Study Protocol

The Effect of Hydralazine on the early Stages of Alzheimer's disease: a raNndomized clinical trial (EHSAN)

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www.ehsan-study.com

Introduction

Ehsan Project (EHSAN) has been funded in 2018 by National Institute for Development of Medical Sciences Research (NIMAD) with participation of Shahid Sadoughi University of Medical Sciences and Yazd Cardiovascular Research Center. This two arms triple blinded (patient, evaluator and researcher) placebo controlled clinical trial has been conducted since 2020. This project assess the effect of hydralazine and its side effects in 424 people aged 50 and over with diagnosis of early stages of Alzheimer's disease.

Execution process

Method:

The study population in this study will be all patients who have a possible diagnosis of Alzheimer's disease according to the National Institute of Neurological and Communicable Diseases and Stroke - Alzheimer's disease and Related Disorders (NINCDS-ADRDA) and will be screened for eligibility before entering the study. The complete inclusion and exclusion criteria are listed below:

NINCDS-ADRDA criteria [1] for the diagnosis of Alzheimer's disease include:

- Dementia is established by a clinical examination and documented by a Mini-Mental Test, or some similar examination confirmed by neuropsychological tests.

- Deficits in two or more areas of cognition.

- Progressive worsening of memory and other cognitive functions.

- No disturbance of consciousness.

- The onset between the ages of 40 and 90, most often after the age of 65.

- Absence of systemic disorders or other brain diseases could account for the progressive deficits in memory and cognition.

- Dementia with variations in onset or course

- Single progressive cognitive deficit

- Presence of systemic or other brain disorders

Inclusion criteria:

1. Diagnoses of possible or probable Alzheimer's disease (NINCDS-ADRDA).

2. The presence of a caregiver (friend or relative) who can assume responsibility for medication administrations, accompany the patient to all visits, and rate the patient's condition and log in to the logbook.

3. Informed written consent from the patient (or surrogate) and caregiver

4. The screening test score (MMSE) between 12 and 26.

5. Prescription of a maintenance dosage of donepezil (5-10 mg daily), rivastigmine (3-6 mg daily), galantamine or galantamine ER (16-18 mg daily) for a minimum of 4 weeks before randomization.

6. Agreement not to take hydralazine during the project.

7. Age 50 years and over

The rationale for several of the inclusion criteria are listed below:

1) The MMSE range of total score below 24 inclusive was chosen to target a population of Alzheimer's patients with mild or moderate dementia

2) Dosage adjustment after initiating AChEI treatment is common and usually involves increasing the starting dosage or reducing the dosage in patients who develop side effects. Patients should be considered for the study following a period of dosage adjustment so that they enter the study on a stably maintained dosage that likely remains the same for the duration of the study. If for some reason the patients treated with Hydralazine experienced intolerable side effects, Hydralazine will be removed from their treatment regimen but they will remain in the study.

3) AD is rare before age 50 years in our population.

Exclusion Criteria

1. A non-Alzheimer primary dementia (e.g., vascular dementia, Lewy body dementia, frontotemporal dementia, vitamin B-12 deficiency, hypothyroidism).

2. Current major depression, delirium, alcohol or psychoactive substance abuse or dependency, schizophrenia, or delusional disorder as defined by DSM-V.

3. Presence of any uncontrolled systemic illness that would interfere with participation in the study or a life expectancy of less than one year.

4. Currently being treated with Hydralazine or a history of intolerance to oral therapy with Hydralazine

5. Any intravenous treatment for heart failure, except IV furosemide (e.g. IV inotropes, pressors, nitrates, or nesiritide) at the time of screening.

6. Systolic blood pressure <100 mmHg

7. Reversible etiology of acute heart failure such as myocarditis, acute myocardial infarction-over the past 4 weeks, arrhythmia and pacing device (Acute MI is defined as symptoms and major electrocardiogram (ECG) changes (i.e., ST-segment elevations), and arrhythmia includes unstable heart rates above 120/min or below 50/min).

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.

9. Concurrent use of phosphodiesterase type 5 (PDE5) inhibitors (e.g. Viagra, Sildenafil. Etc.)

10. Have had cardiac revascularization within the last 3 months or are likely to require coronary revascularization within the study period.

11. EGFR< 15ml/min/1.73m2, or on regular dialysis, or planned dialysis within the study period

Procedure

Participants are 424 randomly selected Alzheimer's patients over the age of 50 years. The sampling method is the use of random blocks. For primary diagnosis and sampling, associate neurologists and psychiatrists are first asked to examine the Alzheimer's Disease (AD) patients they have referred to, and patients with primary dementia according to inclusion criteria based on physical examination, laboratory tests and MRI (to rule-out organic factors such as hemorrhage or tumor) will be identified as potentially qualified by neurologists and

psychiatrists. The potential participants will be referred to Adineh Health Center for screening and obtaining informed consent to participate in the study.

In addition, AD patients who previously identified in Yazd Health Study (YaHS) and have the inclusion criteria are invited to join the study. All patients with a possible diagnosis of AD according to the NINCDS-ADRDA criteria will be screened for eligibility according to the inclusion and exclusion criteria. If the patient is unqualified during the initial screening process, the screening process will not be included, but if the patient qualifies based on the initial screening, patient information will be recorded.

The procedure is as follows: Patients and their caregivers will be invited to Adineh Health Center for screening by trained staffs. The patient is first interviewed clinically by a trained psychologist. The psychologists communicate and interview with the patient. Since the AD patient should be treated appropriately for his or her illness, the session begins with a casual conversation, a brief comment about the weather, or a casual talk about everyday events in order to make the clinical specialist look like an ordinary person and to allay the patient's fear of whether he or she can communicate with this psychologist.

This initial brief conversation, usually aimed at calming the situation before talking to the patient, usually provides the basis for a good interview. The most important thing to talk to AD patients is to use understandable language. Have an initial assessment of the patient's background, level of education, and general level of knowledge. Also, words that the patient may misinterpret should not be used. When the clinician suspects that the patient may have a different perception of what s/he is saying, s/he should clarify what s/he means by talking to the patient. After communication, a screening test is taken from the patient.

Then blood pressure, ECG, and blood sample are taken from the patient. Systolic and diastolic blood pressure is taken three times with three minutes interval from patient's right hand, sitting and using an automatic digital blood pressure monitor (Reister, Germany). We set aside the first measurement and the average of the last two measurements will be considered as blood pressure in this study. All measurements will be made in standard positions with calibrated instruments.

Blood sample will be taken from patients at the same time they are invited to the clinic. The blood sample are used for biobanking, blood and biochemistry tests including; triglycerides, cholesterol, LDL, and HDL. Blood samples will then be centrifuged to separate serum. These tests will be performed to determine if the patient has the exclusion criteria, and then the

results will be sent to a specialist to determine which patients qualify for the study. Blood pressure is taken only at the screening session. The blood tests will be taken at the screening time and at the end of the study.

If the first psychological tests were approved by the psychologist, and then the blood tests and blood pressure were approved by the physician, it is determined whether the patients is eligible for the study or not. Fifteen days after the first visit, the patient and caregiver will be again invited to visit Adineh Health Center. At the beginning, a complete explanation will be given about the research plan to the patient and caregiver, and informed consent will be obtained from the patient and caregiver if they wish to participate in the study. The patient will then answer the study questions in one room and the caregiver in another room. At this stage, all the points of the clinical interview and effective communication with the patient and its caregiver will be monitored occasionally. If it is felt that the patient has not noticed the point, it should be repeated to her/him, and misunderstanding should be addressed by the field supervisor.

Biobanking

Developing a biobank— a type of repository storing biological samples—has become an important part of clinical research in the past few decades. If stored under the appropriate conditions, a variety of biological specimens such as blood, urine, stool, hair, nail, etc. can be kept for many years, and be used for different kinds of medical, epidemiological, and genetic researches. The data gathered in EHSAN biobank is ideal for studies needing to test something, such as a new biomarker or gene, in a reasonably large group of AD patients.

Blood and Urine Specimens

Twenty milliliters (10 cc) of blood is taken from each individual (fasted), and is fractioned and stored in -70C freezers in the following way:

- Whole Blood: Two 1.5 ml Cryo Tubes
- Plasma: Two 1.5 ml, Cryo Tubes
- Buffycoat: One 1 ml Cryo Tubes
- Red Blood Cells: One 1 ml Cryo Tube
- Serum: One 1.5 ml Cryo Tubes

Urine is also collected from each individual and 1.5 ml of it is stored in a Cryo Tube, in a section of the -20C freezers, separate from the blood samples.

All samples will be labeled using barcodes unique to each individual, and placed inside Cryo Boxes, prior to placement in freezers. The lab personnel are trained to store the samples in an orderly fashion in -70° deep freezers and record it in EHSAN database, so that retrieving a sample would be efficiently done in a very short period of time.

Hair and Nail Specimens

All participants are asked to make sure that their hair and nails are clean prior to visiting the EHSAN site, and a small sample of each is taken using individual scissors/nail clippers for each person. Samples are placed inside foil wraps, which are then placed in individual zipped bags, and kept in a cool and dry place.

Ethical considerations

Ethics committee of NIMAD (IR.NIMAD.REC.1398.424) available at https://ethics.research.ac.ir/ProposalCertificateEn.php?id=122413&Print=true&NoPrintHeader=true&NoPrintFooter=true&NoPrintPageBorder=true&LetterPrint=true was approved EHSAN protocol. Informed consent will be obtained from all participants. "Parsian Insurance Company" insured all study participants to compensate all medical expenses and also death liability. All information of patients and their caregivers will be confidential and will only be used for research purpose. To look after Alzheimer's patients, a guide is placed at the entrance of Adineh Health Center to take care of them.

The following questionnaires will be completed by trained psychologists for patients and carers accordingly:

MMSE Questionnaire: This questionnaire was developed by Marshall Folestein et al. [2] to screen for dementia in 1975. There were several tests at the time, but all of them were very long and not suitable for clinical examination. The word "MINI" in the MMSE questionnaire refers to the important feature of this test, which is that it is short. The test lasts 13-14 minutes, has 17 questions which include different parts that Fulstein has selected from the previous tests. The selection criteria were the evaluation of different areas of cognitive practice and, according to Fulstein, the ability to memorize and not need tools and equipment. Of all the dementia screening tests available, MMSE is the most widely used tool to assess

the severity of dementia and to document the progression of dementia over time. It has been used worldwide in epidemiological studies and community studies and has been widely used in clinical trials of drugs for AD.

MMSE questions are grouped into seven categories, each representing a different cognitive domain:

1. Timing, which is measured by five questions about time. (5 points),
2. Orientation to the place, which is measured by five questions about time. (5 points),
3. Record three words (3 points) in which the evaluation of those three words is said to the person and he must repeat them.
4. Attention and calculation that in the evaluation of that person should subtract the number desired by the examiner from 100 consecutively (5 points),
5. Calling three words (3 points), we ask the person to say the three words at the beginning of the test Repeat.

   Different language functions have different parts, the person has to say the name of the objects that are shown to her/him, and also s/he has to repeat the sentence that is said to her/him and must execute a 3-step command, read and execute the sentence shown to him/her and write a complete sentence.
6. Language (8 points) and
7. Visual constructions, we ask the patient to draw the image that is shown to him (1 point).

MMSE returns a total score of 0 for a patient who did not respond correctly and a score of 30 for a patient who made no error. A total score below 24 has been used to distinguish dementia from normal cognitive function, but well-educated and younger patients can have higher total scores above this cut-off.

Advantages of using the test: A brief examination of cognitive status is the most common screening tool for cognitive disorders in the world, which has been translated into different languages and standardized in different cultures. This test measures various cognitive functions and provides an overall estimate of the subject's cognitive status. It is short and concise and can be performed in a short time. The test tool is portable. How to implement this questionnaire can be taught in a short time. Many scientific and executive institutions around the world use this test as one of the indicators for classifying cognitive disorders and as an indicator of eligibility for developing new drugs.

Palliative Performance Scale (PPS): Developed by Stern et al [3] to assess the level of assistance needed by Alzheimer's patients. Since its introduction, it has been used as a measure of outcome in two major trials for the efficacy of Alzheimer's patients. This scale is a useful tool for measuring the gradual decline of patients. It has five functional dimensions: 1) Movement, 2) Level of activity and evidence of disease, 3) Self-care, 4) Oral consumption and 5) Level of consciousness. It has 11 levels for scoring from 0% to 100% with a 10% increase. Each 10% decrease significantly indicates a significant reduction in bodily function. This scale serves as a way to connect the professional team and with patients and their caregivers. Because it can be used as a guide to help start and facilitate conversations about patient care or patient transfer to the hospital [4].

ADAS-cog Questionnaire: The Alzheimer's Disease Assessment Scale (ADAS-cog) [5, 6] is a 21-item scale designed to assess the severity of cognitive and non-cognitive-behavioral disorders in Alzheimer's patients. The Cognitive Scale (ADAS-cog) includes 11 items to assess memory, language, and functioning habits. ADAS-cog is widely used as an outcome measure in clinical trials of narcotics in Iran [7, 8] and has been used in recent large RCTs in the United States due to its sensitivity to changes in disease severity. The total ADAS-cog score increased from 0 (no error) to 70 (severe cognitive impairment) and the expected change in the ADAS-cog total score increased by about 5 points per year in untreated Alzheimer's patients. In calculating the sample size, a 20% reduction in expected change (meaning 1 point per year) was considered. PASS V11.08 was used to estimate the sample size in different scenarios [9]. The 11 items or categories that include ADAS-cog are listed in parentheses with a range of scores for each item; Call task (0-10), 2. Sub-commands (0-5), 3. Naming objects and fingers (0-5), 3. 4. Structural action (0-5), 5. Ideation action (0-5), 6. Orientation (0-8), 7. Word recognition (0-12), 8. Calling test instructions (0-5), 9. Speech, language ability (0-5), 10. Understand spoken language (0-5), and 11. Find the difficult word (0-5). The reliability of the questionnaire was estimated to be 0.99 and the reliability of the test-retest was estimated to be 0.92. This scale showed a moderate to high correlation with the information memory test and the dementia ranking scale. This questionnaire was validated in Persian and has been used several times in similar studies in Iran. It is also used in recent large RCTs in the United States and elsewhere. The following are the questionnaires intended for the patient. These are the three questionnaires for the Alzheimer's patient that will be completed by a psychologist. Patient care questionnaires are also specified below [10].

Neuropsychiatric Questionnaire (NPI): This questionnaire was developed by Cummings et al [11]. To assess psychological and behavioral problems in patients with dementia. Based on a structured interview, a caregiver who can answer a series of questions about patient behavior will be interviewed. The NPI is a fully structured interview in which all the questions are presented and used word by word. It is important to note that the caregiver interview is the only source of information for the rating session, as the patient is not present during the interview. To shorten the time, the NPI uses screening questions for each of the ten or twelve domains (latest version). If there is no behavior, follow-up questions will not be asked. This questionnaire is a valid information-based interview that assesses neuropsychiatric symptoms in the previous month. The main NPI included ten areas of psychiatric neuropathy. In the other two cases, behavioral disorders at night and changes in appetite/eating are added. Another recent modification is the addition of the Caregiver Anxiety Scale to assess the psychological impact of reported neurological symptoms. The NPI-Q is designed as a self-administered questionnaire that is completed by those who care for patients. Each of the 12 NPI-Q domains contains a survey question that reflects the main symptoms of that domain. The initial answers to each question are "yes" (present) or "no" (does not exist). If the answer to the domain question is "no", the interviewee goes to the next question. If "yes", the interview then assesses the severity of symptoms in the previous month on a 3-point scale and the common effects of symptoms on them (i.e. anxiety disorder) using a 5-point scale. NPI-Q provides symptom severity. Anxiety scores are reported for each symptom, and total severity and anxiety scores reflect the sum of individual domain scores. The validity and reliability of the NPI questionnaire were confirmed in the research of Tahernia et al. (2015). The reliability of the questionnaire was measured by Cronbach's alpha coefficient and was 0.81. The mean NPI score of the whole study was directly and significantly correlated with the stress and depression score (HADS) so that the correlation coefficient of NPI and stress was 0.62 and NPI and depression was 0.43. The reliability of frequency and intensity of variables was between (0.6) to (0.98) and the reliability of test-retest was between 0.4 and 0.96%. Test validity at the 5% level is between 0.3 and 0.9.

Caregiver Activity Questionnaire (CAS): This questionnaire was developed by Davis et al [12]. To measure the time caregiver activities spent on helping Alzheimer's patients. The CAS consists of six items that estimate the number of requests in hours and minutes when the caregiver spends 24 hours on specific activities; (1) communicating with the patient, (2) assisting the patient in moving, (3) dressing, (4) eating, (5) caring for the patient's

appearance, and (6) monitoring the individual. CAS showed a moderate correlation with the ADAS-cog scale, MMSE, and physical self-preservation. In a clinical trial of Malate and Lagrin, treated patients showed a trend toward a reduction in care time measured by CAS.

The reliability of the total CAS score test was 0.88 between week 1 and week 3. This scale had strong convergence with MMSE test equal to 0.61 and ADAS-Cog test equal to 0.57. This questionnaire is used to collect information about the times when the patient-caregiver is taking care of the patient during 24 hours. The questionnaire covers a wide range of activities that are usually associated with and coordinated with traditional daily activities (e.g. using the toilet, eating, taking care of one's appearance, etc.). It also includes the activities of daily living (e.g. shopping, transportation, money management, etc.) related to caring for an Alzheimer's patient. The tool also included a monitoring case because caregivers must ensure the safety of the AD person during care. The above items change behavior management to some extent from the time of wandering and potentially dangerous behavior due to impaired judgment in the disease. Because AD patients ask the same questions over and over again and/or need to repeat instructions that require nurse attention, also there is a connection. In summary, the main origins of CAS include those that (1) cover a wide range of tasks and (2) apply to a wide range of cognitive and functional disorders. The main items included: communicating with a person, using the toilet, bathing or showering, using transportation, getting dressed, eating, traveling (mobility/mobility), using the phone, managing money, shopping, doing housework, supervising the person, and taking care of appearance (cleaning). Initially, each activity group consisted of two questions: (1) How much caregivers spent performing each activity during the 24 hours. (2) And how difficult or annoying it was for the caregiver to complete these activities that day.

Lawton Worker's Daily Activities Questionnaire: To evaluate the functional abilities of AD patients treated with hydralazine or placebo in daily life activities, the Persian version of eight daily activities of Lawton dementia patients will be used [13]. Carefully complete this questionnaire. The tool includes 8 questions including "Ability to use the phone", "Ability to shop", "Ability to prepare food", "Housework", "Ability to wash clothes", "Ability to travel and use vehicles"," Ability to take medicine "," Ability to account and books, money and finances ". The response spectrum is based on 1 Likert and the Likert response is different based on each domain. In the field of using phone was 3-0 Likert, In the field of buying 3-0 Likert, In the field of preparing food 3-0 Likert, In the field of home activities 4-0 Likert, In the field of washing clothes 2-0 Likert, The scope of transport is 4-0 Likert, the range of drug

use management is 2-0 Likert and the scope of financial management is 2-0 Likert. The score range is between 0-23, where a higher score indicates a better situation. The reliability of this questionnaire has been reported in Taheri et al.'s (2015) research as 0.75 and the internal correlation coefficient as 0.79. Also Hassani Mehraban et al (2013) has been reported the internal correlation coefficient with the test-retest reliability as 0.993 and the inter-rater reliability was 0.961.

Experimental design

Evaluations of the original study should be performed within 30 days of screening evaluations. If the patient and caregiver have signed the informed consent documents and are eligible to participate in the study according to the defined criteria ascertained by the study general practitioner, the evaluation of the participants will be performed on the same day. However, if the main assessment is not performed on the day of screening, it is necessary to review the inclusion and exclusion criteria, as well as the evaluation of screening tests and blood tests to ensure that the patient is eligible to participate in the study. Basic assessments include reviewing the medications the patient is taking, reporting blood tests, and reviewing the results of patient and caregiver tests. All of these assessments should be performed no later than one week before randomization. The patient will be randomized immediately after baseline assessments.

Randomization Procedures

This is a double-armed randomized clinical trial with an allocation ratio of 1-1 to the intervention and placebo arms. All eligible patients who give a written informed consent directly or via a proxy and whose caregiver also gives written informed consent will be randomized to one of two treatment arms: first arm: 75 mg (25 mg TDS) Hydralazine; or the second arm placebo. The patients will start with 37.5 mg (12.5 mg TDS-half pills) and reach 75 mg daily within two weeks. The participant, outcome assessors, and researchers will be blinded to the study arms.

The treatment allocation ratio for the two treatment arms will be 1:1. The random sequence will be extracted and the randomization process will be permuted block randomization. Each block consists of four participants. The treatment scheme will be generated by a sealed envelope (https://www.sealedenvelope.com/) website. The prepackaged study medications

complied by Iran FDA will be delivered at the reception of Adined Health Clinic to each participating patient. The medications will be provided in identical boxes. Each box will bear a unique medication identification number. The site personnel and the patient will be blinded to the medication and treatment assignment.

Site investigators will receive a patient's treatment allocation (box number) by logging into the password-protected part of the EHSAN website (www.ehsan-study.com) hosted by Shahid Sadoughi University (SSU) URL. The EHSAN database will only allow authorized study personnel to access the database and obtain allocated drug/placebo number. The website will require the study coordinator to enter a user name and password to log in. Once the system has verified the user name and password, the study coordinator will go to the randomization section and enter the Patient ID Number, and confirms the patient's eligibility by answering several questions. Once eligibility is confirmed, the EHSAN website will assign the blinded medication box numbers for the patient. The box number will be assigned based on the random treatment scheme generated by the website. For each subsequent refill of study medication, the site will log on to the EHSAN website to obtain a new medication box number, which will also be linked to the randomization scheme. The patient will begin treatment the same day as randomization.

The unique patient ID number will be linked in the randomization file for the treatment assignment for each randomized patient. The randomization file data will remain in a separate file from the rest of the study data on the EHSAN database. The confidentiality of protected health information will be strictly maintained.

Randomization will occur within 30 days of screening tests immediately following the completion of all baseline forms and procedures. When a new patient has been randomized, the appropriate information will be recorded in the patient's medical record. Immediately following randomization, the site will submit baseline forms to the AHC. Source documentation for eligibility criteria will be kept at the site with the patient's study folder and on the password-protected section of the EHSAN website.

Eligible patients who are currently taking an AChEI will be randomly assigned to 75mg (25mg TDS) Hydralazine per day or matched placebo. Dose adjustments will be allowed for two weeks depending on the patient's tolerance for the drug regimen. A daily dosage of 75mg Hydralazine (25mg /three times per day) is permitted because this dosage is far below the pharmacological dosage allowed and show limited side effects in our patients, we will start

with 32.5mg (half 25mg pills (12.5mg) three times a day) and if the patient does not show any side effect, we will increase and maintain it at 75mg after two weeks. If the patient does show side effects they will be maintained at a lower dose.

Hydralazine will be started with half dosage for 14 days and then (or matching placebo) will be titrated over two weeks to a maintenance dosage of 25mg three times a day (75mg daily). During week 1 patient will take 12.5mg Hydralazine tablet (TDS). During week 2 patients will be assessed for hypotension and any known side effects from Hydralazine. From week 3 patients will take 25mg Hydralazine (TDS) or 75mg daily. The matching placebo for Hydralazine will be a tablet that is identical in appearance to Hydralazine prepared by one of the Iranian pharma companies. Patients whose blood pressure dropped to less than 100 mmHg or eGFR< 15ml/min at any time during the trial will be evaluated for side effects by the treating clinician to determine if it is safe for the patient to remain in the trial. For an individual whose systolic BP dropped below 110 mmHg or eGFR declines to less than 30 ml/min but remains above 15 ml/min, the site physician or the treating clinician will determine if it is necessary to reduce the dosage of the study drug to 12.5 mg TDS. If it is determined that it is not safe for the patient to continue to take the study drug, the patient will stop taking Hydralazine (or matching placebo). If a known Hydralazine side-effect develops during the study and is sufficiently distressing or clinically significant, the Hydralazine treatment arm may be reduced, interrupted, or terminated if necessary. If a symptom develops that is not known to be related to Hydralazine, patients should be encouraged to continue treatment without any medication adjustments. In either case, a clinical evaluation will be conducted to determine if an adjustment to the medication is warranted.

Consideration should always be given for each patient to resume the full dosage of treatments as soon as possible after any reduction or discontinuation. If Hydralazine tablets are discontinued or interrupted for any reason and a decision is made to restart treatment, the titration schedule for initiating Hydralazine will be followed if the period of discontinuation is more than two weeks. Consultation with the study PI will be required if it is unclear whether re-titration is necessary. All medication interruptions, changes to dose, and discontinuation of treatment must be recorded on the appropriate electronic case report forms (eCRF) according to the instructions given in the study Operations Manual. The patient, assessors, and study investigators will be blinded to the randomized treatment assignment. For breaking of the blind in life-threatening emergencies, Adineh Health Centre (AHC) reception will be provided with coded envelopes that can identify the patient's treatment

assignment. Coded envelopes should only be opened if the EHSAN physician has been instructed to do so by the study PI or the study supportive cardiologist if the AHC staff is unable to reach one of the parties listed above. In most cases, if there is a concern about patient safety and the study treatment(s), the patient will be removed from the study treatment(s) without breaking the blind. The blind will be broken at the conclusion of the trial for all randomized patients.

The prepackaged study mediations will be delivered by AHC reception to each patient. Randomized patients will obtain a 3-months supply of study medication from the AHC reception at randomization and at each study follow-up clinic visit. If a patient or his/her caregiver is unable to attend a clinic visit, a 3-month supply of study medication will be shipped to the patient as long as contact has been made via phone. Patients will be asked to continue with their randomized treatment until the trial is terminated. For each patient, the study period will be one year and will be ended depending on the time of randomization

Follow-up Schedule

All randomized patients will be followed in the clinic every 3-months for one year (median follow-up of 1.0 years). To encourage treatment adherence and to help patients and caregivers understand the medication titration schedule, it is recommended that patients/caregivers be contacted by phone at one, two, and four weeks after the start of the randomization to go over the medication schedule and to discuss any concerns or questions that the patient/caregiver may have regarding the medications or the study. If a change in inpatient/caregiver status, patient safety, and/or study medication is identified on one of these calls, the appropriate case report forms must be completed according to the instructions given in the study protocol. These telephone contacts are encouraged but are not required. It is up to the site staff to discuss the possible contacts with each caregiver and patient and to make an assessment of whether or not the contacts would be helpful for each individual. Timer-equipped pillboxes will be provided to all patients free of charge to ensure they will take their medication on time at the determined intervals. A daily text message will also be sent to the patients and caregivers to take the medication.

In the final 3 months of the trial, all randomized patients will be requested to come to the clinic for a final study closeout visit and will be taking off-study medication. At the closeout visit (or within 30 days if the visitor cannot be scheduled), the patient will be withdrawn from

the trial. The final study visit will be equivalent to an annual follow-up visit and include an assessment of adverse events, and a physical exam and laboratory tests if not conducted in the past 6 months. The study blind will not be broken at the time a patient is withdrawn from the trial. The blind will be broken at the conclusion of the trial for all randomized patients.

If the patient and their caregiver miss a scheduled follow-up clinic visit, the ADAS-Cog inventory (the primary outcome measure) along with information about serious adverse events will be assessed by telephone. The ADAS-Cog inventory is acquired from a reporting caregiver or a caregiver who lives with the patient or is knowledgeable about the patient's daily activities.

Each of the 5 secondary outcome measures (MMSE, Lawton IADL (instrumental activities of daily living) the Dependence Scale, NPI, and CAS) will be assessed in the clinic every 3-months similar to the primary outcome measure. The Lawton IADL and MMSE are administered to the patient in person and take approximately 25 – 55 minutes depending on the patient's functioning level. For patients who are unable to attend clinic visits due to hospitalization during the trial, study site personnel will travel to the patient whenever possible to collect the data on the Lawton IADL and MMSE. The other secondary outcome measures (the Dependence Scale, NPI, and CAS) will be acquired from a reporting caregiver or caregiver who lives with the patient or is knowledgeable about the patient's daily activities. The total time needed to complete these three measures is approximately 45-90 minutes. These assessments can be completed in person or over the phone. However, because of the time needed to complete the secondary endpoints, phone data collection will focus only on safety and the primary endpoint.

In addition to an assessment of adverse events and the primary and secondary outcome measures, seasonal clinic visits will focus on safety and include a physical examination, an assessment of concomitant medication usage and blood draws for local laboratory safety measures. Blood test for Hydralazine serum concentrations will be collected at month 12 of the trial after an overnight fast to ensure drug adherence (Patients should not fast). The estimated time needed to complete a follow-up visit is approximately 2-3 hours depending on the patient's level of functioning. The primary and secondary outcome inventories including ADAS-Cog [8], Lawton- IADL [14], Mini-Mental State Exam [15], and Neuropsychiatric Inventory [16] were all translated and validated in Persian the two short inventories, CAS and Dependence Scale each with only few questions, will be translated and validated before conduction of the study.

Study Withdrawal

A patient or caregiver may withdraw at any time during this study. If a patient or caregiver withdraws or fails to follow up, a Study Exit Form will be completed. The study will employ an intent-to-treat analysis. Therefore, patients will be followed whether or not they continue study medication. Patients, who wish to withdraw from the study, will be asked to make a final follow-up visit. If a patient or caregiver withdraws consent to be followed, the patient will be withdrawn from the study. Data collected up to that point will be used in the final analyses. If missing data are determined to be informative, sensitivity analyses will be conducted to examine their influence on the treatment comparisons.

Study Termination

NIMAD, as the sponsor of this trial, may stop the study at any time based on funding issues or internal or external evidence that the study is no longer feasible or ethical. The Kermanshah CTC (www.ctc.kums.ac.ir) as the Data and Safety Monitoring Body, the only group that will review the unblinded data, can at any time recommend to the sponsors that the trial be terminated because of efficacy, safety, or futility. If the entire trail is terminated, Adineh Health Center will be notified to cancel the schedule and not continue.

In the final 3 months of the trial, all randomized patients will be scheduled for a final study conclusion visit. At the conclusion visit (or within 30 days if the visitor cannot be scheduled), the patient will be removed from the trial. In most cases, the site personnel will be funded for 30 days following the termination of the study or to clean up and store data and relevant documents. Upon completion of the trial the study blind will be broken and local PIs, if requested, will inform patients, caregivers, and the patient's primary care physician whether the patient was taking Hydralazine or placebo. After the publication of the primary study results, PIs, if requested, will inform patients and caregivers of the study's results.

Sample size and power consideration

A. Primary Outcome

The primary hypothesis of this study is that Hydralazine (compared to placebo) will significantly delay the progression of AD as measured by ADAS-cog (inventory) in patients

with AD on an AChEI. The analysis of the primary endpoint will be by a repeated measures mixed effects model [17, 18].

Table 1below presents sample size estimates based on published data for a range of clinically meaningful treatment effects. The sample size was calculated for a repeated measures mixed model with a linear trend over time. The approximate average yearly change in mild or moderate AD patients is 4-5 points for the ADAS-cog [7, 8] The sample size estimates were calculated using PASS software [9] a sample size program for repeated measures with attrition, under the following assumptions:

1) 0.05 type-I error (2 sided)

2) 80% and 90% power

3) A study duration of one year accrual period and average follow-up of 1.0 years

4) An average of 4 follow-up measures for each patient (based on measurements every 3 months over an average follow-up period of 1.0 years)

5) A loss rate of 2.5% per 3-month follow-up measure (approximately 10% per year)

7) An estimated average rate of increase of approximately 5 units per year in the ADAS-Cog inventory total score with control therapy

8) A common standard deviation of 3- 5 units

9) Hypothesized mean differences of 1 or 1.5 ADAS-cog inventory units at the end of the 1.0 years of median follow-up between the two arms.

Table 1: Total Sample for a repeated measures analysis assuming a 0.05 Type-I Error (2-Sided), 4 follow-up measures, a correlation of 0.50 between repeats, and 10% loss rate

Treatment Differences ADAS-Cog inventory	Common Standard Deviation	80% Power	90% Power
	3	142	191
1.5	4	251	336
	5	391	522
	3	318	424
1.0	4	562	749
	5	1052	880

Based on the estimates in Table 1, we selected a sample size of (424 total patients, or 212 per treatment arm). This sample size will provide 90% power to detect a 1-point mean treatment

difference in the ADAS-cog inventory by the end of the average follow-up period, adjusted for losses, with a standard deviation of 3. The effects to be detected are modest and translate to a 20.0% reduction in the annual rate of increase in the inventory scale, range 0-70 and lower score indicates less impairment, with the intervention. These effects are equivalent to slowing the rate of progression of the disease by nearly 6 months for Hydralazine compare to placebo for the course of one year treatment.

Sensitivity analyses will be conducted to determine what effect an increase in losses will have on the study's power. The results of these analyses will demonstrate that even if the loss rate doubles from the estimated 10% to 20% per year, the trial would still have at least 80% power to detect the hypothesized treatment effects. The trial also will have at least 80% power to detect the hypothesized treatment effects if accrual is 33% less than expected (e.g. 318 instead of 424). Furthermore, a sample size of 424 patients will provide some protection if the treatment effect is lower than expected. For example, the trial will retain at least 80% power if the mean treatment difference is 0.75 units instead of the hypothesized 1 unit.

To achieve the targeted sample size, patients will be recruited over a 1-year period with a follow-up of one year. A total of 8 supporting specialists (neurologists and psychiatrists) will refer an average of approximately one patient every week.

B. Secondary Endpoints

The secondary endpoints for the study are as follows: 1) Lawton Activity of Daily Living Scale (function), 2) Mini-Mental State Examination (cognition); 3) Dependence Scale (function); 4) Nero-Psychiatry Inventory (behavior) and; 5) Caregiver Activity Scale (caregiver time). To provide control for multiple treatment comparisons, a Type I error of 0.25 (two-sided) will be used for the power calculations for the secondary outcomes. As with the primary outcome measure, and overall Type I error of 5% (two-sided) was considered [19].

Table 2 displays the mean detectable differences at the final time point for 80% and 90% power for a repeated measures mixed model with a linear trend (i.e., growth curve) assuming a sample size of 424 total patients. Variability estimates used in the power calculations for the MMSE is based on data from 185 mild or moderate AD patients in a multicenter, randomized clinical trial of ondansetron published in 2002 [20] a very similar study population to the target population of EHSAN. The mean MMSE score at baseline in the 185 patients was 19.3 (range 14-24) with a standard deviation of 3.2. The mean Lawton ADL score reported in

60patients was 2.28 (range 0.52-4.26) with a standard deviation of 1.87. The variability estimates used in the power calculations for the NPI are based on data from 978 AD patients in a randomized trial of galantamine [21]. In that study, the mean NPI score at baseline was 11.9 with a standard deviation of 13.0. Variability estimates for the CAS were based on data from two recent AD studies (101,130). The first study was published in 1996 and was conducted at two medical centers in 42 AD patients and their caregivers. The authors reported a total mean CAS time in hours of 17.2 with a standard deviation of 16.1 [12]. The second study by Marrin et al. conducted in 44 AD patients and their caregivers reported a mean CAS of 13.1 hours with a standard deviation of 9.9 [22]. The average standard deviation across the two studies was 13.0. The average annual change in mild to moderate AD patients is 2 points for MMSE [22], 5 points for NPI [21], and 2.7 hours per day per year for CAS [22].

Table 2: Power Estimates for a Repeated Measure Analysis with 4 Follow-Up Time Points, a 2.5% Attrition at Each Time Point, a 0.50 Correlation between Time Points, a Type-I Error of 0.05 (2-Sided), and a total Sample Size of 424 Patients

Outcome Measure	Average Annual Change	Common Standard Deviation	Mean per Group Difference at the Final Time Point assuming a Linear Trend from Baseline	Approximate Power
Lawton-ADL	-1	1.9	0.8	90%
			0.5	80%
MMSE	-2	3.2	1.1	90%
			0.9	80%
NPI	-5	13	4.3	90%
			3.8	80%
CAS	3	13	4.3	90%
			3.8	80%

Table 2 indicates that the study will have excellent power to detect moderate and clinically relevant differences in each of the secondary endpoints. These effects are equivalent to

slowing the rate of progression of the disease by nearly 3 months

Ability to study (Feasibility)

To assess the feasibility of implementing EHSAN within the Yazd healthcare system, all academic neurologists and psychiatrists of Shahid Sadoughi (Yazd) University of Medical Sciences were approached. Most of them (80% of n=10) agreed to support the study and thus joined the study as Co-PIs. According to their patient's records, they can nominate 2 eligible early-stage AD patients per week for screening.

In addition, a survey was conducted on academic neurologists and psychiatrists to determine if they would be interested in participating in EHSAN and to estimate the number of current and new patients each year who would likely meet the EHSAN's inclusion and exclusion criteria at their clinics. A total of 8 specialists expressed interest in participating in EHSAN. The total number of potential patients comes to 800 over a year. Thus, EHSAN would need to enroll approximately 50% of the eligible patients to reach its recruitment goal. Based on these data, we believe that our recruitment target is reasonable and achieving the target over the recruitment period is feasible.

Monitoring the study

Monitoring Bodies

The groups charged with monitoring the various aspects of the study will be the Clinical Trial Centre (CTC) associated with Kermanshah University of Medical Sciences and NIMAD Human ethics Committee as the sponsor of the study. Kermanshah CTC data monitoring committee will meet prior to the beginning of patient intake, on the first day, 6 months after beginning, and on the last day (closeout day) of the study. The CTC Monitoring, Auditing, and Resource Team will conduct site visits over the course of the trial for protocol and Iran Good Clinical Practice (IR-GCP) adherence. The team will conduct a full audit if required.

The Executive Committee, the management, and the decision-making body for the operational aspects of the study will monitor the performance of the AHC and the quality of data collected. The PI will formulate plans for publications and will oversee the publication and presentation of all data from the study. The PI must grant permission before any study data may be used for presentation or publication.

The Data Monitoring Committee (DMC) of CTC will review the progress of the study and

will monitor CRF to investigate patient intake, outcomes, adverse events, and other issues related to patient safety. The DMC will make recommendations to the NIMAD ethics committee about whether the study should continue or be stopped. The DMC can consider patient safety or any other adverse events as grounds for early termination, including either compelling internal or external evidence of treatment differences or unfeasibility of addressing the study hypotheses (e.g., poor patient intake, poor adherence to the protocol).

The Human Ethics Committee of NIMAD and Shahid Sadoughi University will review the study before initiation and annually to ensure proper protection of patient's rights and safety. The Study Biostatistician and the PI will present the SSUHEC and NIMAD with annual reports on the progress of the study and ethical issues relevant to both ethics committees. In addition, a NIMAD or Shahid Sadoughi Ethics Committee member will site-visit at least once during the course of the study to determine if patients' rights and safety are being properly protected.

CTC will function as a resource for Good Clinical Practices (GCP) in the study. CTC provided IR-GCP training for the PI, Epidemiologist, and some supportive clinicians of the study and will provide GCP training for the EHSAN researchers at the study kick-off meeting and will site visit AHC at least four times during the trial for IR-GCP and study protocol adherence.

Monitoring Patient Intake

The Planning and the Executive Committee will monitor the intake rate and operational aspects of this study. If the recruitment is not proceeding at an adequate pace overall, the Study PI and the Study Biostatistician will scrutinize the reasons for slow intake including the reasons potentially eligible patients were excluded, and report to the Executive Committee. Based on this information the Executive Committee will consider whether to recommend any modifications to the study’s eligibility criteria. The supporting specialist will only be allowed to continue in the study if adequate patient intake is maintained (4 per month). After three months of intake, any supporting specialist who did not refer at least 10 patients will be considered for probation. If a supporting specialist is placed on probation, the PI will confer with the AHC site personnel, to help improve the rate of recruitment. The Executive Committee will only take actions leading to the discontinuation of a supporting specialist with the concurrence of the PI.

Monitoring Data Quality and Protocol Adherence

AHC will be monitored by CTC associated with Kermanshah University of Medical Sciences (www.ctc.kums.ac.ir) and NIMAD for data quality, completeness of follow-up visits, and adherence to the protocol. Strict adherence to the protocol will be expected from AHC. However, if a participating investigator feels that adherence to the protocol will in any way be detrimental to a particular patient's health or well-being, the interest of the patient will take precedence. Documentation will be required for any breach of protocol. In addition, the Executive Committee and the CTC will monitor protocol adherence centrally.

Data quality and the completeness of data retrieval will be closely monitored on an ongoing basis by the CTC. The Study Biostatistician will present interim monitoring reports to the Executive Committee and DMC. Interim reports will include the following types of information:

- Patient Intake
- Randomization errors
- Protocol deviation
- Adherence and compliance with original treatment assignment
- Missed study visits
- Completeness of follow-up
- Data quality
- Data query rate
- Data error rate
- Audit and site visit results

Monitoring Efficacy and Futility of the project

Treatment efficacy on the primary endpoint will be monitored at approximately one and half years after the beginning of EHSAN. No formal futility analyses are planned. However, the DMC at CTC will review all interim analyses and be responsible for determining whether or not to recommend to the PI that the trial be stopped for efficacy, futility, or safety. If the results of the interim analysis demonstrate that one or more of the treatment comparisons is statistically significant, the DMC would have the option of recommending early termination of the trial or continuing the trial as planned. To aid the DMC in their deliberations, results of analyses of secondary outcome measures and other information inside and outside of EHSAN

will be presented.

Monitoring Adverse Events

1. Role of the Local Site Investigator in Adverse Event Monitoring: The local site investigator will be responsible for following adverse event reporting requirements as outlined below in the protocol. These responsibilities include:

a. Reviewing the accuracy and completeness of all adverse events reported

b. Compliance with local IRB policies for reporting adverse events and/or serious adverse events

c. Reporting to the IRB safety issues reported by the sponsor, and

d. Closely monitoring research patients at each follow-up visit and telephone contact for any new Adverse Events (AEs) or Serious Adverse Events (SAEs).

2. Adverse Event Definition: For EHSAN, Adverse Events (AE) will be recorded using the International Conference Harmonization (ICH) for Clinical Safety Data Management (ICH-E2A).

Definition of an adverse event or side effect: An adverse event is "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmacological product which does not necessarily to have a causal relationship with this treatment". An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the study intervention. All adverse events, both those related to the study intervention, and those not related to the intervention will be collected. Relatedness involves an assessment of the degree of causality (attribution) between the study intervention and the event. Site investigators will be asked to provide an assessment of relatedness. The assessment provided by the site investigator is part of the information used by the sponsor to determine if the adverse event presents a patient safety concern. All adverse events with a reasonable causal relationship to the investigative treatment should be considered "related". A definite relationship does not need to be established.

3. Serious Adverse Event Definition: Serious Adverse Events (SAE) collected for EHSAN are those defined by the ICH for Clinical Safety Data Management and CSP Global SOP as any untoward medical occurrence that at any dose:

a. Results in death;

b. Is life-threatening;

c. Requires inpatient hospitalization or prolongation of existing hospitalization;

d. Results in persistent or significant disability or incapacity;

e. Results in a congenital anomaly/birth defect; or

f. Any other condition that, based upon medical judgment, may jeopardize the subject and require medical or surgical treatment to prevent on of the above outcomes.

Serious adverse events are a subset of adverse events. All SAEs will be collected, including those related and not related to the study intervention. All SAEs with a reasonable causal relationship to the investigative treatment should be considered "related". A definite relationship does not need to be established.

4. Adverse Event and Serious Adverse Event Monitoring

EHSAN randomized study patients will be monitored at each clinic visit and telephone contact for adverse events (AEs) and serious adverse events (SAEs). All AEs and SAEs will be recorded on the appropriate event forms. In addition to the AEs and SAEs defined above, EHSAN will specifically capture data on hypotension, syncopal episodes, falls, and heart failure since these events have some association with high dose Hydralazine in previous studies.

Active monitoring of AEs and SAEs will begin as soon as a study patient signs the consent form and will continue until the patient's last follow-up visit regardless of adherence to the study protocol. In addition, the investigator must collect all the reported SAEs for a period of 30 days after a randomized patient's involvement in the trial has ended. For patients who have signed the consent document but who are not randomized, active monitoring will end at the time it is determined that the patient is not eligible or has notified the site they are no longer interested in participating.

5. Expedited Reporting of Serious Adverse Events (SAE)

The site investigators will be instructed to promptly notify the PI, Study Biostatistician, and Adineh Health Clinic's physician within 72 hours of observing any SAE. Prompt notification of the SAE should be made by faxing a copy of the SAE form to the PI and the study cardiologist. The AHC site will be responsible for the evaluation of all adverse events for patient safety concerns. Serious events that are related to the investigative treatment and unexpected will be reported to the EHSAN supportive investigators after reviewing by the PI.

6. Reporting of Adverse and Serious Adverse Events to the DMC

The Study Biostatistician will tabulate all adverse events and present a summary of all AEs and SAEs to the DMC annually or on a schedule set by the Board. The DMC will also determine when they should be unblended to treatment assignment in reviewing adverse event data. The DMC will advise the SSUHEC about whether the study should continue or be stopped for safety reasons.

Archiving Study Records

At the end of the clinical trial, investigators are instructed to keep all records of the trial and registered participants in an archive. No records shall be destroyed without PI authorization. The current data policy is that participating medical centers can, after consultation with the EHSAN PI, discard study files five years after the study is completed. In some cases, it may be necessary to retain study files longer, depending on local policy.

Statistical analysis plan

Interim Monitoring and Analysis

The interim analysis will focus on sample size re-estimation and will be conducted before the scheduled end of recruitment and before the interim efficacy analysis. The DMC will be presented with the observed variance of the mean ADAS-Cog from the mixed-effects model and the observed correlation of the repeated ADAS-Cog measurements within patients overall and by treatment arm. Based on these estimated nuisance parameters from the model, the sample size for the trial will be re-estimated for 80% and 90% power. In addition, bootstrapping techniques will be used to provide estimates of the distribution of the nuisance parameters and a range of possible re-estimated sample sizes based on this distribution. The range will be provided to the DMC to aid in their decision making about whether there is a need to increase sample size, whether the original sample size is adequate, or whether a smaller sample size will still provide acceptable power without requesting an extension of recruitment if the original target sample size is not achievable. To preserve the type-I error and because the treatment effect sizes were based on what was considered minimally clinically important differences, the observed treatment effects will not be used in the sample size re-estimation procedure.

Interim analysis for treatment efficacy of the primary endpoint will be conducted at approximately 18 months after the launch of EHSAN (following the sample size re-estimation procedure) using the methods of Haybittle and Peto. To protect the Type-I error, the three primary treatment comparisons of interest (the active treatment groups vs. the placebo group) will be tested using a single 3 degrees of freedom test. If the joint test is significant ($p<0.001$), the individual treatment comparisons will also be conducted at a 0.001 level of significance. If the joint test is not significant, no further testing will be conducted. If the results of the interim analysis demonstrate that one or more of the treatment comparisons are statistically significant, the DMC would have the option of recommending early termination of the trial or one or more treatment arms, or continuing the trial as planned.

To aid the DMC in their deliberations over early stopping, results of analyses of secondary outcome measures and other information internal and external to EHSAN would be presented. The decision to only test the primary treatment comparisons of interest at the interim analysis is being done because the study should be stopped if an active treatment group is significantly more effective than the placebo. If the active treatment is not more effective than placebo at the interim analysis, then the relative effects of the active treatments against each other would not be a reason to terminate the trial. If the results of the interim analysis demonstrate that one or more of the treatment comparisons is statistically significant, the DMC will have the option of recommending early termination of the entire trial or one or more of the treatment arms or continuing the trial as planned. To aid the DMC in their deliberation, results of analyses of secondary outcome measures and other information inside and outside of EHSAN will be presented. At each meeting, the DMC will also have the option of recommending early termination of the trial based on safety due to adverse events or futility because of inadequate recruitment or recommending continuation of the trial with a possible adjustment to sample size. To aid the DMC in their evaluation of safety, benchmarks will be established whenever possible for key adverse event rates (e.g., hypotension, postural hypotension, falls, heart failure, and heart failure hospitalizations) based on published data.

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