Effect of Hydralazine on Alzheimer's Disease (EHSAN)

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A randomized clinical trial evaluating Hydralazine's efficacy in early-stage Alzheimer's disease: The EHSAN Study

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Alzheimer's Disease (AD), a neurodegenerative disorder escalating worldwide, remains incurable with existing interventions merely mitigating symptoms. Hydralazine, an antihypertensive agent, has displayed neuroprotective potential in AD animal models via amplification of mitochondrial functionality and stimulation of stress management and repair pathways. Nevertheless, its effectiveness and tolerability in human AD cohorts are yet to be confirmed. This study protocol describes the design of an ongoing, single-center, randomized, triple-blind, placebo-controlled trial to assess hydralazine's effects on cognitive function in mild to moderate -stage AD patients. We will enroll 424 patients aged 50 and older, meeting NINCDS-ADRDA criteria for probable AD with Mini-Mental State Examination scores from 12–26. They'll be randomly assigned to receive either hydralazine HCL (25 mg, thrice daily) or a placebo for 12 months. The primary outcome is the Alzheimer's Disease Assessment Scale change from baseline to 12 months. Secondary outcomes include various measures using Lawton instrumental activities of daily living scale, neuropsychiatry inventory, and caregiver activity survey. This trial will explore the potential benefits and risks of hydralazine in mild to moderate AD treatment. It's the first trial examining hydralazine's impact on mild to moderate -stage AD in human and is registered at the Iranian Registry of Clinical Trials (IRCT20200711048075N1, registered 29/07/2020) and ClinicalTrials.gov (NCT 04,842,552AQ, registered 13/04/2021). Ethics approval was granted by the Research Ethics Committee of the National Institute for Medical Research Development (IR.NIMAD.REC.1398.424), following the SPIRIT Statement guidelines. Findings will be disseminated via peer-reviewed publications and conferences. This inaugural human clinical trial evaluates hydralazine's impact on patients in the mild to moderate AD. Executed with a randomized, triple-blind, placebo-controlled methodology, this study incorporates a significant sample size and an extended monitoring duration. Multiple parameters, including cognitive capabilities, will be assessed. Potential limitations include the inherent homogeneity of the AD cohort, the lack of biomarker assays, and the unpredictable progression of the disease. Notably, the study might not elucidate the protracted effects of hydralazine beyond a 12-month period. Another limitation of our clinical trial is that patients were diagnosed with Alzheimer's disease based solely on clinical evaluation and MRI findings, without the inclusion of specific biomarkers, which may impact the accuracy and specificity of the diagnosis.

Trial registration: Iranian Registry of Clinical Trials (IRCT20200711048075N1, registered 29/07/2020) and ClinicalTrials.gov (NCT 04,842,552, registered 13/04/2021).

Keywords Alzheimer's disease, Clinical trial, Hydralazine, ADAS-cog, Peanut butter test, SIRT1/SIRT5, Mitochondrial activation, NRF2-mediated stress response, Autophagy activation, NCT 04,842,552

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Abbreviations

AChEI	Acetylcholinesterase inhibitor
ADAS-cog	Alzheimer's Disease Assessment Scale-Cognitive
AEs	Adverse events
AHC	Adineh Health Clinic
BUN	Blood Urea Nitrogen
CAS	Caregiver Activity Survey
CTC	Clinical Trial Center
Cr	Creatinine
DMC	Data Monitoring Committee
DSMC	The Data and Safety Monitoring Committee
eCRF	Electronic Case Report Form
HDL	High-Density Lipoprotein
LDL	Low-Density Lipoprotein
Lawton IADL	Lawton Instrumental Activities of Daily Living Scale
MMSE	Mini-Mental State Examination
MPTP	Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NIMAD	National Institute for Medical Research Development
NINCDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NPI	Neuropsychiatric Inventory Questionnaire
PPS	Palliative Performance Scale
SAEs	Serious Adverse Events
SSU	Shahid Sadoughi University
TDS	Three times a day
eCRF	Electronic Case Report Form

There's no cure for AD, and current treatments only slightly improve symptoms without halting disease progression. Thus, new therapies that can prevent or slow down AD are needed. A potential strategy involves enhancing the impaired mitochondrial function in AD, associated with increased oxidative stress and plaque and tangle formation.

Hydralazine, a vasodilator, has an unclear action mode. Our research showed that hydralazine directly binds to cAMP-dependent protein kinase, improving mitochondrial function and metabolic balance via the SIRT1/SIRT5 axis, mechanism which extends longevity and stress resistance in *C. elegans*[1]. Another study from our team confirmed hydralazine as an activator of the NRF2 signaling pathway, also impaired in AD[2]. Unpublished results from our lab also suggest hydralazine activates autophagy, vital for removing of plaque and tangle components.

Elevated cerebral concentrations of neurotoxic $A\beta_{1-42}$ peptide correlate with cognitive impairment in AD patients. Angiotensin-converting enzyme (ACE) can degrade $A\beta_{1-42}$[3]. A large group of vasodilators are ACE inhibitors. In transgenic APPSWE/PS1ΔE9 mouse model of AD (AD^{+}), ACE inhibitor ramipril increased Aβ levels in AD^{+}mice compared with the levels induced by the ACE-independent vasodilator hydralazine proving hydralazine safety[4].

Acrolein, a highly reactive aldehyde produced as a byproduct of oxidative stress and inflammation. Elevated levels of reactive aldehydes, such as malondialdehyde and 4-hydroxy-2-nonenal, are associated with Alzheimer's disease. These aldehydes are products of lipid peroxidation and can contribute to oxidative stress, neuronal damage, and neuroinflammation, which are key features of Alzheimer's pathology[5,6]. Studies have shown that these reactive aldehydes accumulate in the brains of individuals with Alzheimer's disease, leading to impaired cellular functions and contributing to the progression of the disease[6]. Hydralazine, has been shown to reduce neuropathic pain behaviors and offer neuroprotection in a rat model of spinal cord injury[7].

In Alzheimer's disease, degeneration of the olfactory cortex is a notable feature and contributes to the impairment of the sense of smell, which is often one of the earliest symptoms observed in patients. The olfactory cortex, responsible for processing olfactory information, experiences significant neuronal loss and amyloid-beta deposition, which correlates with the overall progression of Alzheimer's pathology. This degeneration disrupts the normal processing of olfactory stimuli, leading to a decline in the ability to detect and differentiate odors[8].

Hydralazine has also been shown to be a reactivator of the Nrf2/ARE pathway in various ages and types of mouse or human lens epithelial cells and mice lenses in-vitro/in-vivo leading to cytoprotection, and extended lens healthspan by delaying/preventing lens opacity against aging/oxidative stress[9].

This study addresses two primary questions: First, is hydralazine (25 mg, thrice daily) effective in managing mild to moderate -stage AD in patients 50 years and older? Second, is the peanut butter test clinically sensitive for evaluating mild to moderate -stage AD progression[10]?

Methods

All methods for the EHSAN phase three clinical trial were conducted in strict adherence to the CONSORT statement, ensuring standardization and reproducibility as per the 25-item checklist that guides the design, analysis, and interpretation of randomized control trials.

Design

This is a first in human, single-center, double-armed, randomized, triple-blind, placebo-controlled ,1–1 allocation ratio, clinical trial to evaluate hydralazine's effect on cognitive function in mild to moderate -stage AD patients of a long term (52 weeks) treatment compared to placebo, called Effect of Hydralazine on mild to

moderate -Stage Alzheimer's disease (EHSAN) study. The intervention and placebo groups, will be randomly allocated to one of two regimens: (1) Hydralazine at 75 mg (25 mg thrice daily) or (2) A placebo.

It is recognized that sections of the olfactory cortex are often the initial locales of AD pathology, with patients frequently exhibiting a greater degree of degeneration in their left hemisphere. Given that the olfactory epithelium chiefly projects to the corresponding olfactory cortex, patients with AD display an asymmetric decrement (left surpassing right) in their capacity for odor detection[11]. A retrospective, case–control investigation aimed at evaluating a rapid olfactory examination as a potential diagnostic tool for AD showed that this non-invasive, economical left–right nostril odor detection assay is a sensitive and specific method for the probable diagnosis of AD[10].

Participants

This single-center, triple-blind, randomized superiority trial involves 424 mild to moderate -stage AD patients for one year at Adineh Health Clinic (AHC), Yazd, Iran. The intervention will last 52 weeks, and the SPIRIT protocol will guide the trial's reporting[12]. Patients are enrolled in the trial through several methods: 1) Referrals from neurology and psychiatry specialists who are co-PIs of the study, 2) Advertisements at Shahid Sadoughi University (SSU) and AHC neurology and psychiatry clinics in Yazd, Iran, 3) A preliminary screening survey on social media, 4) Collaborations with AD NGOs such as Sayeban Salamat, 5) Recommendations from patients' relatives, 6) Health insurance records of individuals prescribed AD medications in the past year, 7) Public announcements via city billboards, and 8) Yazd residents over the age of 50 with signs of dementia were contacted via text and invited to come for screening. Additionally, we access databases from two ongoing prospective cohort studies that have recorded family histories and current AD[13,14].

Assessments

1) Cognitive evaluation:

Interview and filling the questionnaire: The interview sessions will be conducted according to Table 1 and data will be entered in EHSAN eCRF immediately.

The baseline cognitive evaluation will be conducted by trained researchers who will screen potential participants. During the initial visit, the study procedure will be explained, informed consent will be obtained, and the Mini-Mental State Examination (MMSE) and the Palliative Performance Scale (PPS) will be completed. The PPS is a validated and reliable tool used to assess a patient's ability to function and determine their progression toward the end of life. We will complete the PPS along with the MMSE at the beginning to estimate the likelihood of participants completing the study.

PPS questionnaire will be completed for each participant. The MMSE, developed by Folstein and colleagues, is a widely used tool for assessing cognitive status in psychiatric patients with cognitive impairment. It evaluates seven cognitive domains and provides a total score ranging from 0 to 30, with scores below 24 indicating possible dementia. However, well-educated or younger dementia patients may score higher.

Participants with MMSE scores between 12 and 26 and PPS test results below level 3 (80% ability) will undergo further assessments, including body assessments, blood pressure measurement, blood tests, EKG, brain MRI, and psychological evaluations using SADS to assess major depression, psychosis, and illicit drug abuse. Throughout the study, participants will be encouraged to continue their regular physician-prescribed treatments.

2) Physical health assessment:

Blood Sample Collection and Storage: Between 8:00 a.m. and 12:30 p.m., 15 ml venous blood samples will be drawn from participants at the onset and conclusion of the six-month intervention. These samples will be subdivided into two serum, one plasma, one buffy coat, and one whole blood aliquots. They will be stored in 1-mL DNase- and RNase-free microtubes at −80°C pending analysis. Initial and final phase serum samples will undergo biochemical analyses to evaluate creatinine levels for exclusion determinations and other necessary tests for documentation.

Laboratory Analysis: Serum samples will be evaluated for biochemical markers such as glucose, triglycerides, cholesterol, LDL, HDL, BUN, creatinine, urea, and uric acid using standard test kits.

Medication Monitoring: Participants are instructed to maintain their medication regimen and inform researchers of any changes, as some drugs may impact biochemical markers. For tracking, participants should present all medications they're using at each visit.

Adverse Event Monitoring: Although no significant side effects are anticipated from the intervention pills, any adverse events will be evaluated by the trial steering committee and reported to the SSU ethics subcommittee overseeing clinical trials.

Inclusion criteria

Eligibility Criteria: Participants should be 50 years or older, diagnosed with mild to moderate AD (evidenced by MMSE scores between 12–26) and meet the NINCDS-ADRDA criteria for possible or probable AD. They must have a consistent caregiver capable of medication management, accompanying them to appointments, and evaluating their condition and updating the logbook. Both the participant (or surrogate) and caregiver must give written informed consent. Prior to randomization, participants should be prescribed a stable dose of either donepezil (5–10 mg daily), memantine (5–21 mg daily), rivastigmine (3–6 mg daily), or galantamine/ER (16–18 mg daily) and must abstain from using hydralazine during the study.

Enrollment Procedure: After a 2-week wait for blood test and MRI outcomes, the study physician will determine participant eligibility, excluding those with non-Alzheimer dementia based on MRI results. Once approved, patients with direct or surrogate consent, along with caregiver's consent, will be registered. They'll then receive a unique drug code through the Sealed Envelop™ integrated within EHSAN's secure eCRF (www.

Measured variables	Screening of study (pre-enrollment)	run-in period (Month 0)	phase 1 (Month 3)	phase 2 (Month 6)	Phase 3 (Month 9)	Close down visit phase 4 (Month 12)
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Eligibility criteria assessment	*					
Medical history	*					
Informed consent	*					
MMSE[1]	*					
SADS[2]	*					
PPS[3]	*					
EKG[4]	*					*
Blood pressure	*	*	*	*	*	*
Blood tests	*			*		
MRI prescription	*					
ADAS-cog[5]		*	*	*	*	*
NPI[6]		*	*	*	*	*
Lawton IADL[7]		*	*	*	*	*
CAS[8]		*	*	*	*	*
Peanut butter test		*	*	*	*	*
Biobank sampling	*					
Blood pressure	*	*	*	*	*	*
Blood sampling	*			*		
Biochemical assessment			*	*	*	*
Blood glucose (BS)	*			*		
Triglyceride (TG)	*			*		
High-density lipoprotein (HDL)	*			*		
Serum hydralazine level test				*		

Table 1. Overview of administered assessments and their scheduling. This table provides a comprehensive list of the various assessments conducted, detailing each measure's name and the corresponding schedule. The assessments include: (1) Mini-Mental State Examination, (2) Schedule for Affective Disorders and Schizophrenia, (3) Palliative Performance Scale, (4) Electrocardiogram, (5) Alzheimer's Disease Assessment Scale-Cognitive, (6) Neuropsychiatric Inventory Questionnaire, (7) Lawton Instrumental Activities of Daily Living Scale, and (8) Caregiver Activity Survey.

ehsan-study/eCRF) (Fig. 1). All participants will benefit from Persian RCT insurance throughout the study. Centralized activities, like questionnaires and evaluations, are detailed in Table 1.

Exclusion criteria

These exclusion criteria have been established to ensure a homogeneous and relevant study population, enhancing the validity and interpretability of the research findings.

1. Non-Alzheimer primary dementia (e.g., vascular dementia, Lewy body dementia, frontotemporal dementia, vitamin B-12 deficiency, hypothyroidism) to ensure a focus on AD.
2. Presence of current major depression, delirium, alcohol or psychoactive substance abuse or dependency, schizophrenia, or delusional disorder as defined by DSM-V, as these conditions may confound study results.
3. Presence of any uncontrolled systemic illness that could interfere with study participation or a life expectancy of less than one year, to ensure participants can actively engage in the research.
4. Current treatment with hydralazine or a history of intolerance to oral therapy with hydralazine, as this medication could interfere with the study's objectives.
5. Any intravenous treatment for heart failure, except IV furosemide (e.g. IV inotropes, pressors, nitrates, or nesiritide) at the time of screening, to maintain consistency in the study population.
6. Systolic blood pressure < 100 mmHg, as lower blood pressure levels might affect the study outcomes.
7. Reversible etiology of acute heart failure, such as myocarditis, acute myocardial infarction within the past 4 weeks, arrhythmia, and pacing device, as these conditions may not represent chronic heart failure.
8. Known severe congenital heart disease (such as uncorrected tetralogy of Fallot or transposition of the aorta) and severe aortic or mitral stenosis or severe rheumatic mitral regurgitation, as these conditions may significantly affect heart function.
9. Concurrent use of phosphodiesterase type 5 inhibitors (e.g., Viagra, Sildenafil, etc.), to minimize potential drug interactions.

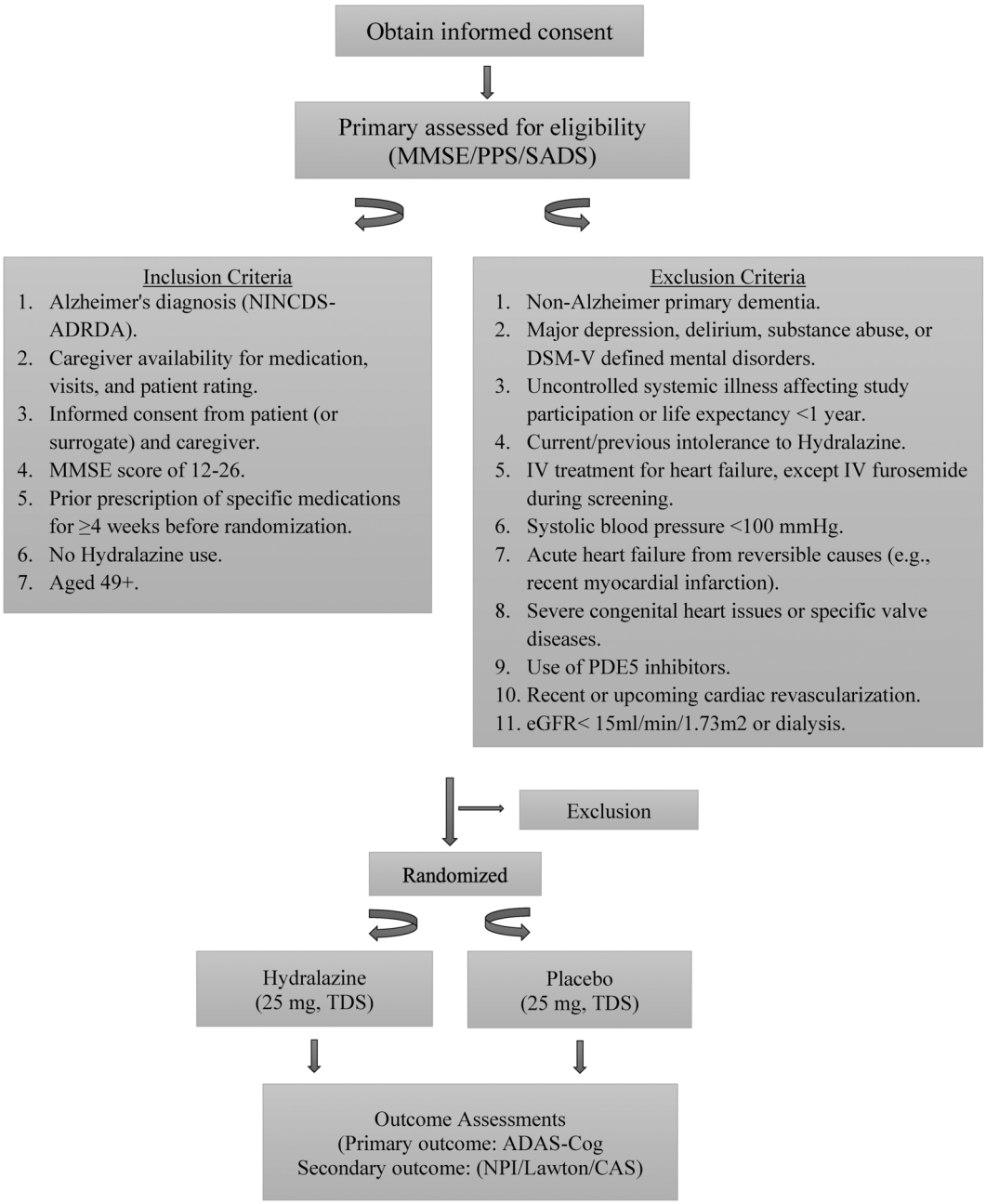


Fig. 1. The study flowchart. Participants will be randomized into one of four rolling sequences to receive a specified intervention.

10. History of cardiac revascularization within the last 3 months or likely to require coronary revascularization within the study period, to exclude individuals with recent or anticipated interventions that might impact the study's parameters.
11. eGFR < 15ml/min/1.73m2, or on regular dialysis, or planned dialysis within the study period, as kidney function might influence study outcomes.

Intervention

The hydralazine HCL of medical grade was imported from the Guangdong Wengjiang Chemical Regent Co. Hydralazine 25 mg tablets and matching placebo for hydralazine, identical in appearance, will be prepared by the Sobhan Pharma Company, which are then packed into 90-pill bottles, intended to provide a 30-day supply of either drug or placebo.

The intervention pills used in the study have undergone chemical analysis. The pill contents were sourced directly from the manufacturer, accompanied by an endorsed certificate of analysis. To investigate the content of hydralazine HCL in the patients' serum, a random sample of 20 will be evaluated using gas chromatography with

a flame ionizer detector during the course of the study. The time between the intervention pills' production and delivery to the participants, as well as their consumption period, will be within the expiration date of the pills.

Hydralazine Dosage Protocol: Hydralazine is typically initiated at a dose of 10 mg orally every 6 h for 2–4 days, with gradual increases of 10–25 mg per dose every 2–5 days, up to a maximum of 50 mg every 6 h. In Iran, the average dose is approximately 25 mg three times daily (TDS) due to available tablet formulations. A study by Gonzalez-Fierro et al. reported that a daily dose of 83 mg hydralazine caused only transient side effects in slow acetylators, who constitute the majority of the Iranian population, indicating that this regimen may be safe[15].

Treatment starts at 37.5 mg daily (split from a 25 mg pill, taken thrice daily). If well-tolerated, this is increased to 75 mg after two weeks. Otherwise, a lower dose is sustained.

Hydralazine Dosage Regimen: Over two weeks, hydralazine (or its placebo) will be adjusted to a steady dose of 75 mg daily (25 mg thrice daily). Initially, patients will consume 12.5 mg thrice daily for a week. In week two, they'll be assessed for hypotension and potential side effects. From the third week onwards, the dosage will be 75 mg daily, split into three 25 mg doses.

Intervention Pill Distribution: Participants will receive intervention pills (Fig. 1) and must return the previous pill bottle at every follow-up. They'll then receive a new coded bottle for the next 12 months (52 weeks). In the latter half of the follow-up, the hydralazine serum level will be randomly measured in 20 patients.

Randomization

Randomization will be stratified based on age (either under 65 years or 65 and older), sex (male or female), and MMSE score (between 12–18 or 19–26) (Fig. 2). The allocation ratio for this process will be 1:1. The sequence of randomization will be created through a computer program using random blocks of varying sizes. To ensure allocation concealment, a random assignment page in the eCRF will be used. Blinding will be maintained throughout the study, with participants, investigators, outcome assessors, data analysts, and sponsors unaware

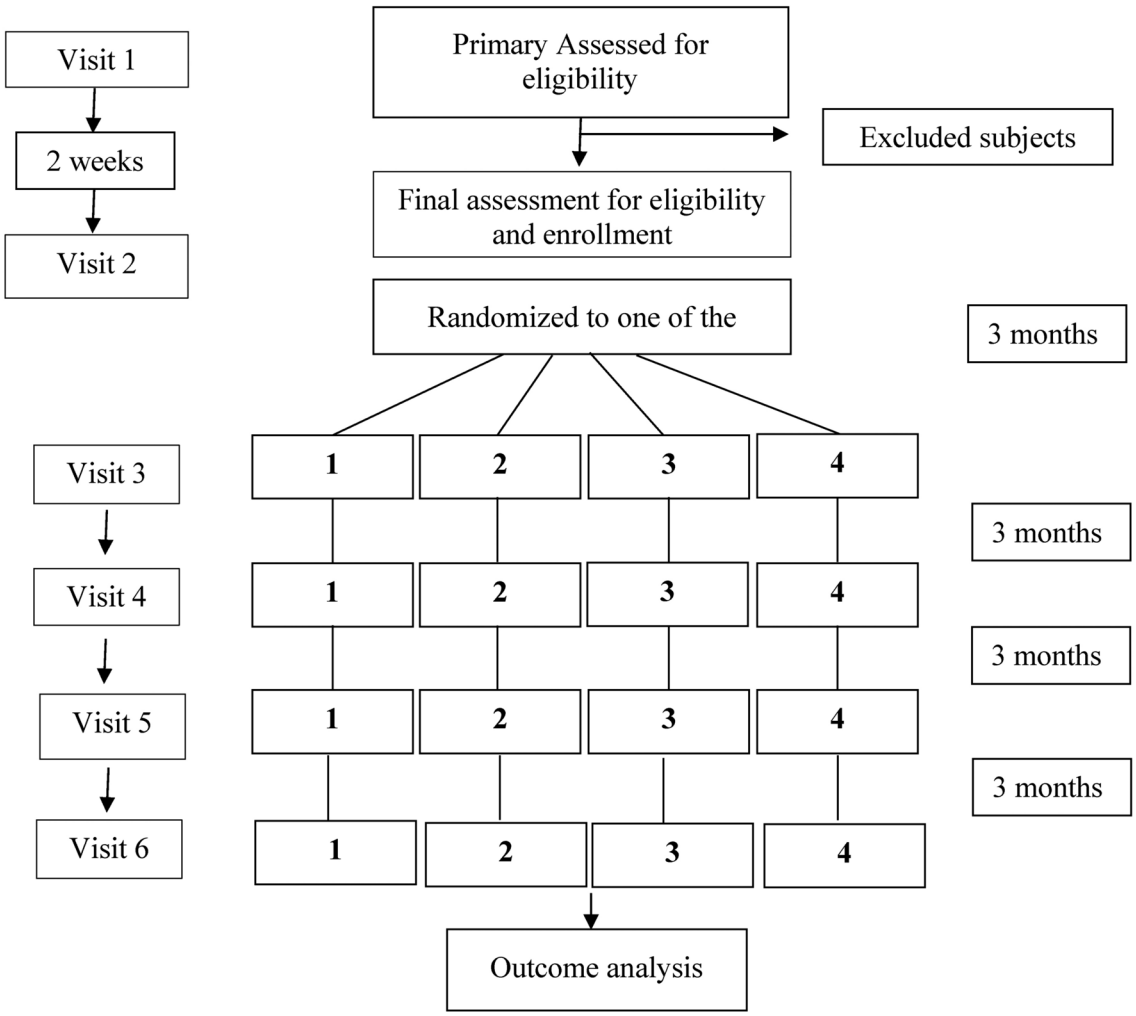


Fig. 2. CONSORT diagram of the EHSAN study. This diagram depicts the flow of participants in the EHSAN study. It begins with the number of participants assessed for eligibility, detailing exclusions and the reasons for them. Following the initial assessment, the diagram illustrates the randomization process, showing the allocation of participants to each intervention group.

of group assignments. Following stratification, participants in each block will be randomly assigned to one of four sequences of the rolling process to administer the two interventions. To ensure a balanced and random order of treatment administration for all participants, computer-generated random numbers will be utilized. The allocation sequence and the randomization process will be conducted using the sealed envelope software embedded in the study software. This entails inputting each participant's randomly assigned sequence of interventions into the appropriate section of the EHSAN software (www.ehsan-study.com/eCRF).

This study uses a double-armed, randomized format with equal allocation to either: (1) Hydralazine, dosed at 75 mg (25 mg three times daily), or (2) A placebo. The pharmaceutical company will blind and code the bottles (001 or 002). These codes won't be revealed until post-analysis to ensure blinding of participants, staff, and statisticians. In emergencies, only the trial manager and data coordinator will access group assignments, documenting any unblinding occurrences.

The participants, outcome assessors, and researchers will be blinded to the treatment groups. The allocation ratio for the two treatment groups will be 1:1. The random sequence will be produced, and permuted block sampling randomization will be used for the randomization process, with each block consisting of four participants (Fig. 2). The treatment scheme will be generated through the Sealed Envelope ™ website embedded in EHSAN e-CRF.

The study medication, which is approved by the Iranian FDA, will be provided to each participant at the AHC. The medications will come in identical bottles, each containing 90 pills. Every bottle will carry a unique medication identification code, which will either correspond to the drug or the placebo. Both the site personnel and the patient will be blinded to the medication type and treatment assignment.

Eligible patients currently prescribed an acetylcholinesterase inhibitor (AChEI) or NMDA blockers will be randomly assigned to receive either a daily dosage of 75 mg hydralazine (25 mg three times a day) or a matched placebo. Dose adjustments are permitted during the initial two weeks, depending on the patient's tolerance of the drug regimen. If a lower dose is sustained and the participant completes the study with the same dose, the analysis will be adjusted accordingly.

Primary outcome measure

The study's primary endpoint is the Alzheimer's Disease Assessment Scale Version 11.0 (ADAS-cog)[16,17], a 21-item scale to gauge the severity of cognitive and behavioral impairments in AD patients. This scale, with a scoring range from 0 (no errors) to 70 (severe impairment), is frequently used in drug trials due to its sensitivity to changes in disease severity. The ADAS-cog includes tasks like word recall, command following, object naming, praxis tasks, orientation, word recognition, instruction recall, spoken language ability, comprehension, and word-finding difficulty. The ADAS-cog is widely used as an outcome measure in clinical drug trials both in Iran, validated in Persian language[18,19], and in recent large RCTs in the US[20] because of its sensitivity to change in disease severity. With an inter-rater reliability estimate of 0.99 and test–retest reliability estimate of 0.92, the scale correlates modestly to highly with the Memory-Information Test and Dementia Rating Scale. Validated in Farsi and used in numerous similar Iranian and international studies, the ADAS-cog is a trusted measure of cognitive function in AD patients.

Secondary outcome measures

1. The trial will employ the eight-item Persian version of the Lawton Instrumental Activities of Daily Living Scale, originally developed by Lawton and colleagues[21]. This scale will be used to measure the functional abilities of AD patients, who are either being treated with hydralazine or a placebo, in performing their daily living activities.
2. Furthermore, the Neuropsychiatric Inventory (NPI), introduced by Cummings and colleagues, will be used to examine psychological and behavioral issues in dementia patients[22]. This inventory is based on a structured interview conducted by a trained clinician with a caregiver who is knowledgeable about the patient's behaviors. The CAS will be used for this purpose, which includes the following: communicating with the person, using transportation, dressing, eating, looking after one's appearance, and supervising the person. By knowledgeable, we mean someone familiar with these aspects of the patient's life. The NPI is a fully scripted interview, with all questions being asked verbatim. Notably, the caregiver interview serves as the sole information source for the rating session, implying that the patient will not be present during this session. The NPI employs screening questions for each of its ten or twelve domains (in its most recent version) to minimize administration time. If a particular behavior is absent, no follow-up questions are asked.
3. The study will utilize the caregiver activity survey (CAS), developed by Davis and colleagues, to evaluate the amount of time caregivers spend assisting Alzheimer's patients with daily activities[23]. The CAS is a six-item questionnaire that asks caregivers to estimate, in hours and minutes, the time spent performing specific activities for the patient over the past 24 h. These activities include 1) communicating with the patient, 2) assisting the patient with movement, 3) helping the patient dress, 4) aiding the patient with eating, 5) maintaining the patient's appearance, and 6) supervising the individual. The CAS has shown moderate correlations with the ADAS-cog, and Physical Self-Maintenance Scale. In a clinical trial involving the drug velnacrine maleate, patients under treatment displayed a trend toward decreased caregiving time, as assessed by the CAS.

All inventories including; ADAS-cog[19], Lawton-IADL[24], CAS[25], and Neuropsychiatric Inventory[26], have all been translated and validated in Persian.

Staff training

In this trial, two psychologists and two nurses were specifically employed to conduct interviews and other interventions as part of the study (Table 1). Before treating patients, they underwent practice sessions, interviewing two patients each, and receiving feedback from the investigators. Eligible participants who have provided informed consent will undergo baseline assessments within one week prior to randomization. These assessments will include a review of current medications, blood test results, and participant and caregiver assessments. Randomization will follow these baseline assessments (Table 1).

To ensure competence in conducting the research, the psychologists and nurses will receive training according to the study protocol from the education committee of the PIs. With participants' consent, interview sessions may be recorded for quality checks and feedback purposes.

Throughout the study, participants will continue their standard care for AD, which may involve cholinesterase inhibitors and/or NMDA receptor antagonists. However, they will be advised not to change their medication dosage or type during the study, except when medically necessary. Any such changes will be documented as protocol deviations. Additionally, participants will be instructed not to take any other medications that could potentially interact with hydralazine or impact cognitive function, except when medically necessary. Concurrent medication use will be carefully documented and reported as adverse events.

Logistical procedure

Throughout the trial, all enrolled patients will be regularly followed up in the clinic every three months for one year (Table 1). In the event of a missed appointment, caregivers will be contacted for a phone interview three months after randomization to report any adverse events, and a logbook will be provided to aid in tracking such events.

If a closeout visit cannot be arranged at the end of the trial or within 30 days, the patient will be withdrawn from the study. The final visit will mirror an annual follow-up appointment, including assessments of adverse events, a physical examination, and laboratory tests if not conducted within the past six months. The study's blind will remain intact when a patient withdraws from the trial, and it will only be broken for all patients at the study's conclusion.

The three secondary outcome measures (Lawton IADL, NPI, and CAS) will be evaluated every three months during clinic visits, similar to the primary outcome measure. The Lawton IADL will be assessed directly with the patient and typically takes 5 to 10 min, depending on their level of functioning. For patients unable to attend clinic visits due to hospitalization, study site staff will attempt to gather Lawton IADL data at the patient's location if feasible. The other secondary outcome measures (NPI and CAS) will be collected from reporting caregivers or caregivers knowledgeable about the patient's daily activities. Completing the three secondary outcome measures takes about 15–20 min in total and can be done in person or over the phone if necessary. However, phone data collection will primarily focus on safety concerns and the primary outcome due to the time commitment required for the secondary measures.

During seasonal clinic visits, in addition to assessing adverse events and primary and secondary outcomes, safety evaluations will be conducted, including physical examinations, concurrent medication use evaluations, and blood draws for local laboratory safety measures. Blood samples to measure hydralazine serum concentrations will be taken at the 6 and 12-month marks to verify medication adherence. A follow-up visit is expected to last approximately one hour, depending on the patient's level of functionality.

Pretrial statistical analysis

Sample size calculation and power analysis: The primary hypothesis of this study posits that hydralazine, when compared to a placebo, will significantly delay AD progression as measured by the ADAS-cog inventory in patients with Alzheimer's who are also taking an AChEI. The primary endpoint analysis will utilize a repeated measures mixed effects model[27].

The calculation was done for a mixed model with repeated measures featuring a linear trend over time. Typically, patients with mild to moderate AD show an average annual change in ADAS-cog scores of 4–5 points[18,19]. The PASS software[28], a sample size program for repeated measures factoring in attrition, was employed to compute sample size estimates. The following assumptions were considered: (1) A type-I error of 0.05 (two-sided); (2) 80% and 90% statistical power; (3) A study duration of one year with an average follow-up of 1.0 years; (4) An average of 4 follow-up measures per patient, based on quarterly assessments over a mean follow-up period of 1.0 years; (5) A loss rate of 2.5% per quarter (approximately 10% annually); (6) An estimated average annual increase of about 5 units in the ADAS-Cog total score with standard therapy; (7) A common standard deviation ranging from 3 to 5 units; (8) Hypothesized mean differences of 1 or 1.5 units in the ADAS-cog score at the end of a median follow-up of 1.0 years between the two study arms.

This research will involve a total sample for a repeated measures analysis, considering a 0.05 Type-I error (2-sided), 4 follow-up measures, a correlation of 0.50 between repeats, and a 10% attrition rate. The study's secondary endpoints are: (1) Lawton Activity of Daily Living Scale (function); (2) Neuropsychiatric Inventory (behavior); (3) Caregiver Activity Scale (caregiver time).

Considering multiple treatment evaluations, the study will use a Type I error of 0.25 (two-sided) for secondary outcome power calculations. For the primary outcome, a Type I error of 5% (two-sided) is applied[29].

For a repeated measures mixed model with a linear trend (growth curve), the detectable mean differences at the final time point for both 80% and 90% power will be established, based on a sample size of 424 patients including 212 in the intervention arm and 212 in the control arm who will be analyzed for primary and secondary outcomes.

For mild to moderate Alzheimer's patients, the average annual change is 25 points for the NPI[30], and 2.7 h per day per year for the CAS[31].

Post-trial statistical analysis

Interim monitoring in this study will emphasize adherence to protocol, baseline equality of treatment groups, data retrieval completeness, total patient enrollment, and safety and efficacy of the treatment in relation to the primary endpoint, which is the change on the ADAS-Cog inventory. The interim analysis will concentrate on re-estimating sample size. This analysis will be performed before the scheduled recruitment end and the interim efficacy analysis.

The DMC will receive data on the observed variance of the mean ADAS-cog from the mixed effects model, and the correlation of repeated ADAS-cog measurements within patients in all treatment arms. These estimated model parameters will be used to re-estimate the sample size required to achieve 80% and 90% power.

Bootstrapping techniques will be used to predict the distribution of these parameters and to generate a range of possible re-estimated sample sizes. The DMC will use this range to decide if there's a need to increase the sample size, if the initial sample size is adequate, or if a smaller sample size would still offer acceptable power without needing an extension of recruitment if the initial target sample size is unattainable.

To preserve the type-I error and since treatment effect sizes rely on minimally clinically important differences, we won't use observed treatment effects for sample size re-evaluation. Approximately 18 months after EHSAN's initiation, an interim analysis on primary endpoint treatment efficacy will be conducted, following sample size re-estimation, using Haybittle and Peto's approaches[32].

To protect the type-I error, the primary treatment comparisons (active treatments vs. placebo) will undergo a single test with 2 degrees of freedom. A significant joint test result ($p\text{-value} < 0.05$) indicates a meaningful difference in treatment outcomes.

Statistical analysis will utilize STATA software (version 14). Quantitative data distribution will be verified with the Kolmogorov–Smirnov test. Non-normally distributed variables will be transformed for normalization prior to evaluation.

The effects of the treatment pills will be compared using the linear mixed method with the rolling process between-subject factors. Adjustments will be made for potential confounders, such as age, sex, baseline BMI, the number of intervention pills consumed per subject, physical activity measured in metabolic equivalent-min/day, and the amount of calorie intake.

A subgroup analysis for sex and hypertension will also be performed. Protocol non-adherence will be managed using an intention-to-treat method. To control for the carryover effect, a clinically appropriate washout period of one month will be used. Additionally, the carry-over effect will be statistically tested through the mixed effect model.

If there are missing data, they will be addressed using multiple imputations in line with Harrell's guidelines.

Data management

Data collection, coding, and entry for the study will be manually carried out by the research staff. All data will be kept by the principal investigator (PI) of the research. Each study participant will be assigned a unique code number to ensure their privacy. Access to identifying participant information will be restricted to authorized personnel only. The study's protocol, dataset, and statistical code are available through the corresponding author. While the data is approved solely for this analysis and won't be public, supporting data for the protocol can be provided on request.

Treatment adherence protocol

Ensuring treatment adherence within this clinical trial is facilitated by the EHSAN website, which employs a text reminder system to dispatch messages to patients and caregivers at specified intervals. Moreover, patients/caregivers will be contacted via telephone at weeks one, two, and four post-randomizations, enabling the resolution of any queries related to medication or the study protocol. Should changes in patient/caregiver status, patient safety, or study medication occur during these calls, the appropriate case report forms must be diligently completed according to the guidelines outlined in the study's Operations Manual. While these telephonic interactions are actively encouraged, they are voluntary and are subject to the discretion of the site staff, who will consider the unique needs of each patient and caregiver.

Attrition rate

Based on analogous clinical trials, an anticipated attrition rate of 10% has been considered. The study will be conducted in a city of moderate size, comprising a population of 825,000. Emergency contact details for all participants will be documented, enabling prompt communication in the event of attrition. Rigorous measures will be adopted to mitigate attrition, encompassing facilitation of complimentary return taxi transportation to the study site, telephonic interviews, and potential medication delivery to participants' homes as stipulated by the protocol.

Trial management and oversight committee

Trial management and oversight are meticulously established within this study. Collaborative consultation with academic neurologists and psychiatrists from SSU resulted in the engagement of the majority (80% out of 10) who concurred to endorse the study, subsequently participating as Co-PIs.

Given the recruitment goal, the EHSAN trial aims to enroll roughly 50% of eligible patients. However, due to the disruptive impact of eight waves of COVID-19 on patient recruitment, the completion date has been deferred by two years, as reflected in the updated information on ClinicalTrials.gov.

The PI and the Executive Committee are vested with the responsibility of overseeing the recruitment pace and operational aspects of the study. If recruitment encounters delays, the Study PI and study biostatistician will investigate the reasons, including the exclusion of potentially eligible patients, and subsequently provide a report

to the executive committee. Upon analyzing these findings, the executive committee may propose adjustments to the study's eligibility criteria.

Executive committee
Ten investigators will be participating in the execution of the clinical trial. Their tasks will include recruiting study participants, conducting participant visits, dispensing intervention pills, collecting blood samples, taking EKGs, biobanking and managing data collection and entry. Regular weekly meetings with the PI will be held to ensure that the study remains in line with the planned protocol. Additionally, weekly meetings with the trial's steering committee, which consists of the PI and four research staff members, will be held at AHC to monitor and support the trial's progress.

Monitoring data quality and protocol adherence
The Clinical Trial Center (CTC) linked with Yazd University of Medical Sciences (yazd-ctc.ssu.ac.ir) and NIMAD will monitor the AHC for data quality, follow-up completeness, and protocol adherence. Ensuring strict protocol adherence at AHC is paramount, yet patient well-being takes precedence if protocol adherence poses risks. Protocol deviations will be diligently documented.

The Executive team and CTC will centrally oversee protocol compliance, with ongoing stringent monitoring of data quality and retrieval. The study biostatistician will compile interim monitoring reports encompassing patient intake, randomization errors, protocol deviations, treatment assignment adherence, missed visits, follow-up completion, data quality, query rate, error rate, and audit/site visit findings. Treatment efficacy and futility will also be assessed.

The primary endpoint's effectiveness will be evaluated about 1.5 years into EHSAN. Formal futility analyses are not planned, but the Data Monitoring Committee (DMC) at the CTC will review interim analyses and decide on trial continuation based on efficacy, futility, or safety concerns. Statistically significant treatment comparison results in interim analysis could prompt DMC recommendations for early termination or continuation. Secondary outcome measures and relevant external information will aid the DMC's decision-making.

Safety monitoring
The Data and Safety Monitoring Committee (DSMC), comprising independent experts in Clinical Neurology, Psychiatry, Cardiology, Biochemistry, and Epidemiology, will assess the study protocol pre-implementation for potential concerns. They will periodically review participant safety, study progress, and conduct, furnishing recommendations on trial continuation, modification, or termination. Operating autonomously from the sponsor, the DSMC maintains conflict-free status. Bi-monthly written and verbal reports will be provided, with additional meetings scheduled if necessitated by data or adverse event occurrences.

In the EHSAN study, randomized patients will undergo vigilant monitoring for adverse events (AEs) and serious adverse events (SAEs) during clinic visits and phone interactions. Beyond the defined AEs and SAEs, EHSAN will specifically capture hypotension, syncopal episodes, falls, and heart failure data, as these are associated with high-dose hydralazine in prior research. Active monitoring of AEs and SAEs will commence upon patient consent, persisting until their final follow-up, irrespective of protocol adherence. Furthermore, for 30 days following a randomized patient's trial conclusion, the investigator will document reported SAEs. If patients who've consented but not been randomized are ineligible or disinterested, active monitoring will cease accordingly.

Discussion

Our upcoming investigation will represent the inaugural human, randomized controlled trial studying the influence of hydralazine HCL on mild to moderate -stage AD. Prior research has been confined exclusively to animal models. This will be a single-center, three-armed randomized clinical trial. We aim to mitigate potential sources of bias, including selection bias, confounding variables, and ascertainment bias, by employing conventional techniques of randomization, allocation concealment, and blinding.

The intervention period, extending over 52 weeks, provides a sufficient duration to monitor fluctuations in cognitive function among participants, as quantified by the ADAS-cog inventory. Furthermore, with an ample cohort of 424 participants and abundant biological samples, our study affords an exploration of the gender-segregated impact of the intervention tablets and various biomarkers with robust statistical power. This participant population additionally empowers us to investigate potential gene-disease interplays in future studies. A distinctive element of this study resides in our comparison of hydralazine to standard treatments currently accessible in the local market, including galantamine, donepezil, memantine and rivastigmine.

Each participant visit will entail the documentation of medication usage, thereby equipping investigators with the ability to validate the sensitivity of observed effects to medication alterations, specifically through the exclusion of individuals reporting modifications in their therapeutic regimen.

The limitations of this trial stem primarily from its single-center design, which could potentially limit the broad applicability of its findings across heterogeneous populations. The protracted intervention duration could present challenges in participant recruitment. To mitigate this, we plan to underscore the prospective benefits of participation, accentuating the study's implications for public health and pharmaceutical advancements. The involvement of neurologists and psychiatrists, whose endorsement holds considerable influence in Iran, may further enhance participation rates.

This trial aims to replace conventional antibody-based therapies for mild to moderate Alzheimer's disease with a more accessible, cost-effective, and safer small molecule treatment. FDA-approved antibody drugs like Aducanumab (Aduhelm) have shown mixed results in slowing Alzheimer's progression and can cause side

effects such as brain swelling and small brain bleeds, particularly at high doses. Moreover, the high cost of Aducanumab limits its[33].

The FDA's approval of Aducanumab in June 2021 was controversial due to mixed evidence regarding its efficacy. It was approved through the accelerated approval pathway, designed for serious illnesses when a drug shows potential clinical benefits. In January 2023, the FDA approved another drug, Lecanemab, which modestly preserves cognitive abilities in early-stage Alzheimer's patients. However, both Aducanumab and Lecanemab pose risks of cerebral edema or hemorrhage, requiring regular brain scans, and their high costs remain uncertain in terms of financial coverage by Medicare and private insurers[34].

Eisai, in collaboration with Biogen, has priced Lecanemab at $26,500 annually. Administered biweekly through intravenous injections, the drug targets amyloid removal from the brain, a key feature of Alzheimer's disease. However, many previous amyloid-targeting treatments have failed to slow cognitive decline.

In the wake of our Phase III clinical trial examining the effects of hydralazine on AD patients, our future research direction will vary based on the trial's outcome. If unsuccessful, we will investigate potential reasons for ineffectiveness, examining patient profiles, dosage variations, and possible interactions with other medications or underlying conditions. If the trial demonstrates success across the board, our next step will be to explore the applicability of hydralazine in treating other neurodegenerative diseases, such as Parkinson's, to understand its broader therapeutic potential. However, if hydralazine proves effective only for a subset of Alzheimer's patients, we will focus on identifying the specific genetic or biochemical markers that correlate with positive responses, aiming for more targeted treatment strategies in future iterations. We are open to collaborations with interested scientists regarding above and are eager to explore novel hypotheses using the data and samples collected in this study. These future endeavors have the potential to uncover new insights and advance our understanding of AD.

Conclusion

Building on prior research linking hydralazine HCL to enhanced lifespan and healthspan in animal models, our study seeks to investigate its effects on cognitive function, blood pressure, in AD patients. Employing a triple-blind, two-arms clinical trial design, we will bio-bank blood samples from participants, creating a vital resource for subsequent studies into the multifaceted influences of hydralazine HCL on human health.

Ethics approval and consent to participate

We will recruit subjects in adherence to the Declaration of Helsinki and in line with the ethical standards set by the Research Ethics Committee of the Iranian National Institute for Medical Research Development (NIMAD). We have received ethics approval from NIMAD's Ethics Committee (IR.NIMAD.REC.1398.424). Informed consent was obtained from all subjects and/or their legal guardian(s) prior to their participation in the study.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

MM, HM, AMA, and MHS conceptualized the study. The study protocol was formulated by MM, HM, NA, and BB. Participant recruitment will be overseen by AMA, SMYA, AR, RB, FHK, GHD, and MA. MHS will provide insights on possible cardiovascular implications. Data gathering will be managed by MM, NA, and BB, with BB and MM also supervising laboratory assessments and bio-banking of blood samples. The manuscript's initial draft was penned by MM, HM, and NA. HM, as the corresponding author, undertook a critical review. All authors have endorsed the final manuscript and accept responsibility for the study's entirety.

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Declarations

Consent for publication

All authors have reviewed the final manuscript and have provided their consent for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.

Additional information
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