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Selection of factors, associated with Alzheimer Disease, for the design of an Epidemiological cross-sectional survey

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Authors’ contributions
Lopez Ricardo Yudisleidy: Participation in the design of the protocol. Literature search and review, writing of the manuscript.
Claudio Rodríguez Martinez: Project leader. Design of the project, critical revision of the manuscript, approval of the final version.
Mary Carmen Reyes Zamora: Critical revision of the Protocol and the manuscript.
Janet Perodin Hernández: Literature review, critical revision and editing of the manuscript.

All authors read and approved the final manuscript.
What is already known on this subject

- Alzheimer’s disease is the most common type of dementia worldwide;
- To date, there is no homogeneous criterion of which factors, associated with the disease, should be included in epidemiological prevalence studies;
- Studies, in several cases, do not reflect the diversity of scientifically proven criteria related with the onset of the disease nor the influence of its course.

What this study adds

- An epidemiological study protocol design of the prevalence of possible Alzheimer’s disease is presented. It includes a significant diversity of factors that can influence on its prevalence and severity. Among those factors, it can be mentioned the demographic, economic, social, eating and non-eating habits, lifestyles, protective and risk factors, comorbidities, family health history, and neuropsychological status;
- Four appropriate neuropsychological tests and two scales are selected for conducting large studies in urban and rural populations.
Abstract:

Objective: to identify the factors that influence in the prevalence of possible Alzheimer Disease (AD) in different groups of elderly Cuban population; and design the suitable epidemiological study protocol.

Setting: The study will be conducted at a primary care level in three different municipalities in Cuba with the intervention of doctors specialized in health care of third age populations and family doctors.

Participants: will include 1092 subjects, both genders, all ethnic groups. Inclusion criteria: aged ≥ 60, same town permanence of residence ≥ 10 years, principle of voluntariness and written consent. Exclusion criteria: Long hospitalized period of the adult; other disabilities related to neurological disorders; severe visual deficit, hearing impairment and motor disabilities that obstruct the execution of tests; severe alcohol dependence and psychotropic drugs dependence.

Primary outcome measures: evaluation of possible Alzheimer’s disease scores obtained from four different neuropsychological tests and two scales, prevalence of the disease.

Results: It was designed a protocol for epidemiological cross-sectional study, which permit to recognize the association of risk and protective factors with the disease in different municipalities. The study will comprise inhabitants from rural and urban areas and will include demographic, social, and economic factors, health condition and familial health history, eating and non-eating habits, neuropsychological status, sexual activity, and physical conditions. To explore comorbidity, a comprehensive list of diseases was incorporated. The protocol involves five neuropsychological tests and two scales adapted for portable devices and suitable diagnostic tests for the Cuban population.

Conclusions: Design of a protocol for the study of the possible correlation among risk and protective factors and the prevalence of Alzheimer disease in the Cuban elderly population. The designed and proposed application, for mobile devices, will permit an appropriate assessment of the neuropsychological tests and scales outcomes of the studied subjects.
Strengths and limitations of this study

- The protocol includes the most significant diversity of factors that can influence on the prevalence and severity of AD
- It comprises occurrence study in rural and urban areas
- It will permit to select the most appropriate neuropsychological tests and scales, to be applied in the population, for diagnosing possible Alzheimer's disease.
- The study does not include populations under 60 years of age, because the prevalence of the disease, before that, is mostly attributed to familial Alzheimer's
- The study does not incorporate biochemical markers of the disease

Ethics

This research followed the ethical and relevant codes of experimentation and legislation principles of the Declaration of Helsinki. The Research Scientific Council of the Promoting Research Institution (Centro Nacional de Biopreparados), the Health Authorities and Ethic Committees of each municipality approved the designed documents as part of the developed protocol.

Funding

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Data sharing

There is still no data collected from the study. The only data provided is derived from the necessary calculation of the population, to be included in the study, to demonstrate the possible differences in the prevalence of the disease.
Patient and Public Involvement statement

The selection of subjects will be defined once the initial date is settled. The protocol includes the proper written informed consent from subjects to the included in the study. They will accept that cannot be identified via any further paper.

The selection of the participating subjects will be done by the family doctors of the selected areas of each municipality. These doctors will enroll the participants and will previously inform each subject, of relevant aspects such as the objectives of the study, its importance, risks, personal and social benefits, the dates, place and the conditions in which the study will be performed, the disclosure of data and the commitment of not to disclose personal data.

When the designated subjects accept to participate in the survey, the team of medical researchers will inform them about the details of the execution of the protocol and will provide them with the informed consent form.

Subjects will also be informed of their willingness, not only to participate in the study, but to abandon it when they deem it, appropriate.

Cohort Description

At the beginning of the design of the study protocol, certain aspects included in the survey, were consulted with people residing in the selected municipalities. A list of commonly daily diet foods, consumed by different populations, especially in rural zones, was formulated. Participants were also inquired about the frequent daily activities, since the references from other countries did not always coincide with the reality of the Cuban populations. For example, the use of electronic devices in rural populations. A group of subjects was consulted about the monetary income and the correlation with the Cuban living standards. Thus, it could be achieved a nearer and accurate classification, since the international indicators were not comparable as they are expressed in USD equivalent.

Mostly, the proposal of subjects, to be included in the study, were consulted and presented by the family doctors of each area. However, in some cases, relatives or associates were the ones who propose subjects to be submitted as they meet the inclusion criteria. In some other cases, the same volunteer subjects contacted family members and friends to be agreed in participating in the study.

The results of the surveys will be delivered to the Health Authorities of each municipality. At the end of the study, the collected information and date will be available in booklet. especially protective
habits and activities. Likewise, they will be published on the internet sites of the municipal health authorities with free access.

The physicians participating in the study, in all cases, will be recognized as authors and the health authorities of the municipalities, experts who contributed ideas to the design of the protocol, will appear in the acknowledgments in future publications and in public outreach sites. General acknowledgments will be expressed to the participating subjects.
Introduction

Dementia is a syndrome characterized by the progressive decline in memory, executive functions, language, and other areas of cognition. It is usually associated with behavioral symptoms, which interfere with the normal development of the individual at their familial and social environment.

Alzheimer's disease (AD) is the most common of dementias. There is currently no effective treatment to prevent, stop, or reverse the pathological processes of AD.[1]

Around the world, there are almost 900 million people ≥ 60 years old. The increase in life expectancy contributes to the rapid growth of the elderly population and that is related to the prevalence of chronic diseases such as dementia.[1] According to the World Alzheimer's Report 2019, about 50 million people in the world suffer from dementia. This number will practically double every 20 years, reaching 152 million by 2050.[2] The total cost of health assistance for treating dementia is estimated to be around USD 1 trillion worldwide.[2-3]

In Latin America and the Caribbean, approximately 3.4 million people ≥60 aged is diagnosed with dementia.[4] This figure will increase up to 7.6 million by 2030,[5] thus the estimate prevalence of AD in the elderly group will be around 11 percent of the world elder population.[6] Cuba has one of the highest life expectancy rates of the region, with an individual average of 77 years old.[7] The incidence and prevalence of people suffering AD is of approximately 160,000 from the total elderly population.[8] Due to the fact that advanced age is considered the main risk factor for developing the disease, AD represents one of the major health problems the country is dealing with, today.[9]

Several reports on population-based observational studies demonstrate the existence of a number of factors associated with the onset and progression of AD.[9-12] The accumulation of amyloid beta peptide (Aβ) plaques and neurofibrillary tau protein tangles in the brain of the patient, are generally recognized among the main triggers of AD.[13-16] The amyloid beta peptide is produced through the proteolytic cleavage of the amyloid precursor protein (APP), a transmembrane protein found in
neurons.[15] In the AD, an aggregation of amyloid beta peptides occurs, forming β-amyloid plaques. These plaques are accumulated in the synaptic terminals of the brain, preventing communication among the neurons and finally leading to neuronal death.[17] Tau protein is responsible for maintaining the structure of microtubules, which permits to stabilize the cytoskeleton of neurons and also contributes to intracellular transport.[13] In AD, tau is hyper-phosphorylated and provokes a loss of affinity of the microtubules. The damage of this function induces the formation of neurofibrillary tangles that interrupt the transport of substances within the cell and cause the neuronal death. [15, 18]

A theory for the late-onset of AD is the cholinergic hypothesis, which states that a reduced synthesis of the neurotransmitter acetylcholine is related to the deterioration of cognitive function and the pathogenesis of AD.[19, 20] Other reports consider the disorders in glucose metabolism as responsible for the development of AD, given the strong correlation between insulin resistance in the brain and the progression of the disease.[21, 22] More recently studies show a growing evidence of the contribution of the gut microbiota to the pathogenesis of AD.[23-25] Longitudinal studies and research reports indicate an association of AD with vascular risk, hypertension, hypercholesterolemia, diabetes mellitus (type II), oxidative stress, and genetic background. In addition, there are lifestyle factors associated with AD, such as active smoking, inadequate nutrition, alcoholism, sedentary life, low educational level, or exposure to toxic elements such as aluminum and silico.[9, 26-29]

In Cuba, it has been performed few studies concerning the main risk factors related to the prevalence of AD. The most frequently factors included in those assessments were hypertension, diabetes mellitus, heart failure, smoking, obesity, advanced age, sexual activity, level of education, and genetic background.[30-33] However, the identification of protective factors against AD risk such as adequate eating habits and healthy lifestyles could impact on the prevention of the disease.[34] At present, there are no homogenous criteria on which tests or markers could be used to establish the prevalence of
possible AD. Hence, there is an urgent need to correctly select a set of possible risk factors for conducting population prevalence studies.

The present work is aimed at selecting the main factors that could significantly influence on the prevalence of possible Alzheimer's disease in the elderly Cuban population, residing in urban and rural regions. This project also designs the suitable epidemiological cross-sectional survey study which allows to reveal the epidemiological status of possible AD in a country that is experiencing a progressive aging on its population.

**Materials and methods**

Authors consulted and examined previous issued literature on epidemiological studies on AD and their associated risk factors, conducted worldwide. The exhaustive revision of bibliography regarding neuropsychological criteria, tests, and scales revealed that there are no international homogenous principles for the early detection and evaluation of memory impairment and possible AD. Published studies adapted and validated in Spanish-speaking countries were particularly revised.

The Short Portable Mental Status Questionnaire (SPMSQ) is a 10 questions test and one of its versions allows correcting the score obtained by the subject according to its level of schooling. The Mini-Mental Status Examination (MMSE) is one of the best known and most used instruments for detecting cognitive impairment.[35] It comprises 11 queries and explores elements such as orientation, fixation memory, concentration and calculation, memory of evocation, perception, language, and praxis. The Clock Drawing Test (CDT) is an old simple easy test to be applied for the evaluation of cognitive skills: listening comprehension, visual memory, reconstruction of a graphic image, and visuospatial skills. A study done by specialists on Psychology and Medicine in Galicia (Spain) reported the MMES as the most used test, followed by SPMSQ and CDT.[35]

The Alzheimer's Disease Assessment Scale (ADAS) test is specially designed for identifying the stages of the AD.[36] and together with the MMSE are the most widely worldwide used. The test
consists of two subscales: cognitive (ADAS-cog) and behavioral (ADAS-noncog). ADAS-cog includes assessment of memory, language, orientation, praxis, and visual construction. ADAS-noncog is based on observations or questions about behavioral symptoms. The test has been adapted to many languages and countries such as Brazil,[37] Asian region,[38] Slovenia,[39] Argentina,[40] Colombia[41] and Spain,[42] just to name a few.

The Clinical Dementia Rating (CDR) scale establishes five possible stages for deterioration, explores fields such as memory, orientation, judgment and problem solving, social life, home and hobbies; and personal care. The Global Deterioration Scale (GDS) scale describes seven global stages of dementia according to the degree of cognitive and functional impairment observed. Both scales are among the most practiced by specialists to define the stages of severity of AD).[43]

It is known that the Information and Communication Technologies (ICTs) offer a great volume of reports on AD, but only 16% of them are focused on the diagnostic applications.[44] An automated system for data collecting and scoring guarantees an efficient and swifter bibliography revision, reduces the result statistical process time and therefore, permits a more rapidly study progress.[45] A recent study reported the application of an electronic version of the standard ADAS-cog for the evaluation of AD in patients diagnosed with the disease. The effective usage of this automatic tool in population studies and clinical trials demonstrated that computerized neuropsychological tests and scales provide greater reliability in their outcome qualifications.[46]

A lately published study done by Quispe-Ramírez et al. (2020)[47] identified, as a risk factor for neurocognitive disorders, the necessity of older adults to be accompanied by others to accomplish advanced activities such as keeping contact with family, friends, and others through different means, caring for someone else, working as volunteers, traveling, exercising, organizing dinners or meetings at home, execute manual activities, go out for pleasure, drive a vehicle and use a computer.[47] Authors also found as risk factors the dependency to perform daily technological actions as the
frequent money withdrawing from automatic money machines, purchases with cards, usage of a mobile phone, electronic health devices and the tools of internet.[47]

Based on the aforementioned and revised bibliography and reports, and with the objective of establishing the prevalence of possible Alzheimer's disease in different groups of elderly Cuban population, the present study included the following features: demographic and socioeconomic information, educational level, occupation, health and neuropsychological status of subjects, familial pathological history, risk factors for developing AD, comorbidity and lifestyles. In addition, it was taken into consideration the protective factors associated with the lesser prevalence of the disease and the selection of neuropsychological tests and scales best adapted to the particular characteristics of the population, object of this study.

Due to the importance attributed to the consumption of certain kind of food and derived products as protective factors for avoiding the development of the AD, eating habit features were emphasized. The selected products were cocoa, fish, and coconut. From the latter, the consumption of coconut oil, rich in middle chain fatty acids, is analyzed. To obtain the best suitable information on eating habits, it was decided to include data on whether the above-mentioned products are consumed or not in the usual diet, ways of consumption, amount, and frequency in at least the last 10 years. The frequency of consumption was classified as daily, weekly, monthly and sporadically. The way of measuring quantities varied from one food to another. For example, coconut oil should - as the quantity of tablespoons consumed; cocoa (chocolate) - in number of servings; fish – in number of portions (converted latter in grams) and coffee - in number of cups per day. Questions about the habitual feasting of other sources of protein, fruits, vegetables, and coffee were also incorporated.

The chosen territories to conduct the study were: Baracoa, located at Guantánamo province, Santa Cruz del Norte and Bejucal in Mayabeque province. (Figure 1). Baracoa was selected because of its environmental, socioeconomic, and cultural factors converge as
a set of possible protective factors against AD. It is known that Baracoa is the main producer of coconut and cocoa in the country. Its industries process by-products such as coconut oil and chocolate. Besides, the artisanal processing and consumption of these products are part of the nutritional traditions of the region. Baracoa is a town where mountains prevailed and the limited narrow coastal plain area with its fast-flowing rivers permit fishing activity, which constitutes an important economic and dietary source for its population. Furthermore, up to date, there was no significant evidence of prevalence of AD in that population. Santa Cruz del Norte town is a coastal zone where fish consumption is high but has a low consumption of coconut and cocoa. Contrasting, Bejucal municipality does not have any of those geographical characteristics; the consumption of fish, coconut and cocoa is very low or actually null, and there is an incidence of AD in adult population.

The number of subjects of each town was selected proportionally to the demographic distribution (rural and urban areas) and gender of the population. Demographic data was collected from the last edition of the Cuban National Survey of Population and Housing in 2011.[48-50] The margin of acceptance of sample size was of 70 %. The assent criterion for considering the compliance with the planned sample size was of 75 % for each location/area/gender.

The sample size was calculated according to Morales-Vellejo (2012)[51] using the formula [1]:

\[ n = \left( \frac{N}{1 + \frac{2z^2(N - 1)}{e^2}} \right) \]

[1]

\( n = \) simple size,
\( N = \) known population size,
\( z = \) confidence level,
\( pq = \) variance of the population,
\( e = \) margin of error
The determined sample size allowed obtaining reliable results on the influence of the main factors, with a 5% maximum margin of error, and a 95% of confidence level.

To identify the studied population sample, we selected the following criteria:

Inclusion criteria:
- Adults aged ≥ 60, both genders
- Town permanence of residence ≥ 10 years
- Principle of voluntariness and written consent

Exclusion criteria:
- Long hospitalized period of the adult (≥1 year)
- People with disabilities related to neurological disorders. (Schizophrenia, mental retardation, aphasia and psychiatric treatment at Psychotic level)
- Persons with severe visual deficit, hearing impairment and motor disabilities that obstruct the execution of tests
- People with severe alcohol dependence and psychotropic drugs dependence

Withdrawing criteria:
- People, who for any reason, do not complete the survey and tests
- Non-completion of relevant data (age, permanence of residence and other data related to exclusion criteria)

The specialist from General Medicine area and leader of the Adult Program of each territory, in collaboration with the Family Medical Offices (CMF) and a team of 5 to 10 doctors, will be in charge of performing the study.

Ethics: The research reported in this paper followed the ethical and relevant codes of experimentation and legislation principles of the Declaration of Helsinki. The Research Scientific Council, from the
Promoting Research Institution, from the Health Authorities and Ethic Committees of each municipality approved the documents designed as part of the developed protocol.

Consent: Once started, the protocol will include the proper written informed consent from subjects to the included in the study. They will accept that cannot be identified via any further paper.

**Results**

The determined total sample size comprise 1092 subjects, aged $\geq 60$, from the three selected zones of the country and distributed as it is described in Table 1. After being gathered the demography, social and all the necessary information on subjects, and been studied the existing bibliography; authors designed the final survey and the detailed interview protocol for subjects and/or their caregivers.

The designed prospective cross-sectional study followed the descriptive inclusion, exclusion, and withdrawing criteria and it was coordinated by the National Center of Biological Products as the Promoting Organization, in collaboration with the Direction of the Ministry of Public Health of the three selected municipalities of the country.

The survey employed the Spanish version of the SPMSQ;[52-53] MMSE;[54-55] CDT tests,[56-57] the Assessment of Alzheimer's Disease Scale;[36, 58], the Clinical Dementia Rating Scale.[59]and the Global Deterioration Scale,[60] to identify the presence of cognitive impairment on subjects. For exploring comorbidity in the enrolled subjects, it was included a comprehensive list of their current diseases. The list comprised psychiatric, neurological, vascular, immunological, genetic, and metabolic illnesses; lapse of time suffering from the diseases and the indicated treatments. In addition, the familial pathological history which could represent a risk for developing AD, was incorporated (Table 2).

Physical activity such as sports, exercises, or physical efforts related to the profession of subjects, their intensity and frequency were also taken in consideration. Some compensatory activities that older adults could perform in their leisure time and that could contribute to stimulating neuronal or brain
activity, as protective factors, were incorporated in the survey, as well. Risks factors like smoking (number of cigarette, the frequency of smoking, and number of years smoking cigars or cigarettes), consumption of alcoholic beverages (frequency and quantity) as well as the exposure to toxic elements were also listed.

A supplementary section of the survey was dedicated to exploring the neuropsychological status of the subjects (Table 3). This section dealt with questions about the frequency of the appearance of deterioration symptoms of certain areas such as cognition or behavior, the ability of acquiring and remembering new information, reasoning and managing complex tasks, impoverished judgment, visuospatial abilities, language functions and changes in personality. If subjects presented evident advanced neuropsychological deterioration, their caregivers or the interviewer himself should declare the physical status of the subject.

The questionnaire contained the following items:

- General information on subjects (name, surnames, address, birth date, ID number).
- Demographic data (gender, age, educational level, skin color, marital status, residence permanence)
- Social and economic data (quantity of members who live in the same house as the subject, family functionality, loss of a relative and of a close friend, family relationships, detach from a close relative or a close friend, past and current employment status, income level)
- Health condition: current illness (age of onset and treatment), familial pathological history (Table 2)
- Eating habits: especially of the foods of interest (coconut, cacao, fish, selfish and derived products) and considering the past and current form of consumption, frequency and quantity). Subject was also asked about the feasting of animal and plant proteins, vegetables, fruits, water supply and water quality used to drink and cooking
- Physical activities (type, frequency and intensity)
- Risk factors: smoking and alcoholic consume, exposure to toxic elements (past and current consumption, frequency and quantity)

-Sleeping (hours, quantity, quality and disorders)

-Neuropsychological status (Table 3)

-Sexual activity (frequency and orgasms)

- Interviewer's observations and/or caregivers responses regarding the physical status of the subject with severe symptoms of the AD, expressed in the inability of pronouncing words, speak but just growls, urinary incontinence, caregivers dependence to go to the bathroom, to eat, the inability to perform psychomotor activities, delusive behavior and loss of will

-Compensatory activities: hobbies, reading, writing, dance, physical exercises, sports, crafts, studio/workshops, playing musical instruments, participation in activities as a volunteer, films/documentaries/series/novels, board games, membership in cultural associations, travel

The research team designed all the necessary documentation for conducting the neuropsychological test study: the inclusion and exclusion registration form, the documented agreement letter, instructions for researchers, paper sheets, envelops and any other additional datasheet.

Subjects will be considered to be diagnosed with AD if it exists documented clinical evidence of the AD, issued by an authorized health institution or if the subject is classified as possible or probable candidate to suffer from the disease.

The BioMer.apk for portable mobiles with Android operating system was designed, in collaboration with the Free Software Development Center (CESOL) of the University of Computer Sciences (UCI) in Havana City. The application is part of the epidemiological study and comprises data on the general information of the studied subjects, the survey and the neuropsychological tests and scales outcomes.

The collected statistics allow recording images of drawings and texts performed as part of different
neuropsychological tests, and results from different laboratory examinations. All recorded information could be exported to an Excel worksheet for further statistical analysis.

Discussion

In Cuba, prevalence studies on Dementia have shown that the most related afflictions with the prevalence of AD are the chronic diseases such as hypertension and diabetes mellitus. Smoking and consumption of alcoholic beverages, age, gender and educational level are factors that should also be taken in consideration.[30, 61-63] Several studies, performed worldwide,[64-65] corroborated this fact and even demonstrated the association of loss of teeth and decreased chewiness,[66] insomnia,[67] expositions to different types of anesthesia,[68] skin color,[69] eating habits and ethnic groups,[70] to the prevalence of AD.

The present study focused on the consumption of coconut oil, due to its possible protective and therapeutic effects on AD. Numerous reports stated cognitive improvement in patients treated with coconut oil as an alternative to traditional treatments.[71-73] Coconut oil contains a high concentration of medium chain fatty acids (MCFA) which is metabolized in the liver yielding ketone bodies,[74] and constitute an alternative source of energy for the brain of AD patients, because of their impossibility of metabolizing glucose at their brain level.[75] Coconut oil also shows antioxidant properties (polyphenols components) that could reduce the toxicity of beta amyloid plaques in the brain of patients with AD.[75-77]

The consumption of cocoa and its derivatives, selected for the study, provide flavonoids known as potent antioxidant.[78] The beneficial effect of flavonoids on the cognitive status could be explained by the positive results in preventing the oligomerization of the Aβ protein.[79] Furthermore, recent studies suggested that regular consumption of chocolate is associated with the improvement of the cognitive function.[90] Fish and shellfish are two of some other essential nutrients for the body as
vitamins, amino acids and omega 3 fatty acids.[81] A recent study was able to significantly correlate
the higher consumption of fish with the lower prevalence of AD, lower density of beta amyloid
plaques and neurofibrillar tangles.[82] Authors suggested that consuming moderate amount of fish
and shellfish could have a protective effect in subjects having specific genetic risk of AD.[81-82]

Although excessive consumption of coffee and especially caffeine is considered harmful to health, in
normal quantity, it could positively influence in certain areas of knowledge, including memory and
learning, just to mentioned some.[83] As the analysis was not conclusive, it will be noteworthy to
accomplish further studies on the Cuban population.

It is known that a sedentary lifestyle, commonly increased in the elderly population, and a risk factor
for the manifestations of numerous diseases as hypertension, diabetes mellitus, and obesity, represents
also a threat for developing dementia (AD included).[84] Regular practice of physical exercise is a
form of having a healthy lifestyle and counteracts the risk factors for the appearance of disease in
older adults. A large number of studies have attempted to demonstrate that physical exercise can
improve neurocognitive functions in elderly. Moon et al. (2016)[85] suggested that there is a
correlation between the practice of physical exercise and the hippocampal-dependent memory
function. In trial, they revealed that the experimental group practicing treadmill routine during 4
months, showed in plasma significantly higher levels of Cathepsin B (CTSB), a protein able to destroy
beta-amyloid plaques, when comparing with the control group.[86] Other studies related the practice
of aerobic exercises to the increase of the brain volume,[87] or with the improvement of mild cognitive
impairment in older adults.[88] Tao et al. (2017)[89] and Kasai et al. (2010)[90] reported that the
practice of Tai Chi Chuan showed similar results by increasing gray matter volume and significantly
improving mild cognitive impairment in older adult patients. In our criteria, yet, not all reported
results have been able to demonstrate remarkable differences between test groups and controls.[91-
93]
Many older adults adopt calm and monotonous lifestyles that lead to their cognitive functions reduce.

The assumed way of life could be origin by the physical limitations because of age, lack of motivations, or mental disorders like depression. In contrast, compensatory cognitive, social and leisure activities are considered beneficial for older adults. In our current survey, the inclusion of this type of activities measures the cognitive stimulation managed as non-pharmacological therapies for patients in advanced stages of AD and acting as protective factors against this disease. The efficacy and effectiveness of the compensatory activities are demonstrated by their characteristic of delaying the progression of dysfunction in patients with dementia.[94] This beneficial outcome is due to the neuroplasticity quality of the brain that is capable of been restructured from its "prior" experience, execution of different tasks and cognitive stimulation.[95] Based on this evidence, several studies focused on the design of memory, cognitive, sensory, and emotional stimulation programs for patients with AD,[96-97] or suggest to use them as part of non-pharmacological therapies.[98]

The first and more relevant manifestations of AD in subjects are the behavioral changes and memory loss. These symptoms are also associated with cognitive deterioration, typical of aging, or other types of dementia.[99-101] The analysis of physical and neuropsychological status of the studied subjects permitted the early identification of AD patients.[102] AD is a degenerative and progressive disease, that show aggravated manifestations in the cognitive area such as forgetting the names of relatives and close people, not recall past or recent life events or important dates and inability to recognize faces. Subjects in a more advanced stage of the disease can lose themselves in familiar places, loss the interest in hobbies and daily household activities, fail at reasoning and solving problems of daily life.[98] In the behavioral area, AD patients show symptoms in their behavior and personality, such as sudden changes in mood, demotivation, apathy, compulsive or obsessive behavior and physical or verbal aggressiveness towards other people. These patients may suffer from hallucinations, anxiety, phobia, depression, and desire of crying without reason.[103] Although, most of these behavioral
indicators are explored in different neuropsychological tests, we decided to include them in a separate
set of questions in the survey, in order to clearly determine the stage of the disease (moderate or
severe) in the studied subjects.

Physical condition of subjects diagnosed with advanced AD reveals changes related to the neuronal
deterioration, such as the loss of visuospatial capacity. This was expressed by the inability to find
objects in sight, despite having good visual acuity and difficulty for operating simple utensils or
dressing.[104] Language function alterations, as the difficulty of finding suitable words, while
speaking or forgetting words, are worsened to the extent of expressing merely few words, grunting,
or even no speaking at all.[105] At more severe stages of the disease, patients are unable to control
their sphincters or execute any psychomotor activity, consequently leading to the death of the
patient.[98] Causes of death may vary depending on the level of cognitive decline of the patient and
the concomitant diseases they suffer from.[106]

Consulted studies stated that diseases like bipolar impairment, depression and schizophrenia, showing
similar symptoms, might be considered as risk factors for AD or as concomitant diseases during AD
progress.[107-109] A comprehensive review done by Dutie et al. (2011)[84] found a large number of
non- psychiatric comorbidities with AD: vascular diseases (vascular dementias included),
hypertension, diabetes mellitus, obesity, hypothyroidism and/or hyperthyroidism, sleep apnea,
osteoporosis, and glaucoma. The same review outlined a less association among AD, cancer and
rheumatoid arthritis.[68]

For the design of the current epidemiological study, the neuropsychological tests and scales were
selected according to the well-defined criteria that they should be previously validated in some of the
Spanish-speaking countries or previously used in Cuba, to diagnose AD, within the National
Healthcare System. Moreover, the selected tests should be of easy and quick application, but they
should allow collecting a wide spectrum of the cognition area of the subjects.
Climent and Molinero (2017)[110] designed a similar study to our current project in which they performed the MMSE, CDT, and SPMSQ tests to trace symptoms of early cognitive decline in the population and analyze the associated risk factors. Authors included a questionnaire that explores demographic, anthropometric data, educational level, occupation, lifestyles such as smoking, alcohol consumption, weekly physical exercises, hobbies, hours of daily television, reading, hours of sleep, and also chronic pathologies such as diabetes, hypertension, hyperglycemia, and depression.

As conclusion remarks we highlight that an epidemiological cross-sectional survey was designed for identifying the possible association of risk and protective factors with the Alzheimer's disease in different groups of the Cuban elderly populations.

The designed study will be applied to more than 1000 inhabitants of both genders residing in rural and urban areas, selected because of their different geographic characteristics. Subject sample was selected due to the diversity of lifestyle, eating habits and average usage of electronic and computer devices. The survey comprises demographic, social, and economic factors, health condition and familial health backgrounds, eating and non-eating habits, neuropsychological status, sexual activity, and physical conditions. Furthermore, the project includes the most widely used neuropsychological tests and scales, all adapted for portable devices.

The Promoting Organization Group and the Ethics and Scientific Committees of the Public Health Direction of the selected territories approved the protocol. The designed and proposed application for mobile devices will permit a suitable assessment of the neuropsychological tests and scales outcomes of the studied subjects.

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Table 1. Sample size by locations

| Location            | Urban area | Rural area | Total |
|---------------------|------------|------------|-------|
|                     | Men        | Women      | Men   | Women |
| Baracoa             | 101        | 106        | 88    | 79    | 374   |
| Bejucal             | 132        | 131        | 50    | 43    | 356   |
| Santa Cruz del Norte| 121        | 130        | 61    | 53    | 365   |
| **Total**           | **354**    | **367**    | **199**| **175**| **1095** |
Table 2. Health condition and Familial pathological history

| Psychiatric and neurological diseases | Vascular diseases | Immunological diseases | Genetic diseases | Metabolic diseases | Others diseases and conditions |
|--------------------------------------|------------------|------------------------|-----------------|-------------------|-------------------------------|
| Alzheimer Disease**                  | Ischemic heart disease** | Asthma**              | Down’s Syndrome * | Type I diabetes** | Severe respiratory insufficiency** |
| Parkinson*                          | Hypertension**    | Arthritis**           | Autism*         | Type II diabetes** | Cancer **                     |
| Schizophrenia*                      | Cardiac arrhythmia** |                        |                 |                   | Avitaminosis (Vit A, B, C, …)** |
| ICTUS**                             |                  |                        |                 |                   | Hyperlipidemia*               |
| Dementia**                          |                  |                        |                 |                   | Hypercholesterolemia*         |
| Chronic depressive status**         |                  |                        |                 |                   | Hyperthyroidism*              |
| Mental retardation*                 |                  |                        |                 |                   | Glaucoma**                   |
| Psychosis*                          |                  |                        |                 |                   | Alcoholism**                 |
| Aphasia*                            |                  |                        |                 |                   | Smoking**                    |
| Frequent headaches**                |                  |                        |                 |                   | Obesity**                    |
| Cranial trauma**                    |                  |                        |                 |                   | General anesthesia***         |
| Hydrocephalus**                     |                  |                        |                 |                   | A severe blow to the head***  |
| Bipolar disorder*                   |                  |                        |                 |                   | Headache***                  |

*- familial pathological history questions; **- questions concerning both, the subject and familial pathological history, *** questions concerning only the subject
| Cognitive or behavioral symptoms | Impairment of the ability to acquire and remember new information | Deterioration of reasoning and management of complex tasks, impoverished judgment | Impairment of visuospatial capabilities | Impairment of language functions | Changes in personality, conduct or behavior | Neuropsychological status |
|--------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------|
| Deterioration in the functional capacity at work | Decrease in the interest of executing hobbies and daily activities at home | Subject’s perception of risks, without realizing a possible danger | Difficulty to find visible objects, even having a good visual acuity | Dysfunction of the language tasks. Difficulty in finding the right words while speaking or forgetting words | Sudden mood swings | Sadness |
| Deterioration in the functional capacity to perform usual activities | Asking same questions or repetitive conversations | Inability to manage finances or confusion to get accounts | Difficulty in operating with simple utensils | Errors in speech, in spelling words or in writing | Feeling unmotivated or apathetic to do things that once caused pleasure | Nervous tenseness |
| Decrease in levels of functionality and performance, compared to the past behavior of the subject | Mistakenly place personal belongings or forgetting their location | Difficulty in making decisions | Difficulty in getting dressed | Isolation or loss of empathy towards known people | Irritableness |
| Unable to remembering recent or past events in personal life or daily life | Incapacity to plan complex activities | Compulsive or obsessive behaviors, physical or verbal | Pessimism |
| Loss in a family place | Hallucinations anxiety, phobia, depression, desire to cry for no reason | Suicidal ideas |
|------------------------|------------------------------------------------------------------|---------------|
| Fail to recall prior remembered names | Loss of auto confidence |               |
| Difficulty to remember a fact or book the subject just read |                       |               |
| Retention of the name of the investigator |                       |               |
Figure 1. Location of the three selected municipalities for conducting the epidemiological study in Cuba
# Prevalence of Alzheimer Disease in rural and urban areas in Cuba. Epidemiological cross-sectional Protocol.

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Prevalence of Alzheimer Disease in rural and urban areas in Cuba. Epidemiological cross-sectional Protocol.

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ABSTRACT:

Introduction: according to the World Alzheimer's Report 2019, around 50 million people suffer from dementia, worldwide. Observational analysis revealed the existence of particular factors associated with the onset and progression of the Alzheimer Disease (AD). There are no international homogenous principles for the early detection and evaluation of memory impairment and possible AD. This work aimed at (i) designing an epidemiological study protocol to determine the prevalence of possible AD in different groups of elderly rural and urban Cuban population and (ii) identifying the factors that influence on that occurrence. Methods and analysis: authors reviewed literature on AD and selected the most appropriated items. Results: the protocol consisted of four neuropsychological tests (Clock Drawing Test, Mini-Mental Status Examination, Short Portable Mental Status Questionnaire, Cognitive and Non Cognitive Alzheimer’s Disease Assessment Scale) and two scales (Clinical Dementia Rating and Global Deterioration Scale). In addition, the protocol includes a survey with demographic and socioeconomic information, educational level, occupation, health and neuropsychological status of subjects, familial pathological history, comorbidities and life styles. The survey will be conducted by licensed physicians from the primary care level in three municipalities and will comprise a total of 1092 subjects aged ≥ 60, both genders and from every ethnic group living in rural and urban areas. Primary outcomes: prevalence of possible AD. Secondary outcomes:
correlation among risk and protective factors and AD, and comparison of the performance of
neuropsychological tests and scales. **Ethics and dissemination:** This research followed the ethical
codes of the Declaration of Helsinki. The Scientific Research Council of the Promoting Research
Institution and the Ethic Committee of the Health Authorities approved the protocol. The
correspondent written informed consents were also incorporated. The results will be published in
scientific papers and will be shared with the Health Authorities of each municipality.

**STRENGTHS AND LIMITATIONS OF THIS STUDY**

- The protocol includes the most significant diversity of factors that could influence on the
  prevalence and severity of AD,
- It comprises occurrence study in rural and urban areas,
- It will permit selecting the suitable neuropsychological tests and scales to be applied in the
  population, for diagnosing possible AD,
- The study does not include populations under 60 years of age, because the prevalence of the
disease, prior to that oldness is generally attributed to familial Alzheimer's,
- The study does not incorporate biochemical markers of the disease

**INTRODUCTION**

Dementia is a syndrome characterized by the progressive decline in memory, executive functions,
language, and other areas of cognition. It is usually associated with behavioral symptoms, which
interfere with the normal development of the individual at their familial and social environment.
Alzheimer's disease (AD) is the most common of dementias. At present, there is no effective treatment
to prevent, stop, or reverse the pathological processes of AD [1].

According to the World Alzheimer's Report 2019, about 50 million people suffer from dementia,
worldwide and this figure will practically double every 20 years, reaching 152 million by 2050 [2].
The total cost of health assistance for treating dementia is estimated to be around USD 1 trillion
globally [2-3]. In Latin America and the Caribbean, approximately 3.4 million people ≥60 aged is
diagnosed with dementia [4]. This amount will increase up to 7.6 million by 2030 [5] thus, the estimate
of AD in the elderly group will be around 11 percent of the older population worldwide [6]. Cuba has
one of the highest life expectancy rates of the region, with an individual average of 77 years old [7].
The prevalence of people suffering AD is of approximately 160,000 of the older population [8]. Due
to the fact, that advanced age is considered the main risk factor for developing the disease, AD
represents one of the major health problems [9].
Several reports on population-based observational studies revealed the existence of a number of
particular factors associated with the onset and progression of AD [9-12]. The accumulation of
amyloid beta peptide (Aβ) plaques and neurofibrillary tau protein tangles in the brain of the patient,
are generally recognized among the main triggers of AD [13-16].
Longitudinal studies and research reports indicated an association of AD with vascular risk,
hypertension, hypercholesterolemia, diabetes mellitus (type II), oxidative stress, and genetic
background [9, 17-20]. In addition, there are other lifestyle factors associated with AD, such as active
smoking, inadequate nutrition, alcoholism, sedentary life, low educational level, or exposure to toxic
elements such as aluminum and silico [9, 17-20].
In Cuba, few studies concerning the main risk factors for AD has been performed. The most
commonly factors included in those assessments were hypertension, diabetes mellitus, heart failure,
smoking, obesity, advanced age, sexual activity, level of education, and genetic background [21-24].
However, the identification of protective factors against AD threat, such as adequate eating habits and
healthy lifestyles could impact on the prevention of the disease [25]. At present, there are no
homogenous criteria on which tests or markers could be used to establish the prevalence of possible
AD. Hence, there is an urgent need to correctly select a set of possible risk factors for conducting
population prevalence studies.
A study done by Quispe - Ramírez et al. (2020) [26] identified, as a risk factor for neurocognitive disorders (i) the need of older adults to be assisted by others, for undertaking common regular activities and (ii) performing certain social actions that involve the use of technology.

Some of the tools generally used for performing AD neuropsychological diagnosis are: (i) the Short Portable Mental Status Questionnaire (SPMSQ), a 10 questions test which, one of its versions allows amending the score obtained according to the level of schooling of subjects; (ii) the Mini-Mental Status Examination (MMSE) for detecting cognitive impairment, which explores elements such as orientation, fixation memory, concentration and calculation, memory of evocation, perception, language, and praxis; and (iii) the Clock Drawing Test (CDT), which evaluates cognitive skills (listening comprehension, visual memory, reconstruction of a graphic image, and visuospatial skills [27]. A study performed by specialists on Psychology and Medicine in Galicia (Spain) reported the MMES as the most functional one, followed by SPMSQ and CDT [27].

The Alzheimer’s Disease Assessment Scale (ADAS) is specially designed for identifying the stages of the AD and, together with the MMSE are the most frequently used worldwide [27]. The former consists of two subscales: cognitive (ADAS-cog) and behavioral (ADAS-noncog). ADAS-cog includes assessment of memory, language, orientation, praxis, and visual construction [28]. ADAS-noncog is based on observations or questions about behavioral symptoms. The scale has been adapted to many languages and countries [29-34].

The Clinical Dementia Rating (CDR) scale explores fields such as memory, orientation, judgment and problem solving, social life, home and hobbies; and personal care [34]. The Global Deterioration Scale (GDS) scale describes seven global stages of dementia, according to the degree of cognitive and functional impairment observed. Both scales are among the mainly proficient tools for defining the stages of severity of AD [43].
The aim of the present study protocol aimed to determining the prevalence of possible AD in elderly residing in urban and rural regions in Cuba, and identify the main factors that could significantly influence on its occurrence.

**METHODS AND ANALYSIS**

**Selection of factors**

Based on the aforementioned and revised bibliography and reports, the study comprised the following features: demographic and socioeconomic information, educational level, occupation, health and neuropsychological status of subjects, familial pathological history, comorbidity and lifestyles. In addition, protective factors associated with the lesser prevalence of the disease such as sports, physical activity and hobbies were included; as well as the best suitable selection of neuropsychological tests and scales.

Due to the importance, attributed to the consumption of certain specific food or products (fish, cocoa and coconut) as protective factors, eating habit aspect was highlighting. The consumption of coconut oil, rich in middle chain fatty acids, was also analyzed. Questions about the habitual feasting of other sources of proteins, fruits, grains, vegetables, and coffee were also incorporated. For obtaining the proper information on eating habits, the query consisted of whether the products were consumed or not as the usual diet, custom, amount, and frequency of consumption within the last 10 years, minimum. Parameters for frequency of intake were daily, weekly, monthly and sporadically. The approach of measuring quantities varied according to the food; for example, coconut oil – consumed quantity of tablespoons; cocoa (chocolate) - amount of servings; fish – amount of portions (adjusted to grams) and coffee - amount of cups, per day.

**Study design**

The chosen territories to conduct the study were Baracoa, located at Guantánamo province, Santa Cruz del Norte and Bejucal situated at Mayabeque province. (Figure 1).
Baracoa location was selected because of its environmental, socioeconomic, and cultural factors converge as a set of possible protective factors against AD. It is known that Baracoa town is the main producer of coconut and cocoa in the country. Moreover, the artisanal processing and consumption of those products are part of the nutritional traditions of the region. Baracoa has also an intense fishing activity, it constitutes thus an important economic and dietary source in its population. Furthermore, there is no significant evidence of prevalence of AD in its inhabitants up to date. Santa Cruz del Norte town is a coastal region where fish consumption is high, but it has a low consumption of coconut and cocoa. Contrasting, population settled in Bejucal does not have a high intake of fish, coconut and cocoa ingestion is very low or actually null, and there is a manifest incidence of AD in the adult population.

The amount of subjects of each town was proportionally selected to the demographic distribution (rural and urban areas) and gender of the population. Demographic data was collected from the last edition of the Cuban National Survey of Population and Housing [36-38]. The assent criterion for considering the compliance with the planned sample size was of 75 % for each location/area/gender.

The sample size was calculated according to Morales-Vellejo (2012) [39] using the formula [1]:

\[
n = \left( \frac{N}{\frac{e^2(N-1)}{Z^2pq}} \right)
\]

[1]

in which:

- \( n \) = simple size,
- \( N \) = known population size,
- \( z \) = confidence level,
- \( pq \) = variance of the population,
The determined sample size comprised 1092 subjects aged $\geq 60$. The sample will allow reliable results on the influence of the main factors, with a 5% maximum margin of error, and a 95% of confidence level (Table 1).

The following selection criteria will be taken into account:

**Inclusion criteria:**
- Adults aged $\geq 60$, both genders
- Town permanence of residence $\geq 10$ years
- Principle of voluntariness and written consent

**Exclusion criteria:**
- Long hospitalized period of the adult ($\geq 1$ year)
- Schizophrenia, mental retardation, aphasia and psychiatric treatment at Psychotic level
- Severe visual deficit, hearing impairment and motor disabilities that obstruct the tests
- Severe alcohol dependence and psychotropic drugs dependence

**Patient and Public Involvement statement**

The selection of subjects will be defined once; the initial date is settled and will be done by the family doctors of the selected areas of each municipality. These doctors will enroll the participants and will previously inform each subject, of relevant aspects such as the objectives of the study, its importance, risks, personal and social benefits, the dates, place and the conditions in which the study will be performed, the disclosure of data and the commitment of not disclosing personal data.

When the designated subjects accept to participate in the survey, the team of medical researchers will provide them with the informed consent form.

Subjects will be also informed of their willingness to abandon the study when they deem it, appropriate.
For subjects who show severe cognitive impairment or with AD, the informed consent from the recognized care family member will be requested.

The results of the surveys will be published in scientific papers and will be shared with the Health Authorities of each municipality.

**Outcome measures**

Primary outcomes: to identify the presence of cognitive impairment and possible AD on subjects, and the prevalence of the disease in rural and urban areas. The protocol included the Spanish versions of the SPMSQ [40, 41]; MMSE [42, 43]; CDT tests [44, 45]; the ADAS test [28, 46]; the CDR Scale [47] and the GDS [48].

For exploring secondary outcomes such as comorbidities, it was added a comprehensive aspect list that comprised (i) current diseases; (ii) psychiatric, neurological, vascular, immunological, genetic, and metabolic illnesses; (iii) lapse of time suffering from those diseases and the indicated treatments; (iv) familial pathological history, which could represent a risk for developing AD (Table 2); and (v) practice, frequency and intensity of performing physical activity (sports, exercises, or physical labors related to the profession of subjects).

As protective factors, listing was enlarged with the inclusion of some leisure time activities of elderly, which could contribute to stimulating the neuronal or brain activity. Risks factors like smoking (quantity of cigarettes, frequency and period of smoking cigars or cigarettes), consumption of alcoholic beverages (frequency and quantity) as well as the exposure to toxic elements were also registered.

A supplementary section of the survey was dedicated to exploring the neuropsychological status of the subjects (Table 3). This section dealt with questions on the frequency of the appearance of deterioration symptoms of certain areas such as cognition or behavior, the ability of acquiring and remembering new information, reasoning and managing complex tasks, impoverished judgment,
visuospatial abilities, language functions and changes in personality. If subjects presented evident advanced neuropsychological deterioration, their caregivers or the interviewer should declare the physical status of the subject.

The questionnaire

The questionnaire contained the following items:

- General information on subjects (name, surnames, address, birth date, ID number).
- Demographic data (gender, age, educational level, skin color, marital status, residence permanence).
- Social and economic data (quantity of members who live in the same house as the subject, family functionality, losing or detaching from a relative or a close friend, family relationships, past and current employment status, income level).
- Health condition: current illness (age of onset and treatment), familial pathological history (Table 2).
- Eating habits of coconut, cacao, fish, selfish and derived products (past and current consumption, frequency and quantity). Subject will also be asked about the feasting of animal and plant proteins, vegetables, grains, fruits, water supply and water quality used for drinking and cooking.
- Physical activities (type, frequency and intensity).
- Other risk factors: smoking and alcoholic consume, exposure to toxic elements (past and current consumption, frequency and quantity).
- Sleeping (amount of hours, quantity, quality and disorders).
- Neuropsychological status (Table 3).
- Sexual activity (frequency and orgasms).
- The surveillance of the interviewer and/or responses of the caregivers, regarding the physical status of the subject with severe symptoms of the AD; expressed in the inability of pronouncing words,
speaking but just growls, urinary incontinence, dependence to complete routine actions, the inability
to perform psychomotor activities, delusive behavior and loss of will.
-Compensatory activities: hobbies, reading, writing, dance, physical exercises, sports, crafts,
studio/workshops, playing musical instruments, participation in activities as a volunteer,
films/documentaries/series/novels, board games, membership in cultural associations, travel.
Subjects will be deemed with AD if it exists documented clinical evidence of the AD, issued by an
authorized health institution or if the subject is classified as possible or probable candidate to suffer
from the disease.
The BioMer.apk for portable mobiles was designed, in collaboration with the University of Computer
Sciences (UCI) in Havana City. The application includes data on the general information, results of
the survey, neuropsychological tests and scales outcomes. In addition, it allows recording the images
of drawings and texts from the different neuropsychological tests. All recorded information could be
the exported to an Excel worksheet for further statistical analysis.

Data analysis plan
Descriptive statistics based on means, standard deviations, percentages, and 95% confidence intervals
will be used for describing the studied population. A logistic regression model will be employed to
analyze the association between AD and studied parameters. Chi-square, Student t-test, or Mann-
Withney test will be performed for selecting parameters related to the outcome measures (p≤0.05).
Several other statistical methods will be also applied: multiple logistic regression models, selecting
variables for inclusion in the model based on the backward elimination procedure; exploratory factor
analysis and multiple component analysis, which models enable to explaining correlations between
the independent variables and prevalence of AD, as dependent variables. Linear and nonlinear
regression analysis, as well as the ROC curves will be used for comparing different
neuropsychological tests and scales.
Strengths of this study

In Cuba, prevalence studies on Dementia have revealed that the most related conditions with AD are the chronic diseases such as hypertension and diabetes mellitus. Smoking and consumption of alcoholic beverages, age, gender and educational level are factors that should also be taken in consideration [21, 49-51]. A number of studies, performed worldwide [52-54], corroborated this fact and even demonstrated the association of insomnia [55], expositions to different types of anesthesia [56], skin color [57], eating habits and ethnic groups [58], with the prevalence of AD.

The present study focused on the consumption of coconut oil, due to its possible protective and therapeutic effects on AD [59-61]. The consumption of cocoa and its derivatives, selected for the study, provide flavonoids known as potent antioxidant [62]. Fish and shellfish, rich in vitamins, amino acids and omega 3 fatty acids are two of some other essential nutrients for humans [63]. A study significantly correlates the higher consumption of fish with the lower prevalence of AD [64].

Although, excessive consumption of coffee and especially caffeine is considered harmful to health; it could positively influence in appropriate amount, in certain areas of knowledge, including memory and learning, just to mentioned some [65]. As the analysis was not conclusive, it will be noteworthy to accomplish further studies on the Cuban population.

It is known that a sedentary lifestyle represents also a threat for developing dementia (AD included) [66]. Regular practice of physical exercise is a way of ensuring a healthy lifestyle and counteracts the risk factors for the appearance of the disease in elderly. Moon et al. (2016) [67] suggested that there is a correlation between the practice of physical exercise and the hippocampal-dependent memory function. Other studies related the practice of aerobic exercises with the increase of the brain volume [68] or with the improvement of mild cognitive impairment in older adults [69]. In our criteria, none of all reported results [70-72] has been able to demonstrate remarkable differences between test groups and controls, thus far.
Contrary, some older adults adopt calm and monotonous lifestyles which lead to diminishing their
cognitive functions. The assumed attitude could be originated by physical limitations, due to age,
lack of motivations, or mental disorders like depression. However, compensatory cognitive, social
and leisure activities are considered beneficial for elderly. In the current survey, the inclusion of this
type of activities measures the cognitive stimulation, as non-pharmacological therapies for patients in
advanced stages of AD, and act as protective factors against this disease. The efficacy and
effectiveness of the compensatory activities are demonstrated by their characteristic of delaying the
progression of dysfunction in patients with dementia [73]. This beneficial outcome is due to the
neuroplasticity characteristic of the brain, which is capable of been restructured from its "prior"
experience, execution of different tasks and cognitive stimulation [74]. Based on this evidence, several
studies focused on the design of memory, cognitive, sensory, and emotional stimulation programs for
patients with AD [75, 76] or suggest to use them as part of non-pharmacological therapies [77].

The first and more relevant manifestations of AD in subjects are the behavioral changes and memory
loss. These symptoms are also associated with cognitive deterioration, typical of aging, or other types
of dementia [78-80]. The analysis of physical and neuropsychological status of the studied subjects
permitted the early identification of AD patients [81]. AD is a degenerative and progressive disease,
which shows aggravated manifestations in the cognitive area such as forgetting the names of relatives
and close people, dismissing past or late life events or important dates and inability to recognize faces.
Subjects in a more advanced stage of the disease can lose themselves in familiar places, loss the
interest in hobbies and daily household activities, fail at reasoning and solving problems of daily life
[77]. In the behavioral area, AD patients show symptoms in their behavior and personality, such as
sudden changes in mood, demotivation, apathy, compulsive or obsessive behavior and physical or
verbal aggressiveness towards other people. These patients may suffer from hallucinations, anxiety,
phobia, depression, and desire of crying without reason [82]. However, many of these behavioral
indicators are explored in different neuropsychological tests, we decided to include them in a separate
set of questions in the survey, in order for clearly determining the stage of the disease (moderate or
severe) of the studied subjects.

Physical condition of subjects diagnosed with advanced AD revealed changes related to the neuronal
deterioration, such as the loss of visuospatial capacity expressed by the inability of finding objects in
sight, despite having good visual acuity and difficulty for operating simple utensils or dressing [83].

Language function alterations, as the difficulty of finding suitable words, while speaking or forgetting
words, are worsened to the extent of expressing merely few words, grunting, or even no speaking at
all [84]. At more severe stages of the disease, patients are unable to control their sphincters or execute
any psychomotor activity, leading consequently to the death of the patient [73]. Causes of death may
vary depending on the level of cognitive decline of the patient and the concomitant diseases they suffer
from [85].

Consulted studies stated that diseases like bipolar impairment, depression and schizophrenia, showing
similar symptoms, might be considered as risk factors for AD or as concomitant diseases during AD
progress.[86-88] A comprehensive review done by Dutie et al. (2011) [66] found a large number of
non- psychiatric comorbidities with AD: vascular diseases (vascular dementias included),
hypertension, diabetes mellitus, obesity, hypothyroidism and/or hyperthyroidism, sleep apnea,
osteoporosis, and glaucoma. The same review outlined a less association among AD, cancer and
rheumatoid arthritis [56].

For the design of the current epidemiological study, the neuropsychological tests and scales were
selected according to the well-defined criteria that they should be previously validated in some of the
Spanish-speaking countries or previously used in Cuba, to diagnose AD, within the National
Healthcare System. Moreover, the selected tests should be of easy and quick application, yet they
should allow collecting a wide spectrum of the cognition area of subjects.
Climent and Molinero (2017) [89] designed a similar study to the current project in which they performed the MMSE, CDT, and SPMSQ tests to trace symptoms of early cognitive decline in the population and analyze the associated risk factors. Authors included a questionnaire that explores demographic, anthropometric data, educational level, occupation, lifestyles such as smoking, alcohol consumption, weekly physical exercises, hobbies, hours of daily television, reading, hours of sleep, and chronic pathologies such as diabetes, hypertension, hyperglycemia, and depression.

**Limitations of the study**

The study does not include population-aged $\leq 60$, because the prevalence of the disease, prior to that time is mostly attributed to familial Alzheimer's [90-92]. Several epidemiological studies have demonstrated that the prevalence of familial AD frequently ranges from 2 and 10 % of the total prevalence of the disease [90, 92]. Commonly the studies performed worldwide, used as inclusion criteria, older adults aged $\geq 60$, and majorly of 65 years old [93, 94].

Another limitation of the study is the fact of not adding biochemical and images markers. It is known the concession, among researchers, on the relation of blood draw and AD, at population level [95]. A considerable group of analysis correlates generally the amyloid beta levels and the hyperphosphorylated tau protein in the cerebrospinal fluid. Therefore, it will be necessary to execute highly invasive procedures [96, 97] that do not justify the population epidemiological study, and do not foresee a therapeutic intervention. On the other hand, it is not feasible to perform, in large rural population, advanced images studies like Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PEC), which are the adequate test for determining changes in the structure and volume of the different regions of the brain, related to the disease [98, 99].

Lastly, we emphasize the design of an epidemiological cross-sectional survey for identifying the possible association of risk and protective factors with AD, in different groups of the Cuban elderly population.
The designed study will be applied to more than 1000 inhabitants of both genders residing in rural and urban areas. Sample selection was based on their different geographic characteristics, diversity of lifestyle and eating habits. The survey comprises demographic, social, and economic factors, health condition and familial health backgrounds, neuropsychological status, sexual activity, and physical conditions. The project includes also the most commonly used neuropsychological tests and scales, all adapted for portable devices.

As the present investigation is not conclusive, it will be noteworthy to accomplish further studies on the Cuban population.

ETHICS AND DISSEMINATION

This research followed the ethical codes of the Declaration of Helsinki. The Scientific Research Council from the Centro Nacional de Biopreparados, as Promoting Research Institution approved the protocol (No. 2.12.12.17), and also the Health Authorities and Ethic Committees of the Health Authority (No.12.12.17 and No.1.12.17). The correspondent written informed consents were also incorporated. The results will be published in scientific papers and will be shared with the Health Authorities of each municipality.

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Table 1. Sample size by locations

| Location                  | Urban area | Rural area | Total |
|---------------------------|------------|------------|-------|
|                           | Men        | Women      |       |
|                           |            |            |       |
| Baracoa                   | 101        | 106        | 374   |
| Bejucal                   | 132        | 131        | 356   |
| Santa Cruz del Norte      | 121        | 130        | 365   |
|                           | 354        | 367        | 1095  |

| Men | Women | Men | Women | Total |
|-----|-------|-----|-------|-------|
| 88  | 79    | 50  | 43    | 374   |
| 61  | 53    | 55  | 52    | 356   |
| 199 | 175   |     |       | 1095  |
Table 2. Health condition and Familial pathological history

| Psychiatric and neurological diseases | Vascular diseases | Immunological diseases | Genetic diseases | Metabolic diseases | Others diseases and conditions |
|-------------------------------------|------------------|------------------------|------------------|-------------------|-------------------------------|
| Alzheimer Disease**                | Ischemic heart disease** | Asthma**          | Down’s Syndrome * | Type I diabetes** | Severe respiratory insufficiency** |
| Parkinson*                         | Hypertension**    | Arthritis**          | Autism*          | Type II diabetes** | Cancer **                     |
| Schizophrenia*                     | Cardiac arrhythmia** |                        |                  | Avitaminosis       | AIDS**                        |
| ICTUS**                            |                  |                        |                  |                   | (Vit A, B, C, …)**            |
| Dementia**                         | Hyperlipidemia*   |                        |                  | Hypercholesterolemia* | Gingivitis**                 |
| Chronic depressive status**        |                  |                        |                  | Hyperthyroidism*   | Rosacea**                     |
| Mental retardation*                |                  |                        |                  | Hypothyroidism*    | Glaucoma**                    |
| Psychosis*                         |                  |                        |                  | Osteoporosis*      | Alcoholism**                  |
| Aphasia*                           |                  |                        |                  |                   | Smoking**                     |
| Frequent headaches**               |                  |                        |                  |                   | Obesity**                     |
| Cranial trauma**                   |                  |                        |                  |                   | General anesthesia***        |
| Hydrocephalus**                    |                  |                        |                  |                   | A severe blow to the head*** |
| Bipolar disorder*                  |                  |                        |                  |                   | Headache***                   |

*- familial pathological history questions; **- questions concerning both, the subject and familial pathological history, *** questions concerning only the subject
| Cognitive or behavioral symptoms | Impairment of the ability to acquire and remember new information | Deterioration of reasoning and management of complex tasks, impoverished judgment | Impairment of visuospatial capabilities | Impairment of language functions | Changes in personality, conduct or behavior | Neuropsychological status |
|----------------------------------|---------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------|---------------------------------|--------------------------------------|-------------------------|
| Deterioration in the functional capacity at work | Decrease in the interest of executing hobbies and daily activities at home | Subject’s perception of risks, without realizing a possible danger | Difficulty to find visible objects, even having a good visual acuity | Dysfunction of the language tasks. Difficulty in finding the right words while speaking or forgetting words | Sudden mood swings | Sadness |
| Deterioration in the functional capacity to perform usual activities | Asking same questions or repetitive conversations | Inability to manage finances or confusion to get accounts | Difficulty in operating with simple utensils | Errors in speech, in spelling words or in writing | Feeling unmotivated or apathetic to do things that once caused pleasure | Nervous tenseness |
| Decrease in levels of functionality and performance, compared to the past behavior of the subject | Mistakenly place personal belongings or forgetting their location | Difficulty in making decisions | Difficulty in getting dressed | Isolation or loss of empathy towards known people | Irritableness |
| | Unable to remembering recent or past events in personal life or daily life | Incapacity to plan complex activities | Compulsive or obsessive behaviors, physical or verbal | Pessimism | |
| Loss in a family place | Hallucinations anxiety, phobia, depression, desire to cry for no reason | Suicidal ideas |
|------------------------|-----------------------------------------------------------------------|---------------|
| Fail to recall prior remembered names | Loss of auto confidence |
| Difficulty to remember a fact or book the subject just read |
| Retention of the name of the investigator |
Figure 1. Location of the three selected municipalities for conducting the epidemiological study in Cuba
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AUTHORS’ CONTRIBUTIONS

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Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: All authors read and approved the final manuscript - Yudisleidy López Ricardo, Mary Carmen Reyes Zamora, Claudio Rodriguez Martínez and Janet Perodin Hernández

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Authors declare no competing interests
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Prevalence of Alzheimer’s Disease in rural and urban areas in Cuba and factors influencing on its occurrence. Epidemiological cross-sectional Protocol.

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Abstract:

Introduction: according to the World Alzheimer’s Report 2019, around 50 million people suffer from dementia, worldwide. Observational analysis revealed the existence of particular factors associated with the onset and progression of Alzheimer’s Disease (AD). There are no international homogenous principles for the early detection and evaluation of memory impairment and possible AD. This work aimed at (i) determining the prevalence of possible AD in the elderly residing in urban and rural regions in Cuba and (ii) identifying the main factors that could significantly influence on its occurrence. Methods and analysis: the study is composed of four neuropsychological tests (Clock Drawing Test, Mini-Mental Status Examination, Short Portable Mental Status Questionnaire, Cognitive and Non-Cognitive Alzheimer’s Disease Assessment Scale) and two scales (Clinical Dementia Rating and Global Deterioration Scale). Moreover, the protocol included a survey with
demographic and socioeconomic information, educational level, occupation, health, and neuropsychological status of subjects, familial pathological history, comorbidities, and lifestyles. The survey will be conducted by licensed physicians from the primary care level in three municipalities. It will comprise a total of 1092 subjects aged ≥ 60, both genders, and from each ethnic group living in rural and urban areas. Primary outcomes: prevalence of possible AD. Secondary outcomes: correlation among risk and protective factors and AD, and comparison of the performance of neuropsychological tests and scales. **Ethics and dissemination:** This research followed the ethical codes of the Declaration of Helsinki. The Scientific Research Council of the Promoting Research Institution and the Ethics Committee of the Health Authorities approved the protocol. The appropriate written informed consent is also incorporated. The results of the survey will be published in scientific papers and will be shared with the Health Authorities of each municipality.

**Strengths and limitations of this study**

- The protocol will include a significant diversity of factors that could influence on the prevalence and severity of AD.
- The study will include the suitable neuropsychological tests and scales to be applied for diagnosing possible AD,
- The protocol will comprise occurrence study in rural and urban areas,
- The study will not incorporate population under 60 years of age, because the prevalence of the disease prior to that oldness, is generally attributed to familial AD.
- The study will not include biochemical markers of the disease.

**Introduction**

Dementia is a syndrome characterized by the progressive decline in memory, executive functions, language, and other areas of cognition. It is usually associated with behavioral symptoms, which interfere with the normal development of the individual in their familial and social environment.
Alzheimer's disease (AD) is the most common of dementia. There is currently no effective treatment to prevent, stop, or reverse the pathological processes of AD.[1]

According to the World Alzheimer's Report 2019, about 50 million people in the world suffer from dementia. This number will practically double every 20 years, reaching 152 million by 2050.[2] The cost of health assistance for treating dementia is estimated in 1 trillion USD worldwide.[2-3]

In Latin America and the Caribbean, approximately 3.4 million people ≥ 60 aged are diagnosed with dementia.[4] This figure will increase up to 7.6 million by 2030.[5] Thus, the estimated prevalence of AD will of the elder population in Latin America.[6] In Cuba life expectancy is of 77 years, both female and male.[7] The prevalence of people suffering from AD is of approximately 160,000 of the elder population.[8] Consequently, elderly is considered the main risk factor for developing the disease and represents one of the main health problems.[9]

Several reports on population-based observational studies revealed the existence of particular factors associated with the onset and progression of AD.[9-12] The accumulation of amyloid beta-peptide (Aβ) plaques and neurofibrillary tau protein tangles in the brain of the patient, are mostly recognized among main triggers of AD.[13-16] Longitudinal studies and research reports show an association of AD with vascular risk, hypertension, hypercholesterolemia, diabetes mellitus (type II), oxidative stress, and genetic background. [9, 17-20] Moreover, lifestyle factors such as active smoking, inadequate nutrition, alcoholism, sedentary life, and low educational level, exposure to toxic elements (aluminum and silicon) are associated with AD.[9, 17-20] Few studies concerning AD risk factors have been performed in Cuba. They included items like hypertension, diabetes mellitus, heart failure, smoking, obesity, advanced age, sexual activity, level of education, and genetic background.[21-24]

Yet, the identification of protective factors against AD threat, such as adequate nutritional habits and healthy lifestyles, might prevent the occurrence of the disease.[25] At present, there are no homogenous criteria on which tests or markers used to establish the prevalence of possible AD. Hence,
there is an imperative need to correctly select a set of likely risk factors for conducting population
prevalence studies. A revision done by Quispe - Ramírez et al. (2020) [26] identified, as risk factors
for neurocognitive disorders, (i) the need of elder adults to be supported for executing regular
activities, and (ii) performing certain social actions involving technology usage. Some commonly
tools used for performing AD neuropsychological diagnosis are: (i) Short Portable Mental Status
Questionnaire (SPMSQ), a 10 question test; which one of its versions allows amending the score
obtained according to the level of schooling of subjects; (ii) Mini-Mental Status Examination (MMSE)
for detecting cognitive impairment, which explores elements such as orientation, fixation memory,
concentration and calculation, the memory of evocation, perception, language, and praxis; and (iii)
Clock Drawing Test (CDT), that permits evaluating cognitive and visuospatial skills.[27] A study
performed by specialists on Psychology and Medicine in Galicia (Spain) reported the MMES as the
mainly functional test, followed by SPMSQ and CDT.[27] Alzheimer’s Disease Assessment Scale
(ADAS) is specially designed for identifying stages of AD, and together with the MMSE are the most
frequently used, worldwide.[27] The former consists of two subscales: cognitive (ADAS-cog) and
behavioral (ADAS-noncog). ADAS-cog includes assessment of memory, language, orientation,
praxis, and visual construction.[28] ADAS-noncog is based on observations or questions about
behavioral symptoms. The scale has been adapted to many languages and countries.[29-35] The most
proficient tools for defining the stage of AD are (i) Clinical Dementia Rating (CDR) [36] scale which
explores fields such as memory, orientation, judgment, and problem-solving and features of social life
and (ii) Global Deterioration Scale (GDS) [37] scale that describes seven global stages of dementia,
according to the degree of cognitive and functional impairment observed.[38]

Method and analysis

Objectives
Primary objective: determining the prevalence of possible AD in elderly residing in urban and rural regions in Cuba.

Secondary objectives:
- to compare the performance of different neuropsychological tests and scales to determine the prevalence of AD in the Cuban population,
- to identify the main factors that could significantly influence on its occurrence.

**Study design**

The study is designed as three-stage protocol. The correspondent physicians will apply the protocol in a single session at patient places. Protocol includes four neuropsychological tests, two scales and a survey: SPMSQ,[39, 40] MMSE,[41, 42] CDT,[43-44] ADAS,[28, 34] CDR Scale [36] and GDS.

In the first stage, the standard MMSE and ADAS tests will permit calculating the prevalence of AD for each area, municipality and local population, once the data from the tests, scales, and survey have been compiled. In the second stage, it will be determined the correlation between SPMSQ and CDT tests, CDR, and GDS scales in respect to MMSE and ADAS for defining whether they are appropriate for the Cuban population. A third stage will implement several statistical tests to analyze the influence of risk and protective factors on the already calculated prevalence of the disease.

**Selection of factors for the survey**

After analyzing the bibliography and published reports, authors agreed on designing a protocol based on a proper selection of neuropsychological tests and scales. It will also comprise the following features: demographic and socioeconomic data, educational level, occupation, health and neuropsychological status, familial pathological history, comorbidity, and lifestyles. In addition, it will include protective factors associated with a minor prevalence of the disease such as sports, physical activity, and hobbies.
Considering the significance of the consumption of particular food as protective factors, nutritional habits will be incorporated in the questionnaire, as well. Questions will include (i) products such as fish, cocoa, coconut and coconut oil, (ii) habitual eating of proteins, fruits, grains, vegetables, and coffee, (iii) quantity (serving size as appropriate) and frequent occurrence (daily, weekly, monthly, and sporadically) within the last 10 years, minimum.

**Study setting**

Pre-intervention stage: training and validation

Physicians taking part in the study, had a one-week training course on the techniques of the neuropsychological tests, the scales, and survey. In the interest of validating the designed protocol, experts from the sponsoring institution participated themselves as respondents. This procedure permitted inquiring into some of the original questions and correct them for a better outcome.

After this initial validation, a small group of subjects was selected to perform a pilot study. The aforesaid study was implemented in rural areas from Baracoa municipality, and the feasibility of the survey was analyzed in details. It was concluded that subjects felt comfortable at answering the questionnaire and the survey could be completed in two hours, maximum.

The pre-intervention and validation phases of the study started in 2018, but the study had to be stopped in 2019 due to COVID-19 epidemic in Cuba. Yet, it will be restarted when the epidemiological situation permits a safe process and contact with elder adults. The closure of the study is planned for December 2022, and data processing should be finished by March 2023.

The study will be conducted at: Baracoa, located at Guantánamo province, Santa Cruz del Norte and Bejucal in Mayabeque province. (Figure 1).

It is generally known that Baracoa town is the main producer of coconut and cocoa in the country. Moreover, the artisanal processing and consumption of those products are part of the nutritional traditions of the region. Baracoa has also an intense fishing activity, thus constitutes an important
economic and dietary source for the population. Additionally, there is no significant evidence of AD prevalence in its inhabitants up to date. Santa Cruz del Norte town is a coastal region where fish consumption is high, but it has a low consumption of coconut and cocoa. Contrasting, the population settled in Bejucal does not have a high consumption of fish, and coconut. The cocoa ingestion is very low or almost null, and there is a manifest incidence of AD in adult population.

**Participant selection**

To study the AD prevalence, the number of subjects of each town will be selected proportionally to the demographic distribution (rural and urban areas) and gender of the population. Demographic data was collected from the last edition of the Cuban National Survey of Population and Housing in 2011.[45-47] The assent criterion for considering the compliance with the planned sample size will be 75% for each location/area/gender.

The sample size was calculated according to Morales-Vallejo (2012) [48] using the formula [1]:

\[ n = \frac{N}{1 + \frac{z^2(N - 1)}{x^2pq}} \]  

[1]

in which:

- \( n \) = simple size,
- \( N \) = known population size,
- \( z \) = confidence level (95%),
- \( pq \) = variance of the population,
- \( e \) = margin of error (5%)

Based on data provided by the participant family doctors, and the inclusion criteria, the calculated sample size will comprise 1095 subjects aged \( \geq 60 \) (Table 1). The study will not include any invasive procedures, therefore, it is expected to have a scope of at least 75%.
Additionally, the estimation of the sample size for determining the influence of the independent variables (age, level of schooling, consumption of certain foods, among others) on the prevalence of AD (dependent variable) will be performed for each of the statistical tests applied (chi-square test, Student t-test, Mann-Whitney test, multiple logistic regression models).

To calculate the fraction of expected subjects to be detected with AD, the closer national registered $P_o$ value of 0.07 was used, (160,000 subjects among 2,386,280 adults over 60 years).[8] For the proportion of subjects whose lifestyles or consumption habits favor the decrease in prevalence ($P_1$) a lower value of 50% in relation to $P_o$ was taken as valid. Values (two-sided alternative hypothesis) of $\alpha=0.05$, of $\beta=0.2$, $Z_{\alpha}=1.96$, $Z_{\beta}=0.84$ were assumed.

The result of the estimation [49] revealed that 350 subjects will be needed for applying the aforementioned statistic tests. This figure is inferior to the estimated one for the prevalence study of the disease for both the municipality town and the total population; hence the sample size calculated for both the prevalence study and all the statistic tests (1095 subjects) will be adopted.

Physicians will execute the recruitment of subjects, in each area.

**Participant selection criteria**

**Inclusion criteria:**

- Adults aged $\geq 60$, both genders
- permanent residency $\geq 10$ years
- voluntariness and written consent

**Exclusion criteria:**

- prolonged length of hospital stay ($\geq 1$ year)
- Schizophrenia, mental retardation, aphasia and psychiatric treatment at psychotic level
- severe visual deficit, hearing impairment and motor disabilities
- severe alcohol and psychotropic drugs dependence
**Patient and Public Involvement statement**

Progress of research questions and outcome measures will be informed to physicians of the selected areas of each municipality and not directly to subjects. Physicians (family doctors) attend the population from those areas and are familiar with patient records (customs, lifestyle, comorbidities, and family situation, among others). Given that patients will not be directly involved in the process of recruitment and management of the study, physicians will individually inform them, of pertinent aspects (objectives and importance of the study, risks, personal and social benefits, relevant dates, place, commitment of not disclosing personal data and conditions of the study). The information will be provided verbally to subjects, relatives, or caregivers at their correspondent home as well as the implementation of tests and surveys.

When the designated subjects accept to participate in the survey, the team of medical researchers will provide the informed consent form. Each subject will be also advised of their willingness to abandon the study when they deem, it, appropriate. For subjects who show severe cognitive impairment or AD, it will be requested to a family member or caregiver to fill out the informed consent.

The research team will communicate the study results to the corresponding physician; which will transmit it to subjects, family members or legally recognized caregivers. If, derived from the results of neuropsychological tests, AD occurrence, sign of memory impairment, or other pathologies not previously declared, occurs; the researchers will be obliged to share such information with the physician so, they may adopt the corresponding medical posture.

Members of the research team, experts, and physicians were involved in the design of the study. As it will not foresee any treatment, no patient intervention will be considered.

**Outcome measures**
Primary outcomes: identification of the presence of cognitive impairment and possible AD in subjects, and prevalence of the disease in rural and urban areas applying the Spanish versions of SPMSQ;[39, 40] MMSE;[41-42] CDT tests;[43-44] ADAS test;[28, 34] CDR Scale [36] and GDS.[37]

For exploring secondary outcomes such as comorbidities a comprehensive list will be added (i) current diseases; (ii) psychiatric, neurological, vascular, immunological, genetic, and metabolic illnesses; (iii) time frame of suffering from those diseases and indicated treatments; (iv) familial pathological history (risking factor for developing AD) (Table 2); and (v) practice, frequency, and intensity of performing physical activity (sports, exercises, or physical labors related to the profession of subjects).

Regarding protective factors, the listing will be enlarged with the inclusion of some leisure time activities of the elderly, which could contribute to stimulating neuronal or brain activity. Risk factors like smoking (quantity, frequency, and time frame), consumption of alcoholic beverages (frequency and quantity) as well as the exposure to toxic elements will be also registered.

A supplementary section of the survey will be dedicated to examine the neuropsychological status of the subjects (Table 3). This section will deal with recognition of signs of deterioration symptoms of certain areas (cognition or behavior), the ability to acquire and remember new information, reasoning and managing complex tasks, impoverished judgment, visuospatial abilities, language functions and personality changes. If subjects will present evident advanced neuropsychological deterioration, their caregivers, or the interviewer, should declare the physical status of the subject.

**The questionnaire**

The questionnaire contained the following items:

- general information of subjects (name, surnames, address, birthdate, ID number),
- demographic data (gender, age, educational level, skin color, marital status, permanent residency),
- social and economic data (quantity of members living in the same house, family functionality, losing or detaching from a relative or a close friend, family relationships, detach from a close relative or a close friend, past and current employment status, income level),

- health condition: current illness (age of onset and treatment), familial pathological history (Table 2),

- nutritional habits (past and current consumption, frequency, and quantity). It includes ingesting of coconut, cacao, fish, selfish and derived products, animal and plant proteins, vegetables, fruits, and water quality used for drinking and cooking,

- physical activities (type, frequency, and intensity),

- other risk factors: smoking and alcohol, exposure to toxic elements (past and current consumption, frequency, and quantity),

- sleeping (hours, quantity, quality, and disorders),

- neuropsychological status (Table 3),

- sexual activity (frequency, and orgasms),

- surveillance of the interviewer and/or responses of the caregivers, regarding the physical status of the subject with severe symptoms of the AD; expressed in the inability to pronounce words, speaking but just growls, urinary incontinence, caregivers’ dependence to routine actions, the inability to perform psychomotor activities, delusive behavior and loss of will,

- compensatory activities: hobbies, reading, writing, dance, physical exercises, sports, crafts, studio/workshops, playing musical instruments, participation in activities as a volunteer, films/documentaries/series/novels, board games, membership in cultural associations, and travel.

Subjects will be considered to be diagnosed with AD if documented clinical evidence of the AD exist or if the subject is classified as candidate to suffering the disease. This clinical certificate should be issued by an authorized health institution.
Additionally, a portable mobile application named BioMer.apk was designed, in collaboration with the Free Software Development Center (CESOL) of the University of Computer Sciences (UCI) in Havana City. The application included general information, results of the survey, neuropsychological tests and scales outcomes. It allows recording images of drawings and texts from different neuropsychological tests. All recorded information could be the exported to an Excel worksheet for further statistical analysis.

**Data analysis plan**

Descriptive statistics based on means, standard deviations, percentages, and 95% confidence intervals will be used for describing the studied population. A logistic regression model will be employed to analyze the association between AD and studied parameters. Chi-square, Student t-test, or Mann-Whitney test will be performed for selecting parameters related to the outcome measures (p≤0.05).

Several other statistical methods will be also applied: multiple logistic regression models, selecting variables for inclusion in the model based on the backward elimination procedure; exploratory factor analysis and multiple component analysis, which models enable to explain correlations between the independent variables and prevalence of AD, as dependent variables. Linear and nonlinear regression analysis, as well as the ROC curves, will be used for comparing different neuropsychological tests and scales.

**Strengths of this study**

In Cuba, prevalence studies on Dementia have revealed that chronic diseases like hypertension and diabetes mellitus are strongly related to AD. Smoking and alcoholic consumption, age, gender and educational level are factors that should be also considered.[21, 50, 51] Studies, performed worldwide,[52-54] corroborated this fact and even demonstrated the association of insomnia.[55]
expositions to different types of anesthesia, skin color, nutritional habits and ethnic groups with the prevalence of AD.

Based on previous studies conducted in different countries, the present study will analyze the effect on AD, of ingesting certain foods. This exploration will comprise the use of coconut oil, a source of medium-chain fatty acids; cocoa, a source of powerful antioxidants; fish and shellfish, rich in vitamins and omega-3 fatty acids; coffee, and its caffeine content.

A sedentary lifestyle also represents a threat to developing dementia (AD included). Moon et al. (2016) suggested an association between the practice of physical exercise and the hippocampal-dependent memory function. Some other studies relate aerobic exercise with the increase of brain volume or the improvement of mild cognitive impairment in elder adults. Our criteria are that none of all reported results has been able to demonstrate remarkable differences between test groups and controls, thus far.

The efficacy and effectiveness of compensatory activities are demonstrated by their characteristic of delaying the progression of dysfunction in patients with dementia. In the current survey, the inclusion of compensatory cognitive, social, and leisure activities will intend to measure cognitive stimulation as non-pharmacological therapies for subjects in advanced stages of AD. Survey will contain relevant elements on the assessment of the onset or progress of AD disease, as it is reported in scientific literature.

Though behavioural indicators will be explored in different neuropsychological tests; authors decided to include them in a separate set of questions to determine the stage of the disease (moderate or severe) in subjects. Behavioural features will comprise unexpected changes of mood, demotivation, apathy, compulsive or obsessive conduct, physical or verbal aggressiveness towards other people, hallucinations, anxiety, phobia, depression, and crying without reason. Symptoms, like loss of visuospatial capacity, difficulty in operating utensils or dressing, language alterations,
inability to control sphincters, or executing any psychomotor activity [73] will be also considered for exploring signs of advanced stage of the AD. Cognitive decline and concomitant disease in patients may lead to different causes of death.[82] The occurrence of psychiatric and non-psychiatric diseases like bipolar impairment, depression, [83-85] vascular diseases, hypertension, diabetes mellitus, obesity, hypothyroidism and hyperthyroidism, sleep apnoea, osteoporosis, and glaucoma, cancer, and rheumatoid arthritis [66] will be registered.

Questionnaire will explore demographics, anthropometric data, educational level, occupation, lifestyles (smoking and alcohol consumption, physical exercises, daily routine of individuals) and chronic pathologies (diabetes, hypertension, hyperglycemia, and depression). MMSE, CDT, and SPMSQ tests were used in a similar study performed by Climent and Molinero (2017)[86] for tracing symptoms of early cognitive decline and analyzing the associated risk factors.

For selecting the neuropsychological tests and scales to be used in the protocol, authors meet the criteria that those should be previously validated in some of the Spanish-Speaking countries or used in Cuba beforehand, for diagnosing AD within the National Healthcare System. Designated tests will be of easy and quick application, and will allow collecting a wide spectrum of the cognition area of the subjects.

Limitations of the study

The study will not include population aged \( \leq 60 \), since the prevalence of the disease prior to that age, is regularly attributed to familial Alzheimer's.[87-89] Several epidemiological studies have demonstrated that the prevalence of familial AD frequently ranges from 2 to 10 % of the total prevalence of the disease.[87, 89] Globally, studies commonly used as inclusion criteria, elder adults aged \( \geq 60 \), and majorly of 65 years old.[90, 91] Biochemical and image markers will not be considered. It is known the position among researchers, concerning blood draw and AD, at population level.[92] A considerable group of analyses generally
correlates the amyloid-beta levels and the hyperphosphorylated tau protein in the cerebrospinal fluid. Therefore, it will be necessary to execute highly invasive procedures [93, 94] which will not justify the population epidemiological study and will not foresee therapeutic interventions. Alternatively, it will not be feasible to perform, in a large rural population, advanced image studies like Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PEC), which are the adequate test for determining changes in the structure and volume of different regions of the brain, related to the disease.[95, 96]

Authors highlight the design of an epidemiological cross-sectional survey to identify the possible association of risk and protective factors with AD in different groups of the Cuban elderly population. Since this analysis will be no conclusive, it would be noteworthy to accomplish further studies on Cuban population.

**Ethics and dissemination**

This research followed the ethical codes of the Declaration of Helsinki. The Scientific Research Council from Centro Nacional de Biopreparados, as Promoting Research Institution, approved the protocol (No. 2.12.12.17). Health Authorities and Ethic Committees of the Health Authority provided also their consent (No.12.12.17 and No.1.12.17). Correspondent written informed consent will be also incorporated.

The results will be published in scientific papers and will also be shared with the Health Authorities of each municipality. Additionally, outcomes will be accessible via presentations in domestic and international scientific meetings. Authors will create guidance documents with detailed instructions for the appropriate selection of tests and scales for further studies.

**Ethics statements**

Patient consent for publication:
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Claudio Rodríguez Martínez: Project leader. Design of the project, critical revision of the manuscript, approval of the final version.
Mary Carmen Reyes Zamora: Critical revision of the Protocol and the manuscript.

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Table 1. Sample size by locations

| Location               | Urban area | Rural area | Total |
|------------------------|------------|------------|-------|
|                        | Men        | Women      | Men   | Women |
| Baracoa                | 101        | 106        | 88    | 79    | 374   |
| Bejucal                | 132        | 131        | 50    | 43    | 356   |
| Santa Cruz del Norte   | 121        | 130        | 61    | 53    | 365   |
| **Total**              | **354**    | **367**    | **199** | **175** | **1095** |
Table 2. Health condition and Familial pathological history.

| Psychiatric and neurological diseases | Vascular diseases       | Immunological diseases | Genetic diseases | Metabolic diseases | Others diseases and conditions |
|---------------------------------------|-------------------------|------------------------|-----------------|-------------------|-----------------------------|
| Alzheimer Disease**                  | Ischemic heart disease**| Asthma**               | Down’s Syndrome *| Type I diabetes**  | Severe respiratory insufficiency** |
| Parkinson*                           | Hypertension**          | Arthritis**            | Autism*         | Type II diabetes** | Cancer**                    |
| Schizophrenia*                       | Cardiac arrhythmia**    |                        |                 |                   | Avitaminosis (Vitamins A, B,….)** |
| ICTUS**                              |                         |                        |                 | Hyperlipidemia*   | Crohn's disease**           |
| Dementia**                           |                         |                        |                 | Hypercholesterolemia* | Gingivitis**                |
| Chronic depressive status**          |                         |                        |                 | Hyperthyroidism*  | Rosacea**                   |
| Mental retardation*                  |                         |                        |                 | Hypothyroidism*   | Glaucma**                   |
| Psychosis*                           |                         |                        |                 | Osteoporosis*     | Alcoholism**                |
| Aphasia*                             |                         |                        |                 |                   | Smoking**                   |
| Frequent headaches**                 |                         |                        |                 |                   | Obesity**                   |
| Cranial trauma**                     |                         |                        |                 |                   | General anesthesia***       |
| Hydrocephalus**                      |                         |                        |                 |                   | A severe blow to the head***|
| Bipolar disorder*                    |                         |                        |                 |                   | Headache***                 |

*- familial pathological history questions; **- questions concerning both, the subject and familial pathological history, *** questions concerning only the subject.
Table 3. Neuropsychological status of the subject.

| Cognitive or behavioral symptoms | Impairment of the ability to acquire and remember new information | Deterioration of reasoning and management of complex tasks, impoverished judgment | Impairment of visuospatial capabilities | Impairment of language functions | Changes in personality, conduct, or behavior | Neuropsychological status |
|---------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------|----------------------------------|------------------------------------------|---------------------------|
| Deterioration in the functional capacity at work | Decrease in the interest of executing hobbies and daily activities at home | Subject’s perception of risks, without realizing a possible danger | Difficulty to find visible objects, even having a good visual acuity | Dysfunction of the language tasks. Difficulty in finding the right words while speaking or forgetting words | Sudden mood swings | Sadness |
| Deterioration in the functional capacity to perform usual activities | Asking same questions or repetitive conversations | Inability to manage finances or confusion to get accounts | Difficulty in operating with simple utensils | Errors in speech, in spelling words or in writing | Feeling unmotivated or apathetic to do things that once caused pleasure | Nervous tenseness |
| Decrease in levels of functionality and performance, compared to the past behavior of the subject | Mistakenly place personal belongings or forgetting their location | Difficulty in making decisions | Difficulty in getting dressed | Isolation or loss of empathy towards known people | Irritableness |
| Unable to remembering recent or past events in personal life or daily life | Incapacity to plan complex activities | Compulsive or obsessive behaviors, physical or verbal | | | | Pessimism |
| Loss in a family place | Hallucinations | Suicidal ideas |
|------------------------|---------------|---------------|
|                        | anxiety, phobia, depression, desire to cry for no reason |               |

| Fail to recall prior remembered names | Loss of auto confidence |
|--------------------------------------|-------------------------|
| Difficulty to remember a fact or book, the subject just read | |
| Retention of the name of the investigator | |
Figure 1. Location of the three selected municipalities for conducting the epidemiological study in Cuba
Prevalence of Alzheimer’s Disease in rural and urban areas in Cuba and factors influencing on its occurrence. Epidemiological cross-sectional Protocol.

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Prevalence of Alzheimer’s Disease in rural and urban areas in Cuba and factors influencing on its occurrence. Epidemiological cross-sectional Protocol.

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Abstract:

Introduction: According to the World Alzheimer's Report 2019, around 50 million people suffer from dementia, worldwide. Observational analysis revealed the existence of particular factors associated with the onset and progression of Alzheimer's Disease (AD). There are no international homogenous principles for the early detection and evaluation of memory impairment and possible AD. This work aimed at (i) determining the prevalence of possible AD in the elderly residing in urban and rural regions in Cuba and (ii) identifying the main factors that could significantly influence on its occurrence. Methods and analysis: The study includes four neuropsychological tests (Clock Drawing Test, Mini-Mental Status Examination, Short Portable Mental Status Questionnaire, Cognitive and Non-Cognitive Alzheimer’s Disease Assessment Scale) and two scales (Clinical Dementia Rating and Global Deterioration Scale). Moreover, the protocol includes a survey with demographic and
socioeconomic information, educational level, occupation, health, neuropsychological status of
subjects, familial pathological history, comorbidities, and lifestyles. The study will comprise a total
of 1092 subjects aged ≥ 60, of both genders, and from every ethnic group settled in rural and urban
areas. Primary outcomes: prevalence of possible AD. Secondary outcomes: correlation among risk
and protective factors and AD, and comparison of the performance of neuropsychological tests and
scales. **Ethics and dissemination:** This research met the ethical codes of the Declaration of Helsinki.
The Scientific Research Council of the Promoting Research Institute and the Ethics Committee of the
Health Authorities approved the protocol. The proper written informed consent is also incorporated.
The results of the survey will be published in scientific papers and shared with the Health Authorities
of each municipality.

**Strengths and limitations of this study**

- The protocol will include a significant diversity of factors that could influence on the prevalence
  and severity of AD,
- The study will include the suitable neuropsychological tests and scales for diagnosing possible
  AD,
- The protocol will comprise occurrence study in rural and urban areas,
- The study will not incorporate population under 60 years old; generally, the earlier prevalence of
  AD is associated with familial AD,
- The study will not include biochemical markers of the disease.

**Introduction**

Dementia is a syndrome characterized by the progressive decline in memory, executive functions,
language, and other areas of cognition. It is usually associated with behavioral symptoms, which
interfere with the normal development of the individual at familial and social environment.
Alzheimer's disease (AD) is the most common of dementia. There is currently no effective treatment to prevent, stop, or reverse the pathological processes of AD. [1]

According to the World Alzheimer's Report 2019, about 50 million people in the world suffer from dementia. This number will practically double every 20 years, reaching 152 million by 2050. [2] The cost of health assistance for treating dementia is estimated in 1 trillion USD worldwide. [2-3]

In Latin America and the Caribbean, approximately 3.4 million people ≥ 60 aged are diagnosed with dementia. [4] This figure will increase to 7.6 million by 2030. [5] Thus, the estimated prevalence of AD will be around 11 percent of the older population in Latin America. [6] In Cuba, life expectancy is of 77 years, both female and male. [7] The number of people suffering from AD is approximately 160,000. [8] Consequently, elderly is considered a key risk factor for developing the disease and represents one of the major health problems. [9]

Several reports on population-based observational studies revealed the existence of particular factors associated with the onset and progression of AD. [9-12] The accumulation of amyloid beta-peptide (Aβ) plaques and neurofibrillary tau tangles in the brain of patients has often been shown to be a trigger for AD. [13-16] Longitudinal studies and research reports show an association of AD with vascular risk, hypertension, hypercholesterolemia, diabetes mellitus (type II), oxidative stress, and genetic background. [9, 17-20] Moreover, lifestyle factors such as active smoking, inadequate nutrition, alcoholism, sedentary life, low educational level, and exposure to toxic elements (aluminum and silicon) are associated with AD. [9, 17-20] In Cuba few studies have been performed concerning AD risk factors. Those studies have included variables such as hypertension, diabetes mellitus, heart failure, smoking, obesity, advanced age, sexual activity, level of education, and genetic background. [21-24] Yet, the identification of protective factors (adequate nutritional habits and healthy lifestyles) to elude AD threat might prevent the occurrence of the disease. [25] There are no homogenous criteria establishing proper tests or markers for prevalence of possible AD, thus far. Hence, there is an
imperative need to select a set of possible risk factors for conducting population prevalence studies.

A revision done by Quispe - Ramírez et al. (2020) [26] identified, as risk factors for neurocognitive disorders, the necessity of elder adults of being supported to fulfill regular activities, and certain social actions involving technology.

Commonly tools for performing AD neuropsychological diagnosis are: (i) Short Portable Mental Status Questionnaire (SPMSQ), a 10 question test version which allows amending the score obtained according to the level of schooling of subjects; (ii) Mini-Mental Status Examination (MMSE) for detecting cognitive impairment, and exploring elements such as orientation, fixation memory, concentration and calculation, memory of evocation, perception, language, and praxis; and (iii) Clock Drawing Test (CDT), that permits evaluating cognitive and visuospatial skills. [27] A study done by specialists on Psychology and Medicine in Galicia (Spain) reported the MMES as the mainly functional test, followed by SPMSQ and CDT. [27] Alzheimer’s Disease Assessment Scale (ADAS) is specially designed to identify stages of AD, and combined with the MMSE are frequently used, worldwide. [27] ADAS consists of two subscales: cognitive (ADAS-cog) and behavioral (ADAS-noncog). ADAS-cog includes assessment of memory, language, orientation, praxis, and visual construction. [28] ADAS-noncog is based on observations or questions about behavioral symptoms. ADAS has been adapted to many languages and countries. [29-35] The mainly proficient tools for defining the stage of AD are (i) Clinical Dementia Rating (CDR) scale [36] which explores memory, orientation, judgment, problem-solving and aspects of social life; and (ii) Global Deterioration Scale (GDS) scale [37] that describes seven global stages of dementia, according to the degree of cognitive and functional impairment observed. [38]
Method and analysis

Objectives

Primary objective: to determine the prevalence of possible AD in elderly from urban and rural regions of Cuba.

Secondary objectives:

- To compare the performance of different neuropsychological tests and scales to establish the prevalence of AD in the Cuban population,
- To identify the main factors that could significantly influence on its occurrence.

Study design

The study is designed as three-stage protocol. The correspondent physicians will apply the protocol in a single session at subject’s places. Protocol includes four neuropsychological tests, two scales and a survey: SPMSQ; [39, 40] MMSE; [41, 42] CDT; [43-44] ADAS; [28, 34] CDR Scale [36] and GDS.

In the first stage, the standard MMSE and ADAS will permit to calculate the prevalence of AD in each area, municipality and local population. In the second stage, it will be determined the correlation between SPMSQ and CDT tests, CDR, and GDS scales in respect to MMSE and ADAS to define whether they are or not appropriate for the Cuban population. A third stage will include several statistical tests for analyzing the influence of risk and protective factors on the already calculated prevalence of the disease.

Selection of factors for the survey

After analyzing the bibliography and published reports, authors agreed on designing a protocol based on a proper selection of neuropsychological tests and scales. Thus, it will also comprise the following variables:
Demographic and socioeconomic data (gender, age, educational level, occupation, skin color, marital status, permanent residency, number of members living in the same house, family functionality, losing or detaching from a relative or a close friend, family relationships, past and current employment status, income level),

- Health (Table 1) and neuropsychological status (Table 2), sexual activity (frequency, and orgasms), sleeping (hours, quantity, quality, and disorders),

- Pathology of familial history,

- Lifestyles, sports, physical activity (type, frequency, and intensity), and compensatory activities (hobbies, reading, writing, dance, crafts, studio/workshops, playing musical instruments, participation in activities as a volunteer, habit of watching films/documentaries/series/novels, board games, membership in cultural associations, and travel).

Considering the impact of the consumption of specific food as protective factors, nutritional habits will be incorporated in the questionnaire, as well. Questions will include the frequency (daily, weekly, monthly, and sporadically) and quantity of the different types of food (fish, cocoa, coconut, coconut oil, habitual eating of proteins, fruits, grains, vegetables, and coffee) eaten in last 10 years.

Personal data will be collected (general information of subjects: name, surnames, address, date of birth, ID number).

**Study setting**

The present protocol corresponds to a cross sectional study including qualitative and quantitative variables.

The study will be conducted at Baracoa, located in Guantánamo province, Santa Cruz del Norte and Bejucal in Mayabeque province. (Figure 1)
Baracoa is the main producer of coconut and cocoa in the country. The artisanal processing and consumption of those crops are part of the nutritional traditions of that region. Baracoa has also an intense fishing activity; therefore, it constitutes a solvent and dietary source for the population. Additionally, there is no significant evidence of AD prevalence in its inhabitants, thus far. Santa Cruz del Norte is a coastal region in which fish consumption is high; however, consume of coconut and cocoa is low. In contrast, the population settled in Bejucal does not have a high consumption of fish and coconut; cocoa ingestion is very low or almost null and presents a palpable incidence of AD in adult population.

**Study population and sample size determination**

To study the AD prevalence, the selected number of subjects will be proportionally to the demographic distribution (rural and urban areas) and gender of the population. Demographic data was collected from the last edition of the Cuban National Survey of Population and Housing in 2011. [45-47] The assent criterion for considering the compliance with the planned sample size will be 75 % for each location/area/gender.

The sample size was calculated according to Morales-Vallejo (2012) [48]:

\[
n = \frac{N}{1 + \frac{z^2(N - 1)}{2\epsilon^2pq}}\]

in which:

- \(n\) = simple size,
- \(N\) = known population size,
- \(z\) = confidence level (95 %),
- \(pq\) = variance of the population,
- \(\epsilon\) = margin of error (5 %)
Based on data provided by the participant family doctors, and the inclusion criteria, the calculated sample size will comprise 1095 subjects aged \( \geq 60 \) (Table 3).

To calculate the fraction of expected subjects to be detected with AD, it was used the closer national registered \( P_o \) value of 0.07 (160,000 subjects among 2,386,280 adults over 60 years). [8] For the proportion of subjects whose lifestyles or consumption habits favor the decrease in prevalence \( (P_1) \) a lower value of 50\% in relation to \( P_o \) was taken as valid. Values (two-sided alternative hypothesis) of \( \alpha=0.05, \beta=0.2, Z_\alpha=1.96, Z_\beta=0.84 \) were assumed.

The result of the estimation revealed that 350 subjects would be required to apply the aforementioned statistic tests. This figure is lower than the estimated for the prevalence study; for this reason, the calculated sample size of 1095 subjects will be used for both the prevalence study and the statistic correlation tests.

Physicians will execute the recruitment of subjects, in each area.

**Participant selection criteria**

**Inclusion criteria:**
- Adults aged \( \geq 60 \), both genders
- Permanent residency \( \geq 10 \) years
- Voluntariness and written consent

**Exclusion criteria:**
- Prolonged length of hospital stay \( \geq 1 \) year
- Schizophrenia, mental retardation, aphasia and psychiatric treatment at psychotic level
- Severe visual deficit, hearing impairment and motor disabilities
- Severe alcohol and psychotropic drugs dependence
Patient recruitment and communication

Specialists from the research team will inform physicians (family doctors) and not subjects directly, about the progress of research questions and outcome measures.

Medical doctors (family doctors) look at every resident in the context of family and community and keep their medical records (customs, lifestyle, comorbidities, and family situation) to mention few. Neither patients nor healthy subjects will be directly involved in the process of recruitment and management of the study. Clinical research team will notify subjects of the pertinent aspects (objectives and importance of the study, risks, personal and social benefits, relevant dates, place, commitment of not disclosing personal data and conditions of the study). Likewise, information regarding implementation of tests and surveys will be given to subjects, relatives, or caregivers.

Once the selected subjects accept to participate in the survey, the clinical research team will provide them with the informed consent form. Subjects will be advised of their willingness to abandon the study whenever they consider it, appropriate. If any of the subjects show a severe cognitive impairment or AD, a family member or caregiver will complete the informed consent.

If, derived from the results of neuropsychological tests, AD occurrence, sign of memory impairment, or other pathologies not previously declared, occurs; clinical researcher team will be obliged to share that information with physicians (family doctors); hence, they might follow the corresponding health care protocol established in the country.

Patient and Public Involvement statement

Members of the research team, experts, and physicians participated in the scheme of the study. No patient intervention is included in the design since the study will not expect any treatment. For specific questions regarding sexual relation queries, older adults of both genders were previously asked about
their willingness or discomfort in answering these types of questions. Once their criteria were analyzed, the designing team decided to include the corresponding additional consent. Selection and insertion of subjects will be done at the advanced execution phase of the study. The clinical research team will communicate the results of the study to the corresponding physician (family doctors) and they will transmit the information to subjects, family members or legally recognized caregivers.

**Outcome measures**

Primary outcomes: Identification of the presence of cognitive impairment and possible AD in subjects, and prevalence of the disease in rural and urban areas applying the Spanish versions of SPMSQ; [39, 40] MMSE; [41-42] CDT tests; [43-44] ADAS test; [28, 34] CDR Scale [36] and GDS. [37] Subjects will be diagnosed with AD if clinical documented evidence of the AD exists or if the subject is classified as candidate for suffering the disease. The clinical certificate should be issued by an authorized health institute. For exploring secondary outcomes such as comorbidities an enlarge list will be added (i) current diseases; (ii) psychiatric, neurological, vascular, immunological, genetic, and metabolic illnesses; (iii) time frame of suffering from those diseases and indicated treatments; (iv) pathology of familial history (risking factor for developing AD) (Table 2); and (v) practice, frequency, and intensity of performing physical activity (sports, exercises, or physical labors related to the profession of subjects). Regarding protective factors, the listing will be broaden with the inclusion of some leisure time activities of the elderly, which could contribute to stimulating neuronal or brain activity. Risk factors like smoking (quantity, frequency, and time frame), consumption of alcoholic beverages (frequency and quantity) as well as the exposure to toxic elements will be also registered. A supplementary section of the survey will be dedicated to examine the neuropsychological status of the subjects (Table 2). This section will deal with recognition of signs of deterioration symptoms of
certain areas (cognition or behavior), the ability to acquire and remember new information, reasoning
and managing complex tasks, impoverished judgment, visuospatial abilities, language functions and
personality changes. If subjects present evident advanced neuropsychological deterioration, their
caregivers, or the interviewer, should declare the physical status of the subject.

**Data management and analysis plan**

Descriptive statistics based on means, standard deviations, percentages, and 95% confidence intervals
will be used for describing the studied population. To determine the influence of the independent
variables (age, level of schooling, and consumption of certain foods, among others) on the prevalence
of AD (dependent variable) different statistical test will be performed (chi-square test, Student t-test,
Mann-Whitney test, multiple logistic regression models based on the backward elimination
procedure; exploratory factor analysis and multiple component analysis).

Linear and nonlinear regression analysis, as well as the ROC curves, will be used for comparing
different neuropsychological tests and scales.

A portable mobile application named BioMer.apk was designed in collaboration with the Free
Software Development Center (CESOL) of the University of Computer Sciences (UCI) in Havana
City. The application included general information, results of the survey, neuropsychological tests and
scales outcomes. BioMer.apk allows recording images of drawings and texts from different
neuropsychological tests. Every recorded data could be exported to an Excel worksheet for further
statistical analysis.

**Status of the Study**

Physicians participating in the study did a one-week training course on the techniques of the
neuropsychological tests, the scales, and survey. In the interest of validating the designed protocol,
experts from the sponsoring institute, participated themselves as respondents. The procedure inquired into original questions and permitted to correct them for an improved outcome.

After this initial validation, a small group of subjects from rural areas in Baracoa, was selected to perform a pilot study. It was concluded that subjects felt comfortable at answering the questionnaire and that the survey could be completed in two hours, maximum.

The pre-intervention and validation phases of the study started in 2018; however, the study stopped in 2019 due to COVID-19 epidemic in Cuba. Yet, it will restart when the epidemiological situation permits a safe process and contact with older adults. The closure of the study is planned by December 2022, and data processing should be finished by March 2023.

**Strengths of this study**

The study includes a large number of variables (190) partly reported by different authors, as factors that influence the onset or progression of Alzheimer's disease [49-60]. Amongst them, aspects such as psycho-affective relationship and family environment, sexuality [61], sleep disorders [62], quality of drinking water [63] highlighted among the least studied at a population scale.

To select the neuropsychological tests and scales for the protocol, the authors employed tests and scales previously validated in some Spanish-speaking countries or used in Cuba to diagnose AD in the national healthcare system. Selected tests will be of easy and quick application, and will allow collecting a wide spectrum of the cognition area of the subjects.

Comparisons between different tests and scales will allow selection of correct tests and scales for the studied population and recommend them for future population studies.

Protocol will comprise occurrence study in rural and urban areas. In Cuba, AD prevalence in rural areas has not been particularly studied and rarely analyzed, worldwide.
Limitations of the study

The study will not include population aged \( \leq 60 \), since the earlier prevalence of the disease, is often associated with familial Alzheimer's. [64-66] Several epidemiological studies have shown that the prevalence of familial AD is typically between 2 to 10 % of the overall prevalence of the disease. [64-66] Globally, studies usually used as inclusion criteria, older adults aged \( \geq 60 \), and majorly of 65 years old. [67, 68]

Biochemical and image markers will be not considered. At population level, the position of researcher worldwide, regarding blood draws and AD are well known [69]. A large group of analysis generally correlates the amyloid-beta levels and the hyperphosphorylated tau protein in the cerebrospinal fluid. Therefore, it will be necessary to execute highly invasive procedures, [70, 71] which will not justify the population epidemiological study and will not anticipate therapeutic interventions. Alternatively, it will not be practicable to perform in rural population, advanced image studies like Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PEC), the right tests for determining changes in the structure and volume of different regions of the brain, related to the disease [72, 73].

Authors highlight the design of an epidemiological cross-sectional survey to identify the possible association of risk and protective factors with AD in different groups of the Cuban elderly population. Since this analysis will be no conclusive, it would be noteworthy to accomplish further studies on Cuban population.

Ethics and dissemination

This research followed the ethical codes of the Declaration of Helsinki. Protocol was accepted by the Scientific Research Council from Centro Nacional de Biopreparados, as Promoting Research Institution (No. 2.12.12.17). Health Authorities and Ethic Committees of the Health Authority
provided also their consent (No.12.12.17 and No.1.12.17). Subjects, caregivers or relatives will provide corresponding written informed consent.

The results will be published in scientific papers and will be also shared with the Health Authorities of each municipality. Additionally, outcomes will be accessible via presentations in domestic and international scientific meetings. Authors will create guidance documents with detailed instructions for the appropriate selection of tests and scales for further studies.

**Ethics statements**

Patient consent for publication:

Not applicable.

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586

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Table 1. Health condition and Familial pathological history.

| Psychiatric and neurological diseases | Vascular diseases | Immunological diseases | Genetic diseases | Metabolic diseases | Others diseases and conditions |
|--------------------------------------|------------------|------------------------|-----------------|------------------|--------------------------------|
| Alzheimer Disease**                  | Ischemic heart disease** | Asthma** | Down’s Syndrome * | Type I diabetes** | Severe respiratory insufficiency** |
| Parkinson*                           | Hypertension**    | Arthritis**            | Autism*         | Type II diabetes** | Cancer ** |
| Schizophrenia*                       | Cardiac arrhythmia** |                      |                 | Avitaminosis      | AIDS** (Vitamins A, B,…) ** |
| ICTUS**                              |                  |                        |                 | Hyperlipidemia*   | Crohn's disease** |
| Dementia**                           |                  |                        |                 | Hypercholesterolemia* | Gingivitis** |
| Chronic depressive status**          |                  |                        |                 | Hyperthyroidism*  | Rosacea** |
| Mental retardation*                  |                  |                        |                 | Hypothyroidism*   | Glaucoma** |
| Psychosis*                           |                  |                        |                 | Osteoporosis*     | Alcoholism** |
| Aphasia*                             |                  |                        |                 |                  | Smoking** |
| Frequent headaches**                 |                  |                        |                 |                  | Obesity** |
| Cranial trauma**                     |                  |                        |                 |                  | General anesthesia*** |
| Hydrocephalus**                      |                  |                        |                 |                  | A severe blow to the head*** |
| Bipolar disorder*                    |                  |                        |                 |                  | Headache*** |

*- familial pathological history questions; **- questions concerning both, the subject and familial pathological history, *** questions concerning only the subject.
| Cognitive or behavioral symptoms | Impairment of the ability to acquire and remember new information | Deterioration of reasoning and management of complex tasks, impoverished judgment | Impairment of visuospatial capabilities | Impairment of language functions | Changes in personality, conduct, or behavior | Neuropsychological status |
|---------------------------------|-------------------------------------------------|-------------------------------------------------|---------------------------------|-----------------------------|---------------------------------|--------------------------|
| **Deterioration in the functional capacity at work** | **Decrease in the interest of executing hobbies and daily activities at home** | **Subject’s perception of risks, without realizing a possible danger** | **Difficulty to find visible objects, even having a good visual acuity** | **Dysfunction of the language tasks. Difficulty in finding the right words while speaking or forgetting words** | **Sudden mood swings** | **Sadness** |
| **Deterioration in the functional capacity to perform usual activities** | **Asking same questions or repetitive conversations** | **Inability to manage finances or confusion to get accounts** | **Difficulty in operating with simple utensils** | **Errors in speech, in spelling words or in writing** | **Feeling unmotivated or apathetic to do things that once caused pleasure** | **Nervous tenseness** |
| **Decrease in levels of functionality and performance, compared to the past behavior of the subject** | **Mistakenly place personal belongings or forgetting their location** | **Difficulty in making decisions** | **Difficulty in getting dressed** | **Isolation or loss of empathy towards known people** | **Irritableness** |  |
| | **Unable to remembering recent or past events in personal life or daily life** | **Incapacity to plan complex activities** | | **Compulsive or obsessive behaviors, physical or verbal** | | **Pessimism** |
| Loss in a family place | Hallucinations, anxiety, phobia, depression, desire to cry for no reason | Suicidal ideas |
|------------------------|---------------------------------------------------------------------|---------------|
| Fail to recall prior remembered names | Loss of auto confidence | |
| Difficulty to remember a fact or book, the subject just read | |
| Retention of the name of the investigator | |
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### Table 3. Sample size by locations

| Location               | Urban area | Rural area | Total |
|------------------------|------------|------------|-------|
|                        | Men | Women | Men | Women |       |
| Baracoa                | 101 | 106 | 88 | 79 | 374 |
| Bejucal                | 132 | 131 | 50 | 43 | 356 |
| Santa Cruz del Norte   | 121 | 130 | 61 | 53 | 365 |
| Total                  | 354 | 367 | 199 | 175 | 1095 |
Figure 1. Location of the three selected municipalities for conducting the epidemiological study in Cuba.
