2
The Biochemical Kits used in the phase III of BEH Program

Biochemical Factors	**Company**	**Biochemical Factors**	**Company**
Fasting Blood Sugar (FBS)	MAN	Free Thyroxine (T4)	Pishtaz Teb
Hemoglobin A1C (HbA1c),	Paadco	Vitamin D (25OH)	Pishtaz Teb
Total Cholesterol (TC)	MAN	Iron	Biomed
Triglycerides (TG)	MAN	Total iron-binding capacity (TIBC (	MAN + Paadco
High-density lipoprotein cholesterol (HDL-C)	MAN + Audit	Phosphate	MAN
Low-density lipoprotein cholesterol (LDL-C)	MAN + Audit	Calcium	MAN + Audit
Alkaline Phosphatase (ALP)	MAN	Urea	MAN
Alanine Transaminase (ALT)	Audit	Uric Acid	MAN
Aspartate Aminotransferase (AST)	Audit	Creatinine	Audit
Parathyroid Hormone (PTH)	Roche	Albumin	Biomed
Thyroid-stimulating hormone (TSH)	Pishtaz Teb		

4. Training and quality assurance

To ensure the validity of the measured variables, the personnel were trained at the beginning of the study based on the study protocol and standard procedures. The agreement between the interviewers and the trainer were evaluated.

5. Monitoring and supervision

Monitoring of the accurate implementation of the study (in accordance with the current protocol) was conducted by inspectors visiting the data collection process and using monitoring checklists. During

study monitoring by inspectors, all processes were evaluated, and any deviations from the protocol and related issues were addressed.

6. Data Management

Collected data were recorded in an eCRF. The eCRF was an electronic questionnaire and data collection form designed to help researchers collect data from each participant and record the results of the questionnaires and other tests and measurements (physical examinations and diagnostic laboratory tests). To ensure minimal data loss, each participant's eCRF was completed during every visit and within one week of the availability of diagnostic tests results. The data file was updated every three months and was shared with the project manager in EMRI-TUMS, Tehran. The interviewer responsible for conducting visits and completing the eCRFs had access to these forms. A physician was also permitted to complete eCRFs if individuals were referred for further clinical evaluations. All collected data, including questionnaires, physical examination results, BMD and body composition assessments, and diagnostic laboratory tests, were treated as confidential information. The members of the research team who had permission to access such data were identified. A data protection expert was appointed to monitor the data management process and was responsible for providing periodic reports on this matter. Each participant was assigned the unique code of the previous phase of BEH program, so that their identity remains unknown during any future use of their data. All printed copies of questionnaires and data collection forms were stored in Persian Gulf Biomedical Sciences Research Institute of Bushehr University of Medical Sciences. A digital file of the collected data was created and updated every month. Additionally, a copy of the data file was saved to an external drive every week.

7. Planned statistical analyses

The following statistical approaches are planned for future analyses of Phase III data, in alignment with the study’s specific objectives. Continuous variables will be reported as mean (standard deviation) or median (interquartile range), while categorical variables will be reported as frequency and percentage. In cases of non-normal distributions for continuous variables, transformation of the data will be considered, or non-parametric tests will be used. When exploring the associations in cross-sectional data, if the outcome variable is continuous, depending on the hypothesis being tested, a T-test, analysis of variance (ANOVA), analysis of covariance (ANCOVA), or linear regression models will be employed. If the outcome variable is categorical, a chi-square test or appropriate regression analysis will be performed. Pearson or Spearman correlation coefficients will be used to assess the correlation between continuous or ordinal variables. Partial correlation will be conducted to control the effects of confounding variables. Multiple linear regression analysis will be performed to investigate the relationship between continuous dependent variables and their potential risk factors. In the case of dichotomous dependent variables, logistic regression will be used. To account for within-subject correlation in the longitudinal data due to repeated measurements, methods such as mixed models, generalized estimating equations (GEE), or repeated-measures ANOVA will be employed. For survival

analysis, the life table and Kaplan-Meier methods will be used. To compare groups, the log-rank test will be employed. Cox proportional hazards models and other appropriate multiple survival analysis methods will be used to adjust for potential confounders. Risk Ratio, Rate Ratio, Odds Ratio, and Hazard Ratio will be used to illustrate the relationship between the relevant risk factors and the study outcomes.

We will apply the inverse probability weighting (IPW) approach to correct for potential bias from non-random attrition in the phase III. The propensity score for responding at the phase III measurement will be modeled for the entire baseline cohort using logistic regression, with baseline demographics as predictors. The final weight for each respondent in phase III will be the inverse of their predicted probability of participation.

Results

Participation status

The third phase of the BEH program's first recruitment was conducted from January 2022 to March 2023. During this phase, about 2063 subjects who participated in the second phase (stage 2) of the study and were still alive and residing in Bushehr were invited. Finally, 1345 participants who met the study's criteria were enrolled in the third phase (a response rate of 65.2%). The details of participation status and the reasons for non-participation are presented in Fig. 2.

Figure 2

Participation Flow Diagram for Phase III of the BEH Program

Demographic characteristics of the participants compared to nonparticipants and previous phases

Among the participants, 667 (49.6%) were female. The age range of the study participants was 66 to 100 years, with a mean (standard deviation) age of 74.3 (4.6) years. Female participants had an age range of 66 to 93 years, while male participants ranged from 68 to 100 years. The mean (standard deviation) age for females was 74.2 (4.3) years and for males was 74.5 (4.9) years. The demographic characteristics of the study participants are detailed in Table 3, with comparisons made to non-participants and previous phases. Compared to non-participants, Phase 3 participants were more likely to be male, younger (by an average of four years), and have a higher education level.

When comparing across the BEH Program phases, the average participant age increased by approximately 1.5 years from Phase 1 to 2 and by 4 years from Phase 2 to 3. Phase 3 also had a slightly higher proportion of male participants. Furthermore, the cohort showed a retention of more highly educated individuals over time, alongside an increase in the proportion of widowed participants (Table 3).

Table 3
Demographic characteristics of Phase III participants and non-participants (those invited from Phase II who did not enroll), compared with participants from earlier phases

Variables	**Phase III**			**Phase II**	**Phase I**
	Participants n = 1345	**Nonparticipants n = 718**	**P-value [c]**	**n = 2426**	**n = 3000**
Gender (Male) n (%)	678 (50.4)	286 (39.8)	< 0.001	1166 (48.1)	1455 (48.5)
Age [year, Mean (SD)]	74.3 (4.6)	78.3 (6.7)	< 0.001	69.3 (6.4)	67.9 (7.1)
Age (year) n (%)					
60–64	0 (0)	0 (0)	< 0.001	598 (24.6)	1284 (42.8)
65–69	160 (11.9)	38 (5.3)		952 (39.2)	697 (23.2)
70–74	751 (55.8)	258 (35.9)		379 (15.6)	430 (14.3)
75–79	277 (20.6)	157 (21.9)		282 (11.6)	348 (11.6)
>=80	157 (11.7)	265 (36.9)		215 (8.9)	241 (8)
Education n (%) [a]					
Illiterate	343 (25.8)	300 (41.8)	< 0.001	800 (33)	1089 (36.3)
Primary school	499 (37.6)	252 (35.1)		885 (36.5)	1061 (35.4)
Secondary school	351 (26.4)	128 (17.8)		550 (22.7)	645 (21.5)
Academic	135 (10.2)	37 (5.2)		189 (7.8)	205 (6.8)
Marital status n (%) [b]					
Married	941 (70)	497 (69.2)	0.48	1864 (76.8)	2262 (75.4)

[a] The analysis is based on the Phase 1 measurements

[b] In nonparticipants of phase 3, the analysis is based on the phase 2 measurements

[c] P-values refer to comparisons between Phase III participants and non-participants

Variables	Phase III			Phase II	Phase I
	Participants n = 1345	Nonparticipants n = 718	P-value [c]	n = 2426	n = 3000
Widowed	381 (28.3)	207 (28.8)		523 (21.6)	687 (22.9)
Divorced/Separate with partner	17 (1.3)	7 (1)		20 (0.8)	26 (0.9)
Single	6 (0.4)	7 (1)		19 (0.8)	25 (0.8)
[a] The analysis is based on the Phase 1 measurements					
[b] In nonparticipants of phase 3, the analysis is based on the phase 2 measurements					
[c] P-values refer to comparisons between Phase III participants and non-participants					

Biobanking of biological samples

The biobank stored a total of 4,035 serum samples (three 1 cc vials per participant) and 1,345 EDTA plasma (EDP) samples (one 1 cc vial per participant) from 1,345 participants at the Endocrinology and Metabolism Research Institute (EMRI) of Tehran University of Medical Sciences. An additional serum and EDP vial for each participant was stored at the Persian Gulf Biomedical Sciences Research Institute of Bushehr University of Medical Sciences.

To create a new microbiota biobank, stool and saliva samples were collected from a subsample of participants. Due to the difficulties of stool sampling at home and its proper transport to the study site, this was fully achieved for a sub-sample of 499 participants. These samples included two cryotubes containing 1 gram of stool and one 250 mg cryotube of stool, along with a 1 cc saliva sample in a 15 cc Falcon tube for each participant. In total, 1,497 stool samples and 564 saliva samples were stored at the EMRI biobank. Samples were immediately frozen and stored at -80°C to preserve microbial composition and integrity, as this is the gold standard for long-term microbiome biobanking.

Discuss the significance

The BEH program has comprehensive data collection, which includes demographic information, general health status, and medical history, to achieve a better understanding of elderly health. Additionally, the precise design using multi-stage cluster sampling ensures that the samples are representative of the larger population, providing more generalizable data. This plan for repeated measurements allows researchers to examine changes in key variables and health status over time. Also, the longitudinal design of the BEH program helps uncover the complex interplay between conditions like cardiovascular, musculoskeletal, and cognitive health over time. This comprehensive assessment provides a clearer

path forward for healthcare providers and policymakers in developing public health strategies aimed at promoting healthy aging and increasing community awareness.

What makes this program stand out are a few key points, including geographical and ethnic uniqueness, focus on older adults and geriatric syndromes (e.g., frailty, osteoporosis, sarcopenia, cognitive and functional status), comprehensive phenotyping (e.g., BMD) and unique assets (e.g. microbiota biobank and genome-wide association studies (GWAS) data). Also, by comparing the questionnaires, we can see how the current phase's improved design addresses the weaknesses of earlier phases.

The BEH biobank of biological samples is of great importance and provides critical opportunities for various future wet lab analyses. With the new collection of stool and saliva samples, further evaluation of the microbiota profile, related microbial assessments, and their associations with health conditions is possible. For example, investigating the gut-brain axis and the association of the gut microbiota with cognitive function; exploring the role of the oral and gut microbiota in the development of geriatric diseases and syndromes like frailty, osteoporosis, and sarcopenia; and studying how the microbiota interacts with environmental and genetic factors in this southern Iranian elderly population (31–33).

Not many similar population-based prospective cohort studies on distinct older adults have collected such comprehensive geriatric biomedical data, which increases the BEH program's importance for both national and international scientific communities (34–40).

However, the BEH program has some limitations. A decrease in the participation rate in phase III due to reasons such as death, migration, declining to participate, and physical disability, coupled with a shift in the sample towards men and individuals with higher education, can lead to a slight selection bias and should be considered in the interpretation of results. This bias may limit the generalizability and internal validity of the results. However, such limitation can be partially addressed by applying methodological approaches, such as weighting based on the probability of dropout, to account for potential biases and to improve the internal and external validity of the results. This approach effectively makes the respondents at phase III more representative of the original baseline cohort again, by giving more weight to the types of people who were lost.

Also, there were some challenges in data collection from participants and engaging in assessments, due to physical or mental health issues.

Conclusion

In conclusion, the third phase of the first recruitment of the BEH program serves as a repetition of the measurements from the previous phases. The data collected will be used to assess changes in the health-related variables of interest among participants over this time interval. This study will help to identify health trends among older adults and develop effective strategies for the prevention and management of NCDs in the elderly, as well as improve their health and quality of life.

Abbreviations

BEH
Bushehr Elderly Health
NCDs
Non-communicable diseases
DALYs
Global disability-adjusted life years
ICF
Informed consent form
eCRF
electronic case report form
IPAQ
International Physical Activity Questionnaire
GLFS-5
Geriatric Locomotive Function Scale-5
EQ-5D
Health-Related Quality of Life Questionnaire
SF-12
12-item Short Form Health Survey
ADL
Activities of Daily Living
BADL
Basic Activities of Daily Living
IADL
Instrumental Activities of Daily Living
6CIT
Cognitive Impairment Test
PHQ-9
Patient Health Questionnaire-9
MNA
Mini Nutritional Assessment
PSQI
Pittsburgh Sleep Quality Questionnaire
ESS
Epworth Sleepiness Scale
BMI
Body Mass Index
WC
Waist circumference

HC
Hip circumference
SPPB
Short physical performance battery
ECG
Electrocardiogram
WHO
World health organization
BMD
Bone Mineral Density
DXA
Dual-energy X-ray absorptiometry
BCA
Body composition analysis
VFA
Vertebral fracture assessment
TBS
Trabecular bone score
BMC
Bone mineral content
ASM
Appendicular skeletal muscle mass
CBC
Complete Blood Count
FBS
Fasting Blood Sugar
HbA1c
Hemoglobin A1C
TG
Triglycerides
HDL-C
High-density lipoprotein cholesterol
LDL-C
Low-density lipoprotein cholesterol
ALP
Alkaline Phosphatase
ALT
Alanine Transaminase
AST
Aspartate Aminotransferase

PTH
Parathyroid Hormone
TSH
Thyroid-stimulating hormone
T4
Free Thyroxine (Tetraiodothyronine)
TIBC
Total iron-binding capacity
SOPs
Standard Operating Procedures
EQA
External Quality Assessment
ANOVA
Analysis of variance
ANCOVA
Analysis of covariance
GEE
Generalized Estimating Equations
IPW
Inverse Probability Weighting
EMRI
Endocrinology and Metabolism Research Institute

Declarations

Ethics approval and Consent to participate

The third phase of the BEH program study was approved by the Ethics Committee of the Endocrinology and Metabolism Research Institute, Tehran University of Medical Sciences (IR.TUMS.EMRI.REC.1396.00223). Participants were fully informed about the study procedures and their rights. Written informed consent was obtained from all participants for all components of the study, including data and biological sample collection, utilization, secondary data analysis, and potential publication of findings. This study was conducted in accordance with the Declaration of Helsinki and adhered to Iranian national guidelines for ethics in research.

Competing Interests

The authors declare that they have no conflict of interest.

AI use statement

We used LLMs only for English language editing.

Patient and Public Involvement statement

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

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Author Contribution

KKH and ZHT share equally the first authorship.KKH, ZHT, HSE, GSH, FR, VH, MJM, DJ, RH, KV, NF, BL, AO and IN: Conceptualization and study design. KKH, ZHT, DJ, ZS, EH, SH, MS, AF, KV and IN: Data collection. KKH, ZHT, NF and AO: Drafting of the manuscript. HSE, GSH, FR, VH, MJM, DJ, ZS, EH, SH, MS, AF, RH, KV, BL and IN: Critical review and intellectual revision. All authors provided their final approval of the version to be published. Furthermore, all authors take responsibility for the integrity of the work presented and endorse the accuracy of the study profile as reported.Corresponding authors: Dr. Noushin Fahimfar AND Dr. Afshin Ostovar

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Data Availability

The datasets used in this study are available from the corresponding author upon reasonable request. Due to licensing restrictions, the data are not publicly available.

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Figures

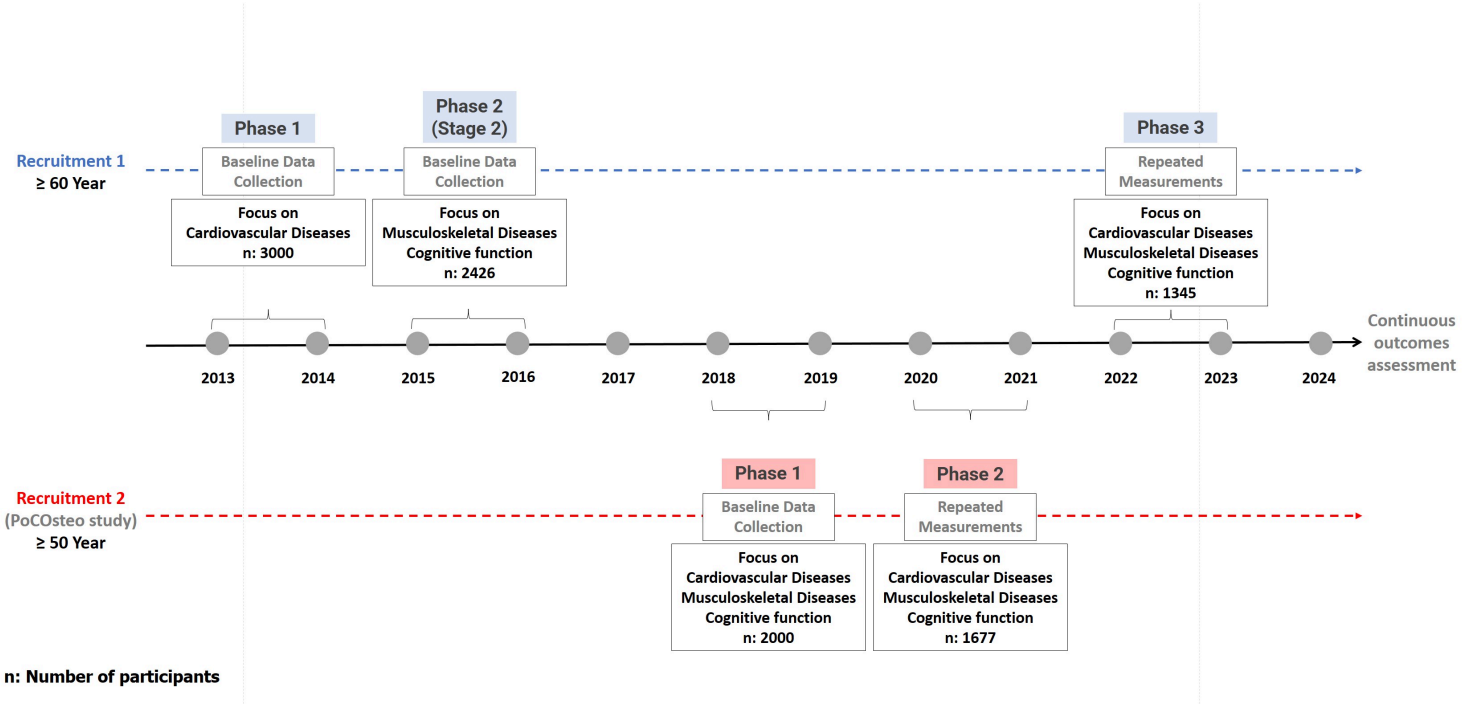


Figure 1

Study Timeline and Key Phases of the Bushehr Elderly Health (BEH) Program

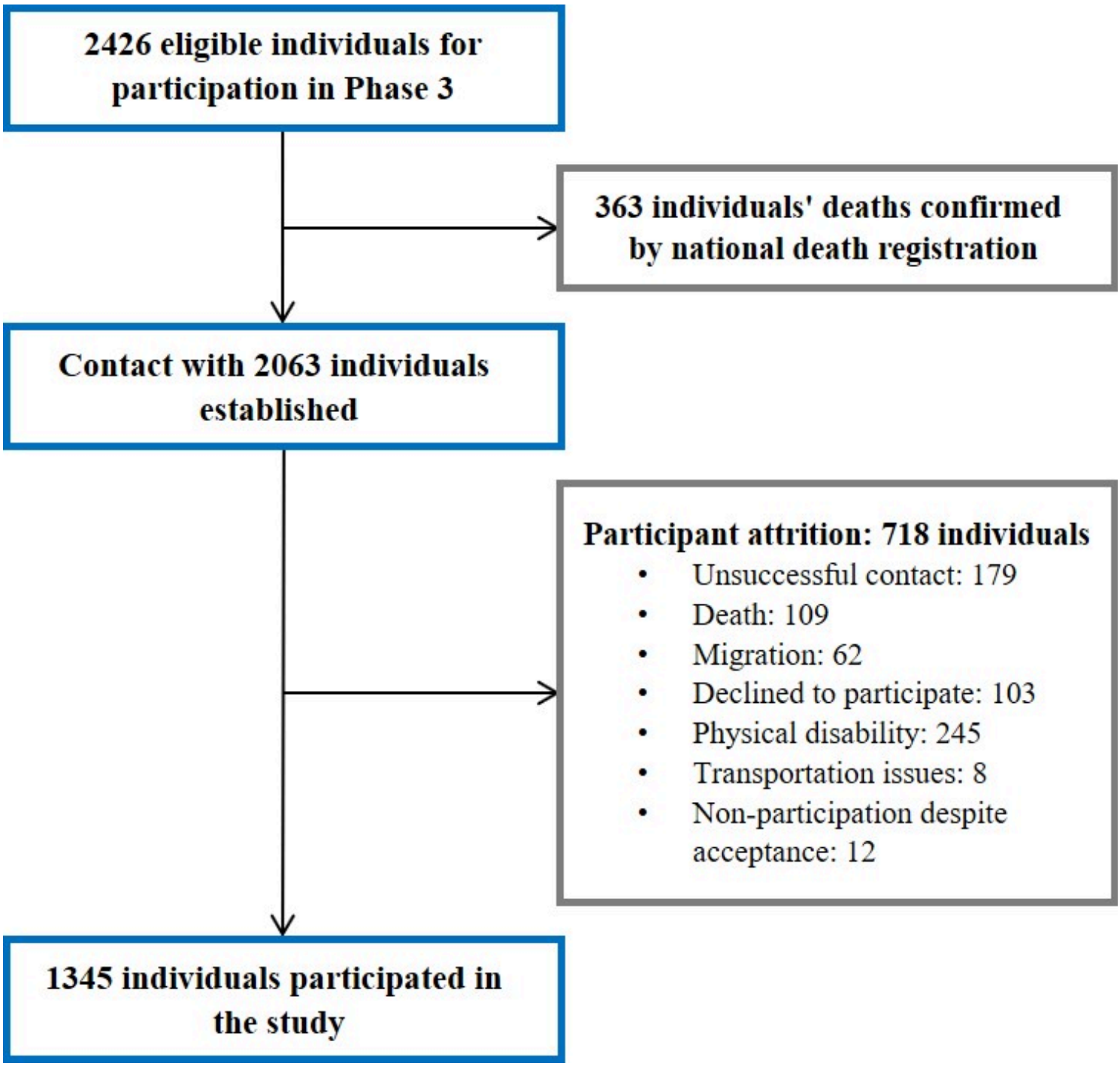


Figure 2

Participation Flow Diagram for Phase III of the BEH Program