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CORRELATION BETWEEN BIOMARKERS OF PAIN IN SALIVA AND PAINAD SCALE IN ELDERLY PEOPLE WITH COGNITIVE IMPAIRMENT AND INABILITY TO COMMUNICATE: STUDY PROTOCOL

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ABSTRACT

Introduction

Pain is an under-diagnosed problem in elderly people, especially in those with cognitive impairment who are unable to verbalize their pain. Although the PAINAD scale is a tool recognized for its clinical interest in this type of patients, its correlation with the saliva biomarkers reinforced its utility. The aim of this research will be to correlate the scores of this scale with the levels of biomarkers of pain found in saliva samples of patients with cognitive impairment and inability to communicate.

Methods and analysis

This is an observational study. The level of pain will be evaluated using the PAINAD scale. Moreover, pain biomarkers, in particular secretory immunoglobulin A (sIgA) and soluble tumor necrosis factor receptor type II (sTNF-RII), will be determined in saliva. Both assessments will be conducted in 75 patients aged over 65 years with advanced cognitive impairment and inability to communicate. The PAINAD scores will be correlated with the levels of these biomarkers of pain. A control group consisting of 75 healthy subjects aged over 65 years will be included in the study. Moreover, sociodemographic variables and variables related to pain, dementia and other clinical conditions will be recorded. The analysis will be performed with the statistical package SPSS v22 and the software R.

Ethics and dissemination

The study has been reviewed and approved by the Andalusian Human Research Ethics Committee. In addition, this study has been financed by the Junta de Andalucía through a regional health research fund (Research code: PI-0357-2017). The results will be actively disseminated through a high-impact journal in our study area, conference presentations and social media.

Keywords: cognitive impairment, dementia, pain, PAINAD, biomarkers, nursing

Strengths and limitations of this study

- The study will provide a reliable and reinforced tool (the PAINAD scale) to assess pain in elderly people with cognitive impairment and inability to communicate which will facilitate the correct pain management.
- The determination of biomarkers of pain in saliva is a promising tool because it is a noninvasive and inexpensive method that provides information and complements the information provided by the PAINAD scale.
- The results of the present study will lead to an improvement in quality of life of these patients and their families thanks to a better diagnosis of pain using the PAINAD scale and the salivary biomarkers.
- The correlation of the information provided by the PAINAD scale and the values of the salivary biomarkers will allow health institutions to make clinical recommendations with a higher level of evidence.

INTRODUCTION

The assessment of pain in elderly people with cognitive impairment is often insufficient and, consequently, pain treatment is inadequate (1-4).

Although elderly patients with cognitive impairment are not the only underdiagnosed and undertreated group, they are undoubtedly one of the most affected. The reasons are mainly two:

1. Elders are more likely to experience pain (5).
2. Elders usually have difficulty communicating the level of pain they are suffering to their caregivers and healthcare providers (3,6-8).

Pain is a frequent experience for many elderly people (5). Most common chronic diseases afflicting the elderly, such as depression, cardiovascular disease, cancer and osteoporosis, involve a larger risk of developing chronic pain (4,9). Approximately 50% of community-dwelling adults and up to 80% of institutionalized older adults are estimated to experience considerable pain (7,10).

In addition, pain has important consequences in the elderly population, because it affects them both physically and psychologically and, often, pain can lead to dependence situations. Pain causes long-term problems affecting, in general, to quality of life (6).

However, despite being a frequent and important problem, studies have shown that pain is often not evaluated or treated correctly, especially in the elderly.

Numerous studies have also shown that older people with cognitive impairment, particularly with dementia, often suffer from painful illnesses, and that they are usually prescribed fewer analgesics than patients with intact cognitive abilities (2,3,11).

This undertreatment is undoubtedly related to the difficulty of detecting pain in this population, mainly due to the loss of verbal abilities to express pain (3), or to insufficient instruction or training of professionals and caregivers to identify pain (12).

The tools used to assess pain can be classified into: 1) self-reports of pain; 2) direct observation of the person's behavior; and 3) biomarkers.

Self-reports are considered the most reliable and refined measure of the presence and intensity of pain, even in patients with moderate dementia (13). However, when the capacity for abstract reasoning is low, the use of such scales, even though they are very simple, becomes very complicated, because patients do not understand the concepts used. This

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3 complexity becomes impossible to use when, in addition, the ability of verbal communication
4 is affected.
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6 As an alternative to verbal evaluation (or self-report), a significant number of observational
7 scales have been developed in the last 15 years. Several literature review studies describe
8 more than 24 tools of this type (14-21) and although none of the tools can be recommended
9 based on existing evidence, several studies advocate the inclusion of any of them within a
10 comprehensive pain care protocol (1,10,22-25).
11

12 Among them, the PAINAD scale is recommended by the National Nursing Home Pain
13 Collaborative as a clinically useful tool (26), and several authors describe the PAINAD as
14 the most practical and promising scale (8,19,27-30). The PAINAD scale has a convergent
15 validity and a moderate internal consistency (31,32). The scores obtained with the PAINAD
16 scale vary when performing a potentially painful activity (32,33); and the scores decrease
17 after the administration of analgesics (2). Despite this, the validation of the PAINAD scale
18 to Spanish is not complete.
19

20 The determination of biomarkers of pain is the third of the potential tools. Their
21 determination in saliva would be an enormously useful, noninvasive and economic tool. In
22 fact, some pain biomarkers have already been determined in saliva (34,35), such as salivary
23 cortisol, its salivary levels correlate strongly with the level of pain (36), salivary α -amylase
24 (sAA) (37), secretory immunoglobulin A (sIgA) (38), testosterone (39), or tumor necrosis
25 factor receptor type II (sTNF-RII) (40) The saliva levels of sTNF-RII correlate significantly
26 with its levels in plasma (41). However, authors such as Sobas et al. (42) point to sIgA and
27 sTNF-RII, out of all these biomarkers, as potential salivary markers of pain in healthy people,
28 since they presented the highest intra-individual reproducibility.
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30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 **METHODS AND ANALYSIS**

46 **Aim and objectives**

47 The aim of this research will be to correlate the scores of the PAINAD scale with the levels of pain
48 biomarkers in saliva samples obtained from patients with cognitive impairment. The objectives of the
49 study will be to:
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- 51 1. Evaluate the pain level through the PAINAD scale.
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2. Determine in saliva the values of the pain biomarkers sNTF α RII and sIgA in a population with cognitive impairment and communication inability.
 3. Identify the possible relation between sociodemographic and clinical variables and the PAINAD score and the values of pain biomarkers in saliva.

Research hypothesis

We anticipate that the PAINAD score will correlate with the salivary levels of sTNF-RII and sIgA in the sample. The correlation level will be established between the final score on the scale and the presence of significant differences in these biomarkers.

Study design

This is an observational study which began in May 2018 and will end in June 2020. The design followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations.

This study is funded by the Health Department of the Regional Government of Andalusia (PI-0357-2017).

Study setting

A health district of an Andalusian province, through its network of Primary Healthcare centers, and an institution dedicated to the care of patients with dementia, specifically, with Alzheimer's disease.

Participants and selection criteria

The sample size has been calculated for a correlation magnitude of $r=0.3$, a statistical confidence of 95%, a statistical power of 80%, with a unilateral approach and a 10% of losses. The result sample size is 75 subjects.

The inclusion criteria for participants were the following:

- Age \geq 65 years.
- Cognitive impairment, dementia, Alzheimer's disease, global deterioration scale (GDS) score between 5 and 7 (43).

- Being unable to communicate verbally.
- Having a relative or legal representative that can sign the informed consent for the participation of the patient in the study.
- Being included, at least, for three months in the listings of the dementia process. In the case of the institution dedicated to the care of patients with Alzheimer's disease, patients who have used this service for at least three months will be included.

The recruitment of the participants will be conducted consecutively by the interventional nurses belonging to the different participating Primary Healthcare Centers, among the subjects that attend their usual nurse consultation based on the lists of the dementia process of the urban Health Centers (HC) and an institution dedicated to the care of Alzheimer's patients.

Subjects without cognitive impairment, within the same age range, who voluntarily wish to participate in this study, will be investigated to correlate the values obtained in the PAINAD with the determination of pain biomarkers. These 75 control subjects will be recruited from the environment close to the subjects (family, relatives and other users of the same HC).

Study measures

The main variables will be the scores of the Spanish version of PAINAD and the determinations of the biomarkers sTNF-RII and IgAs. Other study variables will be the scores of the GDS scale; sociodemographic variables (age, sex); clinical data related to pain (duration, frequency, location, etiology, type of analgesic treatment, adjuvant treatment and pain control at the current time), and level of autonomy in basic activities of daily living (Table 1).

Table 1. Study measures

Sociodemographic data	Clinical history
Health variables	Clinical history
Pharmacological treatments	Clinical history
Cognitive impairment	GDS
Autonomy in activities of daily living	Barthel Index
PAINAD	Observational scale
Biomarkers	Saliva collection

Data collection

A specific Book for Data Collection (BDC) will be provided to each researcher. An explaining manual for the BDC has been developed for standardized data collection to ensure the quality of data collection. In addition, the professionals will receive face-to-face training about data collection and the tools or assessment scales to ensure the validity and reproducibility.

The professionals will also be individually instructed on how to perform the saliva collection using the passive secretion method (44):

1. One hour prior to sample collection, subjects should not eat, drink (except water), chew gum, brush their teeth, consume caffeine, or do physical exercise.
2. Five minutes prior to sample collection, the subject should rinse their mouth with clean water to reduce the contamination of saliva with food debris.
3. All existing saliva in the mouth should be swallowed before starting the sample collection.
4. Subsequently, intermittently deposit the accumulated saliva for a period of 5 min in a collection tube, requiring at least 1 ml. If the 5 ml collection tube is filled before 5 minutes, the amount of time that has elapsed is recorded.

The researcher will obtain voluntary informed consent in writing. If the patient agrees, the researcher will collect the sociodemographic and clinical data of the patient. The PAINAD

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3 scale will be completed along with the other selected measures. In addition, saliva will be
4 collected.
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6 Samples will be collected in a clinical setting, under supervision, between 09:00 a.m. and
7 10:00 a.m. in the morning and always before the morning medication is taken. The samples
8 collected will always be refrigerated, and will be collected always in the same room, where
9 a temperature and humidity will be recorded. After collection, the samples will be frozen at
10 -80 ° C until analysis.
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12 Data will be collected by qualified personnel previously instructed under the aforementioned
13 protocol.
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15 **Determination of sTNF-RII and IgA**

16 The determination of sTNF-RII and IgAs levels will be performed using an enzyme-linked
17 immunosorbent assay (ELISA). The sTNF-RII levels will be determined using the Human
18 sTNF-RII Quantikine ELISA kit (R & D Systems, Minneapolis, MN) and the IgAs using the
19 Secretary Immunoglobulin A ELISA kit (Salimetrics LLC, State College, PA, USA). The
20 collection period will be determined because IgA levels depend on the flow of saliva
21 secretion. The quantification of total proteins in saliva will be performed using Bradford's
22 method, using bovine serum albumin as standard.
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36 **Data analysis**

37 For the description of the sample, the number of observations, mean, standard deviation,
38 minimum, maximum, median, interquartile range and 95% confidence interval for the mean
39 value will be used for quantitative variables; whereas the absolute and relative frequencies
40 will be used for categorical variables (for this type of variables, the lost data will appear as
41 another category, with its absolute frequency and its percentage).
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48 In order to determine the degree of relationship between the PAINAD scale and the values
49 of the biomarkers considered, the matrix of polyserial correlations with their corresponding
50 hypothesis contrasts will be used. A logit model will be used to determine which biomarker
51 most affects the pain condition and multinomial logistic models will be considered to
52 determine which of the two biomarkers has the strongest influence on pain gradation.
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3 Next, to determine the possible relationships between demographic and clinical variables
4 with PAINAD scores and biomarkers, Pearson and polyserial correlations will be obtained
5 depending on the scales considered (continuous and ordinal), as well as the chi-square
6 coefficient or predictive coefficients lambda (nominal and ordinal), with their corresponding
7 significances levels. Finally, in the case of detecting some type of relationship between these
8 variables, they will be introduced in the logit and logistic multinomial models to determine
9 their relative influence on the appearance and degree of pain.
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16 This analysis will be carried out using the statistical package SPSS v22 for most calculations
17 and the software R for the determination of the coefficients of polyserial correlations. For all
18 cases, a significance of 5% will be assumed.
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23 **DISCUSSION**

24 The severe aging of global population has caused a significant increase in the prevalence of
25 cognitive impairment. However, the importance of these figures is not due solely to the
26 current transcendence of this public health problem, but also it is due to the fact that multiple
27 studies indicate that the percentage of people with dementia will increase substantially in the
28 coming years (45).
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33 In this context, healthcare systems, particularly in Western countries, face new challenges in
34 the care of patients with cognitive impairment, among them the assessment and adequate
35 management of pain are significant, needing solutions to provide quality care to this
36 population.
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40 Pain in patients with cognitive impairment is an unresolved problem due to the progression
41 of the neurological disorders that they present and that entails the loss of the ability to
42 communicate, which makes it difficult to manage pain and, therefore, it leads to pain
43 underdiagnosis and undertreatment (46).
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47 The PAINAD scale is a tool of acknowledged utility for the assessment of pain in people
48 with cognitive impairment and communication inability (47).
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51 The determination of pain biomarkers in saliva is a tool of maximum utility because of its
52 non-invasiveness and improved accessibility to the biological sample in comparison with
53 blood determinations, becoming a rigorous and accurate assessment tool thanks to which
54 patient's pain can be detected and addressed.
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3 The correlation of the results of both tools will allow health professionals, as well as relatives
4 and informal caregivers of these patients, to know that pain can be evaluated correctly,
5 allowing a better use of analgesia.
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10 **Limitations**

11 The selection of the subjects studied may be affected by consecutive sampling. However, no
12 previous list of patients, not even an estimate of the total number of patients meeting the
13 established inclusion criteria, was available for the studied location.
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15 In addition, the recruitment process may be affected by the non-acceptance of those relatives
16 of patients who do not want to participate in the study.
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18 Finally, it is necessary to point out that the test-retest has the limitation of the changing nature
19 of pain. Although the study is designed for the chronic pain condition, it is inevitable that
20 cases of acute pain are found and that, therefore, the score changes, even when there is a
21 narrow margin between the two measurements. These cases of acute pain will undoubtedly
22 represent a distortion in the verification of this feature.
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30 **ETHICS AND DISSEMINATION**

31 The study will be conducted following the ethical principles of the Helsinki Declaration. In
32 addition, both the design and the development of the work will meet the standards of good
33 clinical practice (CPMP/ICH/135/95, July 2002, European Medicines Agency).
34

35 The study protocol has been evaluated by a peer-review process and funded by a grant
36 awarded under the call for proposals in Biomedical and Health Sciences R+D+I of the
37 Regional Government of Andalusia (22 December 2017). The proposal has been approved
38 (5 December 2017) by the Research Ethics Committee of the University Hospital Reina Sofia
39 of Cordoba (Spain).
40

41 All participants will be informed of the general aspects of the study. Participants have the
42 right to withdraw consent to participate at any time.
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44 Moreover, all the participants must sign a written informed consent before participating in
45 the study. Confidentiality is guaranteed.
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47 The study will comply with the requirements set out by the Regulation (EU) 2016/679 of the
48 European Parliament and of the Council of April 27, 2016 on Data Protection (GDPR).
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3 The plans to disseminate the results of this observational study include publishing at least a
4 research paper in peer-reviewed journals in our study area. Also, if possible, the results of
5 this research will be presented in conference presentations.
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10 **CONCLUSION**

11 The results of this research will allow elucidating the concentrations of pain biomarkers in
12 saliva, which would be an objective tool of enormous utility to confirm the possible diagnosis
13 of pain in this population. The correlation between the values of the PAINAD scale and those
14 of the pain biomarkers obtained in saliva samples will reflect the complementarity of both
15 tools for the assessment of pain and will reinforce the suitability of the scale in relation to the
16 evaluation of pain in this population.
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AUTHORS' CONTRIBUTIONS

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V-C-H, M-C-G and M-R-R conceptualized the project and conceived the study design. V-C-H drafted the manuscript. M-C-G, M-R-R, M-M-C and J-Q-G reviewed and edited the draft protocol. All authors read and approved the final manuscript.

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CORRELATION BETWEEN BIOMARKERS OF PAIN IN SALIVA AND PAINAD SCALE IN ELDERLY PEOPLE WITH COGNITIVE IMPAIRMENT AND INABILITY TO COMMUNICATE: DESCRIPTIVE STUDY PROTOCOL

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RESEARCH PROTOCOL

CORRELATION BETWEEN BIOMARKERS OF PAIN IN SALIVA AND PAINAD SCALE IN ELDERLY PEOPLE WITH COGNITIVE IMPAIRMENT AND INABILITY TO COMMUNICATE: DESCRIPTIVE STUDY PROTOCOL

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ABSTRACT

Introduction

Pain is an under-diagnosed problem in elderly people, especially in those with cognitive impairment who are unable to verbalize their pain. Although the PAINAD scale is a tool recognized for its clinical interest in this type of patients, its correlation with the saliva biomarkers reinforced its utility. The aim of this research will be to correlate the scores of this scale with the levels of biomarkers of pain found in saliva samples of patients with cognitive impairment and inability to communicate.

Methods and analysis

This is an observational study. The level of pain will be evaluated using the PAINAD scale. Moreover, pain biomarkers, in particular secretory immunoglobulin A (sIgA) and soluble tumor necrosis factor receptor type II (sTNF-RII), will be determined in saliva. Both assessments will be conducted in 75 patients aged over 65 years with advanced cognitive impairment and inability to communicate. The PAINAD scores will be correlated with the levels of these biomarkers of pain. A control group consisting of 75 healthy subjects aged over 65 years will be included in the study. Moreover, sociodemographic variables and variables related to pain, dementia and other clinical conditions will be recorded. The analysis will be performed with the statistical package SPSS v22 and the software R.

Ethics and dissemination

The study has been reviewed and approved by the Andalusian Human Research Ethics Committee. In addition, this study has been financed by the Junta de Andalucía through a regional health research fund (Research code: PI-0357-2017). The results will be actively disseminated through a high-impact journal in our study area, conference presentations and social media.

Keywords: cognitive impairment, dementia, pain, PAINAD, biomarkers, nursing

Strengths and limitations of this study

- The study will provide a reliable and reinforced tool (the PAINAD scale) to assess pain in elderly people with cognitive impairment and inability to communicate which will facilitate the correct pain management.
- The determination of biomarkers of pain in saliva is a promising tool because it is a noninvasive and inexpensive method that provides information and complements the information provided by the PAINAD scale.
- The results of the present study will lead to an improvement in quality of life of these patients and their families thanks to a better diagnosis of pain using the PAINAD scale and the salivary biomarkers.
- The correlation of the information provided by the PAINAD scale and the values of the salivary biomarkers will allow health institutions to make clinical recommendations with a higher level of evidence.

INTRODUCTION

The assessment of pain in elderly people with cognitive impairment is often insufficient and, consequently, pain treatment is inadequate (1-4).

Although elderly patients with cognitive impairment are not the only underdiagnosed and undertreated group, they are undoubtedly one of the most affected. The reasons are mainly two:

1. Elders are more likely to experience pain (5).
2. Elders usually have difficulty communicating the level of pain they are suffering to their caregivers and healthcare providers (3,6-8).

Pain is a frequent experience for many elderly people (5). Most common chronic diseases afflicting the elderly, such as depression, cardiovascular disease, cancer and osteoporosis, involve a larger risk of developing chronic pain (4,9). Approximately 50% of community-dwelling adults and up to 80% of institutionalized older adults are estimated to experience considerable pain (7,10).

In addition, pain has important consequences in the elderly population, because it affects them both physically and psychologically and, often, pain can lead to dependence situations. Pain causes long-term problems affecting, in general, to quality of life (6).

However, despite being a frequent and important problem, studies have shown that pain is often not evaluated or treated correctly, especially in the elderly.

Numerous studies have also shown that older people with cognitive impairment, particularly with dementia, often suffer from painful illnesses (specifically, it is estimated that between 80-85% of them suffer pain) and that they are usually prescribed fewer analgesics than patients with intact cognitive abilities (2,3,11).

This undertreatment is undoubtedly related to the difficulty of detecting pain in this population, mainly due to the loss of verbal abilities to express pain (3), or to insufficient instruction or training of professionals and caregivers to identify pain (12).

The tools used to assess pain can be classified into: 1) self-reports of pain; 2) direct observation of the person's behavior; and 3) biomarkers.

Self-reports are considered the most reliable and refined measure of the presence and intensity of pain, even in patients with moderate dementia (13). However, when the capacity for abstract reasoning is low, the use of such scales, even though they are very simple,

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3 becomes very complicated, because patients do not understand the concepts used. This
4 complexity becomes impossible to use when, in addition, the ability of verbal communication
5 is affected.
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8 As an alternative to verbal evaluation (or self-report), a significant number of observational
9 scales have been developed in the last 15 years. Several literature review studies describe
10 more than 24 tools of this type (14-21) and although none of the tools can be recommended
11 based on existing evidence, several studies advocate the inclusion of any of them within a
12 comprehensive pain care protocol (1,10,22-25).
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15 Among them, the PAINAD scale is recommended by the National Nursing Home Pain
16 Collaborative as a clinically useful tool (26), and several authors describe the PAINAD as
17 the most practical and promising scale (8,19,27-30). The PAINAD scale has a convergent
18 validity and a moderate internal consistency (31,32). The scores obtained with the PAINAD
19 scale vary when performing a potentially painful activity (32,33); and the scores decrease
20 after the administration of analgesics (2). Despite this, the validation of the PAINAD scale
21 to Spanish is not complete.
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24 The determination of biomarkers of pain is the third of the potential tools. Their
25 determination in saliva would be an enormously useful, noninvasive and economic tool. In
26 fact, some pain biomarkers have already been determined in saliva (34,35), such as salivary
27 cortisol, its salivary levels correlate strongly with the level of pain (36), salivary α -amylase
28 (sAA) (37), secretory immunoglobulin A (sIgA) (38), testosterone (39), or tumor necrosis
29 factor receptor type II (sTNF-RII) (40) The saliva levels of sTNF-RII correlate significantly
30 with its levels in plasma (41). However, authors such as Sobas et al. (42) point to sIgA and
31 sTNF-RII, out of all these biomarkers, as potential salivary markers of pain in healthy people,
32 since they presented the highest intra-individual reproducibility.
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46 **METHODS AND ANALYSIS**

47 **Aim and objectives**

48 The aim of this research will be to correlate the scores of the PAINAD scale with the levels of pain
49 biomarkers in saliva samples obtained from patients with cognitive impairment. The objectives of the
50 study will be to:
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- 54 1. Evaluate the pain level through the PAINAD scale.
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2. Determine in saliva the values of the pain biomarkers sNTF α RII and sIgA in a population with cognitive impairment and communication inability.
3. Identify the possible relation between sociodemographic and clinical variables and the PAINAD score and the values of pain biomarkers in saliva.

Research hypothesis

We anticipate that the PAINAD score will correlate with the salivary levels of sTNF-RII and sIgA in the sample. The correlation level will be established between the final score on the scale and the presence of significant differences in these biomarkers.

Study design

This is an observational study which began in May 2018 and will end in June 2020. The design followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations.

This study is funded by the Health Department of the Regional Government of Andalusia (PI-0357-2017).

Study setting

A health district of an Andalusian province, through its network of Primary Healthcare centers, and an institution dedicated to the care of patients with dementia, specifically, with Alzheimer's disease.

Participants and selection criteria

The sample size has been calculated for a correlation magnitude of $r=0.3$, a statistical confidence of 95%, a statistical power of 80%, with a unilateral approach and a 10% of losses. The result sample size is 75 subjects.

The inclusion criteria for participants were the following:

- Age \geq 65 years.
- Medical diagnosis of dementia or Alzheimer's disease with a global deterioration scale (GDS) score between 5 and 7 (43).

- Being unable to communicate verbally.
- Having a relative or legal representative that can sign the informed consent for the participation of the patient in the study.
- Being included, at least, for three months in the listings of the dementia process. In the case of the institution dedicated to the care of patients with Alzheimer's disease, patients who have used this service for at least three months will be included.

The recruitment of the participants will be conducted consecutively by the interventional nurses belonging to the different participating Primary Healthcare Centers, among the subjects that attend their usual nurse consultation based on the lists of the dementia process of the urban Health Centers (HC) and an institution dedicated to the care of Alzheimer's patients.

Subjects without cognitive impairment, within the same age range, who voluntarily wish to participate in this study, will be investigated to correlate the values obtained in the PAINAD with the determination of pain biomarkers. These 75 control subjects will be recruited from the environment close to the subjects (family, relatives and other users of the same HC).

Study measures

The main variables will be the scores of the Spanish version of PAINAD and the determinations of the biomarkers sTNF-RII and IgAs. Other study variables will be the scores of the GDS scale; sociodemographic variables (age, sex); clinical data related to pain (duration, frequency, location, etiology, type of analgesic treatment, adjuvant treatment and pain control at the current time), and level of autonomy in basic activities of daily living (Table 1).

Table 1. Study measures

Sociodemographic data	Clinical history
Health variables	Clinical history
Pharmacological treatments	Clinical history
Cognitive impairment	GDS
Autonomy in activities of daily living	Barthel Index
PAINAD	Observational scale
Biomarkers	Saliva collection

Data collection

A specific Book for Data Collection (BDC) will be provided to each researcher. An explaining manual for the BDC has been developed for standardized data collection to ensure the quality of data collection. In addition, the professionals will receive face-to-face training about data collection and the tools or assessment scales to ensure the validity and reproducibility.

The professionals will also be individually instructed on how to perform the saliva collection using the passive secretion method (44):

1. One hour prior to sample collection, subjects should not eat, drink (except water), chew gum, brush their teeth, consume caffeine, or do physical exercise.
2. Five minutes prior to sample collection, the subject should rinse their mouth with clean water to reduce the contamination of saliva with food debris.
3. All existing saliva in the mouth should be swallowed before starting the sample collection.
4. Subsequently, intermittently deposit the accumulated saliva for a period of 5 min in a collection tube, requiring at least 1 ml. If the 5 ml collection tube is filled before 5 minutes, the amount of time that has elapsed is recorded.

Voluntary written informed consent will be obtained from a family member or their legal representative in the case of patients with cognitive impairment, and directly from the control group subjects. Once the consent is received, the researcher will collect the

sociodemographic and clinical data of the patient. The PAINAD scale will be completed along with the other selected measures. In addition, saliva will be collected.

Samples will be collected in a clinical setting, under supervision, between 09:00 a.m. and 10:00 a.m. in the morning and always before the morning medication is taken. The samples collected will always be refrigerated, and will be collected always in the same room, where a temperature and humidity will be recorded. After collection, the samples will be frozen at -80°C until analysis.

Data will be collected by qualified personnel previously instructed under the aforementioned protocol.

Determination of sTNF-RII and IgA

The determination of sTNF-RII and IgAs levels will be performed using an enzyme-linked immunosorbent assay (ELISA). The sTNF-RII levels will be determined using the Human sTNF-RII Quantikine ELISA kit (R & D Systems, Minneapolis, MN) and the IgAs using the Secretory Immunoglobulin A ELISA kit (Salimetrics LLC, State College, PA, USA). The collection period will be determined because IgA levels depend on the flow of saliva secretion. The quantification of total proteins in saliva will be performed using Bradford's method, using bovine serum albumin as standard.

Data analysis

For the description of the sample, the number of observations, mean, standard deviation, minimum, maximum, median, interquartile range and 95% confidence interval for the mean value will be used for quantitative variables; whereas the absolute and relative frequencies will be used for categorical variables (for this type of variables, the lost data will appear as another category, with its absolute frequency and its percentage).

In order to determine the degree of relationship between the PAINAD scale and the values of the biomarkers considered, the matrix of polyserial correlations with their corresponding hypothesis contrasts will be used. A logit model will be used to determine which biomarker most affects the pain condition and multinomial logistic models will be considered to determine which of the two biomarkers has the strongest influence on pain gradation.

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3 Next, to determine the possible relationships between demographic and clinical variables
4 with PAINAD scores and biomarkers, Pearson and polyserial correlations will be obtained
5 depending on the scales considered (continuous and ordinal), as well as the chi-square
6 coefficient or predictive coefficients lambda (nominal and ordinal), with their corresponding
7 significances levels. Finally, in the case of detecting some type of relationship between these
8 variables, they will be introduced in the logit and logistic multinomial models to determine
9 their relative influence on the appearance and degree of pain.

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12 This analysis will be carried out using the statistical package SPSS v22 for most calculations
13 and the software R for the determination of the coefficients of polyserial correlations. For all
14 cases, a significance of 5% will be assumed.

21 22 23 **PATIENT AND PUBLIC INVOLVEMENT**

24 The research question for this study was developed based on a synthesis of the recent
25 literature, therefore patients and the public were not involved in the design of the study,
26 including the research question, outcomes measures, recruitment to or conduct of the study.
27 The results of the study will be actively disseminated and made available to the heads of the
28 participating centers.

29 30 31 32 **DISCUSSION**

33 The severe aging of global population has caused a significant increase in the prevalence of
34 cognitive impairment. However, the importance of these figures is not due solely to the
35 current transcendence of this public health problem, but also it is due to the fact that multiple
36 studies indicate that the percentage of people with dementia will increase substantially in the
37 coming years (45).

38 In this context, healthcare systems, particularly in Western countries, face new challenges in
39 the care of patients with cognitive impairment, among them the assessment and adequate
40 management of pain are significant, needing solutions to provide quality care to this
41 population.

42 Pain in patients with cognitive impairment is an unresolved problem due to the progression
43 of the neurological disorders that they present and that entails the loss of the ability to

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3 communicate, which makes it difficult to manage pain and, therefore, it leads to pain
4 underdiagnosis and undertreatment (46).

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6 The PAINAD scale is a tool of acknowledged utility for the assessment of pain in people
7 with cognitive impairment and communication inability (47).

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10 The determination of pain biomarkers in saliva is a tool of maximum utility because of its
11 non-invasiveness and improved accessibility to the biological sample in comparison with
12 blood determinations, becoming a rigorous and accurate assessment tool thanks to which
13 patient's pain can be detected and addressed.

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16 The correlation of the results of both tools will allow health professionals, as well as relatives
17 and informal caregivers of these patients, to know that pain can be evaluated correctly,
18 allowing a better use of analgesia.
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23 **Limitations**

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25 The selection of the subjects studied may be affected by consecutive sampling. However, no
26 previous list of patients, not even an estimate of the total number of patients meeting the
27 established inclusion criteria, was available for the studied location.

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29 In addition, the recruitment process may be affected by the non-acceptance of those relatives
30 of patients who do not want to participate in the study.

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32 Finally, it is necessary to point out that the test-retest has the limitation of the changing nature
33 of pain. Although the study is designed for the chronic pain condition, it is inevitable that
34 cases of acute pain are found and that, therefore, the score changes, even when there is a
35 narrow margin between the two measurements. These cases of acute pain will undoubtedly
36 represent a distortion in the verification of this feature.
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50 **ETHICS AND DISSEMINATION**

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52 The study will be conducted following the ethical principles of the Helsinki Declaration. In
53 addition, both the design and the development of the work will meet the standards of good
54 clinical practice (CPMP/ICH/135/95, July 2002, European Medicines Agency).
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3 The study protocol has been evaluated by a peer-review process and funded by a grant
4 awarded under the call for proposals in Biomedical and Health Sciences R+D+I of the
5 Regional Government of Andalusia (22 December 2017). The proposal has been approved
6 (5 December 2017) by the Research Ethics Committee of the University Hospital Reina Sofia
7 of Cordoba (Spain).
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11 All participants will be informed of the general aspects of the study. Participants have the
12 right to withdraw consent to participate at any time.
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15 Moreover, all the participants must sign a written informed consent before participating in
16 the study. Confidentiality is guaranteed.
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19 The study will comply with the requirements set out by the Regulation (EU) 2016/679 of the
20 European Parliament and of the Council of April 27, 2016 on Data Protection (GDPR).
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22 The plans to disseminate the results of this observational study include publishing at least a
23 research paper in peer-reviewed journals in our study area. Also, if possible, the results of
24 this research will be presented in conference presentations.
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29 **CONCLUSION**

30 The results of this research will allow elucidating the concentrations of pain biomarkers in
31 saliva, which would be an objective tool of enormous utility to confirm the possible diagnosis
32 of pain in this population. The correlation between the values of the PAINAD scale and those
33 of the pain biomarkers obtained in saliva samples will reflect the complementarity of both
34 tools for the assessment of pain and will reinforce the suitability of the scale in relation to the
35 evaluation of pain in this population.
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AUTHORS' CONTRIBUTIONS

44 V-C-H, M-C-G and M-R-R conceptualized the project and conceived the study design. V-
45 C-H drafted the manuscript. M-C-G, M-R-R, M-M-C and J-Q-G reviewed and edited the
46 draft protocol. All authors read and approved the final manuscript.
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COMPETING INTEREST

None declared.

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