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CIÊNCIAS BIOMÉDICAS

Periodontal disease and its risk factors in a Portuguese elderly population

João Tiago da Silva Botelho

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

João Tiago da Silva Botelho

PERIODONTAL DISEASE AND ITS RISK FACTORS IN A PORTUGUESE ELDERLY POPULATION

Tese de Candidatura ao grau de Doutor em Ciências Biomédicas, submetida ao Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto

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Para a Vanessa,

a minha Mãe,

o meu Pai

e a minha irmã Mariana

"Real change, enduring change, happens one step at a time."

Justice Ruth Bader Ginsburg

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In compliance with the University of Porto Ethical Code of Academic Conduct, approved in December 2017, and in particular consideration for its Article 14: I declare that this thesis is my own and has not been previously used in another Program or Curricular Unit, at the University of Porto or another institution. References to other authors (statements, ideas, thoughts) scrupulously respect the rules of attribution, and are duly indicated in the text and in the bibliographic references, in accordance with the referencing norms. I realize that the practice of plagiarism and self-plagiarism is an academic offense.

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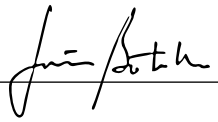

(João Tiago da Silva Botelho)

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Scientific Output

According to "*Artigo 31º, n.º do Decreto-lei n.º 74/2006 de 24 de Março, aditado pelo Decreto-Lei no. 230/2009, de 14 de Setembro*", I declare to have participated in the design and accomplishment of the experimental work, as well as in the interpretation of the results and in the writing of the following works published and in the phase of publication that integrate this thesis.

Articles published in journals indexed in ISI Web of Knowledge:

1. **João Botelho**, Vanessa Machado, Luís Proença, Maria João Oliveira, Maria Alzira Cavacas, Luís Amaro, Artur Águas & José João Mendes. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: findings from a cross-sectional survey. *BMC Oral Health* **2020**, 20, 199.
   Doi: 10.1186/s12903-020-01183-7.
   Author contribution: João Botelho conceived and designed the experiment, collected data, performed data analysis and interpretation, prepared figures and tables, drafted the work and critically revised the final work to be published.

2. **João Botelho**, Vanessa Machado, Paulo Mascarenhas, Ricardo Alves, Maria Alzira Cavacas, José João Mendes. Fine-tuning multilevel modeling of risk factors associated with nonsurgical periodontal treatment outcome. *Brazilian Oral Research* **2019**, vol.33, e081.
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   Author contribution: João Botelho collected data, contributed with statistical analysis and interpretation, prepared tables, authored drafts of the paper, and approved the final draft.

3. **João Botelho** *, Vanessa Machado *, Luís Proença, Ricardo Alves, Maria Alzira Cavacas, Luís Amaro, José João Mendes. Study of Periodontal Health in Almada-Seixal (SoPHiAS): A Cross-Sectional Study in the Lisbon Metropolitan Area. *Scientific Reports* **2019** 29, 9, 15538.
   Doi: 10.1038/s41598-019-52116-6.
   * Equally first author

Author contribution: João Botelho has created the project design and the main conceptual ideas and technicalities, computed sample size calculation, performed clinical examination, executed data analysis and interpretation, and wrote and approved the final work.

4. Vanessa Machado *, **João Botelho** *, Catarina Ramos, Luís Proença, Ricardo Alves, Maria Alzira Cavacas, José João Mendes. Psychometric properties of the Brief Illness Perception Questionnaire (Brief-IPQ) in Periodontal Diseases. *Journal of Clinical Periodontology* **2019**, 46, 1183-1191.
   Doi: 10.1111/jcpe.13186.
   * Equally first author
   Author contribution: João Botelho conceived, designed the experiments, and had a substantial contribution to acquisition of data. Furthermore, contributed for interpretation of data, drafted the article including the preparation of figures and table, and approved the final draft.

5. **João Botelho** *, Vanessa Machado *, Luís Proença, João Rua, Leonardo Martins, Ricardo Alves, Maria Alzira Cavacas, Daniele Manfredini, José João Mendes. Relationship Between Self-Reported Bruxism and Periodontal Status: Findings From a Cross-Sectional Study. *Journal of Periodontology* **2019**, 8.
   Doi: 10.1002/JPER.19-0364.
   * Equally first author
   Author contribution: João Botelho designed the study, applied the questionnaire and the periodontal measurements, processed the experimental data, was involved in bioinformatic analysis, assisted in the interpretation of results and was a major contributor in writing the manuscript.

6. Vanessa Machado, **João Botelho**, António Amaral, Luís Proença, Ricardo Alves, João Rua, Maria Alzira Cavacas, Ana Sintra Delgado, José João Mendes. Prevalence and extent of chronic periodontitis and its risk factors in a Portuguese subpopulation: a retrospective cross-sectional study and analysis of Clinical Attachment Loss. *PeerJ* **2018** 6, e5258. Doi:10.7717/peerj.5258.

Author contribution: João Botelho conceived and designed the experiment, collected data, performed data analysis and interpretation, prepared figures and tables, drafted the work and critically revised the final work to be published.

7. **João Botelho**, Vanessa Machado, Paulo Mascarenhas, João Rua, Ricardo Alves, Maria Alzira Cavacas, Ana Sintra Delgado, José João Mendes. Stress, Salivary Cortisol and Periodontitis: A Systematic Review and Meta-analysis of Observational Studies. *Archives of Oral Biology* **2018**, 96, 58-65.
   Doi: 10.1016/j.archoralbio.2018.08.016.
   Author contribution: João Botelho conceived the systematic review protocol, selected the included studies, extracted the data from each included article, conducted the meta-analysis, prepared figures and tables, authored the paper draft and approved the final work.

8. Vanessa Machado, **João Botelho**, Paulo Mascarenhas, Ricardo Alves, João Rua, Maria Alzira Cavacas, Ana Sintra Delgado, José João Mendes. Partial recording protocols performance on the assessment of periodontitis severity and extent: bias magnitudes, sensibility, and specificity. *Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial*, **2018** 59, 145-153.
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   Author contribution: João Botelho conceived and designed the experiment, collected data, contributed with statistical analysis and interpretation, prepared figures and tables, authored draft and approved the final draft.

Abstract

Periodontal disease is a polymicrobial inflammatory and plaque-induced process of the periodontium that can progress from gingivitis to periodontitis, if not stopped. Periodontal disease comprises two of the most prevalent diseases worldwide – periodontitis and gingivitis – and contributes to the global burden of chronic diseases.

The average life span has been increasing due to medical progression, healthcare, and socioeconomic growth. The prevalence of periodontitis among older people endures a substantial epidemiological challenge, while estimates presented in recent years have been very dissimilar, even in countries with similar socio-economically backgrounds. Alarmingly, the majority of systemic risk factors associated with periodontitis are precisely increased in elder inhabitants, which contributes to the complexity of these patient's treatment and management. As a consequence, it is imperative to better characterize periodontal health among elder people in order to determine preventive, diagnostic and therapeutic framework strategies to promote health.

Mindful of the epidemiological difficulties to survey periodontal disease among older people, several studies have reported high prevalence of periodontitis among this group of people. Regarding the Portuguese scenario, epidemiologic studies on the periodontal status are scarce, and so an imperative issue. Beyond its systemic impact in elders, periodontitis worsens perceived quality of life. Remarkably, the clinical burden of periodontitis, as missing teeth and denture use, have a higher impact on oral health-related quality of life (OHRQoL) than the periodontal status itself mainly because this target populations are frequently uneducated for periodontitis but are familiarized with side issues, as teeth loss or denture use. In particular, stress has been linked to both periodontitis and OHRQoL, and xerostomia has been associated to poorer quality of life. Therefore, studying other features and how they influence OHRQoL could improve our knowledge and clarify potential confounding variables, in particular stress and xerostomia that have never been investigated in the elderly context.

To these ends, our main purpose was to investigate the prevalence of periodontal disease and its risk factors in a Portuguese elderly population of the Southern Lisbon Metropolitan Area.

Previous to the epidemiologic survey design and implementation, we conducted a series of preliminary investigations in a Portuguese subpopulation of the Egas Moniz Dental Clinic (EMDC) (**Chapter 3**). Preliminary data confirmed that age was a major risk factor for chronic periodontitis in this subpopulation (**Section 3.2**). Multivariate analysis showed that elderly patients with 65 years old or older presented an Odds Ratio (OR) of 8.70 towards chronic periodontitis, and this result was in line with previous studies. Furthermore, smoking was the other major risk factor, although variables such as obesity and lower educational level warranted consideration. This group of elders evidenced alarming levels of tooth loss and periodontal parameters, which revealed elevated periodontal destruction. Then, due to the discrepancy between national estimates for periodontitis prevalence in elders and other European epidemiologic studies of reference we evaluated bias magnitudes, sensibility, and specificity of particular partial-mouth recording protocols (PRPs) used in the Portuguese national survey. Overall, the PRP used in the Direção Geral de Saúde (DGS) national study showed low sensitivity, specificity, and concordance to estimate periodontal clinical measurements (**Section 3.3**). Consequently, the results supported the decision to undergo a standard full-mouth circumferential examination in our epidemiologic study. Finally, we investigated the influence of known risk factors on nonsurgical periodontal treatment (NSPT) response using a pocket depth fine-tuning multilevel linear model (MLM) (**Section 3.4**). This study evidenced that treating periodontitis is effective and depends on many levels, and this may help to empower health-promoting messages during the cross-sectional study.

On the other hand, we conducted a systematic review that determined an association between stress and periodontitis, through the levels of salivary cortisol (**Section 3.5**). These results sustained the decision to include validated stress questionnaires in the epidemiologic study and consider it in data analysis.

The Study of Periodontal Health in Almada-Seixal (SoPHiAS) revealed a high burden of periodontitis in the population of the southern region of the Lisbon Metropolitan Area, in Portugal (**Chapter 4**). Age, education level, smoking status and diabetes mellitus were identified as significantly potential risk factors towards periodontitis, confirming the previous results of this thesis. In a more detailed analysis, the elderly subgroup presented disturbing levels of prevalence of periodontitis and reported faulty oral hygiene habits (**Chapter 5**). Furthermore, the number of missing teeth, self-reported xerostomia and stress

were heavily reported, and we confirmed that self-perceived xerostomia and stress revealed to be influential on OHRQoL in a population of elders, with a similar magnitude to the extent of periodontitis. Importantly, the periodontal status did not influence the OHRQoL, rather some clinical features did, such as missing teeth or denture use.

Moreover, after confirming the potential role of stress we hypothesized that bruxism (a stress-related condition) and periodontal status might be linked. To test this hypothesis, we used data from the population from SoPHiAS for periodontal status and self-reported bruxism along with other significant confounding variables (**Chapter 6**). We were able to confirm that self-reported bruxism is associated with less prevalence of periodontitis, lower periodontal measures and is a relevant factor for periodontitis. Interestingly, ageing is a contributor factors towards this association between.

Lastly, considering the disturbing unawareness of the target population regarding periodontal disease, its risk factors and potential consequences for health, we intended to validate a short tool of illness perception, the Brief Illness Perception Questionnaire (Brief-IPQ) (**Chapter 7**). Globally, the Brief-IPQ showed validity and reliability for use among patients with PDs (Cronbach's alpha value of 0.80), being in agreement with the original Brief-IPQ study and within the range of similar chronic diseases researches. The factorial invariance concerning gender was also confirmed showing, therefore, that the Brief-IPQ is a reliable instrument to both genders, similarly to previous reports that have employed the IPQ-Revised questionnaire. This validated tool is independent of age and with potentially high interest because is short and easily applicable. Thus, this questionnaire can help dental professionals in realize patients' awareness of their periodontal status and aid during periodontal care, especially in Geriatric Periodontology where communication and comprehension may be challenging.

In conclusion, this Thesis confirmed a high prevalence of periodontal disease in a Portuguese elderly population of the Southern Lisbon Metropolitan Area. This elderly population showed alarming levels of missing teeth, reported faulty oral hygiene habits and an apparent association with self-reported bruxism. Also, self-reported xerostomia and stress were confirmed as important confounding variables to OHRQoL but the periodontal status itself did not, revealing lack of awareness, education for oral health, and ignorance with the potential consequences to oral and systemic health. Also, we validated an illness

perception tool with prospective usefulness in the Geriatric Periodontology daily practice and in Public Health programmes.

Keywords: Periodontal Disease, Periodontitis, Oral Health, Public Health, Epidemiology, Aged

Resumo

A doença periodontal é uma doença inflamatória, de causa polimicrobiana e induzida por biofilme presente ao redor dos dentes, que pode progredir de gengivite para a periodontite. A doença periodontal compreende duas das doenças mais prevalentes em todo o mundo – periodontite e gengivite – e contribui para o impacto global das doenças crónicas.

A esperança média de vida tem vindo a aumentar devido à evolução da Medicina, dos cuidados de saúde e do crescimento socioeconómico. A prevalência de periodontite em populações idosas constitui um importante desafio epidemiológico, já que as estimativas apresentadas nos últimos anos têm sido contraditórias, mesmo em países com padrões socioeconómicos semelhantes. A maioria dos fatores de risco sistémicos associados com periodontite estão aumentados, precisamente, em pessoas mais velhas, contribuindo para a complexidade da gestão e tratamento destes pacientes. Como consequência, é fundamental caracterizar melhor a saúde periodontal dos idosos, a fim de determinar estratégias preventivas, de diagnóstico e terapêuticas para promover a saúde periodontal.

Considerando as dificuldades epidemiológicas na deteção de doenças periodontais em idosos, vários estudos reportaram uma elevada prevalência de periodontite neste grupo de pessoas. Em Portugal, existe uma escassez de estudos epidemiológicos sobre o estado periodontal geriátrico. Além do seu impacto sistémico em idosos, a periodontite piora a qualidade de vida auto-reportada. Por outro lado, o impacto clínico da periodontite, como a falta de dentes e o uso de prótese dentária, tem um maior impacto na qualidade de vida relacionada com a saúde oral (QdVRSO) do que o próprio estado periodontal, principalmente porque estas populações-alvo, frequentemente, não são educadas para a existência da doença periodontal, mas estão familiarizadas para os efeitos colaterais. Por outro lado, o *stress* tem sido associado com a periodontite e a QdVRSO, e a xerostomia tem sido associada com pior QdVRSO. Assim, estudar outras características orais e como elas influenciam a QdVRSO poderia contribuir para clarificar o papel de outras possíveis variáveis de confusão, especialmente porque os níveis de *stress* e de xerostomia estão pouco investigados no contexto geriátrico.

Assim, o nosso principal objetivo foi investigar a prevalência da doença periodontal e seus fatores de risco numa população idosa portuguesa da Região Sul da Área Metropolitana de Lisboa.

Previamente ao desenvolvimento e implementação do estudo epidemiológico, realizámos uma série de investigações preliminares numa subpopulação portuguesa da Clínica Dentária Egas Moniz (EMDC) (**Capítulo 3**). Os resultados preliminares confirmaram a idade como um fator de risco importante da periodontite crónica nesta subpopulação (**Secção 3.2**). A análise multivariada mostrou que pacientes com 65 anos ou acima apresentaram 7,70 vezes maior risco de ter periodontite crónica, e esse resultado está em concordância com estudos anteriores. Além disso, o tabagismo foi o outro fator de risco principal, embora variáveis como obesidade e menor escolaridade justifiquem consideração futura apesar da não associação. Este grupo de idosos evidenciou níveis alarmantes de perda dentária e parâmetros periodontais deteriorados com elevada destruição periodontal. De seguida, devido à discrepância entre as estimativas nacionais da prevalência de periodontite em idosos e outros estudos epidemiológicos europeus de referência, avaliámos a magnitude de enviesamento, sensibilidade e especificidade de determinados protocolos parciais de registo periodontal (PRPs). Globalmente, o PRP utilizado no estudo nacional da Direção Geral de Saúde (DGS) demonstrou baixa sensibilidade, especificidade e concordância para estimar medidas clínicas periodontais (**Secção 3.3**). Consequentemente, os resultados apoiaram a decisão de se utilizar um exame circunferencial completo no estudo epidemiológico em desenvolvimento. Finalmente, investigámos a influência de fatores de risco conhecidos na resposta ao tratamento periodontal não cirúrgico (TPNC) usando um modelo linear multinível (MLM) ajustado para a profundidade da bolsa periodontal (**Secção 3.4**). Este estudo demonstrou que o tratamento da periodontite é eficaz e depende de muitos níveis, e isso pode ajudar a fortalecer as mensagens de promoção da saúde periodontal durante o estudo epidemiológico.

Por outro lado, realizámos uma revisão sistemática que determinou uma associação entre *stress* e periodontite, através da medição do cortisol salivar (**Secção 3.5**). Estes resultados sustentaram a decisão de incluir um questionário de auto-percepção de *stress* no estudo epidemiológico e considerá-lo na análise dos dados.

O *Study of Periodontal Health in Almada-Seixal* (SoPHiAS) revelou uma elevada prevalência de periodontite na população da Região Sul da Área Metropolitana de Lisboa, em Portugal (**Capítulo 4**). Idade, nível de escolaridade, tabagismo e diabetes mellitus foram identificados como fatores de risco significativos para a presença de periodontite, confirmando os resultados anteriores desta tese. Numa análise mais detalhada, o subgrupo de idosos apresentou níveis preocupantes de prevalência de doenças periodontais e relatou hábitos inadequados de higiene oral (**Capítulo 5**). Além disso, o número de dentes perdidos, a xerostomia e o *stress* auto-reportados encontravam-se fortemente associados, e confirmámos que a xerostomia e o *stress* auto-reportados demonstram associação com QdVRSO, com magnitude semelhante à extensão da periodontite. É importante salientar que a presença de periodontite não apresentou associação com a QdVRSO, mas sim algumas características clínicas, dentes perdidos ou uso de prótese dentária.

Não obstante, após a confirmação da associação do *stress* com a periodontite, admitimos a hipótese de poder existir uma associação entre o bruxismo (condição relacionada com o *stress*) e o estado periodontal. Para testar esta hipótese, usámos os dados da população do SoPHiAS relativamente ao estado periodontal e o bruxismo auto-reportado, juntamente com outras variáveis significativas (**Capítulo 6**). Foi possível confirmar que o bruxismo auto-reportado está associado com menor prevalência de periodontite, melhor estado periodontal e pode ser um fator relevante para a periodontite. Curiosamente, verificámos que esta associação é dependente do factor idade.

Por fim, considerando o preocupante desconhecimento da população-alvo em relação à doença periodontal, seus fatores de risco e possíveis consequências para a saúde, pretendemos validar o Brief Illness Perception Questionnaire (Brief-IPQ) (**Capítulo 7**), este que é um instrumento de percepção de doença. Globalmente, o Brief-IPQ apresentou validade e confiabilidade para ser utilizado em pacientes com doença periodontal (valor alfa de Cronbach de 0,80), estando de acordo com o estudo original do Brief-IPQ e com investigações semelhantes noutras doenças crónicas. A invariância fatorial em relação ao género também foi confirmada, mostrando que o Brief-IPQ é um instrumento confiável para ambos os sexos, à semelhança do questionário IPQ-Revised usados noutros estudos de validação. Esta ferramenta validada é independente da idade e tem um benefício potencialmente elevado, pois é curto e de fácil aplicabilidade.

Assim, este questionário pode ajudar os profissionais de Saúde Oral a compreender o estado periodontal dos pacientes e auxiliar durante os cuidados periodontais, especialmente na Periodontologia Geriátrica, onde a comunicação e a compreensão podem ser desafiantes.

Em suma, esta Tese confirmou uma elevada prevalência de doença periodontal numa população portuguesa idosa da Região Sul da Área Metropolitana de Lisboa. Esta população idosa mostrou níveis alarmantes de dentes perdidos, relatou maus hábitos de higiene oral e uma aparente associação da periodontite com bruxismo auto-reportado. Além disso, a xerostomia e o *stress* auto-reportados são factores que apresentam associação com a QdVRSO, apesar do estado periodontal *per se* não ter demonstrado importância, revelando falta de consciência, educação para a saúde oral e desconhecimento sobre as possíveis consequências para a saúde oral e sistémica. Além disso, validámos uma ferramenta de percepção da doença periodontal com eventual utilidade na prática clínica diária de Periodontologia Geriátrica e em futuros programas de Saúde Pública.

Palavras-chave: Doença Periodontal, Periodontite, Saúde Oral, Saúde Pública, Epidemiologia, Idoso

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Acronyms and Abbreviations

AAP – American Academy of Periodontology

ACES – Almada-Seixal Group of Health Centres

BMI – Body Mass Index

BoP – Bleeding on Probing

CAL – Clinical Attachment Loss

CFA – Confirmatory Factor Analysis

CI – Confidence Interval

CPITN – Community Periodontal Index of Treatment Needs

DGS – Direção Geral de Saúde

DM – Diabetes Mellitus

EFA – Exploratory Factor Analysis

EFP – European Federation of Periodontology

EMDC – Egas Moniz Dental Clinic

IPQ – Illness Perception Questionnaire

MLM – Multilevel Modelling

MMPs – Matrix Metallopeptidases

NHANES – National Health and Nutrition Examination Study

NSPT – Nonsurgical Periodontal Treatment

NUTS – Nomenclatura das Unidades Territoriais para Fins Estatísticos

OHRQoL – Oral Health-related Quality of Life

OR – Odds Ratio

PD – Probing Depth

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PRP – Partial Recording Protocols

QdVRSO – Qualidade de Vida Relacionada com a Saúde Oral

SHIP – Studies of Health in Pomerania

SoPHiAS – Study of Periodontal Health in Almada-Seixal

TPNC – Tratamento Periodontal Não-Cirúrgico

USA – United States of America

WHO – World Health Organization

Periodontal Health and Disease in the Elderly

1.1. Periodontium and Periodontal Health

The periodontium or 'the attachment apparatus" is an intricate set of supporting tissues surrounding the tooth. The periodontium encompasses the gingiva, the periodontal ligament, the cementum, and the alveolar bone proper (Figure 1.1). The foremost purpose of the periodontium is to attach the tooth to the bone tissue and, consequently, to ensure integrity of the masticatory mucosa's' surface of the oral cavity [1, 2].

The periodontium's vasculature is a major anatomical feature because is very rich in anastomoses and forms a polyhedral network surrounding the root [1, 3] (Figure 1.2). While the gingiva blood supply derives from supraperiosteal blood vessels, the periodontal ligament is vascularized through ranches from the alveolar bone vessels [4]. Markedly, the vascular network is of utmost importance because warrants the periodontium homeostasis and, consequently, a state of periodontal health.

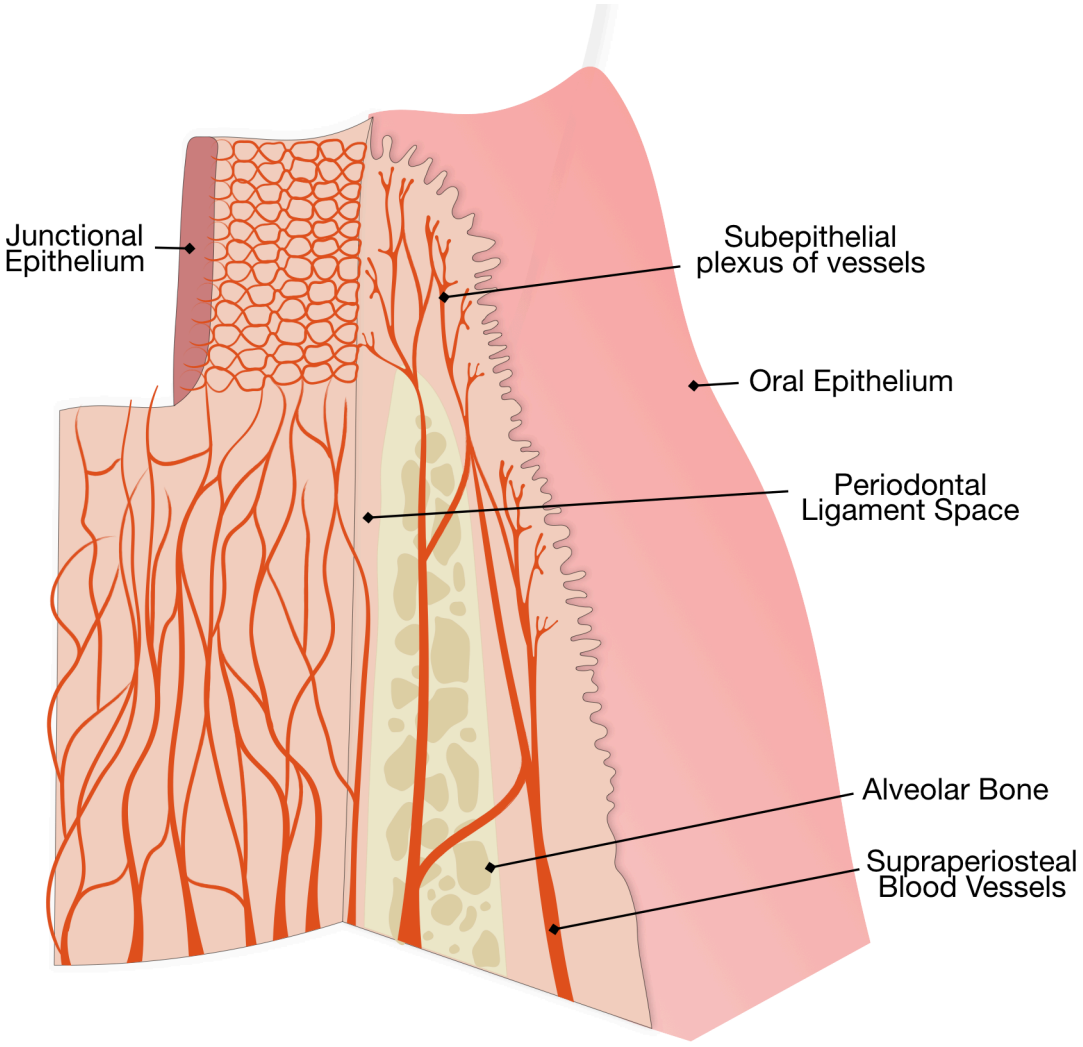

Figure 1.1: Diagram representing the vasculature of the periodontium. (Original image)

The definition of periodontal health remains highly debatable. Hitherto, the recent American Academy of Periodontology (AAP)/ European Federation of Periodontology (EFP) World workshop of 2017 [5] defined periodontal health in two main clinical situations: 1) intact periodontium, whether is pristine or clinically well-preserved; and 2) reduced periodontium, with stable periodontal disease or in remission/control. Interestingly, the main innovation is the notion of a past history of disease in the periodontium, while the main difference between disease stability and remission/control relies in the percentage of Bleeding on Probing (BoP) (Table 1.1).

Table 1.1. Plaque-associated periodontal diseases and clinical features.

	Intact Periodontium			Reduced Periodontium	
	Pristine Periodontal Health	Clinical Periodontal Health	Gingivitis	Periodontal Disease Stability	Periodontal Disease Remission/Control
BoP	No	No/Minimal	Yes	No/Minimal	Significantly reduced
Normal PD	Yes	Yes	Yes	No	No
Bone Loss	No	No	No	Yes	Yes
Modifying factors	Controlled	Controlled	May be present	Controlled	Not fully controlled
Predisposing factors	Controlled	Controlled	May be present	Controlled	Not fully controlled

BoP – Bleeding on Probing, PD – Pocket Depth. Adapted from Lang & Bartold et al. [5].

In any circumstances, early diagnosis, careful oral hygiene and preventive measures are the gold standard strategies to hamper periodontal loss or periodontal disease [6, 7].

1.2. Periodontal Disease

Periodontal disease represents a polymicrobial inflammatory and plaque-induced process of the periodontium that can progress from gingivitis to periodontitis, if not stopped [8–10] (Figure 1.2). Periodontal disease is one of the two most prevalent diseases worldwide and they contribute to the global burden of chronic diseases [11–14]. Conceptually, periodontal disease is often categorized in gingivitis and periodontitis.

Gingivitis is an inflammatory reaction by the gum often caused by dental biofilm without loss of periodontal attachment [15]. Regularly, gingivitis is pain-free, with rare spontaneous bleeding, subtle clinical changes, and patients are unaware of it [16–18].

Others, when this inflammation progresses further causes loss of connective tissue and alveolar bone, resulting in periodontitis [19, 20] (Figure 1.3). Uninterruptedly, there is the formation of crevices between the gingiva and tooth root, the so-called periodontal pocket [21]. The persistence of uncontrolled inflammatory reaction and poor oral health results in pain, discomfort, tooth mobility, impaired mastication and, indeed, tooth loss [22, 23].

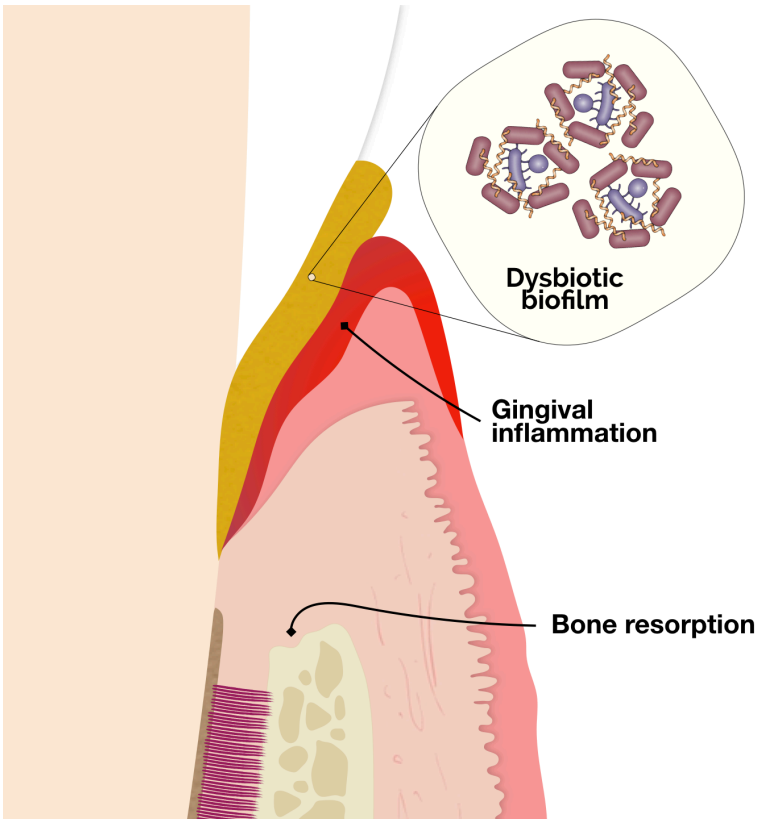

Figure 1.2. Illustration of tissue breakdown in periodontitis. (Original image)

In terms of progression, while gingivitis is a common clinical reaction that can occur throughout life, periodontitis typically starts in early adulthood or later and has a long-stand progression [24, 25].

Remarkably, periodontitis clinical features portray a complex cycle of active-inactive phases that had been long evidenced [26–28]. The disease-active phase depends on the individuals' risk factors such as smoking, alcohol consumption, diabetes or genetic factors [29]. As well, periodontitis activity depends on the immunity ability [30], dietary habits [31], herpesviruses activity [19] or oral self-care [32, 33].

In parallel, oral health-related quality of life (OHRQoL) measures have been progressively introduced in periodontal research. Nowadays, it is known that periodontal disease has a negative impact on OHRQoL [34–36]. Particularly, greater severity and extent of periodontal disease increases impairment [35]. Moreover, we demonstrated that nonsurgical periodontal treatment (NSPT) improves the OHRQoL of periodontitis patients until 3 months after therapy [37], attesting the conclusions of a previous systematic review [38].

The chronicity of periodontitis and its worldwide prevalence are of public concern. Chronic diseases rates are accelerating globally, with a major contribution from periodontitis [25, 39]. Further, periodontitis pandemic is, in some ways, even more worrying because of the close association with several systemic diseases through the rich vascularization of the periodontium [11, 23, 40–42]. Prominently, some of these illnesses affect elderly people, contributing to latent health complications.

1.3. Aging of the periodontium and 'immunosenescence'

1.3.1. Periodontium aging

A wealth of literature has emerged on the role of aging in both healthy and diseased periodontium, since age fosters inherent changes in this functional unit, the so-called age-related modifications [2].

As any tissue in our body, the periodontium undergoes histological age-related modifications. Recently, it was demonstrated that in healthful situations fibroblast density in the human periodontal ligament decreases with age [43],

and the cementum hardness increases with age [44]. Also, a pre-clinical study reported that aging promotes a decline in collagen quality and quantity and bone resorption is increased [45]. All in all, these changes demonstrate the aging effect on periodontal tissues whose clinical impact remains contested.

These age-related modifications are not directly related to the loss of probing attachment or alveolar bone, and research has shown that the absence of plaque ensures the maintenance of sound periodontal tissues throughout life [46–48]. Comprehensively, periodontitis is not a natural result of aging [49] and has been proven accordingly [46, 49, 50].

Nevertheless, the progression of periodontitis is frequently intermittent and slow-paced in the elderly, mainly attributed to the immunosenescence process [51]. Notwithstanding, there are important external factors that contribute to the periodontium clinical shortness, for instance traumatic tooth brushing, tooth malposition, parafunctional habits or excessive occlusal forces [52, 53].

1.3.2. Immunosenescence of the Periodontiium

In a physiologic perspective, the immune system is composed by the innate and adaptive systems. The innate immune system is based on a nonspecific response to a harmful invasion, i.e., it engages an immediate action but not continuing defensive immunity. The inflammatory response is a mechanism of the innate immune system and can be acute or chronic. Still, the adaptive immune system identifies the harmful agent and encompasses cells and molecules ensuring “immune memory” to prevent potential establishment, reproduction and tissue damages by the infection.

Immunosenescence states for progressive changes that occur with aging in the immune system mainly due to continued antigenic stimulation and/or stress responses across lifespan [54, 55]. This on-going deterioration makes elderly individuals more susceptible to infections and is related to inflammatory age-related diseases [56]. Additionally, several lines of evidence suggest an impact of microbiome on immunosenescence, in particular gut dysbiosis [57].

The effect of immunosenescence in both immune systems have been widely investigated. Regarding the innate system, it becomes dysregulated with persistent inflammatory actions, decreased innate cell function, and an age-related basal inflammation that might affect chronic inflammatory diseases,

such as atherosclerosis, Alzheimer's disease or periodontitis [58–61]. Also, these general changes in the innate immune system impairs the induction of the adaptive immune system [59]. In the periodontium, there are important changes underlined [61]: 1) the chemotaxis of innate immune cells is significantly impaired, altering the initiation and/or the resolution of an inflammatory process [59, 62]; 2) decreased phagocytosis activity [63–69]; 3) decrease of antigen-presenting function [59, 70, 71]; and 4) decreased neutrophils ability to kill pathogens [72, 73] (Figure 1.3).

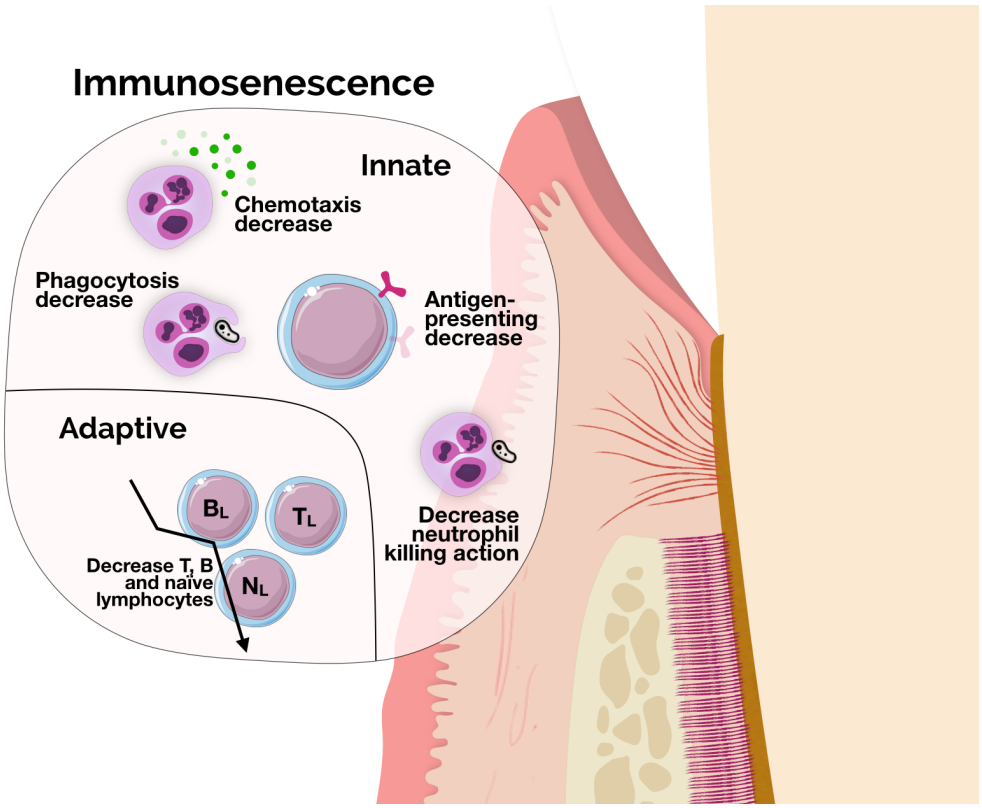

Figure 1.3. Diagram of immunosenescence in the periodontium. (Original image)

While for the adaptive immunity, the main change relies in the decrease of naïve lymphocytes as a result of a reduction in T-cells and progenitor B-cells, causing a drop of antigen-specific immunity [74, 75] (Figure 1.4). These lymphocytes have been suggested as key for homeostasis maintenance between the bacterial plaque and the periodontium [76]. Nevertheless, the humoral immune responses to oral bacteria is generally unaffected by age, but noticeably are linked to the extent of periodontitis [77].

Considering these inherent modifications, aging makes elder people more susceptible to periodontitis although with particular hallmarks. Therefore, periodontitis is an age-related disease. Also, in some situations, this inflammatory reaction can be delayed based on the described decreased of immunity ability. Further, the decrease in the rate of naïve and specific lymphocytes impairs the ability to hold subgingival infection locally in older individuals, leading to probable systemic repercussions.

All in all, these changes may explain the disturbingly high prevalence of periodontal disease among elder populations. Moreover, it contributes to the complex interaction of immunosenescence with a prominent proinflammatory status of the patients, also called 'inflammaging'.

1.3.3. Inflammaging

It has long been known that periodontal tissues changes are caused by inflammation and/or the result of aging [46, 61, 78].

In the beginning of the XXI century, it was proposed the term 'inflammaging' [79]. Inflammaging refers to the long-term result of chronic physiological stimulation of the innate immune system [80] and has been proposed as a "trigger" process for multiple diseases [81].

Theoretically, inflammaging has been proposed to periodontitis [61, 77, 78] and is not surprising that such association can greatly enlighten its pathophysiology relatively to aging [77, 82]. In fact, periodontitis lesions are the result of an immune system action against a complex polymicrobial plaque, whose progression depends on multiple factors of which the immune competence of the individual. Besides, periodontitis is cumulative [53], i.e., is a progressive disease with successive multiple events.

Certainly, aging alone might lead to physiological loss of periodontal tissues and alveolar bone, but without inflammation this loss poses with minor clinical significance. Only the presence of inflammation can promote an exacerbated reaction from the immune system and, eventually, leads to loss of function.

1.3.4. Subgingival microbiota of the aging mouth

A comprehensive investigation was made using data from two centres, the Center for Clinical Research at Guarulhos University (São Paulo, Brazil) and The Forsyth Institute (Cambridge, United States of America [USA]) [83]. From periods longer than 10 years, the authors aimed to study the periodontal microbiota evolution throughout life by means of DNA count of 40 bacterial species.

Overall, the results of this multicentre study revealed that subgingival microbiota composition is not age-related and, therefore, age is not a determinant of the oral microbiome [83]. Expectedly and regardless of age, individuals with most severe periodontitis had significant higher levels of *Tannerella forsythia*, *Porphyromonas gingivalis* e *Treponema denticola*, pathogens that compose the red complex [83]. Besides, a previous investigation also demonstrated this relative microbial community stability across lifespan, though it was evidenced that human microbiota can rapidly and greatly change because of the host lifestyle, for instance travelling to developing countries or enteric infections [84].

1.4. Geriatric Periodontology

Although proposed in 1984 [85], 'Geriatric Periodontology' was recently suggested as a specialty that will invariably emerge soon [86]. With the world population aging rapidly [87], is expected that dental care providers will have to develop competences to treat older adults.

Most geriatric patients include age-related decays that difficult coping mechanisms with stressors, also called frailty syndrome [88]. It is important to highlight that frailty syndrome represents intellectual and physical instabilities that predisposes individuals to more risks [88]. Thereby, elders tend to have higher risk behaviours such as inefficient oral hygiene, difficulties accessing dental care, intolerance to dental services and unhealthy dietary habits [89]. All these aspects considered expose elders to an even higher risk for periodontitis and its complications, plus immunosenescence and inflammaging processes.

Importantly, it is consistently upheld that the number of teeth disturbs longevity and life expectancy in humans [90, 91], through some important longitudinal studies, for instance the Baltimore Longitudinal Study of Aging (USA) [92], the Atherosclerosis Risk in Communities Study (USA) [93], the Örebro and

Östergötland counties Study (Sweden) [94] and the National FINRISK 1997 Study (Finland) [95]. In other words, these investigations proved people with more preserved teeth are more associated with the longer life expectancy. Since periodontitis is one of the biggest causes of tooth loss, we can extrapolate that Geriatric Periodontology will play a key role to prevent tooth loss and, possibly, contributing to greater longevity. Nevertheless, little is known about the periodontal characteristics of elders in the Portuguese population, making this matter paramount.

1.4.1. Epidemiological Periodontal Data in the Elderly

The world population is rapidly aging due to medical progression, healthcare, and socioeconomic growth [84]. The prevalence of periodontal disease among older people endures a substantial epidemiological challenge, while estimates presented in recent years have been very dissimilar, even in countries with alike socio-economically standards [96]. Moreover, the majority of systemic risk factors associated with periodontal diseases are factually increased in older populations, which contributes to the complexity of these patient's treatment and management [97, 98]. As a consequence, it is imperative to better characterize periodontal health among elder people in order to determine preventive, diagnostic and therapeutic framework strategies to promote health [12, 99].

In the National Health and Nutrition Examination Study (NHANES) 2009–2012 (USA), 68.0% of the population ≥ 65 years had periodontitis [100]. In this elderly subset, women more cases of no periodontitis than men (29.5% vs. 19.8%, respectively). Further, almost half of this sample had a periodontal probing depth ≥ 4 mm.

In Europe, some studies have showed interest in the periodontal state of their elderly populations. Krustrup and Petersen [101] reported 93.1% of periodontitis prevalence among Danish individuals with 65-74 years old. Schützhold et al. [102] from the Studies of Health in Pomerania (SHIP) group compared the changes across time in Germany with a national survey, and have concluded that despite the improvement in attachment loss levels, periodontal pocking depths remained the same and higher in the 65-74 years old group. A cross-sectional study in Turin, North Italy, also revealed 87.4% prevalence estimation for the 60-

75 years' group [103]. On the other hand, the study Tromstannen – Oral Health in Northern Norway reported a periodontitis prevalence of 81.3% among the 65-79 years of age group [104].

Currently, there is an insufficiency of periodontal epidemiological data for the Portuguese elderly population. The Portuguese Health General Directorate (DGS) has estimated a prevalence of 15.3% of periodontal diseases in an elderly range (65-74 years old) [105], despite being a clearly optimistic value comparing to other developed countries prevalence. Considering this, the bias effect of the community periodontal index of treatment needs (CPITN), used in the DGS study, and other partial periodontal strategies would be of interest to understand why such low levels of periodontal disease in the Portuguese elderly population were reported.

1.4.2. Periodontitis and risk factors in Elders

The relationship between periodontitis and systemic health can be focussed in the likelihood of a bidirectional association, i.e., periodontitis influences systemic status and vice-versa. For the past decades, a body of literature have evidenced the association of periodontitis with several diseases, such as diabetes, atherosclerotic cardiovascular disease, respiratory diseases, chronic kidney disease, rheumatoid arthritis or cancer, and others [40]. Importantly, all these illnesses have high prevalence and burden among elderly populations [96, 106]. Therefore, because of the inherent immunosenescence (addressed in section 1.3.2) and greater frailty of older people, elders might be more disposed to periodontal disease and to its systemic effects.

Presently, diabetes mellitus (DM) and periodontitis have a very well established two-way relationship [107–109]. According to the World Health Organization (WHO), 422 million adults had diabetes in 2016, a higher prevalence among elderly populations, and with age being an important risk factor [110]. The main explanation for this relationship relies on the inflammatory interplay. While periodontitis leads to an inflammatory environment that disturbs serum glycated haemoglobin (Hba1c) levels of diabetic patients, diabetes itself causes a dysregulation of the inflammatory response and the increase of pro-inflammatory cytokines and matrix metallopeptidases (MMPs) in the periodontium [111]. Therefore, a poor controlled diabetic individual might have

higher levels of periodontal destruction and, consequently, a difficult diabetic status to control.

Nonetheless, a 2015 Cochrane systematic review concluded that NSPT has short-term effects on metabolic control in patients with type 2 DM [112]. Further, a recent trial, with longer follow-up and sample size, evidenced that routine oral health assessment and NSPT might be significant for successful glycaemic control of type 2 DM [113]. Also, the 2017 World Workshop has established, for the first time, diabetes as a modifier risk factor for the periodontitis grade case definition considering serum Hba1c values [114].

Another well-known risk factor towards periodontitis is smoking [29]. In fact, smoking greatly increases the risk of developing periodontitis [115, 116] and periodontal disease severity increases with the number of pack-years smoked [117]. Still, the mechanism by which tobacco influences the periodontal tissues is still not fully understood, although it has been demonstrated that nicotine plays a weakening role in the immune system as well as other toxins embodied inside the cigarette [118, 119]. Likewise, electronic cigarettes have also been pointed as a risk factor for periodontitis with harmful effects, although there is still much to be explored [120, 121].

Newly, a report from the Niigata Elderly Study investigated the impact of lifestyle (expressed as smoking status, physical activity, body mass index [BMI] and diet) in the incident or progression of periodontitis and tooth loss in an elderly Japanese population [122]. Over six years, a healthy lifestyle (i.e., not smoking, good physical activity level, healthy BMI, and high-quality diet) was associated with a lower risk of incidence or progression of periodontitis and tooth loss [122]. These results highlighted that positively controlling some modifying risk factors of periodontitis eventually improve quality of life.

Notwithstanding, DM and smoking habits are two lifelong risk factors, affecting adults as they affect adults regardless of age. Afterward, we must not forget that with aging these factors may become more relevant, just as they were accounted as two key risk factors to the latest grading diagnostic system [114].

Despite these two key risk factors, Persson [106] has summarized how particular systemic diseases can be a complication in periodontal conditions with increasing age, such as cardiovascular disease, osteoporosis, rheumatoid arthritis.

1.4.3. Periodontitis impact on Oral-Health Related Quality of Life in Elders

Currently, it is well-established that periodontitis impacts the Oral-Health Related Quality of Life (OHRQoL) of patients aged 18 years old or older [35]. Two systematic reviews has addressed whether periodontitis is really silent for patients and how much impact the quality of life, since its progression is often slow-paced and symptomless [35, 36]. In fact, periodontitis negatively influences the OHRQoL perception of patients mainly because of the long-term clinical consequences, such as tooth loss, tooth mobility and loss of tissues that hinder oral hygiene [35]. However, the impact of periodontitis in the OHRQoL of elder populations is still poorly studied and only recently has gain some research interest.

Some studies on elder populations have reported that periodontitis is associated with worse perceived quality of life [123, 124] though the contrary had been showed [125]. Remarkably, these studies show that the clinical burden of periodontitis, as missing teeth and denture use, have a higher impact on OHRQoL than the periodontal status itself [123, 126]. The fact that periodontitis symptoms influence less than its clinical consequences is not eccentric, mainly because this target populations are frequently uneducated for periodontitis but are familiarized with side issues as teeth loss or denture use. Therefore, studying other oral features and how they influence OHRQoL could improve our knowledge and clarify potential confounding variables. For instance, stress has been linked to both periodontitis and OHRQoL [127, 128], and xerostomia has been associated to poorer quality of life [129, 130]. The hypothesis is that these confounders together with periodontitis could influence the quality of life of elders, and this has never been investigated.

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Aims

In the introductory section (***1. Periodontal Health and Disease***), a review literature made it clear the pandemic pattern of periodontal disease with manifest burden to the systemic health, quality of life and socioeconomic standards. Is of the utmost importance to characterize the periodontal conditions among all populations, in particular the elderly ones which have a disturbing prevalence. This characterization will bridge stronger preventive, diagnostic and therapeutic framework strategies to promote health. Also, there is lack of appropriate epidemiological data for the Portuguese elderly population.

Ergo, our main purpose was to investigate the prevalence of periodontal disease and its risk factors in a Portuguese elderly population of the Southern Lisbon Metropolitan Area.

To achieve this purpose, our specific aims were divided in two stages:

MILESTONE 1: Studies prior to the Epidemiologic Survey

1. To investigate the periodontal characteristics of periodontitis patients from the Egas Moniz Dental Clinic, its risk factors and the efficacy of nonsurgical periodontal treatment (Paper I, II and III).
2. To perform a systematic review studying the association between salivary cortisol levels and periodontitis (Paper IV).

MILESTONE 2: Epidemiologic Survey

3. To conduct a large-based epidemiologic survey assessing the periodontal status of elders from the health centres of the Almada-Seixal Group of Health Centres (ACES) and its association with sociodemographic and medical characteristics (Paper V).
4. To investigate the impact of periodontitis and other relevant confounding variables on oral-health related quality of life in this specific elder population (Paper VI).
5. To investigate the association of self-reported bruxism and the periodontal condition (Paper VII).
6. To investigate the psychometric properties of the validated Portuguese version of the Brief Illness Perception Questionnaire (Brief-IPQ) in patients with gingivitis and periodontitis (Paper VIII).

3 Periodontal patients at Egas Moniz Dental Clinic, the efficacy of nonsurgical therapy and stress as a risk factor for periodontitis

This chapter was based from the published work:

Paper I *– Machado V, Botelho J, Amaral A, Proença L, Alves R, Rua J, Cavacas MA, Delgado AS, Mendes JJ. Prevalence and extent of chronic periodontitis and its risk factors in a Portuguese subpopulation: a retrospective cross-sectional study and analysis of Clinical Attachment Loss. PeerJ* ***2018*** *6, e5258. Doi:10.7717/peerj.5258.*

Paper II *– Machado V, Botelho J, Mascarenhas P, Cavacas MA, Alves R, Mendes JJ. Partial recording protocols performance on the assessment of periodontitis severity and extent: bias magnitudes, sensibility, and specificity. Revista Portuguesa de Estomatologia, Medicina Dentária e Cirurgia Maxilofacial,* ***2018****, 59, 145-153. Doi: 10.24873/j.rpemd.2018.11.239*

Paper III *– Botelho J, Machado V, Mascarenhas P, Alves R, Cavacas MA, Mendes JJ. Fine-tuning multilevel modeling of risk factors associated with nonsurgical periodontal treatment outcome. Brazilian Oral Research* ***2019****, 33, e081. Doi: 10.1590/1807-3107bor-2019.vol33.0081*

Paper IV *– Botelho J, Machado V, Mascarenhas P, Rua J, Alves R, Cavacas MA, Delgado AS, Mendes JJ. Stress, Salivary Cortisol and Periodontitis: A Systematic Review and Meta-analysis of Observational Studies. Archives of Oral Biology* ***2019****, 96, 58-65. Doi: 10.1016/j.archoralbio.2018.08.016.*

These papers have Copyright Permissions appended as Annexes 2-5.

3.1. Background

Previous to the epidemiologic survey design and implementation, we conducted a series of preliminary investigations in a Portuguese subpopulation of the Egas Moniz Dental Clinic (EMDC). The EMDC is a university clinic, located in the municipality of Almada, in Setúbal Peninsula (a NUTS III subregion, part of NUTS II Lisbon Region). This Clinic provides dental health services to the general public. This subpopulation study was from a suburban area of the Lisbon Region and was forwarded to a periodontal examination, after an initial triage at EMDC.

The first output (Paper I – Section 3.2) yielded the prevalence, severity, and extent of chronic periodontitis, and its association with sociodemographic, behavioral and environmental risk factors. All in all, the results confirmed our expectations, that age was a major risk factor for chronic periodontitis in this subpopulation. Multivariate analysis showed that elderly patients with 65 years old or older presented an Odds Ratio (OR) of 8.70 towards chronic periodontitis, and this result was in line with previous studies [1-5]. Furthermore, smoking was the other prime risk factor, although variables such as obesity and lower educational level warranted consideration.

Moreover, these results evidenced disturbing values of tooth loss and periodontal parameters, which revealed elevated periodontal destruction. These results made clear the need for a large-scale and representative study of the population to understand the prevalence of periodontal disease and its impact on patients' quality of life. Also, the perception of periodontal disease would have to take into account, because 69% of patients failed the periodontal visit despite triage referral.

Continuingly, our second output (Paper II – Section 3.3) intended to evaluate bias magnitudes, sensibility, and specificity of particular partial-mouth recording protocols (PRPs) to estimate periodontal clinical measurements and periodontal status. The main reason for this study was the discrepancy between national estimates for periodontitis prevalence, provided by DGS [6], and other European epidemiologic studies of reference. The reason for such discrepancy might be linked to the PRP used in the DGS study of 2015. Therefore, and to prove this hypothesis, we compared fifteen different PRPs with full-mouth gold standard through periodontal data sourced from Paper I.

Our findings suggested three potential half-mouth PRPs with high sensitivity, specificity, and concordance to estimate periodontal clinical measurements, Probing Depth (PD) and Clinical Attachment Loss (CAL), however with unpleasant bias for epidemiologic purposes. Nevertheless, all full-mouth PRPs failed to estimate pocket depth and clinical attachment loss means, presenting less ability then half-mouth partial protocols. Consequently, the results reinforced the decision to undergo a standard full-mouth circumferential examination in our epidemiologic study.

Interestingly, the CPITN, used in the DGS study [6], displayed substantial variations and the higher bias outcome of all PRPs. Thus, we confirmed our hypothesis that the DGS (2015) results are underestimating the prevalence of periodontitis, and attested the necessity of new studies with improved approaches.

Finally, the abovementioned negligent behaviour towards periodontitis by the patients, by missing or unbooking the periodontal visit despite knowing they might be unhealthy, raised the hypothesis that patients might not believe in the efficacy of its treatment.

Consequently, our third study (Paper III – Section 3.4) aimed to investigate the influence of known risk factors on nonsurgical periodontal treatment (NSPT) response using a pocket depth fine-tuning multilevel linear model (MLM). This study hypothesized that PD and CAL reduction were affected by patient, tooth, and site-level factors after NSPT. The main reason to perform this study was to produce evidence that treatment of periodontitis is effective and depends on many levels, and to empower health-promoting messages during the cross-sectional study.

On the other hand, we have considered stress as a risk factor for periodontitis because the mechanisms supporting this link have been considerably yielded [7]. Periodontal disease and psychological factors are associated [8], and Genco et al. [9] found increased salivary cortisol levels in patients exhibiting severe periodontitis. Hence, we intended to conduct a systematic review to determine if there is an association between salivary cortisol levels and periodontitis and whether salivary cortisol levels are associated with periodontitis severity (Paper IV – Section 3.5). If such link was demonstrated in an evidence-manner, we might include validated stress questionnaires in the epidemiologic study and consider it in data analysis.

3.1. References

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3.2. Prevalence and extent of chronic periodontitis and its risk factors in a Portuguese subpopulation: a retrospective cross-sectional study and analysis of Clinical Attachment Loss

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Abstract

Objectives. To assess the prevalence and extent of chronic periodontitis, and its risk factors in a Portuguese subpopulation referred to periodontal examination.

Methods. This retrospective cross-sectional study used a subset of data from patients who sought dental treatment in a university dental clinic in the Lisbon metropolitan area. The sample consisted of 405 individuals (225 females/180 males), aged 20-90 years. All patients underwent a full-mouth periodontal examination and chronic periodontitis was defined as Clinical Attachment Loss (CAL) ≥ 3 mm affecting two or more teeth. Aggressive periodontitis cases were excluded from the analysis.

Results. Prevalence of chronic periodontitis was 83.5% (95% CI [80.4-86.6%]). For these subjects, CAL≥3 mm affected 86.0% (95% CI [84.7-87.2]) of sites and 83.7% (95% CI [81.7-85.6]) of teeth, respectively. Mean CAL ranged from 3.6 to 4.3 mm, according to age. In the multivariate logistic regression model, smoking (OR = 3.55, 95% CI [1.80-7.02]) and older age (OR=8.70, 95% CI [3.66-20.69] and

OR=4.85, 95% CI [2.57-9.16]), for 65+ and 45-64 years old, respectively, were identified as risk indicators for CAL ≥ 3mm.

Conclusions. This particular Portuguese adult subpopulation had a high prevalence of chronic periodontitis, with severe and generalized clinical attachment loss, and its presence was significantly associated with age and smoking. This data should serve to prepare future detailed epidemiological studies and appropriate public health programs.

3.2.1. Introduction

Chronic periodontitis is an inflammatory disease characterized by a polymicrobial breakdown of host homeostasis and a progressive destruction of tooth-supporting structures [1,2], and its epidemiology and risk factors have been broadly studied [3–6].

Periodontal diseases have a significant impact on oral health-related quality of life, especially with the worsening and extension of the disease in which it presents higher destructive consequences [7]. There are important risk factors/indicators for periodontal disease such as alcohol,[8] overweight and obesity [9], smoking [6] and diabetes [10]. Also, periodontitis can be a risk factor for several systemic diseases [10–18].

Some European epidemiological studies have demonstrated the high prevalence of periodontitis among the populations [19–23]. However, data on the prevalence and risk factors for periodontal disease in the Portuguese population are still missing. According to the latest Portuguese Oral National Health Survey, the prevalence of periodontitis was 10.8% in adults and 15.3% in the elderly [24]. This nationwide survey used the Community Periodontal Index (CPI), with its recognized limitations. To the best of our knowledge, there are no epidemiological studies that used full-mouth periodontal examination (FMEP) methodology to estimate the prevalence of periodontitis regarding Portuguese samples.

The aim of this study was to assess the prevalence, severity, and extent of chronic periodontitis through the full-mouth examination of CAL, and its association with sociodemographic, behavioral and environmental risk factors, in a Portuguese adult subpopulation, of a suburban area of the Lisbon Region, forwarded to periodontal examination.

3.2.2. Material and Methods

The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2013, and approved by the Ethics Committee of Egas Moniz (Ethical Application Ref: 595). A written informed consent was obtained from all participants during the first appointment. After the examination, the participants were informed of their periodontal status, and those with diagnosed periodontal diseases were advised to follow the proper treatment. This protocol followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [25].

Study subjects

All participants were patients of Egas Moniz Dental Clinic (Almada, Portugal). This university clinic, located in the municipality of Almada, in Setúbal Peninsula (a NUTS III subregion, part of NUTS II Lisbon Region), provides dental health services to the general public.

At the first appointment, patients were submitted to a dental triage protocol, with the application of a self-reported health questionnaire and oral and dental examinations, to guide their treatment needs. Regarding periodontal triage, patients were assessed using the Periodontal Screening and Recording (PSR) procedure [131], and, if diagnosed with code 2 or higher, they were forwarded to a periodontology appointment.

Patient selection

This retrospective cross-sectional study analyzed patients who attended the dental clinic between September 2015 and March 2017. From a total of 3648 subjects who sought the first consultation in the university dental clinic during that period, 1501 (41%) patients were referred to the periodontology department, based on their triage status. From these, 459 attended a periodontal consultation and were considered for this study. Fifty-two participants were excluded due to incomplete questionnaires and periodontal data, and two subjects diagnosed with aggressive periodontitis. Hence, a final

sample size of 405 subjects was obtained (11% of the total, 27% of the patients forwarded for periodontal treatment).

Health questionnaire

Before clinical examinations, all patients answered a general and oral health questionnaire that included information such as age, gender, educational level, employment status, general medical history and medication, smoking status and oral hygiene habits.

Periodontal status

Five well-trained and calibrated periodontists (R.A., J.C., C.I., F.J., L.A.) performed all dental and periodontal examinations. Periodontal examinations were performed using CDC/AAP full-mouth periodontal examination (FMEP) methodology [27]. We defined chronic periodontitis as CAL≥3 mm affecting two or more teeth [28]. All permanent fully erupted teeth were examined, excluding third molars, retained roots, and implants. The evaluated parameters were: missing teeth, presence or absence of supragingival biofilm (SB), probing depth (PD), bleeding on probing (BOP), gingival recession (REC) and clinical attachment loss (CAL). SB and BOP were scored on four surfaces of each tooth (mesial, distal, buccal and lingual). At six sites per tooth (mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual and distolingual), PD was measured as the distance from the cementoenamel junction (CEJ) to the bottom of the pocket and REC as the distance from the CEJ to the free gingival margin, and this assessment was assigned a negative sign if the gingival margin was located coronally to the CEJ. CAL was calculated as the algebraic sum of PD and REC. It was used a CP-12 SE (Hu-Friedy, Chicago, IL, USA).

Measurement reproducibility

Prior to the initiation of the study, all examiners were submitted to theoretical and practical training in a total of ten volunteer non-study patients suffering from moderate to severe periodontitis. The inter-examiner correlation coefficients, at subject level, ranged from 0.76 to 0.97 and between 0.91 and 0.99, for mean PD and mean CAL, respectively.

Covariates

Sociodemographic variables and several periodontal disease risk factors were selected as confounding variables. The selected variables were: age, gender, educational level, employment status, smoking status, Body Mass Index (BMI), time elapsed since the last dental appointment, consultation motive and oral hygiene habits.

Educational level was assessed as three categories: elementary (1-4 years), middle (5-12 years) and higher (> 12 years) education. Employment status of each participant was classified as: employed, unemployed or retired. Smoking status was defined as non-smoker or smoker. Active smokers were further divided into three categories: light smokers (< 10 cigarettes per day), medium smokers (10-20), heavy smokers (> 20). The height of the participants was measured in centimeters, using a hard ruler installed vertically and secured with a stable base. Weight was assessed in kilograms using mechanical scales. BMI was calculated as the ratio of the individual's' body weight to the square of their height. Four BMI categories were defined using WHO criteria [29]: underweight (BMI < 18.5 kg/m2), normal weight (BMI 18.5 - 24.9 kg/m2), overweight (BMI 25 - 29.9 kg/m2) and obese (BMI ≥ 30 kg/m2). The time elapsed since last dental consult was classified into five categories (never visited, less than one year, 1-2 years, 3-4 years, 5 years or over). Consultation motives were classified as routine, aesthetics, pain, functional or other. Oral hygiene habits were assessed by information on toothbrush frequency (2-3 times / daily, one time daily, 2-6 times/weekly) and dental floss use.

Data analysis

Data analysis was performed using IBM SPSS Statistics version 24.0 for Windows (IBM Corp., Armonk, NY, USA). Descriptive and inferential statistics methodologies were applied. In the latter, Mann-Whitney and Kruskal-Wallis tests were used to compare the clinical data as a function of the sociodemographic variables. Further, logistic regression analysis was used to model the relationship between chronic periodontitis and several risk indicators. Preliminary analyses were performed using univariate models. Next, a multivariate model was constructed for the outcome variable CAL ≥ 3 mm. Only

variables showing a significance $p \leq 0.25$ in the univariate model were included in the multivariate stepwise procedure. Predictor variables considered in this procedure were: age (years), smoking status, education (years), employment status, last dental visit and dental floss use. The contribution of each variable to the model was evaluated by Wald statistics. Interactions were also analyzed for all tested variables. The final reduced model was obtained with the following predictor variable categories: age (45-64 and ≥65 years) and smoking status (smoker). Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for both univariate and multivariate analyses. The level of statistical significance was set at 5%.

3.2.3. Results

Table 3.2.1 shows the distribution of sociodemographic, behavioral, biometric and oral hygiene data in the studied sample. Ages ranged from 20 to 90 years. The sample had 55.6% of female patients. It is worth to mention that 65.2% of subjects did not smoke and active smokers were mainly medium smokers (66%), followed by light smokers (29%) and heavy smokers (5%). Regarding education and employment status, 77.1% of subjects had elementary or middle education, and 51.9% of the subjects were employed. Approximately 59% were overweight and obese, and only 40% had normal values. Interestingly, 53.1% had a period of over one year without any dental visit and 1.2% never had a dental appointment, whereas functional complaint was the major consultation motive.

Table 3.2.2 shows the periodontal data of this sample according to age, gender, and smoking status. Subjects over 65 years of age had a significantly higher mean number of missing teeth and, in total, this subpopulation presented a mean loss of 8 teeth. Younger individuals (<45 years of age) presented a significantly lower mean number of missing teeth, PD, REC, furcation lesions and teeth with mobility compared to older subjects. Male patients presented a significantly higher mean PD, deep periodontal pockets (≥5 mm) and teeth with furcation lesions than female. Compared to smokers, non-smokers had lower mean SB, PD and CAL, and less deep periodontal pockets.

Table 3.2.1. Sociodemographic, behavioural, biometric and oral hygiene data (n=405).

Variable		n (%)
Gender	Female	225 (55.6)
	Male	180 (44.4)
Age (years)	20–44	90 (22.2)
	45–64	217 (53.6)
	≥65	98 (24.2)
Smoking status	Smoker	141 (34.8)
	Non-smoker	264 (65.2)
Active smokers (cigarettes per day) (n = 141)	Light (<10)	41 (29.1)
	Medium (10–20)	93 (66.0)
	Heavy (>20)	7 (5.0)
Education	Elementary	157 (38.8)
	Middle	155 (38.3)
	Higher	93 (23.0)
Employment status	Employed	210 (51.9)
	Unemployed	63 (15.6)
	Retired	132 (32.6)
BMI (kg/m2)	<18.5	5 (1.2)
	18.5–24.9	162 (40.0)
	25.0–29.9	159 (39.3)
	≥30	79 (19.5)
Last dental visit	<1 year	185 (45.7)
	1–2 years	57 (14.1)
	3–4 years	75 (18.5)
	≥5 years	83 (20.5)
	Never	5 (1.2)
Consultation motive	Routine	125 (30.9)
	Aesthetics	35 (8.6)
	Pain	73 (18.0)
	Functional	157 (38.8)
	Other	15 (3.7)
Dental floss usage	Yes	141 (34.8)
	No	264 (65.2)
Toothbrush frequency	2–3 times/daily	313 (77.3)
	1 time/daily	75 (18.5)

Variable		n (%)
	2-6 times/weekly	17 (4.2)

BMI (kg/m^2): Body Mass Index (kilogram/meter^2)

Chronic periodontitis was diagnosed in 83.5% of the patients (Table 3.2.3), and subjects with chronic periodontitis had CAL≥3 mm, ≥4 mm, ≥5 mm, ≥6 mm and ≥7 mm affecting, on average, 83.7%, 54.4%, 32.1%, 17.8% and 9.2% of their teeth, respectively (Table 3.2.4). Besides, the first lower molar was the most frequently missing tooth, while the lower canine was the least lost but the most severely affected tooth (Fig. 3.2.1).

In the logistic regression analysis, similar results were observed in the univariable (Table 3.2.5) and multivariable models (Table 3.2.6). In the multivariable analysis, smoking (OR=3.55, 95% CI [1.80-7.02]) and older age (OR=8.70, 95% CI [3.66-20.69] and OR=4.85, 95% CI [2.57-9.16]), for 65C and 45-64 years old, respectively, were identified as risk indicators for CAL≥3 mm (Table 3.2.5). Chronic periodontitis was not significantly associated with the remaining variables.

Table 3.2.2. Periodontal clinical data (presented as mean ± standard deviation) as a function of gender, age and smoking status (n=405). (Note: table was divided to fit within the page, and the below part is the continuity of the table).

		SB (%)	BOP (%)	PD (mm)
Gender	Female	34.6 ± 22.8	9.1 ± 12.6	3.1 ± 0.7*
	Male	37.4 ± 23.6	11.3 ± 15.7	3.3 ± 0.8*
Age (years)	20–44	33.3 ± 20.9	9.9 ± 12.3	3.1 ± 0.7
	45–64	35.4 ± 23.5	10.1 ± 14.6	3.3 ± 0.8
	≥65	39.0 ± 24.1	10.1 ± 14.5	3.1 ± 0.7
Smoking status	Smoker	38.7 ± 23.9*	8.6 ± 14.2	3.4 ± 0.8*
	Non-smoker	34.3 ± 22.6*	10.8 ± 13.9	3.1 ± 0.7*
Total		35.8 ± 23.1	10.1 ± 14.1	3.2 ± 0.8

REC (mm)	Missing teeth (*n*)	Teeth w/mobility (*n*)	Teeth w/furcation lesions (*n*)	Deep periodontal pockets (≥5 mm) (*n*)
1.0 ± 0.9	8.5 ± 5.9	5.2 ± 5.0	0.4 ± 0.8*	15.0 ± 18.8*
1.0 ± 0.9	8.1 ± 5.6	4.4 ± 4.2	0.5 ± 0.9*	20.1 ± 19.8*
0.6 ± 0.7**	5.3 ± 5.0**	4.0 ± 4.7**	0.2 ± 0.5**	18.4 ± 19.9
1.0 ± 0.9**	8.5 ± 5.5**	5.4 ± 5.0**	0.5 ± 0.9**	19.1 ± 21.2
1.2 ± 1.0**	10.7 ± 6.0**	4.5 ± 3.6**	0.6 ± 0.9**	12.3 ± 12.8
1.1 ± 1.0	8.2 ± 5.8	5.3 ± 5.2	0.4 ± 0.8	22.5 ± 22.0*
0.9 ± 0.8	8.4 ± 5.8	4.6 ± 4.3	0.5 ± 0.9	14.5 ± 17.3*
1.0 ± 0.9	8.3 ± 5.8	4.8 ± 4.6	0.4 ± 0.9	17.3 ± 19.4

CAL

Total	≥3 mm (%)	≥5 mm (%)	≥7 mm (%)
4.0 ± 1.2	77.6 ± 19.8*	33.2 ± 24.8	11.1 ± 15.8*
4.3 ± 1.5	81.5 ± 17.7*	38.4 ± 27.1	14.8 ± 18.9*
3.6 ± 1.2**	72.5 ± 21.2**	25.6 ± 24.3**	7.6 ± 15.1**
4.3 ± 1.3**	81.5 ± 18.2**	38.5 ± 25.9**	13.9 ± 17.4**
4.3 ± 1.4**	80.8 ± 17.2**	38.0 ± 25.5**	14.7 ± 18.3**
4.5 ± 1.4*	85.5 ± 16.1*	42.9 ± 28.6*	15.8 ± 19.6*
3.9 ± 1.3*	76.0 ± 19.6*	31.6 ± 23.5*	11.1 ± 15.8*
4.1 ± 1.3	79.3 ± 19.0	79.3 ± 19.0	79.3 ± 19.0

Notes.

SB, Supragingival Biofilm; BOP, Bleeding on Probing; PD, Pocket Depth; REC, Recession; CAL, Clinical Attachment Loss.

*Mann–Whitney test ($p < 0.05$).

**Kruskal-Wallis test ($p < 0.05$).

Table 3.2.3. Percentage of patients with 95% confidence interval (95% CI), by threshold of CAL (mm), severity and age group (years). (Note: table was divided to fit within the page, and the below part is the continuity of the table).

CAL (mm)	Subjects with chronic periodontitis							
	20–44 (*n* = 59)		45–64 (*n* = 190)		≥65 (*n* = 89)		Total (*n* = 338)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Prevalence (patients)								
≥3	100	100.0–100.0	100	100.0–100.0	100	100.0–100.0	100	100.0–100.0
≥4	42.4	30.0–54.8	62.1	55.7–68.6	59.6	49.7–69.5	58.0	53.4–62.6
≥5	20.3	10.2–30.4	30.0	23.9–36.1	25.8	17.0–34.6	15.4	12.0–18.8
≥6	8.5	1.515.5	13.2	8.7–17.7	12.4	5.8–19.0	12.1	9.0–15.2
≥7	5.1	0.0–10.6	0.5	0.0–1.4	5.6	1.0–10.2	5.0	3.0–7.1

Notes.
CI, Confidence Interval; CAL, Clinical Attachment Loss.

All subjects							
20–44 (*n* = 90)		45–64 (*n* = 217)		≥65 (*n* = 98)		Total (*n* = 405)	
%	95% CI	%	95% CI	%	95% CI	%	95% CI
65.6	56.1–75.1	87.6	83.5–91.7	90.8	85.3–96.3	83.5	80.4–86.6
27.8	18.8–36.8	54.4	48.3–60.5	54.1	44.6–63.6	48.4	44.2–52.6
13.3	6.5–20.1	26.3	20.9–31.7	23.5	15.4–31.6	12.8	10.0–15.6
5.6	1.0–10.2	11.5	7.6–15.4	11.2	5.2–17.2	10.1	7.6–12.6
3.3	0.0–6.9	4.1	1.7–6.5	5.1	0.9–9.3	4.2	2.5–5.9

Table 3.2.4. Percentage with 95% confidence interval (95% CI), of sites (prevalence) and affected teeth (extent), by threshold of CAL (mm), severity and age group (years). (Note: table was divided to fit within the page, and the below part is the continuity of the table).

CAL (mm)	Subjects with chronic periodontitis							
	20–44 (*n* = 59)		45–64 (*n* = 190)		≥65 (*n* = 89)		Total (*n* = 338)	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Prevalence (sites)								
≥3	85.3	82.7–87.4	86.9	85.3–88.5	84.4	81.7–87.1	86.0	84.7–87.2
≥4	57.0	51.6–62.4	61.3	58.1–64.5	59.2	54.5–63.9	60.0	57.6–62.4
≥5	36.9	30.7–43.0	43.3	39.8–46.8	41.2	36.1–46.3	41.6	39.0–44.2
≥6	21.9	16.4–27.4	27.7	24.5–30.9	26.8	22.2–31.4	26.5	24.1–28.9
≥7	11.7	7.0–16.4	15.8	13.2–18.3	16.1	12.2–20.1	15.2	13.2–17.1
Extent (affected teeth)								
≥3	82.1	77.6–86.6	85.0	82.5–87.7	81.7	77.6–85.7	83.7	81.7–85.6
≥4	49.3	41.9–56.7	56.1	51.9–60.3	54.0	47.8–60.2	54.4	51.3–57.5
≥5	25.0	17.8–32.1	33.4	29.2–37.6	33.8	27.8–39.8	32.1	29.0–35.2
≥6	12.3	6.8–17.8	18.6	15.2–22.0	19.5	14.3–24.7	17.8	15.2–20.3
≥7	5.8	2.1–9.4	10.0	7.5–12.4	9.9	5.8–14.1	9.2	7.4–11.1

All subjects							
20–44 (*n* = 90)		45–64 (*n* = 217)		≥65 (*n* = 98)		Total (*n* = 405)	
%	95% CI	%	95% CI	%	95% CI	%	95% CI
72.5	68.0–76.9	81.5	79.0–83.9	80.8	77.4–84.2	79.3	77.5–81.2
41.5	35.8–47.1	55.2	51.7–58.7	55.3	50.2–60.4	52.2	49.6–54.8
25.6	20.5–30.7	38.5	35.1–42.0	38.0	33.0–43.1	35.5	33.0–38.1
14.7	10.6–18.8	24.5	21.5–27.5	24.6	20.1–29.1	22.4	20.2–24.5
7.6	4.4–10.8	13.9	11.6–16.2	14.7	11.0–18.4	12.7	11.0–14.4
62.1	55.5–68.7	77.3	73.7–80.9	77.0	72.3–81.7	73.9	71.1–76.6
33.5	27.1–39.9	49.8	45.5–54.0	49.5	43.2–55.8	46.1	43.0–49.2
16.0	10.9–21.2	29.5	25.6–33.4	30.8	25.0–36.5	26.8	24.0–29.6
7.9	4.2–11.6	16.3	13.2–19.3	17.7	12.8–22.6	14.7	12.6–16.9
3.7	1.3–6.0	8.7	6.5–10.8	9.0	5.2–12.8	7.6	6.1–9.2

Table 3.2.5. Univariate logistic regression analysis of sociodemographic, behavioural, anthropometric and oral hygiene variables for the outcome variable CAL ≥ 3 mm (N = 405).

Predictor variables		OR (95% CI)	p
Gender	Female	1	-
	Male	1.32 (0.77-2.26)	0.310
Age (years)			<0.001
	20-44	1	-
	45-64	3.70 (2.04-6.69)	<0.001
	≥65	5.20 (2.31-11.70)	<0.001
Smoking status	Smoker	2.06 (1.11-3.81)	0.021
	Non-smoker	1	-
Education (years)			0.107
	1-4	1.40 (0.74-2.66)	0.298
	5-12	2.09 (1.05-4.13)	0.035
	>12	1	-
Employment status			0.246
	Employed	1	-
	Unemployed	1.67 (0.74-3.77)	0.219
	Retired	1.54 (0.84-2.81)	0.163
BMI (kg/m^2)			0.699
	<18.5	1	-
	18.5-24.9	1.06 (0.11-9.79)	0.961
	25.0-29.9	1.48 (0.16-13.82)	0.732
	≥30	1.40 (0.14-13.59)	0.774
Last dental visit			0.026
	<1 year	1	-
	1-2 years	1.39 (0.54-3.57)	0.493
	3-4 years	0.42 (0.22-0.81)	0.009
	≥5 years	0.97 (0.46-2.03)	0.930
	Never	0.24 (0.04-1.54)	0.134
Consultation motive			0.806
	Routine	1	-
	Aesthetics	0.72 (0.29-1.80)	0.483
	Pain	1.09 (0.50-2.35)	0.834
	Functional	1.24 (0.66-2.36)	0.502
	Other	1.39 (0.29-6.60)	0.680
Dental floss use	Yes	1	-
	No	1.66 (0.97-2.82)	0.063

Predictor variables		OR (95% CI)	p
Toothbrush frequency			0.803
	2–3 times/daily	1	–
	1 time/daily	1.27 (0.63–2.56)	0.508
	2–6 times/weekly	–	0.998

BMI (kg/m^2): Body Mass Index (kilogram/meter^2); CI: Confidence Interval; OR: Odds Ratio

Table 3.2.6. Multivariate logistic regression analysis (final reduced model) (*) for the outcome variable CAL ≥ 3 mm (N = 405).

Predictor variables	CAL ≥ 3 mm		
		OR (95% CI)	p
Age (years)	20–44	1	–
	45–64	4.85 (2.57–9.16)	<0.001
	≥65	8.70 (3.66–20.69)	<0.001
Smoking status	Non-smoker	1	-
	Smoker	3.55 (1.80–7.02)	<0.001

Notes. CI, Confidence Interval; OR, Odds Ratio; CAL, Clinical Attachment Loss. (*) The model was statistically significant, χ 2 (3) = 39.507, p < 0.001, explained 15.7% (Nagelkerke R^2) of the variance and correctly classified 83.5% of cases.

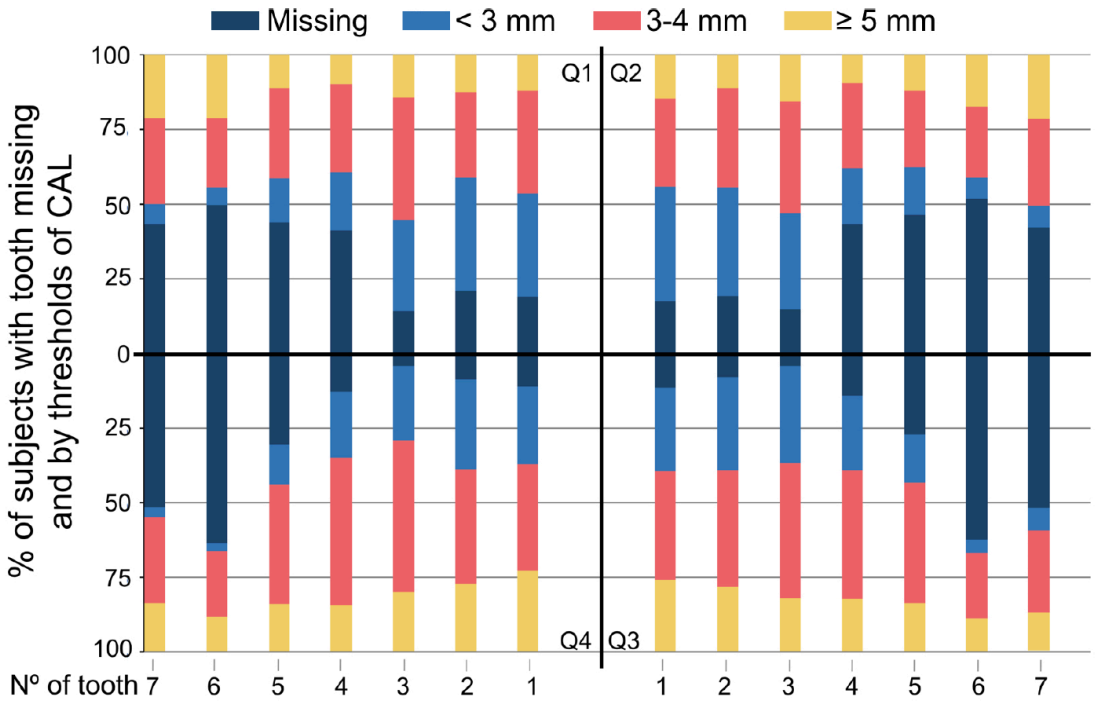

Figure 3.2.1. Percentage of subjects with the respective tooth present and by thresholds of CAL (mm), at each specific position, for all teeth in all quadrants. The black lines indicate the separation by each quadrant. Dark blue, percentage of missing teeth; Blue, percentage of teeth with less than 3 mm of CAL; pink, percentage of teeth with 3_4 mm of CAL; yellow, percentage of teeth over 4 mm of CAL.

3.2.4. Discussion

This retrospective cross-sectional study assessed the periodontal status of forwarded adult subjects who sought dental treatment in a Portuguese university dental clinic, that is located in the metropolitan area of Lisbon. This area has over 2.8 million inhabitants and is the largest Portuguese metropolitan area [29]. This university dental clinic is an important reference dental center in the Lisbon Region and receives patients from all social strata. The absence of complete socioeconomic data constitutes a limitation of this study. Unfortunately, over 70% of patients (data not shown) refused to provide socioeconomic status information.

The results of this retrospective study can't be compared with previous investigations performed in Portugal because in these it was applied the CPITN methodology [24,30–32]. This is the first FMPE protocol used in a Portuguese population and provides direct evidence for estimating periodontal status and results in a better representation of the population [33]. Although FMPE methodology can result in an overestimation of periodontal treatment needs among young adults [21], the partial-mouth examination can miscalculate the prevalence of periodontitis in almost 50% of the population [33]. The overall results demonstrate that this referred subpopulation had a high prevalence of chronic periodontitis (79.3%, 95% CI 77.5-88.1%), and severe extensity of periodontal destruction among the affected subjects (83.7%, 95% CI 81.7-85.6%).

This investigation study design is not an epidemiological study per se, but rather an observational study of patients who were forwarded to a periodontology consultation. Thus, we were only able to estimate the prevalence and extent of our referred subpopulation. However, these results underline the fact that the majority of patients attended the periodontal consultation already in a state of advanced periodontal destruction and only a small percentage appeared in the early stages or healthy. Still, a disturbing percentage of patients did not attend periodontal consultations despite the triage referral with approximately 69% missing or unchecking the appointment.

Regarding tooth loss, the most frequently missed teeth were the lower first molars and the less missed were the lower canines, as with recent European data [21,22]. Additionally, lower canines and incisors were the most affected teeth with CAL and the lower molars the less. The lower arch presented more

periodontal destruction than the upper, and the teeth with more severe CAL levels in the upper arch were the canines.

Concerning periodontal parameters, unlike PD, CAL severity increased with age and can be related to the increase of gingival recession with aging [34]. As in the literature [19–23], age was confirmed in the multivariate analysis as a risk indicator for chronic periodontitis for 45-64 years old (OR=4.85, 95% CI 2.57-9.16) and 65+ years old (OR=8.70, 95% CI 3.66-20.69). However, it's important to highlight that, in the majority of CAL thresholds of subjects with the disease, 45-64 years old group presented worse results for prevalence of chronic periodontitis, while 65+ years old group had worse levels of periodontal destruction extent.

Smoking was strongly associated with chronic periodontitis (OR = 3.55, 95% CI 1.80-7.02). Previous studies reported OR values ranging between 2 and 9 of having periodontitis [21–23,35–38]. Despite not accounting for lifetime smoking exposure, we stratified current smokers according to the number of cigarettes smoked although it was not significantly associated with the severity and progression of the periodontal disease.

Several studies found that obesity was associated with an increased risk of periodontitis [39–41]. Besides that, Suvan et al. [42] concluded that overweight/obese individuals are more likely to suffer from periodontitis compared to normal weight individuals. Although our results show that overweight and obesity have no impact on the aggravation of periodontitis, we emphasize that more than half of this subpopulation was overweight or obese, in agreement with the latest national IAN-AF Food and Activity Survey [43].

In the past, several epidemiological surveys reported that people with lower educational level had higher prevalence and severity of periodontal disease [20,21,23,44,45]. However, other studies have indicated that this impact cannot be seen in a singular way but in a multifactorial view [3,46]. Our results show that despite middle education had significance in the univariable model (OR = 2.09 (95% CI 1.05-4.13), p = 0.035), when analyzed in a multivariable model it had no impact on the probability of having chronic periodontitis.

3.2.5. Conclusion

This specific subpopulation of individuals referred to periodontal examination in a university dental clinic of the Lisbon region presented high prevalence and severe extent of chronic periodontitis. Age and smoking were identified as risk indicators for chronic periodontitis in this referred subpopulation. Within the limitations of this study, these results highlight the importance of developing appropriate public health programs to educate the Portuguese population about the burden of periodontal diseases.

3.2.6. References

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3.3. Partial recording protocols performance on the assessment of periodontitis severity and extent: bias magnitudes, sensibility, and specificity

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Abstract

Objectives: This study aimed to assess bias magnitudes, sensibility, and specificity of particular partial-mouth recording protocols (PRPs) to estimate periodontal clinical measurements and periodontal status.

Methods: Estimates of mean clinical attachment loss (MCAL) and mean probing depth (MPD) were calculated for 15 different PRPs and were compared to full-mouth recording protocol (FRP) data from 402 Portuguese. Biases, relative biases and intra-class correlations for all PRPs were evaluated. Bland–Altman plots and Receiver Operating Characteristic / Area Under the Curve (ROC/AUC) analysis were used to assess the sensitivity and specificity for each PRP periodontal diagnosis.

Results: Regarding MPD, Half RD6 UR/LL and RD6 UL/LR had the lowest bias observed with 0.00 mm (-0.22% and 0.22%, respectively) and all full-mouth PRPs significantly produce an underestimation. Concerning MCAL, the Half MB-B-DL UR/LR had the lowest bias observed with 0.01 mm (0.16%). Excluding CPITN, Full-Mouth PRPs outperforms in average Half Mouth PRPs correlations. The Half RD6 UR/LL had the highest AUC (0.96) with 95.5 and 97.1% of sensitivity and specificity, respectively.

Conclusions: Three half-mouth PRPs (Half MB-B-DL UR/LR, Half RD6 UR/LL and Half RD6 UL/LR) protocols can be used to estimate periodontal clinical measurements with limited bias, and high sensitivity, specificity, and concordance. All full-mouth PRPs failed to estimate pocket depth means, and for clinical attachment loss, they present less ability then half-mouth partial protocols, despite presenting high sensitivity levels.

3.3.1. Introduction

Periodontal diseases are a crucial dental public health problem, since it is the sixth most prevalent disease worldwide and have increased by 57% over the last two decades [1–5]. Periodontitis was recently defined as a microbially-associated and host-mediated inflammation that results in loss of periodontal attachment [6]. Further, periodontal diagnosis is mandatory to screen or stage the extent and severity of periodontitis and should include an assessment of known risk factors [5].

Currently, in clinical research and periodontal practice, the gold standard method for assessing periodontal status involves a full-mouth recording protocol (FRP) conducted on six sites per tooth, possibly involving to at least 168 sites within each person (excluding third molars). However, in large surveys and epidemiological periodontal diseases' studies it is often not feasible to conduct the traditional FRP because it is time and labor intensive for the patients and examiners, possibly leading to dropout rates and measurement errors [7,8].

A partial-recording protocol (PRP) is defined as a clinical assessment of a "representative set" of teeth or sites within the individual [9], that is used to estimate the periodontal status for population-based studies, when budget restrictions and time constraints are found [10]. In epidemiological studies of periodontal disease, several choices for PRPs have been proposed, although such protocols may be inappropriate to allow proper assessment of the level and pattern of periodontal disease. PRPs include either indexes such as the community periodontal index of treatment needs (CPITN) or a subgroup of probing sites and/or teeth like the National Health and Nutrition Examination Survey (NHANES) III and IV protocols [11].

Since 1972, several articles have compared the performance of PRPs [12,13]. In most articles, PRPs have shown inconsistent results in diagnostic estimates of periodontitis, although several PRPs have produced small biases for forecasts of periodontal disease severity [8] and extent [7]. It has been shown that the use of PRPs showed varying degrees of underestimation of disease prevalence (3,4,14). Thus, researchers have not reached consensus on a PRP that should be employed in large-scale epidemiological studies [16–18]. It is essential to standardize the method for assessing the prevalence, severity, and extent of the disease in epidemiological studies in order to be comparable.

For instance, Kingman et al. [8] focused on eight PRPs and have concluded that assessing three specific sites per tooth (mesiobuccal, buccal and distolingual) had a very small bias in estimating disease severity with high sensitivity for estimating disease prevalence.

Nevertheless, the authors raised the need for "more convenience-based databases to determine the probable levels or bounds on the bias produced by the PRPs" [8].

Thereby, this study aimed to evaluate the bias and precision associated with probing depth (PD) and clinical attachment loss (CAL) measurements (taken as estimates of periodontitis prevalence, severity, and extent) obtained from PRP methods against the "gold standard" FRP protocol, in a referenced Portuguese population suffering from periodontitis.

3.3.2. Materials and Methods

The data analyzed in this study was sourced from previous research [19] and had the approval of Egas Moniz Ethics Committee (Ethical Application Ref: 595). This retrospective cross-sectional investigation analyzed data from 405 patients who attended the Egas Moniz Dental Clinic between September 2015 and March 2017.

In the previous study [19], it was performed a full-mouth recording protocol (FRP) using CDC/AAP full-mouth methodology [1]. All permanent fully erupted teeth were examined, excluding third molars, retained roots, and implants. The evaluated parameters were: missing teeth, probing depth (PD), bleeding on probing (BOP), gingival recession (REC) and clinical attachment loss (CAL). At six sites per tooth [mesiobuccal (MB), mid-buccal (B), distobuccal (DB), mesiolingual (ML), mid-lingual (L) and distolingual (DL)] PD was measured as the distance from the cementoenamel junction (CEJ) to the bottom of the pocket and REC as the distance from the CEJ to the free gingival margin, and this assessment was assigned a negative sign if the gingival margin was located coronally to the CEJ. CAL was calculated as the algebraic sum of PD and REC. For the referred clinical measures, it was used a CP-12 SE (Hu-Friedy, Chicago, IL, USA).

Three of the 405 original patients were excluded due to having 2, 3, and 5 teeth in one quadrant, respectively. The PD and CAL data from the FRP were defined as the "gold-standard" and were compared with several half and full-mouth PRP methodologies. The selected half-mouth PRPs were: (i) NHANES III (MB-B measurements) in upper and lower right quadrants (UR/LR); (ii) NHANES III in upper

and lower left quadrants (UL/LL); (iii) NHANES III (MB-B-DB measurements) in UR/LR; (iv) NHANES IV in UL/LL; (v) MB-B-DL measurements in UR/LR; (vi) MB-B-DL measurements in UL/LL; (vii) 6-sites (MB-B-DB-ML-L-DL) in UR/LR; (viii) 6-sites in UL/LL; (ix) 6-sites in UR/LL; (x) 6-sites in UL/LR. Regarding full-mouth PRPs we selected: (xi) 6-sites on ''Ramfjord'' teeth – right maxillary first molar, left maxillary central incisor, left maxillary first premolar, left mandibular first molar, right mandibular central incisor and right mandibular first premolar; (xii) MB-B measurements in all teeth; (xiii) MB-B-DB measurements in all teeth; (xiv) MB-B-DL measurements in all teeth; (xv) 6-sites on Community Periodontal Index of Treatment Needs (CPITN) teeth - right maxillary first and second molar, right maxillary central incisor, left maxillary first and second molar, left mandibular first and second molar, left mandibular central incisor and right mandibular first and second molar.

Data analyses were performed using IBM SPSS Statistics version 24.0 for Windows (Armonk, NY: IBM Corp.). FRP, partial and full-mouth PRPs data were summarized as mean, standard deviation and standard error for PD, CAL and tooth sites with specified disease severity (PD ≥4 or ≥6 mm, CAL ≥4 or ≥6 mm). The prevalence of disease, i.e., the proportion of sites with unsound depths within specified disease threshold (PD ≥4 to ≥7 mm, CAL ≥4 to ≥7 mm), were also determined. FRP and PRPs' means across disease severity groups were compared through paired t-test.

Evaluation of PRPs bias against FRP was made upon patient level summary measures of MPD (Mean Probing Depth) and MCAL (Mean Clinical Attachment Loss) across all 402 subjects in the sample. Bias was defined as the difference between the mean PRP and the mean "gold standard" FPR, for each subjects´ PRP (Kingman et al. 2008, Tran et al. 2013, Tran et al. 2014) [i.e. bias (PRP) = PRP (mean) - FRP (mean)]. For each PRP the relative bias was calculated as the percentage of the respective bias divided by the full-mouth subject mean score [8,17,18] [i.e. relative bias (PRP) = 100 x bias (PRP) / FRP].

The discrepancy of partial-mouth assessment (PMA) was expressed as the following ratio: (FRP mean - PRP mean)/FRP mean. Discrepancy positive outcome was considered underestimation, whereas a negative outcome was considered overestimation. PRP reliability was evaluated through the Intraclass Correlation Coefficients (ICC) who expressed the agreement between FRP and PRP assessments. The degree of correlation between FRP and PRPs was expressed as the Pearson correlation coefficient. A p-value <0.05 was considered statistically significant.

Bland–Altman plots [20] were used to investigate and assess the agreement between the PRP and gold standard FRP mean, by studying the mean difference and constructing limits of agreement. The statistical limits are calculated by using the mean and the standard deviation of the differences between PRP and gold standard FRP. The resulting graph is a XY scatter plot, in which Y axis shows the differences between PRP and FRP and X axis represents the average of the two measures. As recommended, 95% confidence interval was added into the plots 20.

Logistic regression analyses output were used to estimate accuracy and the probability of concordance between FRP-based and each tested PRP-based periodontal disease diagnosis. An FRP-based periodontal disease status (present/absent) was the binary dependent variable (CAL > 3.0 as cutoff), and as an independent factor each tested PRP-based periodontal disease status binary output (same conditions). For each logistic model, potential covariates (sex, age, and the number of missing teeth) contribution for model fitness were hierarchically assessed and if turned out redundant covariates were removed from the final model. Receiver Operating Characteristic / Area Under the Curve (ROC/AUC) with 95% confidence intervals at threshold p=0.5 were used to estimate concordance, sensitivity, and specificity for each tested PRP outcome against the gold standard FRP. The level of statistical significance was set at 5%.

3.3.3. Results

Table 3.3.1 describes the characteristics of the Portuguese sample that hampered this study. A summary for the MPD estimates for each PRP is presented in table 3.3.2, and the “gold standard” FRP was 2.19 mm (±0.77) for this study population. The biases for MPD for the multi-site PRPs are all <0.1 mm in absolute value. The associated relative biases ranged from -12.89% to 0.58%. The bias (relative bias) for the NHANES III and NHANES IV half-mouth PRPs ranged between -0.16 mm (-7.49%) and -0.04 mm (-0.34%), much similar to their full-mouth versions [-0.16 mm (-7.24%) and -0.04 mm (-1.96%)], respectively. The bias and relative biases for the Half 6 sites diagonal (UR/LL and UL/LR) PRP-based MPD are much smaller, 0.00 mm (-0.22% and 0.22%, respectively) for both partial-mouth versions. The MPD estimate based on the Ramfjord PRP had -0.14 mm (-6.55%) negative bias. There were no statistically significant biases for five half-mouth PRPs (Half MB-B-DL UR/LR, Half 6 Sites UR/LR, Half 6 Sites UL/LL, Half

6 Sites UR/LL, and Half 6 Sites UL/LR). All full-mouth PRPs showed a statistically significant underestimation of the "gold standard" FRP.

Table 3.3.1. Characteristics of the Portuguese sample.

Variables	
Age, mean ± SD	55.07 (12.38)
Number of missing teeth, , mean ± SD	8.29 (5.78)
Gender, n(%)	
Female	222 (55.22)
Male	180 (44.78)
Education, n(%)	
Elementary	155 (38.56)
Middle	154 (38.31)
Higher	93 (23.13)
Smoking, n(%)	
Non-smoker	261 (64.93)
Smoker	141 (35.07)
Employment status, n(%)	
Employed	208 (51.74)
Unemployed	63 (15.67)
Retired	131 (32.59)

SD – Standard Deviation

Table 3.3.2. Comparison of means, standard deviations, standard error, bias, and percent relative bias for probing pocket.

	N	Mean	SD	SE	Bias*	Relative Bias (%)	*P*-value
Full Mouth (standard)	402	2,19	0,77	0,04			
Partial Mouth PRPs							
Half NHANES III 1Q/4Q	402	2,04	0,74	0,04	-0,15	-6,92	**<0.001*****
Half NHANES III 2Q/3Q	402	2,03	0,74	0,04	-0,16	-7,49	**<0.001*****
Half NHANES IV 1Q/4Q	402	2,15	0,79	0,04	-0,04	-1,97	**<0.001*****
Half NHANES IV 2Q/3Q	402	2,15	0,78	0,04	-0,04	-1,94	**0,002****
Half MB-B-DL 1Q/4Q	402	2,18	0,81	0,04	-0,01	-0,34	0,518
Half MB-B-DL 2Q/3Q	402	2,16	0,78	0,04	-0,03	-1,55	**0,003****

Half 6 Sites UR/LR	402	2,18	0,81	0,04	-0,01	-0,59	0,232
Half 6 Sites UL/LL	402	2,20	0,80	0,04	0,01	0,58	0,245
Half RD6 1Q/3Q	402	2,19	0,81	0,04	0,00	-0,22	0,693
Half RD6 2Q/4Q	402	2,20	0,82	0,04	0,00	0,22	0,693
Full-Mouth PRPs							
Ramfjord	402	2,05	0,91	0,05	-0,14	-6,55	**<0.001*****
Full MB-B	402	2,03	0,71	0,04	-0,16	-7,24	**<0.001*****
Full MB-B-DB	402	2,15	0,75	0,04	-0,04	-1,96	**<0.001*****
Full MB-B-DL	402	2,17	0,76	0,04	-0,02	-0,94	**<0.001*****
CPITN	402	1,91	1,02	0,05	-0,28	-12,89	**<0.001*****

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols. RD – random diagonal quadrants; CPITN – community periodontal índex of treatment needs. **Paired t-test. P<0.01; ***Paired t-test. P<0.001.

Table 3.3.3 presents mean scores and standard deviations of all recording protocols, besides it has bias and percent relative bias (scores further from zero indicate more relative bias) of each PRP compared with FRP (standard). The true full-mouth mean clinical attachment loss (MCAL) was 4.17 mm (± 1.32) for this study population. Biases for partial mouth PRPs MCAL estimates were all less than 0.02 mm, and the associated relative biases ranged between -5.05% and 9.62%. Further, CPITN relative biases were the highest of all PRPs both in MPD and MCAL. MCAL biases (relative biases) for NHANES III, and NHANES IV half-mouth PRPs ranged between -0.21 mm (-5.05%) and -0.02 mm (-0.45%) away from their corresponding full-mouth versions, who were -0.15 mm (-3.66%) and -0.03 mm (-0.69%), respectively. The Half MB-B-DL UR/LR had the lowest bias observed with 0.01 mm (0.16%) and the bias for its homologous full-mouth estimate was -0.03 mm (-0.62%). The MCAL estimate based on the Ramfjord PRP had 0.05 mm (1.11%) positive bias. There were no significant biases for four half-mouth PRPs (NHANES IV UR/LR, MB-B-DL UR/LR, 6 Sites UR/LR, and 6 Sites UL/LR) and two full-mouth PRPs (Ramfjord and MB-B-DB). The extent and severity of different thresholds of PD and CAL are presented in Supplements S1-S8.

Table 3.3.3. Comparison of means, standard deviations, standard error, bias, and percent relative bias for attachment loss.

	N	Mean	SD	SE	Bias*	Relative Bias (%)	*P*-value
Full Mouth (standard)	402	4,17	1,32	0,07			
Partial Mouth PRPs							
Half NHANES III 1Q/4Q	402	4,05	1,38	0,07	-0,12	-2,83	**<0.001*****

Half NHANES III 2Q/3Q	402	3,96	1,29	0,06	-0,21	-5,05	**<0.001*****
Half NHANES IV 1Q/4Q	402	4,15	1,35	0,07	-0,02	-0,45	0,559
Half NHANES IV 2Q/3Q	402	4,11	1,30	0,06	-0,07	-1,57	**<0.005****
Half MB-B-DL 1Q/4Q	402	4,18	1,37	0,07	0,01	0,16	0,809
Half MB-B-DL 2Q/3Q	402	4,09	1,31	0,07	-0,09	-2,05	**<0.001*****
Half 6 sites UR/LR	402	4,19	1,38	0,07	0,02	0,46	0,461
Half 6 Sites UL/LL	402	4,13	1,35	0,07	-0,05	-1,11	**0.001****
Half RD6 1Q/3Q	402	4,12	1,34	0,07	-0,06	-1,37	**0.006****
Half RD6 2Q/4Q	402	4,20	1,39	0,07	0,02	0,58	0,271
Full-Mouth PRPs							
Ramfjord	402	4,22	1,53	0,08	0,05	1,11	0,225
Full MB-B	402	4,02	1,26	0,06	-0,15	-3,66	**<0.001*****
Full MB-B-DB	402	4,14	1,26	0,06	-0,03	-0,69	0,068
Full MB-B-DL	402	4,15	1,29	0,06	-0,03	-0,62	**0.002****
CPITN	402	4,57	1,62	0,08	0,40	9,62	**<0.001*****

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols. RD – random diagonal quadrants; CPITN – community periodontal índex of treatment needs. **Paired t-test, P<0.01; ***Paired t-test, P<0.001

Figure 1 represents Bland–Altman plots for the half-mouth versions of NHANES III (UR/LR and UL/LL), NHANES IV (UR/LR and UL/LL), MB–B–DL (UR/LR and UL/LL) and six-site (UR/LR, UL/LL, UR/LL, and UL/LR) PRPs. The SDs for the MPD scores were slightly larger than the associated means (coefficients of variation varied from 0.08 in full-mouth MB-B-DL to 0.51 in CPITN). In the CPITN and Ramfjord PRPs there were substantial variations among subject-specific MPD differences compared with those for the MB–B–DL full-mouth PRPs. The SDs for the MPD scores were slightly larger than the associated means (coefficients of variation varied from 0.22 in half six-sites UL/LL and UR/LR to 0.28 in NHANES III UR/LR and NHANES IV UL/LL). The SDs for the MCAL scores were slightly larger than the associated means (coefficients of variation varied from 0.16 in full-mouth MB-B-DL to 1.05 in CPITN). In the CPITN and Ramfjord PRPs, there were substantial variations among subject-specific MCAL differences compared with those for the MB–B–DL full-mouth PRPs. The remaining Bland–Altman plots for MPD and MCAL are as a supplement (S3.3.9 - 3.3.10).

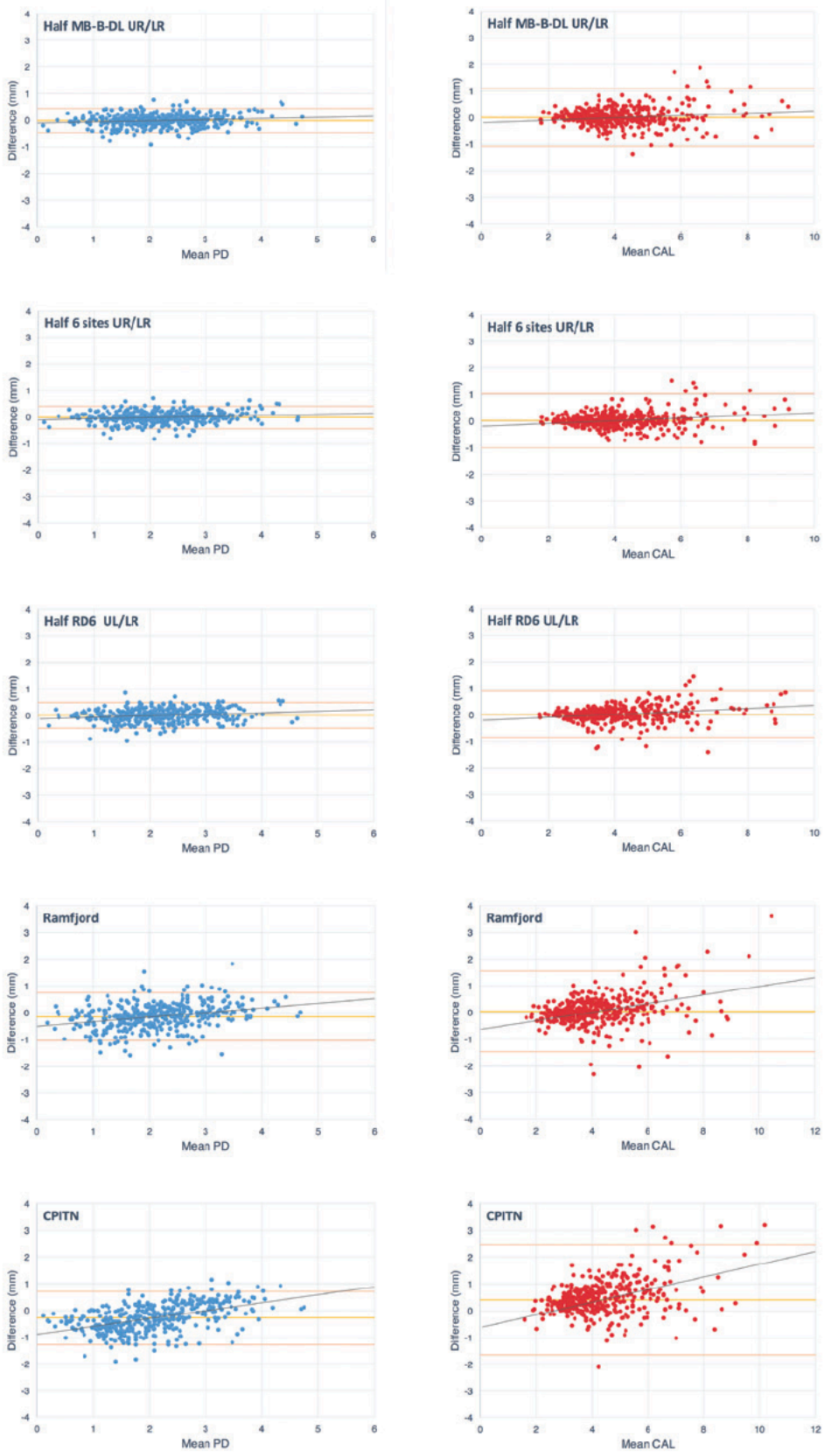

Figure 3.3.1. Bland-Altman plots to evaluate bias between the Mean PD (MPD) and Mean CAL (MCAL) differences for Half MB-B-DL UR/LR, Half 6 sites UR/LR, Half RD6 UL/LR, Ramfjord and CPITN. The area within the upper and lower orange lines sets 95% confidence interval and the yellow line the mean value.

Table 3.3.4 shows the Pearson's correlation as a measure of the relationship between FRP and PRPs MCAL values. In general, all correlations shown in this table are quite high, being indicative of strong relationships. Excluding CPITN, Full-Mouth PRPs outperforms in average Partial Mouth PRPs correlations. Furthermore, the highest correlation was 0.94 for Full MB-B-DL and the lowest 0.58 for CPITN.

Table 3.3.4. Degree of correlation between FRP and PRPs.

	Pearson R
Partial Mouth PRPs	
Half NHANES III UR/LR	0,72***
Half NHANES III UL/LL	0,71***
Half NHANES IV UR/LR	0,77***
Half NHANES IV UL/LL	0,78***
Half MB-B-DL UR/LR	0,82***
Half MB-B-DL UL/LL	0,82***
Half 6 Sites UR/LR	0,87***
Half 6 Sites UL/LL	0,86***
Half 6 Sites UR/LL	0,87***
Half 6 Sites UL/LR	0,85***
Full-Mouth PRPs	
Ramfjord	0,72***
Full MB-B	0,79***
Full MB-B-DB	0,88***
Full MB-B-DL	0,94***
CPITN	0,58***

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols. RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs. ***Pearson correlation, P < 0.001

The ROC/AUC values obtained from the logistic analysis ranged between 73.8% for CPITN and 96.3% for Half RD6 UR/LL (p<0.0001). There was no significant improvement in sensitivity and specificity when sex, age, and the number of missing teeth covariables were hierarchically added to the logistic model (Supplement S3.3.11).

The corresponding sensitivity of PRPs in detecting clinical attachment loss distributions are presented in Table 3.3.5. All PRPs had high sensitivity for mean attachment loss > 3 mm. The MB-B-DL full-mouth protocol was the only PRP that achieved 99% sensitivity. There was a decrease in sensitivity, although the half MB-B-DL UR/LR, half 6 sites UR/LR, MB-B-DB full-mouth protocol maintained reasonably high sensitivity. In contrast, NHANES III PRPs showed the lowest sensitivity values.

On the other hand, most of PRPs had high specificity for mean attachment loss > 3 mm. The half RD6 UR/LL was the only partial recording protocol that achieved a specificity of at least 97%. In opposition, the CPITN and Ramfjord protocols had the smallest specificity (50% and 82.4%, respectively).

Table 3.3.5. ROC/AUC analysis for panel of PRPs.

	Sensitivity (%)	Specificity (%)	AUC (%)	SE	*P*-value
Partial Mouth PRPs					
Half NHANES III UR/LR	89.5	92.6	91.1	0.021	0.000***
Half NHANES III UL/LL	91.6	86.8	89.2	0.025	0.000***
Half NHANES IV UR/LR	96.1	80.9	88.5	0.029	0.000***
Half NHANES IV UL/LL	95.5	83.8	89.7	0.027	0.000***
Half MB-B-DL UR/LR	97.0	85.3	91.2	0.026	0.000***
Half MB-B-DL UL/LL	95.2	91.2	93.2	0.021	0.000***
Half 6 sites UR/LR	98.2	86.8	92.5	0.025	0.000***
Half 6 Sites UL/LL	96.1	94.1	95.1	0.018	0.000***
Half RD6 UR/LL	95.5	97.1	96.3	0.014	0.000***
Half RD6 UL/LR	96.1	92.6	94.4	0.019	0.000***
Full-Mouth PRPs					
Ramfjord	93.4	82.4	87.9	0.028	0.000***
Full MB-B	93.4	92.6	93.0	0.020	0.000***
Full MB-B-DB	98.2	89.7	94.0	0.022	0.000***
Full MB-B-DL	99.4	92.6	96.0	0.019	0.000***
CPITN	97.6	50.0	73.8	0.040	0.000***

AUC – Area under the curve; SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols; RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs. ***Pearson correlation, P < 0.001.

3.3.4. Discussion

The balance of advantages and disadvantages of PRPs, in the assessment of the prevalence and severity of periodontal disease in epidemiologic research, needs

to be carefully evaluated. In large-scale surveys, time and resource demanding are the primary considerations and usually mandates the use of a partial-mouth periodontal examination [7,8,17,21]. The prevalence and severity of estimated periodontal disease produced by these PRPs are necessarily biased [8,22]. However, their bias magnitude depends on the group of teeth/sites examined, and prevalence of the disease in that particular population [8,10,23].

One of the fundamental strengths of this study is the access to full-mouth periodontal examination from a large sample of a Portuguese population, with a considerable variation in periodontal disease severity. This database allowed to investigate the effects of specific PRPs in estimating the prevalence and severity of periodontal disease with bias and relative bias. Moreover, in assessing the usefulness of different PRPs di agnostic methods, both the sensitivity and specificity are essential criteria and must be taken into consideration when selecting a suitable system.

There have been very few studies published about this thematic. The disease severity in this Portuguese study population was substantially higher (full-mouth MCAL=4.17 mm and MPD=2.19 mm) than that reported for Dowsett et al. [24] and Beck et al. [25], in Guatemalan and American populations respectively. The study conducted by Dowsett et al. [24] reported similar findings for the half random diagonal six-site PRP (full-mouth MCAL=2.76 mm and full-mouth MPD=2.88 mm). In contrast, in the US multicentric study of Beck et al. [25], NHANES III, NHANES IV and Ramfjord PRPs have shown higher relative bias for MPD and MCAL (MCAL=1.77 mm and MPD=1.89 mm) comparing with our study. The mouth characteristics of each population and their demographics may explain these contradictory findings [25,26].

The NHANES III and NHANES IV protocols randomly select one maxillary quadrant and one mandibular quadrant at the same side and involve three fixed buccal sites per tooth (MB–B– DB) [27]. In our study, we opted to evaluate both two random options (one upper and one lower) separately for all subjects, allowing the investigation of the effect of randomly choose the quadrants. The current study has indicated that when used in an epidemiological survey, both NHANES III and IV lead to an underestimate of the MCAL and MPD. Also, the half NHANES IV UR/LR was the only one that did not have statistically significant differences with the gold-standard full-mouth examination and had the highest sensitivity (96.1%).

Numerous authors have applied the Ramfjord teeth for evaluating periodontal status, and the results have been acceptable and representative of FME [25,28]. In contrast, other investigators [8,29,30] have reported that examination of the Ramfjord teeth is not suitable for evaluating the extension, degree, or prevalence of both PD or CAL. In fact, Fleiss [29] verified that Ramfjord Teeth are an inadequate surrogate for epidemiologic studies of periodontitis. This limitation of the Ramfjord teeth also becomes evident in our research, since Ramfjord PRP produces one of the more substantial biases for estimating MPD, and, in Bland–Altman plots, there were significant variations among subject-specific in both MCAL and MPD.

The current study demonstrates that partial and full-mouth PRPs underestimated MPD and MCAL. This investigation identified three potential PRPs that better estimated the MPD and MCAL standard values: (1) Half MB-B-DL UR/LR, (2) Half 6 sites UL/LR, and (3) Half RD 6 UL/LR. These results are following previous findings [17].

Furthermore, all full-mouth PRPs presented significant different results for MPD, and CPITN was the protocol that had higher bias and relative bias results of all PRPs (-0.28 and -12,89%, respectively). Whereas concerning MCAL, Ramfjord and Full MB-B-DB did not have statistically different results from the standard, and once again, CPITN had the higher bias and relative bias results (0.40 and 9,62%, respectively). These results comport with previous studies that have highlighted the biasing potential of CPITN in epidemiological surveys [17,31]. Moreover, our results reveal a significant reduction in bias and high sensitivity for periodontal disease severity when using the half-mouth MB–B–DL UR/LR PRP, and this has been previously reported for a Brazilian population [22].

Notwithstanding, regarding the extent and severity of different thresholds of PD and CAL, PRPs tend to fail when the established threshold is low (Supplements S1-S8). About the extent of PD, the Ramfjord PRP was the only protocol that was consistent with gold standard values in all considered thresholds. Concerning CAL extent, Half NHANES IV UR/LR was the protocol that better estimated the extent of attachment loss of the respective standard value. Besides, only one study has addressed this comparison with other variables like age and gender [18]. This is the first time that is assessed the potential bias of PRPs on the extent and severity of periodontal disease. The extent and severity are elements of extreme importance since they have long been used as a critical descriptor of

periodontitis cases [6], and remain highly relevant in the most recent Consensus Report of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [6,32].

With these being said, there is lack of agreement on which PRP should be used in epidemiological surveys. Hereupon and considering the possible effect of the characteristics of each population, as previously mentioned, Kingman et al. [8] have proposed to perform an FMP on a randomly selected subsample of the subjects (5 to 10%) to calculate the magnitude of bias incurred by the proposed PRP. In the future, to apply this method in population surveys, there is the need to appraise the epidemiological impact on the periodontal estimates.

3.3.5. Conclusion

Our findings suggest that a half-mouth three sites and two half-mouth six sites protocols can be used to estimate periodontal clinical measurements and status in Portuguese patients with limited bias. Also, these protocols showed high sensitivity, specificity, and concordance. Nevertheless, although all full-mouth partial protocols had high sensitivity levels, they all failed to estimate pocket depth and clinical attachment loss means, presenting less ability then half-mouth partial protocols.

3.3.6. References

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3.3.7. Supplementary material

Table S3.3.1. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with PD ≥ 4 mm.

	% PD ≥ 4 (n=402)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	30.14	21.46	1.07			
Partial Mouth PRPs						
Half NHANES III UR/LR	22.82	19.21	0.96	-0.07	-24.29	**<0.001*** **
Half NHANES III UL/LL	22.96	18.78	0.94	-0.07	-23.82	**<0.001*** **
Half NHANES IV UR/LR	26.64	20.66	1.03	-0.04	-11.61	**<0.001*** **
Half NHANES IV UL/LL	26.90	21.09	1.05	-0.03	-10.75	**<0.001*** **
Half MB-B-DL UR/LR	28.70	20.84	1.04	-0.01	-4.78	**<0.001*** **
Half MB-B-DL UL/LL	27.43	20.90	1.04	-0.03	-8.99	**<0.001*** **
Half 6 Sites UR/LR	30.79	22.08	1.10	0.01	2.16	**0.038***
Half 6 Sites UL/LL	29.28	22.32	1.11	-0.01	-2.85	**<0.001*** **
Half RD6 UR/LL	29.17	21.77	1.09	-0.01	-3.22	**<0.001*** **
Half RD6 UL/LR	30.75	22.39	1.12	0.01	2.02	**<0.001*** **
Full-Mouth PRPs						
Ramfjord	30.19	23.80	1.19	0.00	0.17	0.925
Full MB-B	22.73	17.98	0.90	-0.07	-24.59	**<0.001*** **
Full MB-B-DB	26.95	19.92	0.99	-0.03	-10.58	**<0.001*** **
Full MB-B-DL	28.23	20.04	1.00	-0.02	-6.34	**<0.001*** **
CPITN	37.78	23.90	1.19	0.08	25.35	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; ***Paired t-test, P<0.001.

Table S3.3.2. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with PD ≥ 5 mm.

	% PD ≥ 5 (n=357)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	15.21	16.33	0.81			
Partial Mouth PRPs						
Half NHANES III UR/LR	10.63	13.402	0.668	-0.05	-30.74	**<0.001*** **
Half NHANES III UL/LL	10.93	13.246	0.661	-0.05	-27.76	**<0.001*** **
Half NHANES IV UR/LR	12.94	15.315	0.764	-0.03	-12.44	**<0.001*** **
Half NHANES IV UL/LL	13.2	15.098	0.753	-0.02	-13.44	**<0.001*** **
Half MB-B-DL UR/LR	14.19	15.666	0.781	-0.01	-4.01	**<0.001*** **
Half MB-B-DL UL/LL	13.75	15.719	0.784	-0.02	-10.46	**<0.001*** **
Half 6 Sites UR/LR	15.59	17.039	0.85	0.00	3.98	0.094
Half 6 Sites UL/LL	14.82	16.726	0.834	0.00	-5.30	0.052
Half RD6 UR/LL	14.73	16.293	0.813	-0.01	-4.07	**0.017***
Half RD6 UL/LR	15.64	17.522	0.874	0.00	2.71	0.053
Full-Mouth PRPs						
Ramfjord	15.52	18.427	0.919	0.00	-1.35	0.443
Full MB-B	10.67	12.429	0.62	-0.05	-30.44	**<0.001*** **
Full MB-B-DB	13.15	14.441	0.72	-0.02	-12.36	**<0.001*** **
Full MB-B-DL	14.06	15.064	0.751	-0.01	-6.44	**<0.001*** **
CPITN	20.67	19.963	0.996	0.06	69.74	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; ***Paired t-test, P<0.001.

Table S3.3.3. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with PD ≥ 6 mm.

	% PD ≥ 6 (n=303)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	7.72	11.14	0.56			
Partial Mouth PRPs						
Half NHANES III UR/LR	5.44	9.839	0.491	-0.03	-37.42	**<0.001*** **
Half NHANES III UL/LL	5.27	9.352	0.466	-0.03	-25.67	**<0.001*** **
Half NHANES IV UR/LR	6.61	10.794	0.538	-0.01	-14.07	**<0.001*** **
Half NHANES IV UL/LL	6.39	10.547	0.526	-0.02	-12.25	**<0.001*** **
Half MB-B-DL UR/LR	7.28	11.249	0.561	-0.01	-6.79	**0.021***
Half MB-B-DL UL/LL	6.83	10.688	0.533	-0.01	-6.99	**<0.001*** **
Half 6 Sites UR/LR	7.91	11.83	0.59	0.00	-0.09	0.255
Half 6 Sites UL/LL	7.44	11.425	0.57	0.00	-2.00	0.086
Half RD6 UR/LL	7.45	11.278	0.562	0.00	-4.79	**0.08***
Half RD6 UL/LR	7.88	11.804	0.589	0.00	3.58	0.309
Full-Mouth PRPs						
Ramfjord	8.28	13.536	0.675	0.01	1.54	0.068
Full MB-B	5.25	8.787	0.438	-0.03	-34.92	**<0.001*** **
Full MB-B-DB	6.55	9.963	0.497	-0.02	-12.70	**<0.001*** **
Full MB-B-DL	7.09	10.48	0.523	-0.01	-5.58	**<0.001*** **
CPITN	11.1	14.753	0.736	0.05	91.61	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; ***Paired t-test, P<0.001.

Table S3.3.4. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with PD ≥ 7 mm.

	% PD ≥ 7 (n=193)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	2.31	4.91	0.25			
Partial Mouth PRPs						
Half NHANES III UR/LR	1.72	5.484	0.274	-0.01	-38.49	**0.001** **
Half NHANES III UL/LL	1.86	6.225	0.31	-0.01	-21.12	**0.026***
Half NHANES IV UR/LR	2.02	5.644	0.281	-0.01	-17.33	**0.049***
Half NHANES IV UL/LL	2.11	6.355	0.317	0.00	-4.71	0.325
Half MB-B-DL UR/LR	2.28	5.761	0.287	0.00	-9.99	0.831
Half MB-B-DL UL/LL	2.13	5.477	0.273	0.00	3.55	0.151
Half 6 Sites UR/LR	2.28	5.517	0.275	0.00	-8.10	0.807
Half 6 Sites UL/LL	2.29	5.189	0.259	0.00	5.86	0.867
Half RD6 UR/LL	2.25	5.203	0.26	0.00	-7.38	0.553
Half RD6 UL/LR	2.39	5.568	0.278	0.00	6.48	0.395
Full-Mouth PRPs						
Ramfjord	2.61	6.605	0.329	0.01	-0.64	0.135
Full MB-B	1.74	5.191	0.259	-0.01	-33.45	**<0.001*** **
Full MB-B-DB	2.07	5.43	0.271	0.00	-10.52	0.051
Full MB-B-DL	2.23	5.16	0.257	0.00	-2.90	0.241
CPITN	3.46	7.237	0.361	0.02	87.93	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; **Paired t-test, P<0.01; ***Paired t-test, P<0.001.

Table S3.3.5. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with CAL ≥ 4 mm.

	% CAL ≥ 4 (n=402)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	52.50	26.60	1.33			
Partial Mouth PRPs						
Half NHANES III UR/LR	50.13	28.05	1.40	-0.02	-4.51	**<0.001*** **
Half NHANES III UL/LL	48.97	27.93	1.39	-0.04	-6.72	**<0.001*** **
Half NHANES IV UR/LR	52.36	27.22	1.36	0.00	-0.27	0.816
Half NHANES IV UL/LL	51.85	27.48	1.37	-0.01	-1.24	0.219
Half MB-B-DL UR/LR	52.76	26.84	1.34	0.00	0.50	0.605
Half MB-B-DL UL/LL	51.20	27.38	1.37	-0.01	-2.48	**0.001****
Half 6 Sites UR/LR	53.10	27.07	1.35	0.01	1.14	0.160
Half 6 Sites UL/LL	51.52	27.71	1.38	-0.01	-1.87	**0.001****
Half RD6 UR/LL	51.55	27.44	1.37	-0.01	-1.81	**0.006****
Half RD6 UL/LR	52.95	27.24	1.36	0.00	0.86	0.226
Full-Mouth PRPs						
Ramfjord	53.33	29.39	1.47	0.01	1.58	0.228
Full MB-B	49.73	26.47	1.32	-0.03	-5.28	**<0.001*** **
Full MB-B-DB	52.27	26.11	1.30	0.00	-0.44	0.531
Full MB-B-DL	52.19	26.05	1.30	0.00	-0.59	0.132
CPITN	60.28	26.66	1.33	0.08	14.82	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
**Paired t-test, P<0.01; ***Paired t-test, P<0.001.

Table S3.3.6. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with CAL ≥ 5 mm.

	% CAL ≥ 5 (n=393)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	35.62	25.80	1.29			
Partial Mouth PRPs						
Half NHANES III UR/LR	34.31	26.41	1.32	-0.01	0.85	**0.029***
Half NHANES III UL/LL	31.86	25.62	1.28	-0.04	-6.52	**<0.001*** **
Half NHANES IV UR/LR	35.72	26.33	1.31	0.00	6.96	0.923
Half NHANES IV UL/LL	34.49	25.54	1.27	-0.01	1.76	**0.031***
Half MB-B-DL UR/LR	36.01	26.19	1.31	0.00	4.93	0.452
Half MB-B-DL UL/LL	34.04	25.97	1.30	-0.02	-2.36	**<0.001*** **
Half 6 Sites UR/LR	36.20	26.73	1.33	0.01	3.11	0.176
Half 6 Sites UL/LL	34.71	26.47	1.32	-0.01	-3.92	**0.004****
Half RD6 UR/LL	34.59	26.17	1.31	-0.01	-5.17	**0.002****
Half RD6 UL/LR	36.29	26.78	1.34	0.01	4.59	0.083
Full-Mouth PRPs						
Ramfjord	37.13	28.47	1.42	0.02	12.62	**0.029***
Full MB-B	33.18	24.54	1.22	-0.03	-2.58	**<0.001*** **
Full MB-B-DB	35.28	24.75	1.23	0.00	4.68	0.292
Full MB-B-DL	35.18	25.04	1.25	0.00	1.65	**0.028***
CPITN	20.67	19.96	1.00	0.09	47.14	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; **Paired t-test, P<0.01; ***Paired t-test, P<0.001

Table S3.3.7. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with CAL ≥ 6 mm.

	% CAL ≥ 6 (n=381)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	22.27	21.83	1.09			
Partial Mouth PRPs						
Half NHANES III UR/LR	21.41	22.84	1.14	-0.01	-2.75	0.140
Half NHANES III UL/LL	18.94	21.12	1.05	-0.04	-12.46	**<0.001*** **
Half NHANES IV UR/LR	22.43	22.83	1.14	0.00	5.26	0.764
Half NHANES IV UL/LL	20.81	21.54	1.07	-0.02	1.46	**0.001****
Half MB-B-DL UR/LR	22.87	22.92	1.14	0.01	6.29	0.173
Half MB-B-DL UL/LL	20.47	21.74	1.08	-0.02	-7.50	**<0.001*** **
Half 6 Sites UR/LR	23.00	23.01	1.15	0.01	4.78	0.057
Half 6 Sites UL/LL	21.38	22.33	1.11	-0.01	-5.80	**0.002****
Half RD6 UR/LL	21.34	21.92	1.09	-0.01	-5.92	**0.002****
Half RD6 UL/LR	22.91	23.08	1.15	0.01	5.51	0.066
Full-Mouth PRPs						
Ramfjord	23.07	24.65	1.23	0.01	7.39	0.165
Full MB-B	20.21	20.49	1.02	-0.02	-7.05	**<0.001*** **
Full MB-B-DB	21.70	20.94	1.04	-0.01	4.17	0.066
Full MB-B-DL	21.72	21.30	1.06	-0.01	-0.17	**0.001****
CPITN	11.10	14.75	0.74	0.08	64.87	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
**Paired t-test, P<0.01; ***Paired t-test, P<0.001.

Table S3.3.8. Comparison of means, standard deviations, standard error, bias, and percent relative bias percentages of sites with CAL ≥ 7 mm.

	% CAL ≥ 7 (n=348)					
	Mean (%)	SD (%)	SE (%)	Bias*	Relative Bias (%)	P-value
Full Mouth (standard)	13.13	17.16	0.86			
Partial Mouth PRPs						
Half NHANES III UR/LR	12.39	18.31	0.91	-0.01	-2.04	0.133
Half NHANES III UL/LL	11.14	16.78	0.84	-0.02	-10.75	**<0.001*** **
Half NHANES IV UR/LR	13.01	18.43	0.92	0.00	6.92	0.779
Half NHANES IV UL/LL	12.10	17.02	0.85	-0.01	0.28	0.008**
Half MB-B-DL UR/LR	13.70	18.71	0.93	0.01	9.97	**0.131**
Half MB-B-DL UL/LL	11.99	17.06	0.85	-0.01	-6.79	**<0.001*** **
Half 6 Sites UR/LR	13.65	18.69	0.93	0.01	6.69	0.112
Half 6 Sites UL/LL	12.53	17.39	0.87	-0.01	-7.83	**0.019***
Half RD6 UR/LL	12.42	16.68	0.83	-0.01	-4.38	**0.01***
Half RD6 UL/LR	13.62	18.86	0.94	0.01	3.89	0.089
Full-Mouth PRPs						
Ramfjord	13.85	19.96	1.00	0.01	11.56	0.123
Full MB-B	11.81	16.18	0.81	-0.02	-5.52	**<0.001*** **
Full MB-B-DB	12.59	16.53	0.83	-0.01	4.34	**0.038***
Full MB-B-DL	12.91	16.81	0.84	0.00	2.25	0.089
CPITN	3.46	7.24	0.36	0.06	77.01	**<0.001*** **

SD – standard deviation; SE – standard error; UR – upper right; LR – lower right; UL – upper left; LL – lower left; MB – mesiobuccal; B – buccal; DB – distobuccal; DL – distolingual; PRPs – partial recording protocols, RD – random diagonal quadrants; CPITN – community periodontal index of treatment needs.
*Paired t-test, P<0.05; **Paired t-test, P<0.01; ***Paired t-test, P<0.001

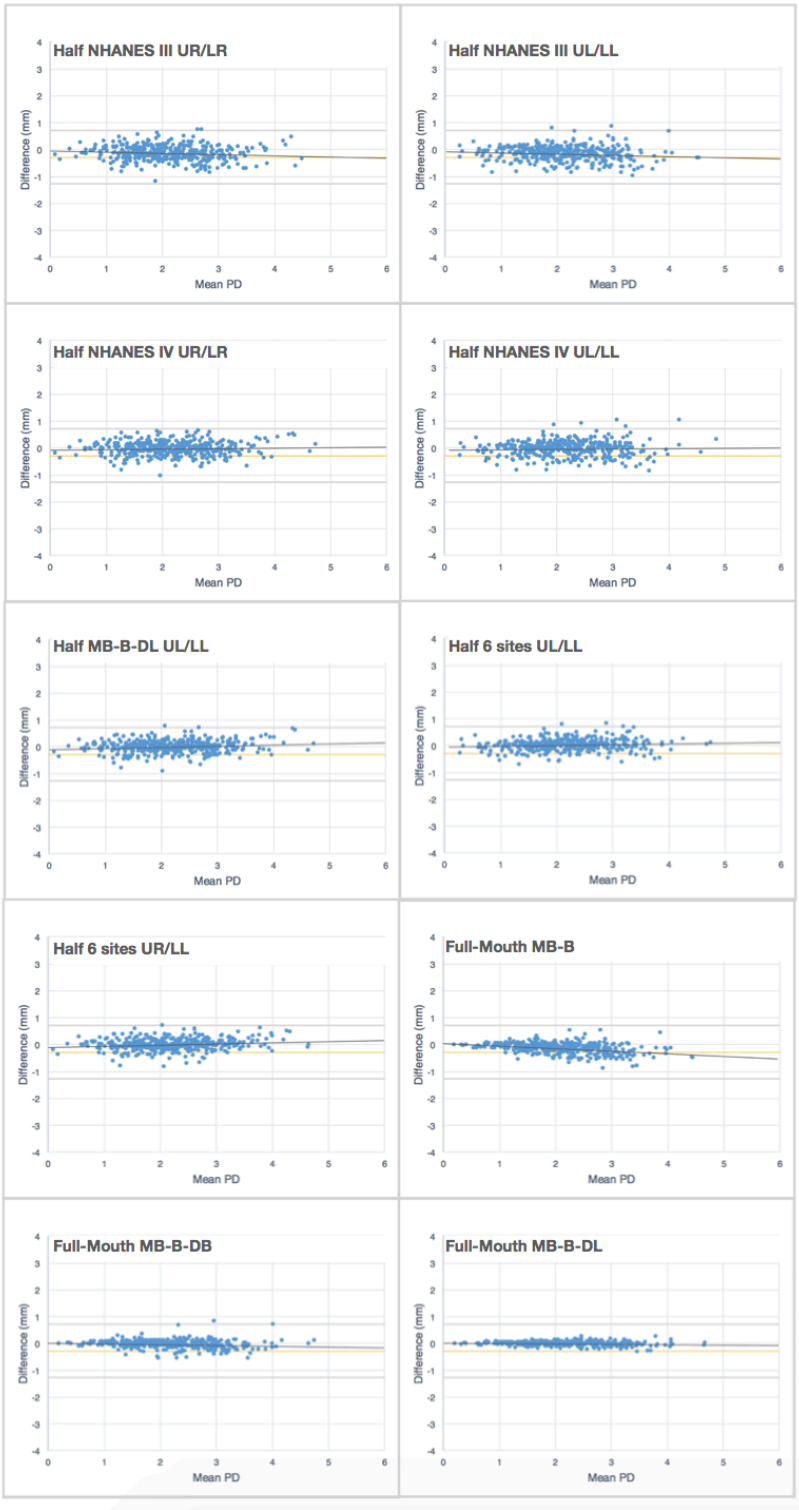

Figure S3.3.9. Bland-Altman plots to evaluate bias between the Mean PD (MPD) differences for Half NHANES III UR/LR, Half NHANES III UL/LL, Half NHANES IV UR/LR, Half NHANES IV UL/LL, Half MB-B-DL UL/LL, Half 6 sites UL/LL, Half RD6 UR/LL, Full-Mouth MB, Full-Mouth MB-B-DB and Full-Mouth MB-B-DL. The area within the upper and lower orange lines sets 95% confidence interval and the yellow line the mean value.

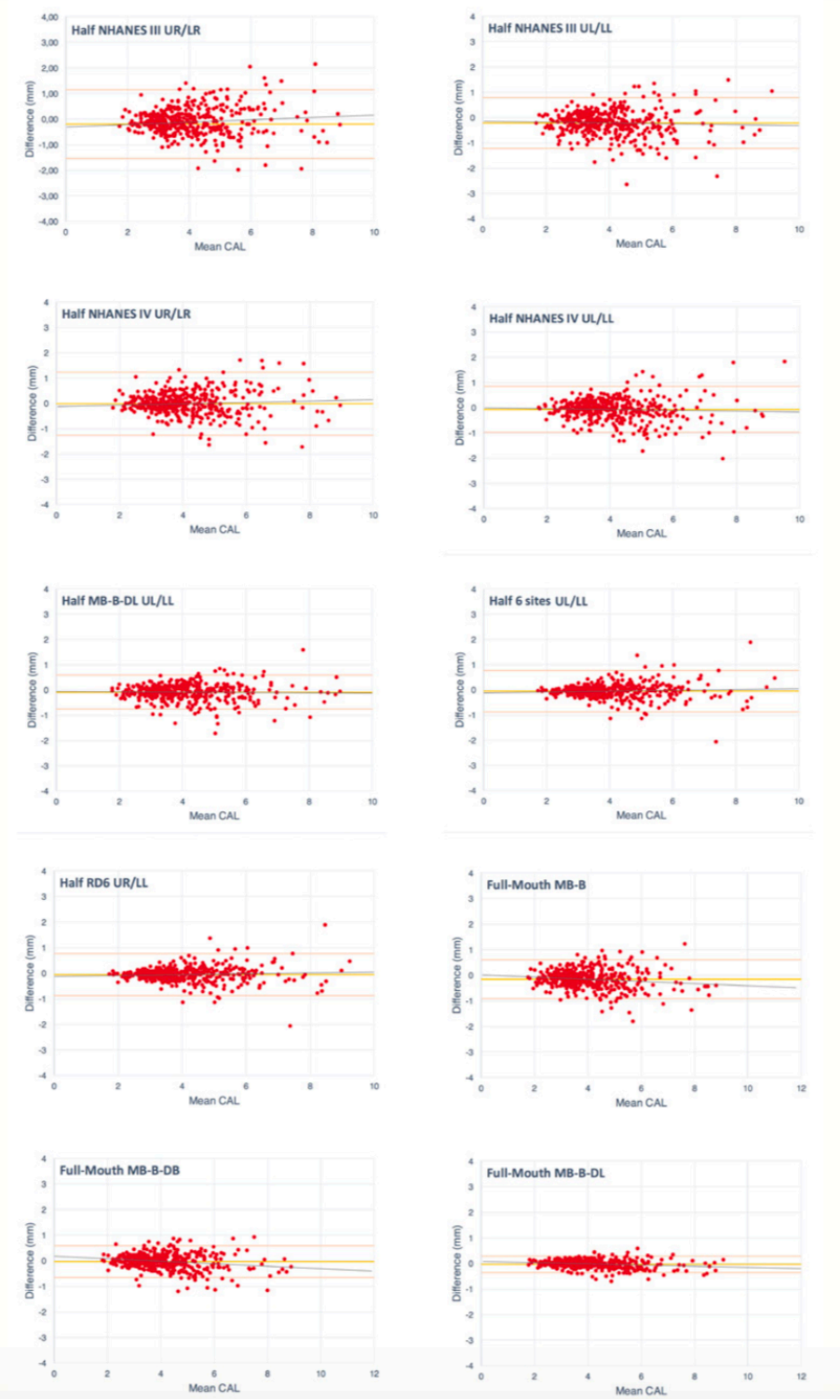

Figure S3.3.10. Bland-Altman plots to evaluate bias between the Mean CAL (MCAL) differences for Half NHANES III UR/LR, Half NHANES III UL/LL, Half NHANES IV UR/LR, Half NHANES IV UL/LL, Half MB-B-DL UL/LL, Half 6 sites UL/LL, Half RD6 UR/LL, Full-Mouth MB, Full-Mouth MB-B-DB and Full-Mouth MB-B-DL. The area within the upper and lower orange lines sets 95% confidence interval and the yellow line the mean value.

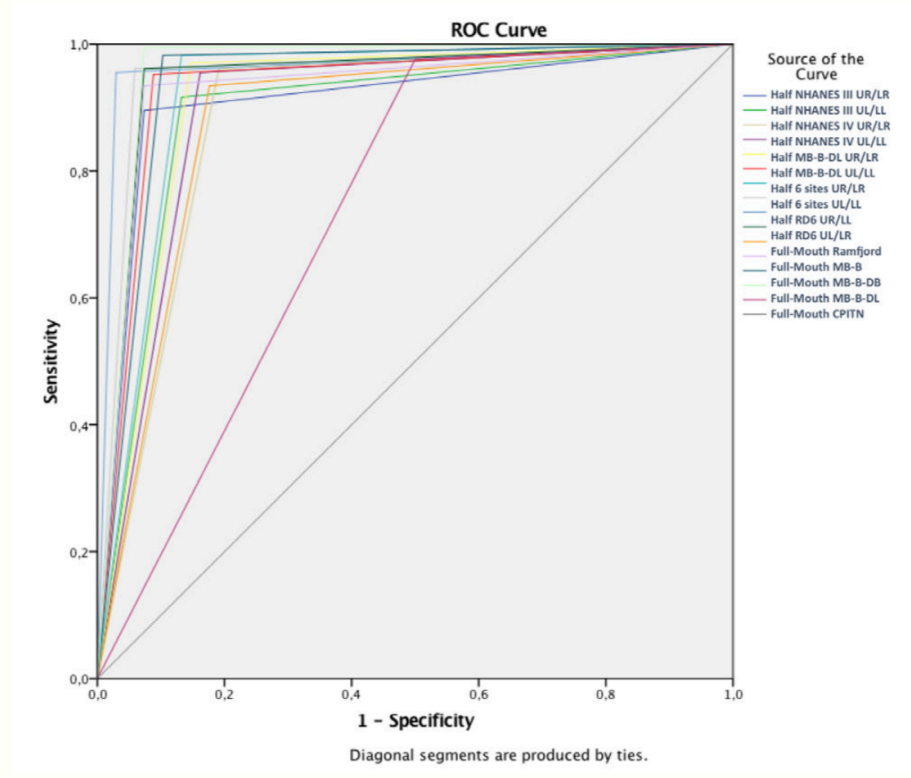

Figure S3.3.11. ROC curves for markers PRPs in discriminating patients with periodontal disease from healthy subject.

3.4. Fine-tuning multilevel modeling of risk factor associated with nonsurgical periodontal treatment outcome

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Abstract

This retrospective study evaluated the influence of known risk factors on nonsurgical periodontal treatment (NSPT) response using a pocket depth fine-tuning multilevel linear model (MLM). Overall, 37 patients (24 males and 13 females) with moderate-to-severe chronic periodontitis underwent NSPT. Follow-up visits at 3, 6, and 12 months included measurements of several clinical periodontal parameters. Data were sourced from a previously reported database. In a total of 1416 initially affected sites (baseline PD ≥ 4 mm) on 536 teeth, probing depth (PD) and clinical attachment loss (CAL) reductions after NSPT were evaluated against known risk factors at 3 hierarchical levels (patient, tooth, and site). For each post-treatment follow-up, the variance component models fitted to evaluate the 3-level variance of PD and CAL decrease revealed that all levels contributed significantly to the overall variance ($p < 0.001$). Patients who underwent NSPT and were continually monitored had curative results. All 3 hierarchical levels included risk factors influencing the degree of PD and CAL reduction. Specifically, the type of tooth, surfaces involved, and tooth mobility site-level risk factors had the strongest impact on these reductions and were highly relevant for the success of NSPT.

3.4.1. Introduction

Periodontitis is an inflammatory disease that progressively destroys tooth-supporting structures and, according to the Global Burden of Disease Study (GBD, 1990–2010), its severe form is the sixth most prevalent disease worldwide, affecting 11% of the overall population [1-6]. The complexity of bacterial biofilms, the "silent pattern" of progression, and poor awareness of periodontal health in individuals hinders its treatment and requires a motivated patient and long-term compliance for a successful treatment outcome [5-10].

Currently, periodontitis treatment approaches consist of nonsurgical (NSPT) and surgical treatments (SPT) that are centered on the patient [11-13]. Conventional NSPT is the mainstay of periodontitis treatment and is shown to have meaningful results [5,12]; however, the presence of residual pockets may jeopardize tooth survival [14,15], requiring NSPT or SPT [13].

The application of multilevel modeling (MLM) to periodontal research was proposed by Albandar and Goldstein [16] in an attempt to integrate explanatory variables in a hierarchical clustering analysis. Numerous articles have subsequently validated the utility of that analysis, which provides clear insights into periodontal research, from disease onset and progression to risk factors to healing response [17-29].

Aside from the extensive literature on NSPT outcomes [11-13], MLM approaches to NSPT upshots are not as commonly reported, but they have shown that smoking habits, tooth type, use of antibiotics, baseline probing depth (PD), baseline clinical attachment loss (CAL), baseline tooth mobility, and frequency of periodontal maintenance are relevant factors for the success of NSPT [21,23,24,28,29]. Notably, this is the first time an MLM analysis has been applied to a Portuguese periodontitis patient sample to highlight the factors influencing the therapeutic result of NSPT.

Therefore, the present retrospective study used pocket depth fine-tuning MLM to evaluate the influence of defined risk factors that may affect NSPT for moderate-to-severe chronic periodontitis (CP) in Portuguese patients. This study hypothesized that PD and CAL reduction are affected by patient, tooth, and site-level factors after NSPT, including age, sex, body mass index (BMI), educational background, smoking, tooth type, specific baseline clinical parameters, and tooth surface location.

3.5.2. Methodology

Ethical considerations

The data analyzed in this study were sourced from a previously reported database [30] on the effect of risk factors in a Portuguese cohort. Our study was approved by the Egas Moniz Ethics Committee (IRB approval number: 595), and informed consent was obtained from all subjects. All data were recorded in a database specifically created for this purpose, where a code number was assigned to each participant. Periodontal intervention was performed according to the approved guidelines and regulations of this retrospective cohort study.

Patient selection

Of the 405 initial patients, a total of 37 were evaluated in our 12-month retrospective clinical study (Figure 3.4.1). The patients were referred to the Department of Periodontology at the Egas Moniz Dental Clinic, Almada (Portugal) between 2015 and 2017. All patients had moderate-to-severe periodontitis according to Page and Eke case definitions [31]. Inclusion criteria were: a) patients aged 35 to 60 years with no previous periodontal or orthodontic treatment; b) at least 6 standing teeth (excluding third molars); and c) no serious mental illness or cognitive dysfunction. Exclusion criteria were: a) patients who did not consent to NSPT or regular follow-up visits; b) a history of systemic antibiotic or periodontal treatment in the previous 3 months; c) pregnant or lactating females; and d) failure to follow up. All eligible participants had previously completed an in-person oral survey.

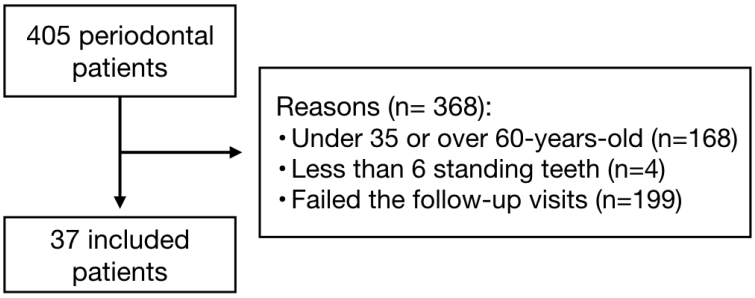

Figure 3.4.1. Flowchart of the included patients and reasons for exclusion.

Clinical procedures

The questionnaire included general information including sex (male/female), age, educational level (elementary/middle/higher), and smoking history. Height of the patients was measured in centimetres, using a vertically installed hard ruler secured to a stable base. Weight was evaluated in kilograms using mechanical scales. BMI was calculated as the ratio of the individual's body weight to their height squared. Self-reported hypertension and diabetes were extracted from the medical questionnaire. All patients received the periodontal diagnosis, NSPT, and follow-up, including oral hygiene instruction on brushing and interdental cleaning, and regular follow-up visits at 3, 6, and 12 months. NSPT was performed by undergraduate students under the supervision of a periodontist, according to the protocol of [32] an average of 4 sessions. Data were collected at baseline and at 3-, 6-, and 12-month follow-up after NSPT. Before the periodontal evaluation, the number of missing teeth was recorded (excluding third molars), and the plaque index (PI) was assessed via the plaque control record (PCR) [33] in 6 sites (mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual, and distolingual). PD, bleeding on probing (BOP), and CAL were determined at the same 6 sites per tooth at baseline and follow-up visits using a manual periodontal probe (CP-12 SE Hu-Friedy, Chicago, USA). Circumferentially, PD was defined as the distance from the cementoenamel junction (CEJ) to the bottom of the pocket and recession (REC) as the distance from the CEJ to the free gingival margin, and this assessment was assigned a negative value if the gingival margin was coronal to the CEJ. CAL was calculated as the algebraic sum of PD and REC. The presence of furcation involvement (FI) was evaluated using a Nabers probe (2N Hu-Friedy) [34], after examining the molars and upper first premolars and tooth mobility [35]. All of the periodontal parameters mentioned above were repeated at each follow-up visit. Teeth extracted during the follow-up period were excluded from the multilevel analysis.

MLM variable assignment

At the patient level, age; BMI; number of missing teeth; and percentage of sites with plaque index, BOP, and PD ≥ 5 mm at baseline were used as continuous

variables, and sex (female = 0, male = 1), smoking habit (yes = 2, former smoker = 1, no = 0), diabetes (yes = 1, no = 0), and hypertension (yes = 1, no = 0) were used as categorical variables. At the tooth level, tooth position (anterior = 1; premolar = 2; molar = 3), mobility (physiologic mobility < 0.2 = 0; mobility ≤ 1 mm = 1; 1 mm < mobility ≤ 2 mm = 2; and mobility > 2 mm = 3) and FI (no involvement = 0; degree I = 1; degree II = 2; degree III = 3) were used as categorical variables. At the site level, PD, CAL, plaque index, and BOP values at baseline were used as continuous variables, and interproximal versus mid surfaces (mesiobuccal/distobuccal/mesiolingual/distolingual = 1; mid-buccal/mid-lingual = 2) and buccal versus lingual surfaces (mesiobuccal/mid-buccal/distobuccal = 1; mesiolingual/mid-lingual/distolingual = 2) were used as categorical variables.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics software, Version 24 (IBM Corp, Armonk, USA). Data were filtered to select only treated sites (baseline PD ≥ 4 mm). Means were reported with standard deviation (SD): mean (± SD). After analyzing the descriptive statistics, we confirmed the hierarchical structure of periodontal disease measurements by performing 3-level (tooth site, tooth, and patient) variance component modeling for both PD and CAL healing response to treatment. Because the site-level treatment response was not truly independent, we tested the data for other MLM assumptions and continued with the MLM analysis once they were met 16,28,29] (Table 3.4.1).

This type of analysis weighs the influence of multilevel nested factors on the reduction of PD and CAL after NSPT. To prevent over-fitness, MLM was reduced from redundant variables through backward stepwise analysis ($p > 0.1$, cut-off for removal). In addition, the treatment outcome at the 3-, 6-, and 12-month follow-up visits was compared via nested, repeated-measures ANOVA using the Greenhouse-Geisser correction. When differences were identified, post-hoc pairwise multiple comparison tests were conducted using the conventional 5% statistical significance via modified Bonferroni adjustment.

Table 3.4.1. Fixed intercept models for reduction in PD and CAL.

Variable	3-month Estimate (SE)	p-value	6-month Estimate (SE)	p-value	12-month Estimate (SE)	p-value	3-month Estimate (SE)	p-value	6-month Estimate (SE)	p-value	12-month Estimate (SE)	p-value
Intercept patient-level												
Age	-0.02 (0.01)	0.141	-0.02 (0.01)	0.222	-0.02 (0.02)	0.238	-0.02 (0.01)	0.107	-0.02 (0.01)	0.181	-0.02 (0.01)	0.239
Gender (female versus male)	0.22 (0.29)	0.691	0.22 (0.29)	0.438	0.11 (0.33)	0.748	0.01 (0.25)	0.981	0.12 (0.24)	0.615	0.01 (0.28)	0.973
Non Smoker (versus smoker)	0.41 (0.24)	0.299	0.41 (0.24)	0.087	0.36 (0.27)	0.173	0.25 (0.26)	0.347	0.16 (0.24)	0.513	-0.21 (0.27)	0.428
No Diabetes (versus diabetes)	-0.02 (0.51)	0.844	-0.2 (0.51)	0.704	-0.50 (0.58)	0.401	0.19 (0.46)	0.688	0.09 (0.43)	0.831	-0.22 (0.5)	0.661
No Hypertension (versus hypertension)	0.25 (0.23)	0.051	0.25 (0.23)	0.268	0.40 (0.25)	0.119	0.47 (0.23)	0.043*	0.24 (0.21)	0.254	0.34 (0.24)	0.161
BMI	0.05 (0.04)	0.081	0.05 (0.04)	0.183	0.06 (0.04)	0.166	0.02 (0.03)	0.508	0.01 (0.03)	0.827	0.02 (0.04)	0.539
Education background	0.52 (0.35)	0.273	0.52 (0.35)	0.141	0.26 (0.39)	0.508	0.26 (0.31)	0.410	0.40 (0.29)	0.183	0.14 (0.34)	0.676
Number of missing teeth	-0.06 (0.03)	0.683	-0.06 (0.03)	0.050*	-0.04 (0.03)	0.250	-0.00 (0.03)	0.922	-0.05 (0.02)	0.050*	-0.03 (0.03)	0.295
% of sites with PD ≥ 5 mm at baseline	-0.00 (0.02)	0.301	-0.00 (0.02)	0.917	-0.00 (0.02)	0.937	-0.01 (0.01)	0.513	0.01 (0.01)	0.542	-0.00 (0.02)	0.761
Tooth-level												
Molars (reference)												
Anteriors	0.36 (0.08)	0.002**	0.36 (0.08)	< 0.001***	0.33 (0.08)	< 0.001***	0.22 (0.09)	0.014*	0.33 (0.08)	< 0.001***	0.29 (0.09)	0.001**
Premolars	0.24 (0.08)	0.035*	0.24 (0.08)	0.005**	0.26 (0.09)	0.004**	0.17 (0.1)	0.069	0.23 (0.09)	0.009**	0.25 (0.09)	0.009**
Degree III (reference)												
Degree 0	0.89 (0.31)	0.001**	0.89 (0.31)	0.004**	0.60 (0.34)	0.075	1.14 (0.36)	0.001**	0.92 (0.32)	0.004**	0.60 (0.35)	0.084
Degree I	0.83 (0.31)	0.006**	0.83 (0.31)	0.008**	0.39 (0.33)	0.244	0.91 (0.36)	0.011*	0.79 (0.32)	0.014*	0.34 (0.35)	0.333
Degree II	0.65 (0.31)	0.018*	0.65 (0.31)	0.036*	0.32 (0.34)	0.344	0.82 (0.36)	0.023*	0.65 (0.32)	0.044*	0.30 (0.35)	0.396
FI	0.20 (0.11)	0.178	0.20 (0.11)	0.080	0.11 (0.13)	0.400	0.14 (0.13)	0.281	0.18 (0.12)	0.144	0.08 (0.13)	0.572
Site-level												
Surface (Interproximal vs. Center)	0.44 (0.07)	< 0.001***	0.44 (0.07)	< 0.001***	0.46 (0.07)	< 0.001***	0.28 (0.08)	<0.001***	0.41 (0.07)	< 0.001***	-0.43 (0.07)	< 0.001***
Surface (B vs. L)	0.16 (0.05)	0.075	0.16 (0.05)	0.001**	0.17 (0.05)	< 0.001***	0.07 (0.05)	0.171	0.15 (0.05)	0.002**	0.15 (0.05)	0.002**
Plaque	-0.00 (0.01)	0.856	-0.00 (0.01)	0.691	-0.00 (0.01)	0.789	-0.00 (0.01)	0.950	-0.00 (0.01)	0.838	-0.00 (0.01)	0.886
BoP	-0.01 (0.01)	0.893	-0.01 (0.01)	0.383	-0.02 (0.02)	0.320	-0.00 (0.01)	0.689	-0.01 (0.01)	0.614	-0.01 (0.01)	0.438
Baseline PD	0.78 (0.03)	< 0.001***	0.78 (0.03)	< 0.001***	0.83 (0.03)	< 0.001***	0.56 (0.04)	< 0.001***	0.70 (0.03)	< 0.001***	0.76 (0.03)	< 0.001***
Baseline CAL	-0.04 (0.02)	0.138	-0.04 (0.02)	0.039*	-0.06 (0.02)	0.014*	0.03 (0.02)	0.221	0.02 (0.02)	0.342	-0.00 (0.02)	0.859
Variance												
Patient	0.57 (0.18)	0.002**	0.50 (0.15)	0.001**	0.65 (0.21)	0.002**	0.33 (0.11)	0.002**	0.28 (0.08)	<0.001***	0.46 (0.15)	0.003**
Tooth	0.20 (0.03)	<0.001***	0.15 (0.03)	<0.001***	0.23 (0.03)	<0.001***	0.20 (0.03)	<0.001***	0.20 (0.03)	<0.001***	0.29 (0.04)	<0.001***
Site	0.85 (0.04)	<0.001***	0.69 (0.03)	<0.001***	0.68 (0.03)	<0.001***	0.67 (0.03)	<0.001***	0.68 (0.03)	<0.001***	0.67 (0.03)	<0.001***
Total variance % change in variance												
Patient	-22.6%		-4.8%	-	28.5%	-	1.9%	-	-36.1%	-	10.3%	-
Tooth	-9.1%		-46.3%		-30.5%		-12.7%		-27.5%		15.0%	
Site	-30.3%		-42.3%		-45.9%		-44.3%		-42.0%		-45.4%	

BMI – Body Mass Index, PD – Pocket Depth, BoP – Bleeding on Probing, B – Buccal, L – Lingual, CAL – Clinical Attachment Loss, SE – Standard Error.

*bold face representative P < 0.05. **bold face representative P < 0.01. ***bold face representative P < 0.001.

This type of analysis weighs the influence of multilevel nested factors on the reduction of PD and CAL after NSPT. To prevent over-fitness, MLM was reduced from redundant variables through backward stepwise analysis ($p > 0.1$, cut-off for removal). In addition, the treatment outcome at the 3-, 6-, and 12-month follow-up visits was compared via nested, repeated-measures ANOVA using the Greenhouse-Geisser correction. When differences were identified, post-hoc pairwise multiple comparison tests were conducted using the conventional 5% statistical significance via modified Bonferroni adjustment.

3.4.3. Results

This clinical study investigated a total of 37 patients. The baseline clinical and periodontal parameters are shown in Table 3.4.2. The mean age was 57.92 ± 10.87 years (range 36–75), and the sample had a higher prevalence of male patients (64.86%). Only 7 patients were smokers. The mean BMI was 26.69 (± 3.97 kg/m2). When assessing socioeconomic status, we identified 13 patients with a monthly income up to 580€ (national minimum wage), 11 earning 581€–900€, and 13 earning more than 900€. Most of the individuals had a high school education or below (78.38%). The patients had an average of 7.24 (± 5.00) missing teeth. Diabetes was reported in 11 (29.73%) and hypertension in 17 patients (45.95%). The overall sample included 758 teeth, including 366 anterior teeth, 221 premolars, and 171 molars, of which 574 had physiologic mobility, 114 had grade 1 mobility, 64 had grade 2 mobility, and 6 had grade 3 mobility. At baseline, plaque was noted at 31.64% ± 20.43% of the sites. At baseline, the mean percentage of sites with BOP was 10.56 ± 13.03 and that with PD ≥ 5 mm was 8.18 ± 9.25.

In response to NSPT, full-mouth mean PD and CAL showed significant reductions from baseline at the 3-, 6-, and 12-month follow-up visits. The mean PD was 4.89 mm (± 1.19) at baseline, 3.61 mm (± 1.32) at 3 months, 3.14 mm (± 1.20) at 6 months, and 3.16 mm (± 1.21) at 12 months. The mean CAL was 5.84 mm (± 2.05) at baseline, 4.60 mm (± 2.16) at 3 months, 4.13 mm (± 2.13) at 6 months, and 4.14 mm (± 2.09) at 12 months.

The mean proportion of sites with plaque was 31.64 (± 20.43) at baseline, 21.20 (± 15.11) at 3 months, 21.02 (± 13.75) at 6 months, and 20.60 (± 10.82) at 12 months. The mean percentage of sites with BOP was 10.56 (± 13.03) at baseline,

4.04 (± 5.81) at 3 months, 4.94 (± 5.70) at 6 months, and 4.10 (± 5.48) at 12 months (Table 3.4.2).

Table 3.4.2. Baseline clinical and periodontal parameters by variables (note: table was divided to fit within the page, and the right part is the continuity of the table).

Variable			
Patient level (n = 37)	Mean (SD)	Diabetes	
Age (years)	57.92 (10.87)	Yes	11 (29.73%)
BMI (kg/m²)	26.69 (3.97)	No	26 (70.27%)
Number of missing teeth (N)	7.24 (5.00)	Smokers	
% of sites with plaque at baseline	31.64 (20.43)	Yes	7 (18.92%)
% of sites with plaque at 3-month follow-up	21.20 (15.11)	Former smokers	0 (0.00%)
% of sites with plaque at 6-month follow-up	21.02 (13.75)	No	30 (81.08%)
%of sites with plaque at 12-month Follow-up	20.60 (10.82)	Tooth level (N = 758)	
% of sites with BOP at baseline	10.56 (13.03)	Tooth position	
% of sites with BOP at 3-month follow-up	4.04 (5.81)	Anterior	366 (48.28%)
% of sites with BOP at 6-month follow-up	4.94 (5.70)	Premolar	221 (29.16%)
% of sites with BOP at 12-month follow-up	4.10 (5.48)	Molar	171 (22.56%)
% of sites with PD ≥ 5 mm at baseline	8.18 (9.25)	Mobility	
Patient level (n = 37)	N (%)	No mobility	574 (75.73%)
Sex		Mobility ≤ 1 mm	114 (15.04%)
Male	24 (64.86%)	1 mm < mobility ≤ 2 mm	64 (8.44%)
Female	13 (35.14%)	Mobility > 2 mm	6 (0.79%)
Education		FI (first premolars and molars) (n = 122)	
Elementary School	21 (56.76%)	No involvement	209 (91.14%)
High School	8 (21.62%)	Degree I	9 (4.05%)
Higher	8 (21.62%)	Degree II	2 (0.90%)
Hypertension		Degree III	2 (0.90%)
Yes	17 (45.95%)	Site level (N = 1416)	
No	20 (54.05%)	Tooth surface	
		Buccal/lingual	640 (45.2%)/ 776 (54.8%)
		Interproximal (mesiocclusion/ distocclusion)/mid	1218 (86.0%)/ 198 (14.0%)

BMI: Body mass index; BoP: Bleeding on probing; FI: Furcation involvement; PD: Pocket depth; REC: Recession.

Multilevel statistical analysis

To assess the amount of variance associated with PD and CAL reduction assigned in each studied level, we started the MLM analysis by fitting a variance component model (Table 3.4.3). This model exhibited an unbalanced, though significant ($p < 0.001$), distribution of variance across all 3 levels, with the major proportion due to within-tooth (site) variations. In addition, the mean marginal

products for PD and CAL reduction were all significantly positive throughout the follow-up visits, increasing within the follow-up time period. Although the model results indicated major improvements in the first 3 months after treatment, a smaller but still significant improvement was also demonstrated in the following 3-month period until the 6-month check-up.

Table 3.4.3. Variance component models for reduction in PD and CAL.

Variance	Variance components (%)	SE	p-value	Marginal mean values (SE)
3-month reduction in PD				
Patient (level 3)	0.465 (24.4%)	0.128		
Tooth (level 2)	0.220 (11.5%)	0.043		1.14 (0.12) [a]
Site (level 1)	1.220 (64.0%)	0.054		
6-month reduction in PD				
Patient (level 3)	0.525 (26.3%)	0.139		
Tooth (level 2)	0.280 (14.0%)	0.047	*< 0.001	1.51 (0.13) [b]
Site (level 1)	1.195 (59.8%)	0.054		
12-month reduction in PD				
Patient (level 3)	0.506 (24.2%)	0.138		
Tooth (level 2)	0.331 (15.8%)	0.052		1.56 (0.13) [b]
Site (level 1)	1.257 (60.0%)	0.057		
3-month reduction in CAL				
Patient (level 3)	0.324 (18.5%)	0.093		
Tooth (level 2)	0.229 (13.0%)	0.043		1.10 (0.10) [c]
Site (level 1)	1.202 (68.5%)	0.054		
6-month reduction in CAL				
Patient (level 3)	0.438 (23.2%)	0.117		
Tooth (level 2)	0.276 (14.6%)	0.046	*< 0.001	1.46 (0.12) [d]
Site (level 1)	1.172 (62.1%)	0.053		
12-month reduction in CAL				
Patient (level 3)	0.417 (21.0%)	0.116		
Tooth (level 2)	0.341 (17.2%)	0.052		1.52 (0.12) [d]
Site (level 1)	1.227 (61.8%)	0.055		

*Nested ANOVA repeated measures, $p < 0.05$; [a,c] Post-hoc test (the different letters signify Bonferroni-adjusted significant differences, $p < 0.001$).

Next, we fitted MLM including all of our selected risk factors for PD and CAL reductions (Table 3.4.1). In this crude model, the continuous variables with significant positive coefficients were associated with recovery, while those with significant negative coefficients represented an unfavourable prognosis. Conversely, the categorical variable coefficients were relative to the reference category, with positive values signifying a better prognosis compared to the reference and negative values representing a worse prognosis. To prevent over-

fitness, these models were reduced through backward stepwise analysis ($p < 0.10$ to remain in the model), and the final model variables and associated coefficients are shown in Table 3.4.4.

Table 3.4.4. Adjusted intercept models for reduction in PD and CAL.

Variable	3-month Estimate (SE)	p-value	6-month Estimate (SE)	p-value	12-month Estimate (SE)	p-value	3-month Estimate (SE)	p-value	6-month Estimate (SE)	p-value	12-month Estimate (SE)	p-value
Intercept patient level												
Age (years)	−0.02 (0.01)	0.141	−0.02 (0.01)	0.222	−0.02 (0.02)	0.238	−0.02 (0.01)	0.107	−0.02 (0.01)	0.181	−0.02 (0.01)	0.239
Sex (female vs. male)	0.22 (0.29)	0.691	0.22 (0.29)	0.438	0.11 (0.33)	0.748	0.01 (0.25)	0.981	0.12 (0.24)	0.615	0.01 (0.28)	0.973
Nonsmoker (vs. smoker)	0.41 (0.24)	0.299	0.41 (0.24)	0.087	0.36 (0.27)	0.173	0.25 (0.26)	0.347	0.16 (0.24)	0.513	−0.21 (0.27)	0.428
No diabetes (vs. diabetes)	−0.02 (0.51)	0.844	−0.2 (0.51)	0.704	−0.50 (0.58)	0.401	0.19 (0.46)	0.688	0.09 (0.43)	0.831	−0.22 (0.5)	0.661
No Hypertension (vs. hypertension)	0.25 (0.23)	0.051	0.25 (0.23)	0.268	0.40 (0.25)	0.119	0.47 (0.23)	0.043*	0.24 (0.21)	0.254	0.34 (0.24)	0.161
BMI	0.05 (0.04)	0.081	0.05 (0.04)	0.183	0.06 (0.04)	0.166	0.02 (0.03)	0.508	0.01 (0.03)	0.827	0.02 (0.04)	0.539
Educational background	0.52 (0.35)	0.273	0.52 (0.35)	0.141	0.26 (0.39)	0.508	0.26 (0.31)	0.410	0.40 (0.29)	0.183	0.14 (0.34)	0.676
Number of missing teeth	−0.06 (0.03)	0.683	−0.06 (0.03)	0.050*	−0.04 (0.03)	0.250	−0.00 (0.03)	0.922	−0.05 (0.02)	0.050*	−0.03 (0.03)	0.295
% of sites with PD ≥ 5 mm at baseline	−0.00 (0.02)	0.301	−0.00 (0.02)	0.917	−0.00 (0.02)	0.937	−0.01 (0.01)	0.513	0.01 (0.01)	0.542	−0.00 (0.02)	0.761
Tooth level												
Molars (reference)												
Anteriors	0.36 (0.08)	0.002**	0.36 (0.08)	< 0.001***	0.33 (0.08)	< 0.001***	0.22 (0.09)	0.014*	0.33 (0.08)	< 0.001***	0.29 (0.09)	0.001**
Premolars	0.24 (0.08)	0.035*	0.24 (0.08)	0.005**	0.26 (0.09)	0.004**	0.17 (0.1)	0.069	0.23 (0.09)	0.009**	0.25 (0.09)	0.009**
Degree III (reference)												
Degree 0	0.89 (0.31)	0.001**	0.89 (0.31)	0.004**	0.60 (0.34)	0.075	1.14 (0.36)	0.001**	0.92 (0.32)	0.004**	0.60 (0.35)	0.084
Degree I	0.83 (0.31)	0.006**	0.83 (0.31)	0.008**	0.39 (0.33)	0.244	0.91 (0.36)	0.011*	0.79 (0.32)	0.014*	0.34 (0.35)	0.333
Degree II	0.65 (0.31)	0.018*	0.65 (0.31)	0.036*	0.32 (0.34)	0.344	0.82 (0.36)	0.023*	0.65 (0.32)	0.044*	0.30 (0.35)	0.396
FI	0.20 (0.11)	0.178	0.20 (0.11)	0.080	0.11 (0.13)	0.400	0.14 (0.13)	0.281	0.18 (0.12)	0.144	0.08 (0.13)	0.572
Site level												
Surface (Interproximal vs. center)	0.44 (0.07)	< 0.001***	0.44 (0.07)	< 0.001***	0.46 (0.07)	< 0.001***	0.28 (0.08)	< 0.001***	0.41 (0.07)	< 0.001***	−0.43 (0.07)	< 0.001***
Surface (B vs. L)	0.16 (0.05)	0.075	0.16 (0.05)	0.001**	0.17 (0.05)	< 0.001***	0.07 (0.05)	0.171	0.15 (0.05)	0.002**	0.15 (0.05)	0.002**
Plaque index	−0.00 (0.01)	0.856	−0.00 (0.01)	0.691	−0.00 (0.01)	0.789	−0.00 (0.01)	0.950	−0.00 (0.01)	0.838	−0.00 (0.01)	0.886
BOP	−0.01 (0.01)	0.893	−0.01 (0.01)	0.383	−0.02 (0.02)	0.320	−0.00 (0.01)	0.689	−0.01 (0.01)	0.614	−0.01 (0.01)	0.438
Baseline PD	0.78 (0.03)	< 0.001***	0.78 (0.03)	< 0.001***	0.83 (0.03)	< 0.001***	0.56 (0.04)	< 0.001***	0.70 (0.03)	< 0.001***	0.76 (0.03)	< 0.001***
Baseline CAL	−0.04 (0.02)	0.138	−0.04 (0.02)	0.039*	−0.06 (0.02)	0.014*	0.03 (0.02)	0.221	0.02 (0.02)	0.342	−0.00 (0.02)	0.859
Variance												
Patient	0.57 (0.18)	0.002**	0.50 (0.15)	0.001**	0.65 (0.21)	0.002**	0.33 (0.11)	0.002**	0.28 (0.08)	< 0.001***	0.46 (0.15)	0.003**
Tooth	0.20 (0.03)	< 0.001***	0.15 (0.03)	< 0.001***	0.23 (0.03)	< 0.001***	0.20 (0.03)	< 0.001***	0.20 (0.03)	< 0.001***	0.29 (0.04)	< 0.001***
Site	0.85 (0.04)	< 0.001***	0.69 (0.03)	< 0.001***	0.68 (0.03)	< 0.001***	0.67 (0.03)	< 0.001***	0.68 (0.03)	< 0.001***	0.67 (0.03)	< 0.001***
Total variance % change in variance												
Patient	−22.6%		−4.8%		28.5%		1.9%		−36.1%		10.3%	
Tooth	−9.1%		−46.3%		−30.5%		−12.7%		−27.5%		15.0%	
Site	−30.3%		−42.3%		−45.9%		−44.3%		−42.0%		−45.4%	

BMI: Body mass index; PD: Pocket depth; BoP: p < 0.01; ***bold face signifies p < 0.001. Bleeding on probing; B:Buccal; L: Lingual; CAL: Clinical attachment loss; SE: Standard error. *bold face signifies p < 0.05; **bold face signifies.

The relationship of the risk factors and PD on healing response

Overall, 1416 sites with baseline PD ≥ 4 mm (31.13% of all sites) from 536 teeth of 37 patients were assessed in this study (Table 3.4.4). The mean PD reductions from baseline at 3, 6, and 12 months were 1.29 mm (± 1.38), 1.75 mm (± 1.46), and 1.74 mm (± 1.49), respectively.

The selected site-level risk factor variables demonstrated 30.3%, 42.3%, and 45.9% of the total PD variance reduction at 3, 6, and 12 months. The mid surfaces showed the best prognosis in the reduction of PD at all follow-up visits ($p < 0.001$). Compared to the lingual tooth surfaces, the buccal surfaces had a significantly higher reduction in PD at 6 and 12 months ($p < 0.01$).

The selected tooth-level risk factor variables reduced the unexplained total variance of PD reduction at this intermediate level by 4.6%, 39.3%, and 24.5%, at 3, 6, and 12 months, respectively. Tooth mobility demonstrated a higher reduction in PD at 3 and 6 months ($p < 0.01$). In addition, the anterior teeth and premolars showed a significant decrease in PD at 3, 6, and 12 months ($p < 0.01$).

The unexplained variance in PD reduction at the patient level decreased 19.3%, 29.5%, and 13.0% at 3, 6, and 12 months, respectively, after including the selected patient-level risk factor variables in MLM. Conversely, the number of missing teeth negatively influenced the decrease in PD at 6 months ($p = 0.024$).

A significant difference in PD reduction from baseline was noted between the first follow-up (3 months) and both the second and third follow-ups (6 and 12 months), but not between the second and third follow-ups, even when adjusting for patient and tooth effects.

The relationship of the risk factors and CAL on healing response

This analysis included the same 1416 sites used in the other analyses (Table 3.4.4). Compared with baseline, mean CAL reductions were 1.24 mm (± 1.34), 1.71 mm (± 1.43), and 1.70 mm (± 1.46) at 3, 6, and 12 months, respectively.

At the site level, an unexplained variance decrease of 30.1%, 42.0%, and 46.2% in CAL reduction was found at 3, 6, and 12 months after including the selected risk factors of the fixed-effects variables to MLM. The mid surfaces of the teeth demonstrated a significantly greater reduction in CAL at 3, 6, and 12 months ($p < 0.001$). The buccal surfaces showed a significantly greater reduction at 6 and

12 months ($p < 0.01$) compared to the lingual surfaces. Baseline PD was significant for CAL recovery at all follow-up visits ($p < 0.001$).

The tooth level variables reduced 27.5% and 15.0% of the unexplained variance regarding CAL reduction at 6 and 12 months. Teeth with mobility had greater CAL reduction at 3 and 6 months ($p < 0.01$). Anterior teeth showed a significantly greater reduction at all follow-up visits, whereas premolars only revealed significant improvement at 6 and 12 months ($p < 0.01$).

The unexplained variance in CAL reduction at the patient level was reduced by 19.8%, 36.1%, and 23.3% at 3, 6, and 12 months, respectively. In addition, mean PD at baseline showed a significant positive effect on CAL reduction at 3, 6, and 12 months ($p < 0.001$); however, mean REC at baseline was not significant. The number of missing teeth significantly affected CAL reduction at 6 months ($p = 0.034$).

3.4.4. Discussion

The results of this retrospective study are consistent with previous studies and show that discounting any level may lead to inaccurate conclusions [19,20,29]. The variance component models were used to weigh and compare the risk factors of moderate-to-severe periodontitis after NSPT.

Since it was proposed for use in periodontology research [16], multilevel analysis has been used to investigate the risk factors of periodontitis onset [17,19,20,25,27] and the effect of risk factors in NSPTs and SPTs [21,23,24,28,29] as well as predict bone and tooth loss in maintained periodontal patients [18,22,26]. Though we assume that all sites in periodontitis-onset risk studies are potentially susceptible, we should focus only on the treated sites in periodontitis treatment studies, to avoid misleading or skewing the results using the combination of initial pathological and non-pathological pocket depth locations. Furthermore, Jiao et al. [28] evaluated the NSPT outcomes of all sites against those sites with baseline PD ≥ 5 mm and identified significant differences between the sites. Consequently, in the present study, we limited our analyses to baseline unreliable PD (PD ≥ 4 mm).

At the patient level, most of the covariates did not indicate any influence on post-NSPT recovery, namely, age, sex, smoking, self-reported systemic diseases, educational background, and select clinical parameters. Conversely, BMI and

number of missing teeth showed uncommon significance. Unlike those in previous studies, these patients demonstrated a decreased tendency for gingival bleeding. Mean baseline BOP was 10.56% and was much lower than in American (26.4%–82.01%), Asian, and European patients [23,24,28,36-40]. This is possibly because all of the patients were referred by a screening department at our clinic. During this triage, patients are educated and instructed on oral hygiene. Therefore, the time between the screening and periodontology appointments could a hypothetically influence reduction of baseline BOP. This decreased tendency may explain why the percentage of BOP did not affect NSPT outcomes as previously reported [23,24,28,29].

At the tooth level, a more significant reduction in PD and CAL was seen in the anterior teeth (incisors and canines) compared to the molars during the follow-up period, but this was seen only between the molars and premolars at 6 and 12 months. These results are consistent with previous studies [21,23,28,29,41], although Jiao et al. [28] compared molar and non-molar teeth, while PD reduction was not as significant in the study by Song et al.29 Molars are well known to have a worse healing prognosis due to anatomical and morphological characteristics such as furcation and dimensions of furcation entrance, root trunk length, bifurcation ridges, root concavities, and cervical enamel projections [12,41,42]. Furthermore, premolars have some characteristics that worsen the prognosis but less so compared to molars [12,41,42]. Moreover, initial hypermobility was associated with worse treatment outcomes but only during the first 6 months after NSPT.

At the site level, the mid teeth surfaces showed more reduction in PD and CAL at 3, 6, and 12 months. Compared to the lingual surfaces, the buccal surfaces had a more significant decrease at 6 months, resulting in a significantly higher recovery. As reported by Song et al. [29] the interproximal surfaces had less improvement compared to the mid surfaces, with more significant values for PD. However, the buccal surfaces demonstrated more substantial recovery, only at 6 and 12 months, as opposed to the results of Wan et al., [23] which demonstrated an improvement on the lingual sites. Although the reason for less recovery on the interproximal surfaces can be explained by a marked history of worse interproximal hygiene, the difference between buccal and lingual surfaces is not easy to explain. In the future, additional studies are needed to understand this matter thoroughly. However, baseline PD mainly influenced the efficacy of NSPT

during the 3 follow-up periods in a progressive manner, showing that the initial PD may guide the treatment outcome as previously demonstrated [21].

A limitation of the present study is its limited sample size, which may lead to unpowered analysis and test results, even though we have identified the same limitation in similar MLM studies [21,23,29]. from $p < 0.05$ to $p < 0.10$ and by fitting the model strictly with data from treated sites. The cost of NSPT is expensive and is not reimbursed by most forms of insurance. In addition, the response rate was quite low (9.1%) despite efforts to ensure patient participation, which can be explained by the poor awareness of dental health and lack of follow-up in this population, highlighted recently by our group.[30] On the other hand, the retrospective nature of the study and various clinicians treating and examining participants can increase the probability of consistent failures.

3.4.5. Conclusion

In the present study, pocket depth fine-tuning MLM showed that NSPT had a significant healing effect for moderate-to-severe CP with considerable reductions in PD and CAL. PD and CAL showed major recovery in the first 3 months after NSPT. The PD fine-tuning MLM analysis found that all 3 levels influenced the reduction of PD and CAL levels. The largest effect on PD and CAL reductions was seen at the site level.

3.4.6. References

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3.5. Stress, Salivary Cortisol and Periodontitis: A Systematic Review and Meta-analysis of Observational Studies

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Abstract

Objective: This meta-analysis aims to systematically assess whether periodontitis has a meaningful effect on salivary cortisol, reflecting changes on free blood cortisol levels.

Design: The Cochrane Handbook and the PRISMA statement were used as reporting guidelines. The MEDLINE-PubMed, Google Scholar, EMBASE, and CENTRAL databases were searched until September 2017 to identify eligible studies, screened by seven independent authors and verified by an eighth. Studies comparing salivary cortisol level of periodontitis cases to controls were included. Data were extracted using a predefined table and since all papers were non-randomized clinical trials they were appraised using Downs and Black tool. DerSimonian random effects meta-analysis was performed using OpenMetaAnalyst.

Results: Six cross-sectional studies were included, with 258 participants with chronic periodontitis and 72 with aggressive periodontitis, in a total of 573 participants. Overall results showed that aggressive periodontitis patients have, on average, 53% higher salivary cortisol levels than healthy controls 1.53 (1.11-2.12). Meta-regression exploring the relationship among salivary cortisol levels and

periodontal measures, i.e., periodontitis severity, showed a global neutral effect, although this result requires future confirmation due to the low power of the model.

Conclusion: Observational studies results suggest that subjects with aggressive periodontitis have higher salivary cortisol levels than healthy ones or patients with chronic periodontitis. Such salivary cortisol response difference may have a negative impact on the periodontium, contributing to worse the burden of aggressive periodontitis disease. In the future, wide and well-designed longitudinal studies should be carried out in order to extensively confirm this possible effect, considering the complex nature of periodontitis and its many confounders factors that may contribute to this outcome.

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3.5.1. Introduction

Periodontitis is a polymicrobial disruption of host homeostasis that induces chronic inflammatory disease of the periodontium and causes the destruction of the supporting structures of the dentition [1]. Each year millions of people are affected by periodontitis, however there is epidemiological evidence that the initiation, progression and severity of periodontal disease do not affect all people in the same way [2–5].

Periodontal diseases have a significant impact on oral health-related quality of life with potentially destructive consequences [6]. Furthermore, periodontitis has also been associated with many systemic diseases and conditions including diabetes, stroke, obesity, rheumatoid arthritis, alcoholism, inflammatory bowel diseases and pancreatic cancer, becoming increasingly important the understanding of the surrounding pathological mechanisms beyond periodontitis development [7–15]. It is known that periodontal disease is more widespread and severe in those extensively exposed to chronic impaired stress [16–18]. The main culprit pointed for is cortisol. Thus, if individual attempts to cope with stress fail recurrently, cortisol levels can stay chronically elevated, consequently downregulating the cellular immune response. This status, across time, would lead to changes in periodontal tissues resistance, raising the

susceptibility towards periodontitis development [17]. In such perceived chronic stress, salivary cortisol, following free blood cortisol, would similarly be elevated after awakening [19–23]. This straightforward relationship connecting stress, blood and salivary cortisol, makes the latter the biomarker of choice in stress investigation for the non-invasive assessment of free cortisol levels [24]. Nevertheless, some modulating factors act as confounders in the perception of blood cortisol through salivary cortisol levels, including age and common hormonal variation in women [23,25,26].

Periodontitis and stress seem to have a bidirectional relationship. The mechanisms supporting this connection are extensively proposed elsewhere [17]. An earlier report points out an association between periodontal disease and psychological factors, specifically the reaction to stressful life events, including workplace stress [27]. Later, Genco et al. [16] found increased salivary cortisol levels in patients exhibiting severe periodontitis, high-level financial strain and coping behaviors. Moreover, further studies have investigated chronically elevated cortisol as a potential risk factor for periodontal disease early onset or severity, although such an association has not been assessed so far in a systematic evidence-based manner [28–42].

On the other hand, a systematic review recently published revealed a positive effect of periodontal disease on psychological measures of stress [18], and further independent studies also reported an increase of blood, urinary, salivary and gingival crevicular cortisol levels in periodontitis patients [37–41]. Regardless the accumulating evidence reported essentially on cross-sectional studies, there is still not enough understanding about the role of periodontitis as a chronic stressor disease, and about the associated blood and salivary cortisol levels backfire on the burden of periodontal disease. For instance, very little is known about the effect of salivary cortisol on the soft and hard tissues surrounding the teeth.

Furthermore, some known handicaps make it difficult for researchers to perform unbiased well-designed observational studies on periodontitis patients. Uncontrolled confounders, lack of adhesion to the treatment appointments and ethical concerns regards to control untreated groups, among other issues, are well-known examples. For those reasons, most studies highlighting the relationship between periodontitis and stress through cortisol biomarkers are somehow faulty regarding research quality guidelines, making urgent to perform

a systematic revision assessing the potential different sources of bias and uncovering individual studies data hidden trends through meta-analysis synthesis. Therefore, the primary aim of this systematic review was to determine if there is an association between salivary cortisol levels and periodontitis, with the main research question being: "Do periodontitis patients have higher salivary cortisol levels than healthy patients?". The secondary aim was to appraise, through meta-regression, whether salivary cortisol levels are associated with periodontal measures, i.e., with periodontitis severity.

3.5.2. Materials and Methods

Protocol and registration

The protocol for this systematic review was made *a priori*, agreed upon by all authors and registered in PROSPERO (ID Number: CRD42017079026). This systematic review was reported according to the PRISMA statement [43] (Table S3.4.1) and its extension for abstracts [44].

In the systematic revision procedure were involved a team that included: three researchers of the Periodontology Department, Clinical Research Unit, CiiEM, [Instituto Universitário Egas Moniz]: JB, VM and RA; one researcher of the Environmental Health Research Line, CiiEM, [Instituto Universitário Egas Moniz]: MAC; two researchers of the Clinical Research Unit, CiiEM, [Instituto Universitário Egas Moniz]: AD, JJM; and one biostatistics expert of the Periodontology Department, Clinical Research Unit, CiiEM, [Instituto Universitário Egas Moniz]: PM.

The review PICO research question was: "Do periodontitis patients have higher salivary cortisol levels than healthy patients?"; with the following statements: Adult patients (Patients – P); Chronic and aggressive periodontitis (Intervention/Exposure – I); Patients without periodontitis (Comparison – C); salivary cortisol (Outcome – O).

Eligibility criteria

Inclusion and exclusion criteria were determined *a priori*. Both randomized controlled studies (RCTs) and non-RCTs that assessed any of the pre-specified

periodontal or oral health outcomes in patients with salivary cortisol levels were included.

Eligibility criteria for Outcome Measure

To be included, salivary cortisol levels using standardized measures needed to be reported in both periodontitis population and non-periodontitis population.

Information sources and search

Electronic general, open access, regional and grey literature databases were systematically searched up to September 2017. MESH terms and relative keywords were used accordingly for each electronic database. No limitations were applied regarding publication year. Only English language papers were selected. The reference lists of included articles and relevant reviews were manually searched. Grey literature was searched through appropriate databases and registers. Authors were contacted when necessary for additional data or clarifications.

We combined keywords and subject headings under the thesaurus of each database and applied exploded subject headings. Our search string consisted of three components: 1) “cortisol” and synonyms, 2) “periodontitis”, 3) “stress”. All searches were confined to studies conducted in humans.

Study selection

Study selection was initially conducted by two authors (JB and VM), who screened the titles and/or abstracts of retrieved studies. Final selection of studies was performed by seven authors independently (JB, VM, PM, JR, MAC, AD and JJM), and verified by an eighth author (RA) by reviewing the full text based on inclusion criteria above. Any disagreements were resolved by discussion.

Data extraction process and data items

Data were extracted onto a predefined data extraction table. Data obtained included: the first author’s name, study design, publication year, country where the study was conducted, mean age at baseline years, number of cases and

participants, gender, smoking history, diagnostic criteria of periodontitis and periodontitis measure. These included percentage with periodontitis, probing depth (PD), plaque index (PI), missing teeth, the proportion of sites with plaque, bleeding on probing (BOP), and clinical attachment loss (CAL). All Data were independently extracted by three reviewers (JB, VM, and PM) with a consensus on all of the aspects.

Quality Assessment

The risk of bias and quality assessment of the selected individual studies (all non-RCTs) were assessed with a Downs & Black checklist [45]. Disagreements between the review authors over the risk of bias in particular studies were resolved by discussion, with the involvement of a seventh review author where necessary.

Summary Measures & Synthesis of results

Median and interquartile range reported in selected studies for salivary glucose for cases and controls were converted to mean and standard deviations following Hozo et al [46] procedure, under the assumption of normal distribution. Next, log scale ratio of means (Log(RM)) effect sizes (ES) and associated standard errors were calculated by applying the method reported in [47] for the decimal logarithm. This ES is similar to other log scale ES like Log(OR) or Log(RR), allowing to take advantage of log scale math properties [48]. All random-effects meta-analysis and forest plots were performed using OpenMetaAnalyst (2016) software [49]. To rank chronic and aggressive periodontitis effect on salivary cortisol we performed two different subgroup meta-analysis. The first one a pairwise meta-analysis and the second one a network meta-analysis including both direct and indirect ES estimate for validation purposes. Pooled results were back converted to mean ratio raw scale for evaluation through direct exponential transformation. Indirect estimates of ES and associated consistency towards homologous direct (pairwise) ES estimates were determined through Bucher´s [50] approach. Quantity I^2 was measured to assess the degree of dispersion of ES estimates and the overall homogeneity statistical significance was calculated through the χ^2 test [51]. All tests were two-tailed with alpha set at 0.05 except for homogeneity test whose significance level

cutoff was considered to be 0.10 due to the low power of the $\chi 2$ test with a limited amount of studies. Publication bias analysis was planned to be performed if, at least, we had 10 or more studies included [52]. Galbraith plot was designed to assess the extent of heterogeneity between the studies [53].

Random-effects meta-regressions were conducted for the comparison of salivary cortisol levels according to the following studies characteristics of (a) mean age difference to control, (b) gender (assessed through the male ratio), (c) smoking difference (percentage of patients smoking), d) Clinical Attachment Loss (CAL), (e) Bleeding on Probing (BoP), (f) latitude and (g) longitude. Partial and overall ES estimates were reported with 95% confidence intervals (CI).

3.5.3. Results

Study selection

A total of 3677 records were identified through the electronic and manual searches, respectively (Fig. 3.5.1). After removal of duplicates, 3652 were judged against the eligibility criteria, and after the previous exclusion process the 27 remaining full-length articles were screened, leaving a final number of 6 papers to be included in the qualitative and quantitative analysis.

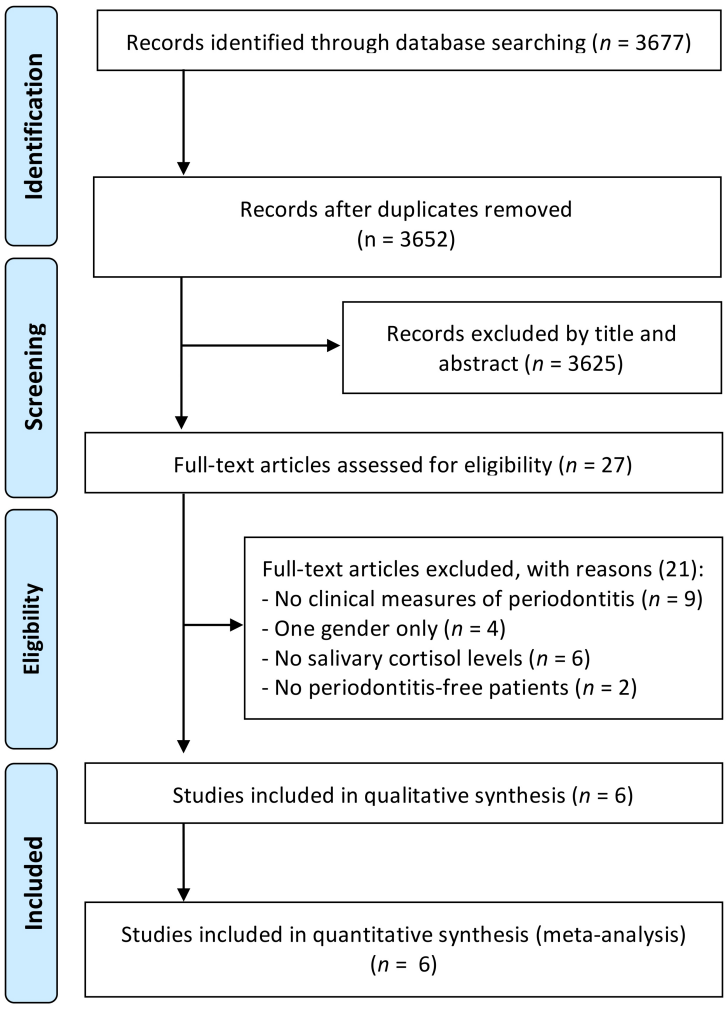

Figure 3.5.1. PRISMA flow-chart that depicts the results of the workflow to identify eligible studies.

Study characteristics

The characteristics of the included studies are shown in Table 3.5.1. Six cross-sectional studies from five different countries across Europe and Asia were included. These studies were published between 2009 and 2017 period. Studies sample sizes ranged from 45 [133] to 171 participants [134]. Globally a total of 573 participants were included in this review, including 258 participants with chronic periodontitis (CP), 72 with aggressive periodontitis (AgP) and 243 participants without periodontitis. Mean age of participants with CP was 53.6 years, whereas with AgP was 36.3 years and controls was 47.4 years, respectively.

Table 3.5.1. Characteristics of included studies.

Author (Year) (Country) (City)	N (Control/CP/AgP)	Mean age (Control/ CP/AgP)	Gender (Female/Male)	Saliva collected (Method)	Time of Saliva Collection	Salivary Cortisol Assessment	Diagnostic for Periodontal Disease	Perio Outcomes	Study Results
Ansai et al (2009) (Japan) (Kitakyushu)	171 (87/84)	67.2/69.5	86/85	Stimulated (paraffin chewing)	11:00 am – 1:00 pm	ELISA	NHANES III, 1999	PD, CAL, %BOP	Positive association between salivary cortisol and periodontitis severity
Haririan et al (2012) (Austria) (Vienna)	88 (30/34/24)	33.3/43.1/31.4	43/45	Stimulated (Greiner Bio-One System)	8:00-11:00 am	Mass Spectrometry	Armitage, 1999	PD, CAL, %BOP	Association of salivary cortisol levels with periodontitis.
Nayak et al (2013) (India) (Manipal)	45 (15/30)	42.5/43.9	15/30	Unstimulated (Drooling)	8:00-10:00 am	ELISA	Armitage, 1999	PD, CAL, %PI	Salivary cortisol levels can be used as biomarker for evaluating part of the etiopathogenesis of chronic periodontitis.
Mesa et al (2014) (Spain) (Granada)	77 (36/41)	37.8/53.7	46/31	Stimulated (Salivette®)	8:00-10:00 am	ELISA	Periodontitis; ≥4 teeth with ≥1 sites with PD ≥ 4 mm and CAL ≥ 3 mm (at the same site).	PD, CAL, %BOP, %PI	Salivary cortisol levels in periodontitis patients were correlated with worse plaque index, higher gingival inflammation, and greater tooth loss.
Cakmak et al (2016) (Turkey) (Ankara)	92 (31/34/27)	36.0/37.0/32.0	45/47	Stimulated (Pooled saliva)	8:00-9:00 am	ELISA	Armitage, 1999	PD, CAL, %BOP, %PI	Salivary cortisol levels were related with more severe and aggressive forms of periodontal disease.
Haririan et al (2017) (Austria) (Vienna)	100 (44/35/21)	35.4/50.0/34.0	43/57	Stimulated (Greiner Bio-One System)	8:00-11:00 am	Mass Spectrometry	Armitage, 1999	PD, CAL, %BOP, %PI	Salivary cortisol levels were different between states of periodontal health and disease.

Risk of bias within studies

Table 3.5.2 shows the risk of bias assessment for the included studies. Quality assessment was hampered by the limited information available in some studies. The studies admitted to this meta-analysis had a clear hypothesis, aims, outcome measures and characteristics of patients clearly described (n = 6, 100%).

Table 3.5.2. Downs & Black's Appraisal.

	1	2	3	4	5	6
Hypotheses/aims/objectives clearly described	✓	✓	✓	✓	✓	✓
Main outcome measures clearly described	✓	✓	✓	✓	✓	✓
Characteristics of patients/subjects clearly described	✓	✓	✓	✓	✓	✓
Interventions of interest clearly described	✓	✓	✓	✓	✓	✓
Distribution of principal confounders in each group clearly described	X	X	✓	✓	X	✓
Main findings clearly described	✓	✓	✓	✓	✓	✓
Estimates of random variability in the data provided	✓	✓	✓	✓	✓	✓
Important adverse events reported	NA	NA	NA	NA	NA	NA
Characteristics of patients lost to follow-up described	NA	NA	NA	NA	NA	NA
Actual probability values reported	✓	✓	✓	✓	✓	✓
Participants approached representative of entire population	✓	X	X	X	X	X
Participants recruited representative of entire population	X	X	X	X	X	X
Staff, places, and facilities were patients treated representative of majority of population	X	X	X	X	X	X
Blinding of study subjects	NA	NA	NA	NA	NA	NA
Blinding of assessors	X	X	X	X	X	X
Data based on data-dredging clearly stated	NA	NA	NA	NA	NA	NA
Time period between the intervention and outcome the same for cases and controls	NA	NA	NA	NA	NA	NA
Appropriate statistical tests used	✓	✓	✓	✓	✓	✓
Compliance to intervention reliable	NA	NA	NA	NA	NA	NA
Main outcome measure reliable and valid	✓	✓	✓	✓	✓	✓
Intervention groups or case-controls recruited from same population	✓	✓	X	✓	✓	✓
Intervention groups or case-controls recruited at the same time	NS	✓	NS	✓	✓	✓
Study subjects randomized to the interventions	NA	NA	NA	NA	NA	NA
Was concealed randomization to allocation undertaken	NA	NA	NA	NA	NA	NA
Adequate adjustment made in the analysis of confounders	✓	X	✓	✓	X	✓
Patient losses accounted for	NA	NA	NA	NA	NA	NA
Sufficiently powered cohort size	NA	NA	NA	NA	NA	NA

NS, not stated; N/A, not applicable. 1, Ansai et al. (16); 2, Haririan et al. (17); 3, Nayak et al. (15); 4, Mesa et al. (18); 5, Cakmak et al. (19); 6, Haririan et al. (20).

Also, the main findings with estimates of random variability and actual probability reported, with reliable outcome measures and clearly reported (n = 6, 100%). However, we point some limitations with partially distribution of principal confounders (n = 3, 50%) [133, 135, 136], limited adequate adjustment for its analysis (n = 4, 66.67%) [133–136], the participants not representative of the population (n = 0, 0%) and the complete absence of blinded assessors (n = 0, 0%).

Synthesis of results

The assessment of salivary cortisol levels in periodontitis patients was sourced from 6 studies (Table 3.5.2, Fig. 3.5.2). All those studies provided data for the CP salivary cortisol response group assessment, while three [136–138] also had data regarding salivary cortisol response to AgP. Global pooled results suggest a slight increase of salivary cortisol levels in periodontitis patients when compared to healthy controls, and that AgP outranks CP regarding salivary cortisol response. Subgroup results show that AgP patients had on average 53% more salivary cortisol than the control cohort (mean ratio [95% CI]: 1.53 [1.11-2.12]). Regarding chronic periodontitis subgroup, we did not find any significant differences against control. The heterogeneity between studies for both groups was considered low. In addition, we compared the differences in salivary cortisol response between CP and AgP patients through a network meta-analysis (Fig. 3.5.3) to validate the previous periodontitis type ranking order on salivary cortisol response. Results show that, although the direct and indirect point estimates are not statistically significant, they are fully consistent with each other according to Bucher´s test for consistency ($p = 0.99$) and both favours AgP against CP for salivary cortisol response. The overall result suggests that cortisol response to AgP is in average 42% above the one present in CP ($p<0.05$), as measured in patient´s saliva (mean ratio [95% CI]: 1.42 [0.97-2.06]).

Additional analyses

Random-effect meta-regressions against potential covariates or factors identified case and control difference in age as modifying effect adding for heterogeneity (Table S3.5.2). Results suggest that an increase in age difference in case sample against control may increase the salivary cortisol response to

chronic periodontitis. No meaningful effect was found for CAL or BoP indexes, latitude, longitude and difference in smokers to control on salivary cortisol outcome. Furthermore, a Galbraith plot evidenced low heterogeneity within both CP and AgP groups of studies (Fig. 3.5.4).

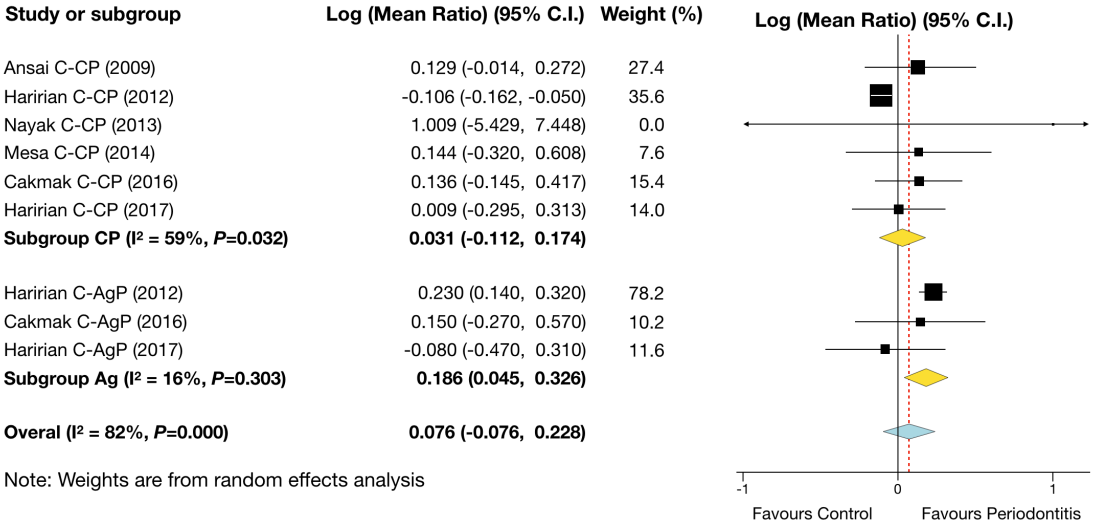

Figure 3.5.2. Subgroup forest plot of studies measuring chronic or aggressive periodontitis effect on salivary cortisol. Studies have been grouped according to the periodontitis type: chronic or aggressive. Logarithm of mean ratio (Log Mean Ratio) effect size estimates have been calculated with 95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Yellow diamonds (the top two) indicates the subgroup pooled estimates while the blue diamond (the further down) and the vertical red dotted line both point to the overall pooled estimate. C-CP: Chronic periodontitis versus control; C-AgP: Aggressive periodontitis versus control.

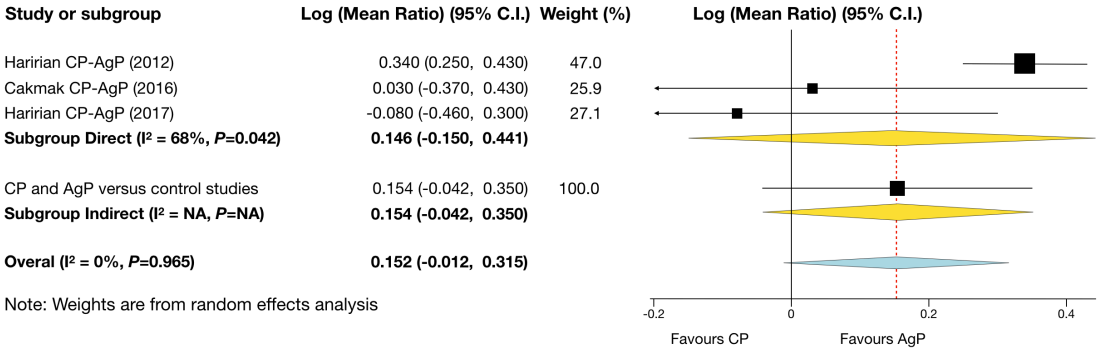

Figure 3.5.3. Subgroup forest plot of studies comparing between chronic and aggressive periodontitis effect on salivary cortisol. Studies have been grouped according to the estimation type: direct or indirect, in a network adjusted meta-analysis. Logarithm of mean ratio effect size estimates have been calculated with

95% confidence intervals and are shown in the figure. Area of squares represents sample size, continuous horizontal lines and diamonds width represents 95% confidence interval. Yellow diamonds (the top two) indicates the subgroup pooled estimates while the blue diamond (the further down) and the vertical red dotted line both point to the overall pooled estimate. CP-AgP: Chronic versus aggressive periodontitis.

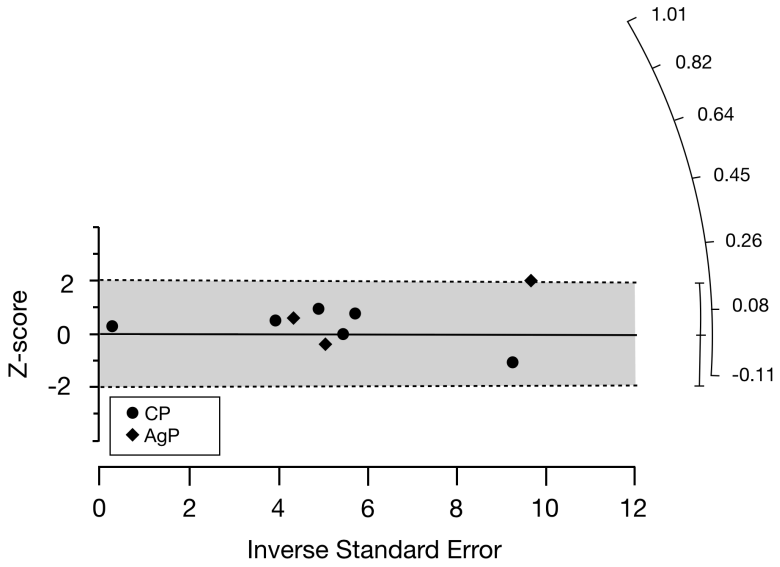

Figure 3.5.4. Radial (Galbraith) plot for exploring the sources of heterogeneity on salivary cortisol levels according to periodontal loss levels. The slope of the central line represents the overall effect. 95% of studies are expected to lie within the area between the upper and lower lines.

3.5.4. Discussion

Summary of evidence

This systematic review is the first attempt to synthesize the effect of periodontal status on salivary cortisol levels and to evaluate if the increase of this salivary biomarker is in concordance with the progress and severity of periodontitis. This effect was assessed from 6 systematically selected observational studies comprising a total of 573 patients.

According to the results of the meta-analyses, aggressive periodontitis patients have, in average, salivary cortisol increased by 53%, in contrast with chronic periodontitis which had levels not much different from controls. Some included studies confirm that salivary cortisol correlates with blood cortisol levels in periodontitis patients [39,40] although meta-regression results could not

establish any trend of salivary cortisol change with periodontitis severity (measured by the studies average CAL/BoP in periodontitis patients). If such increase in salivary cortisol in aggressive periodontitis is enough to result in periodontal damage, is a matter that should be addressed in future research.

Aggressive and chronic periodontitis show different rates of progression and patterns of tissue destruction, with aggressive periodontitis affecting predominantly younger individuals. The role of cortisol as oxidative damage mediator may contribute to those differences since in aggressive periodontitis oxidative stress seems to highly contributes to periodontal pathology [54,55]. On the other hand, hypercortisolism appears to promote bone fragility through the apoptosis of osteocytes, via caspase-3 activation, resulting in bone surface remodeling [56,57]. However, further studies are needed to understand this matter better.

According to current guidelines, late-night salivary cortisol (LNSC), 24-h urine-free cortisol, and the 1-mg overnight dexamethasone suppression test are the golden standard procedures to initially screen Cushing's syndrome [58]. From the included studies in this systematic review, Nayak et al. [37] did not refer any exclusion criteria related to glucocorticoids' treatments for diseases which could have been the reason why this study contributed so much for the observed heterogeneity in this meta-analysis. The menstrual cycle phase and the use of oral contraceptives have been reported to have an impact on the salivary cortisol levels [59–62], with considerable variability. Excluding Ansai et al. [42] which represents an elderly population study, none of the five studies clearly mentioned these confounders, and only one (Mesa et al. 2014) stated as exclusion criterion "treatment with estrogens" although it is not clear if they have considered oral contraceptives within. Also, another absent criterion worth mentioning is physical exercise which seems to induce salivary cortisol changes [62–64]. In the future, we strongly recommend considering the aforementioned in the exclusion criteria to decrease hormonal impact on the results.

Besides, hair cortisol analysis, a recent promising trend using immunoassays or mass spectrometry, can be introduced in future methodology ensuring a retrospective approach of total exposure, assess the baseline cortisol status before an event and screen related pathologies like Cushing's Disease, Addison's Disease, chronic pain and depression [65].

Strengths and limitations

The strengths of the present review include the pre-defined protocol and outcomes, the vast literature search, and the rigorous methodology implemented during every stage of it, according to existing evidence-based guidelines.

However, all studies were made in university periodontology department settings, with no broad representation of the population. Thus, the results can't be broadly generalized to the average patient. Moreover, the heterogeneity of the selected studies regarding the periodontal diagnosis is an important factor to emphasize, since 4 studies [37,39–41] used diagnostic criteria according to Armitage [66], Ansai et al. [42] used NHANES III protocol [67] and Mesa et al. [38] used an alternative case definition.

Furthermore, three of the six studies [37,39,41] took into account the evaluation of stress/psychological measures through stress indexes, despite contradictory results using different sets, whereas the remaining studies simply assumed cortisol as a stress response hormone. Thus, we can only extrapolate the effect of salivary cortisol levels variance on periodontal tissues in as much as can be associated with other causes.

Finally, the fact that only cross-sectional studies were included represents the most important limitation of this study. Hence, we are unable to fully support bidirectional causality in the relationship between salivary cortisol levels and periodontitis severity.

3.3.5. Conclusions

Results of observational studies suggest that subjects with aggressive periodontitis have higher salivary cortisol levels than healthy ones or patients with chronic periodontitis. Such salivary cortisol response difference may have a negative impact on the periodontium, contributing to worsening the burden of aggressive periodontitis disease. However, although the analysis suggested this relationship, periodontitis has a complex nature, and many confounders factors may have contributed to this outcome. Thus, in the future, more robust evidence about this topic should be gathered through the implementation of larger, well-designed longitudinal studies, to confirm this possible association and to elucidate the pathological mechanism beyond. Also, we strongly recommend to perform initial cortisol screening, retrospective analysis of total exposure and

baseline assessment, and take into consideration menstrual cycle-phase, oral contraceptives and physical exercise in exclusion criteria.

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3.5.7. Supplementary material

Table S3.5.1. PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4-5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6-7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6-7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6-7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7-8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9-10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Table S3.5.2. Random-effect meta-regressions against potential covariates or factors identified case and control difference in age difference to control, difference in smokers to control (%), CAL disease Mean, BoP disease Mean, study location (latitude & longitude) and male ratio (%) as modifying effects adding for heterogeneity.

Variable	Coefficients	95% IC	p-value
Age difference to control	**0.024**	**(-0.003; 0.027)**	**<0.001***
Difference in smokers to control (%)	0.000	(-0.007; -0.008)	0.956
CAL Disease Mean	0.061	(-0.345; 0.468)	0.768
BoP Disease Mean	0.001	(-0.004; 0.006)	0.712
Latitude	-0.008	(-0.027; -0.011)	0.395
Longitude	0.001	(-0.002; 0.003)	0.592
Male Ratio (%)	-0.014	(-0.058; 0.031)	0553

*Omnibus p < 0.05 (bold-faced to highlight).

4 Study of Periodontal Health in Almada-Seixal (SoPHiAS): A Cross-Sectional Study in the Lisbon Metropolitan Area

This chapter was based from the published work:

Paper V – *Botelho J, Machado V, Proença L, Alves R, Cavacas MA, Amaro L, Mendes JJ. Study of Periodontal Health in Almada-Seixal (SoPHiAS): A Cross-Sectional Study in the Lisbon Metropolitan Area. Scientific Reports* ***2019****, 29, 9, 15538. Doi: 10.1038/s41598-019-52116-6.*

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4. Study of Periodontal Health in Almada-Seixal (SoPHiAS): a periodontal examination survey in the Lisbon Metropolitan Area

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Abstract

This study aimed to describe the prevalence and extent of periodontal diseases among adults in the southern region of the Lisbon Metropolitan Area. This population-based cross-sectional study included 1,064 randomized participants (aged 20 to 95 years, 617 females/447 males). Sociodemographic, behaviours and medical information were recorded. Periodontal conditions were assessed with a full-mouth circumferential periodontal examination. It was used the American Association of Periodontology/European Federation of Periodontology 2017 case definitions. A logistic regression analysis was applied to ascertain hypothetical risk factors towards periodontitis. The prevalence of periodontitis was 59.9%, with 24.0% and 22.2% of the participants exhibiting severe and moderate periodontitis, respectively. The risk of periodontitis significantly increased with age (OR=1.05, 95% CI: 1.04-1.06), for active and former smokers (OR = 3.76 and OR = 2.11, respectively), with lower education levels (OR = 2.08, OR = 1.86, for middle and elementary education, respectively) and with diabetes

mellitus (OR = 1.53). This study reveals a high burden of periodontitis in the target population. The findings provide a comprehensive understanding that will empower appropriate national public oral health programmes and population-based preventive actions.

4.1. Introduction

Prevalence of periodontal diseases endures a substantial epidemiological challenge, while estimates presented in recent years have been very dissimilar, even in countries with alike socio-economically standards [1-3]. Thus, these has contributed to the lack of comprehensive understanding of the periodontal status worldwide. In addition, periodontitis has a large socioeconomic impact and it is estimated that is responsible for 54 billion USD/year in lost productivity and a major portion of the 442 billion USD/year cost for oral diseases [4]. Also, these polymicrobial inflammatory diseases are extremely impacting on other systemic conditions.

Over the last decades, periodontitis case definitions have undergone paradigmatic changes evolving from a diagnosis based in terms of clinical attachment loss (CAL) and probing depth (PD), as proposed by the CDC Working Group [5] and revised accordingly [6], to a diagnosis proposed in the new American Association of Periodontology (AAP)/European Federation of Periodontology (EFP) based mainly upon CAL and considering the interproximal space as an adjacent common zone [7]. In fact, all efforts made to improve these diagnostic criteria focused on the prevention of underestimation of periodontitis and to reveal the natural history of periodontitis, especially in older subjects.

To date, very few data have provided a comprehensive assessment of the periodontal status of the Portuguese population [8-10]. A single national epidemiological study was conducted, in 2015, by the Portuguese Health General Directorate using the Community Periodontal Index of Treatment Needs (CPITN). The obtained results estimated a prevalence of 10.8% and 15.3% of periodontal diseases in adults and elderly, respectively [9]. These results contrast, specifically, and due to geographical proximity, with the last national Spanish periodontal survey where 38.4% of subjects had periodontal pockets [11], as well with other developed countries studies where found prevalence

ranged from 51.0 to 88.3% in the USA, Italy, Norway or Pomerania [6,12-15] and World Health Organization (WHO) global reports [3].

Due to the recent disclosure of the new periodontal stage consensus [7], there is still limited data coming from epidemiological studies employing these diagnostic criteria in Europe. Also, the available Portuguese national epidemiologic data relies on CPITN methodology which is inadequate to describe the periodontal status of populations [16]. Consequently, it is essential to carry out studies using the new case definitions which will allow a comprehensive understanding of the current periodontal status in the Portuguese population and the assessment of associated risk factors, to allow future international comparability and to serve as a foundation for future national public health strategies.

Therefore, this study was aimed to investigate the distribution of periodontal diseases using a population-based stratified sample of adults from the southern region of the Lisbon Metropolitan Area. The prime purposes of this study were: (1) to comprehensively describe the prevalence and extent of periodontal diseases according to the Workshop in 2017 [7], (2) to evaluate potential periodontal diseases risk indicators.

4.2. Methods

This study was approved by the Research Ethics Committee of the Regional Health Administration of Lisbon and Tagus Valley, IP (Portugal) (Approval numbers: 3525/CES/2018 and 8696/CES/2018). Following examination, each participant was informed of their periodontal status. Patients with diagnosed periodontal diseases were referred to the Egas Moniz Dental Clinic (EMDC) for treatment without additional costs. This survey followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [17].

Study design and sampling procedure

The Study of Periodontal Health in Almada-Seixal (SoPHiAS) was designed as a population-based cross-sectional representative study, geographically stratified, with a target population of subjects over 18 years of age (adults and elderly),

living in the municipalities of Almada and Seixal, in Portugal. Almada and Seixal, are two of the largest municipalities located in the southern part of the Lisbon Metropolitan Area, a NUTS II region (PT17). This region, with over 2.8 million inhabitants, includes 18 municipalities and is the most populated Portuguese Metropolitan Area and the second most populated NUTS II region of the country. In Portugal, all residents are covered by the National Health System and assigned to a General Practitioner of a public Family Health Unit (FHU). FHUs are grouped in Health Centers grouping (ACES), depending on the geographic region. For this study, the ACES Almada-Seixal was defined as the study group. All twenty-two ACES Almada-Seixal FHUs were included to ensure a global geographic and socioeconomic coverage of the Almada and Seixal territory. In September 2018, according to the institutional data provided, the two municipalities had 386,168 inhabitants in the selected age groups (adults and elderly). To achieve an estimate of the periodontitis prevalence in the population, with a margin of error of 3.0%, for a 95% confidence level, a minimum of 962 individuals were needed to be examined, based on the previously reported national prevalence data of 10.8% and 15.3%, for adults and elderly, respectively [9]. The required sample was stratified according to the number of subjects assigned to each FHU, based on the information provided by ACES Almada-Seixal. The invitation to participate in the survey was made by direct contact at the waiting room of the FHU, explaining the purpose of the study and including a description of the clinical examination. After a detailed explanation with the information sheet delivery to the patient, individuals who agreed to participate signed the informed consent form. A questionnaire was completed by each subject and collected before the periodontal examination.

Gingivitis and Periodontitis case definitions

Gingivitis and periodontitis cases were defined according to the new AAP/EFP consensus [7,18].

Clinical periodontal examination

Two calibrated investigators (VM and JB) performed a full-mouth periodontal examination, on an average of 30 minutes. Each clinical examination was performed under proper lighting with the individuals seated on an regular

adjustable stretcher in the FHU's medical office. No radiographic examination was made.

All fully erupted teeth, excluding third molars, implants and retained roots, were examined by means of a daily sterilized dental mirror and a manual periodontal North Carolina probe (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). The number of missing teeth was recorded. Further, dichotomous plaque index (PI), gingival recession (REC), probing depth (PD), and bleeding on probing (BoP) were circumferentially recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). PD was measured as the distance from the free gingival margin to the bottom of the pocket and REC as the distance from the cementoenamel junction (CEJ) to the free gingival margin, and this assessment was assigned a negative sign if the gingival margin was located coronally to the CEJ. CAL was calculated as the algebraic sum of REC and PD measurements for each site. The measurements were rounded to the lowest whole millimeter. Furcation involvement (FI) was assessed using a Nabers probe (2N Hu- Friedy, Chicago, IL, USA) following [19] in molars, and upper first premolars if applicable, and tooth mobility was appraised following.

Sociodemographic and Medical Questionnaires

Information on sociodemographic characteristics and behaviors was collected by self-reported questionnaire. The questionnaire covered questions on the following items: 1) gender, age, marital status, educational level, occupation; 2) monthly family gross income; 3) smoking habits; 4) oral hygiene-related behaviors (tooth brushing frequency, interproximal cleaning, etc.); 5) attitudes and awareness towards oral health; 6) diabetes mellitus (DM) and comorbidities [12].

Education was categorized according to the 2011 International Standard Classification of Education (ISCED-2011) [20]: No education (ISCED 0 level), Elementary (ISCED 1–2 levels), Middle (ISCED 3–4 levels), Higher (ISCED 5–8 levels). Occupation status of each participant was classified as: student, employed, unemployed or retired. Marital status was defined as: married/union of fact, divorced, single or widowed. Smoking status was defined as non-smoker, current smoker or former smoker. Family gross income was categorised in three levels: less or equal to 600, 601 to 1500 and higher than 1500 euros per month.

Measurement Reliability and Reproducibility

Two examiners (VM and JB) were trained under the supervision of an experienced senior periodontist (RA), prior to data collection. For the purpose of measurement reliability and reproducibility, a total of 10 volunteers seeking care at EMDC were randomly selected and evaluated. These patients were not further involved in the study. Volunteers were examined by the senior periodontist, the 'reference examiner', and the two field clinicians. Measurements were repeated one week later in the same volunteers. Measurement reliability and reproducibility were assessed by the intra-class correlation coefficient (ICC). Obtained ICC inter-examiner values were 0.98 and 0.99, for CAL and PD, respectively. The intra-examiner ICC ranged from 0.97 to 0.99, for both PD and CAL.

Data Analysis

Data analysis was performed using IBM SPSS Statistics version 25.0 for Windows (Armonk, NY: IBM Corp.). Descriptive and inferential statistics methodologies were applied. Spearman's rank correlation coefficient (rho) was used to assess correlations between periodontal clinical data and age. Binomial logistic regression analysis was used to model the relationship between periodontitis and several potential risk factors. Preliminary analyses were performed using univariate models. Next, a multivariate model was constructed for periodontal disease estimation. Only variables showing a significance $p \leq 0.25$ in the univariate model were included in the multivariate stepwise procedure. The contribution of each variable to the model was evaluated by Wald statistics. Interactions were also analyzed for all tested variables. The final reduced model was obtained with the following predictor variable categories: age, education, smoking status and diabetes. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for both univariate and multivariate analyses. The level of statistical significance was set at 5% in all inferential analyses.

4.3. Results

Study Sample

The characteristics of the 1,064 subjects included in the study, according to the periodontal diagnosis, are shown in Table 4.1. The mean age of participants was 60.9 (± 16.3) years, 58.0% were women, 63.3% reported having an elementary education level and 52.2% were retired. The prevalence of moderate and severe periodontitis increased with age. Moreover, the majority of the population (81.9%) report not knowing what periodontal disease is, 37.6% brush their teeth once or less daily, and 70.2% of subjects with severe periodontitis have never performed interproximal cleaning.

Table 4.1. Sociodemographic characteristics, behaviors, attitudes towards oral health and medical information (diabetes and comorbidity) of the included participants, presented as n (%), according to the severity of periodontal status (N = 1,064).

	No Disease n (%)	Gingivitis n (%)	Mild n (%)	Moderate n (%)	Severe n (%)	Total n (%)
Gender						
Male	117 (34.2)	23 (27.1)	64 (43.8)	111 (47.0)	132 (51.8)	447 (42.0)
Female	225 (65.8)	62 (72.9)	82 (56.2)	125 (53.0)	123 (48.2)	617 (58.0)
Age (years)						
18-30	34 (9.9)	17 (20.0)	10 (6.8)	1 (0.4)	0 (0.0)	62 (5.8)
31-40	42 (12.3)	7 (8.2)	11 (7.5)	10 (4.2)	5 (2.0)	75 (7.1)
41-50	62 (18.1)	11 (12.9)	21 (14.4)	23 (9.8)	19 (7.4)	136 (12.8)
51-60	50 (14.6)	5 (5.9)	15 (10.3)	32 (13.6)	35 (13.7)	137 (12.9)
61-70	82 (24.0)	26 (30.6)	45 (30.8)	81 (34.3)	94 (36.9)	328 (30.8)
71-80	58 (17.0)	16 (18.8)	38 (26.0)	57 (24.2)	75 (29.4)	244 (22.9)
> 80	14 (4.1)	3 (3.5)	6 (4.1)	32 (13.6)	27 (10.6)	82 (7.7)
Educational level						
No education	8 (2.3)	3 (3.5)	6 (4.1)	11 (4.7)	14 (5.5)	42 (4.0)
Elementary	176 (51.5)	50 (58.8)	94 (64.4)	173 (73.3)	180 (70.6)	673 (63.3)
Middle	94 (27.5)	23 (27.1)	35 (24.0)	38 (16.1)	43 (16.9)	233 (21.9)

Higher	64 (18.7)	9 (10.6)	11 (7.5)	14 (5.9)	18 (7.1)	116 (10.9)
Marital status						
Single	81 (23.7)	23 (27.1)	22 (15.1)	22 (9.3)	22 (8.6)	170 (16.0)
Married/Union of fact	213 (62.3)	49 (57.7)	92 (63.0)	158 (66.9)	172 (67.5)	684 (64.3)
Divorced	25 (7.3)	8 (9.4)	20 (13.7)	25 (10.6)	25 (9.8)	103 (9.7)
Widowed	23 (6.7)	5 (5.9)	12 (8.2)	31 (13.1)	36 (14.1)	107 (10.1)
Occupation						
Student	11 (3.2)	7 (8.2)	1 (0.7)	0 (0.0)	0 (0.0)	19 (1.8)
Employed	138 (40.4)	27 (31.8)	53 (36.3)	52 (22.0)	57 (22.3)	327 (30.7)
Unemployed	63 (18.4)	16 (18.8)	13 (8.9)	35 (14.8)	36 (14.1)	163 (15.3)
Retired	130 (38.0)	35 (41.2)	79 (54.1)	149 (63.1)	162 (63.5)	555 (52.2)
Smoking status						
Non-smoker	238 (69.6)	58 (68.2)	88 (60.3)	122 (51.7)	120 (47.1)	626 (58.8)
Former smoker	71 (6.7)	14 (16.5)	38 (26.0)	76 (32.2)	94 (36.9)	293 (27.5)
Current Smoker	33 (3.1)	13 (15.3)	20 (13.7)	38 (16.1)	41 (16.1)	145 (13.6)
Family income (monthly, €)						
<= 600	74 (22.0)	24 (30.0)	37 (25.5)	61 (26.2)	74 (29.3)	270 (25.8)
601-1,500	194 (57.7)	46 (57.5)	92 (63.4)	137 (58.8)	143 (56.5)	612 (58.4)
> 1,500	68 (20.2)	10 (12.5)	16 (11.0)	35 (15.0)	36 (14.2)	165 (15.8)
Periodontal diseases awareness						
Yes	75 (21.9)	12 (14.1)	26 (17.8)	36 (15.2)	44 (17.3)	193 (18.1)
No	267 (78.1)	73 (85.9)	120 (82.2)	200 (84.8)	211 (82.7)	871 (81.9)
Brushing frequency (daily)						
3+	65 (19.0)	16 (18.8)	24 (16.4)	39 (16.5)	27 (10.6)	171 (16.1)
2	196 (57.3)	36 (42.3)	74 (50.7)	122 (51.7)	132 (51.8)	560 (52.6)
1	78 (22.8)	29 (34.1)	46 (31.5)	65 (27.5)	84 (32.9)	302 (28.4)
0	3 (0.9)	4 (4.7)	2 (1.4)	10 (4.2)	12 (4.7)	31 (2.9)
Interproximal cleaning						
Yes	82 (24.0)	10 (11.8)	31 (21.2)	36 (15.3)	26 (10.2)	185 (17.4)
Occasionally	71 (20.8)	10 (11.8)	25 (17.1)	27 (11.4)	28 (11.0)	161 (15.1)
No	189 (55.3)	65 (76.5)	90 (61.6)	173 (73.3)	201 (78.8)	718 (67.5)

Diabetes Mellitus						
Yes	44 (12.9)	9 (10.6)	31 (21.2)	46 (19.5)	74 (29.0)	204 (19.2)
No	298 (87.1)	76 (89.4)	115 (78.8)	190 (80.5)	181 (71.0)	860 (80.8)
Comorbidity (by Aimetti 2015)						
Yes	260 (76.0)	63 (74.1)	123 (84.2)	206 (87.3)	227 (89.0)	879 (82.6)
No	82 (24.0)	22 (25.9)	23 (15.8)	30 (12.7)	28 (11.0)	185 (17.4)
Total	342 (32.1)	85 (8.0)	146 (13.7)	236 (22.2)	255 (24.0)	1064 (100.0)

Prevalence and Severity of Periodontal Disease

The prevalence of periodontitis was 59.9% (95% CI 56.9-62.8), with a prevalence and correspondent estimates for severe and moderate periodontitis of 24.0% (95% CI 21.4-26.6%) and 22.2% (95% CI 19.7-24.8%), respectively. The prevalence and correspondent estimates of localized and generalized periodontitis amounted to 23.2% (95% CI: 20.7- 25.9%) and 36.7% (95% CI: 33.8-39.6%) respectively (Table 4.2). Further, periodontal health is a well distributed status, whereas periodontal diseases exhibited a distinct scattering (Fig. 4.1).

Table 4.2. Prevalence of localized and generalized periodontitis, stratified by age and gender.

	Females		Males		Total	
	n	Prev. (95% CI) (%)	n	Prev. (95% CI) (%)	n	Prev. (95% CI) (%)
Localized	139	56.3 (50.1-62.5)	108	43.7 (37.5-49.9)	247	23.2 (20.7-25.9)
18-30	5	3.6 (0.5-6.7)	6	5.6 (1.2-9.9)	11	4.5 (1.9-7.0)
31-40	14	10.1 (5.1-15.1)	5	4.6 (0.7-8.6)	19	7.7 (4.4-11.0)
41-50	20	14.4 (8.6-20.2)	10	9.3 (3.8-14.7)	30	12.1 (8.1-16.2)
51-60	20	14.4 (8.6-20.2)	13	12.0 (5.9-18.2)	33	13.4 (9.1-17.6)
61-70	51	36.7 (28.7-44.7)	34	31.5 (22.7-40.2)	85	34.4 (28.5-40.3)
71-80	25	18.0 (11.6-24.4)	28	25.9 (17.7-34.2)	53	21.5 (16.3-26.6)
80+	4	2.9 (0.1-5.7)	12	11.1 (5.2-17)	16	6.5 (3.4-9.5)
Generalized	191	49.0 (44.0-54.0)	199	51.0 (46.0-56.0)	390	36.7 (33.8-39.6)
18-30	0	0.0 (0.0-0.0)	0	0.0 (0.0-0.0	0	0.0 (0.0-0.0
31-40	5	3.6 (1-6.2)	2	1.9 (0.0-3.7)	7	1.8 (0.5-3.1)
41-50	13	9.4 (5.2-13.5)	20	18.5 (13.1-23.9)	33	8.5 (5.7-11.2)

51-60	23	16.5 (11.3-21.8)	26	24.1 (18.1-30.0)	49	12.6 (9.3-15.9)
61-70	71	51.1 (44-58.2)	64	59.3 (52.4-66.1)	135	34.6 (29.9-39.3)
71-80	57	41. (34-48)	60	55.6 (48.7-62.5)	117	30.0 (25.5-34.5)
80+	22	15.8 (10.7-21)	27	25. (19-31.0)	49	12.6 (9.3-15.9)
Molar-Incisor Pattern	330	-	307	-	637	-
No	311	94.2 (91.7-96.8)	295	96.1 (93.9-98.3)	606	95.1 (93.5-96.8)
Yes	19	5.8 (3.2-8.3)	12	3.9 (1.7-6.1)	31	4.9 (3.2-6.5)

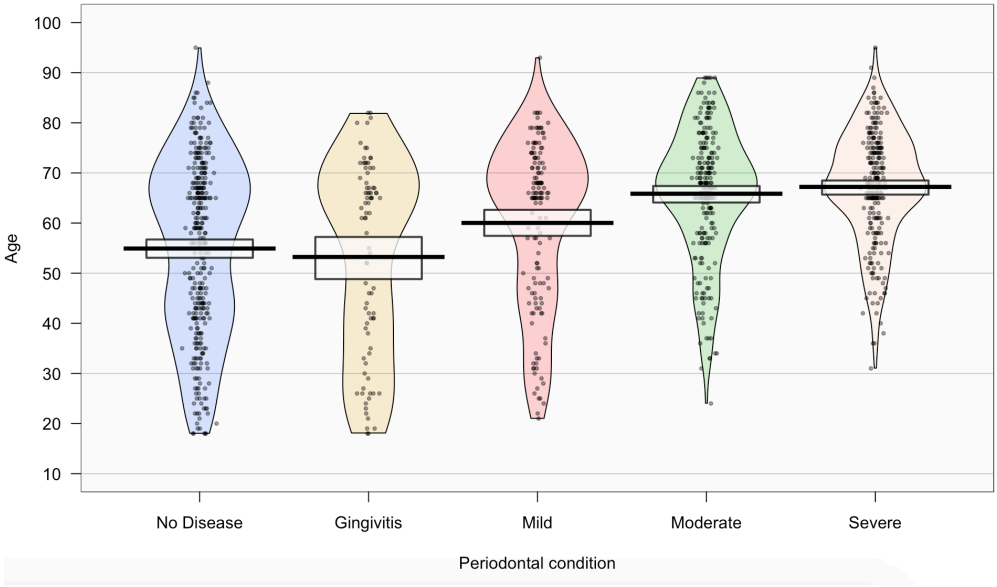

Figure 4.1. Density plot exhibiting the distribution of periodontal conditions over the age range.

Clinical Attachment Loss (CAL) and Probing Depth (PD)

The mean values of PD, CAL, recession (REC), missing teeth and teeth with mobility as well the prevalence and extent of CAL and PD by selected threshold are presented in Table 3. Mean PD and the number of sites with PD ≥ 4 mm and ≥ 6 mm remained similar across all age groups. The average CAL and number of sites with CAL ≥ 4 mm and ≥ 6 mm were unequally distributed in the population for all age groups, increasing with age, while exhibiting a moderate significant correlation. The number of missing teeth is also related to the mean CAL across age groups, that is, the higher the number of missing teeth the greater the CAL average but for PD this is not so evident (Fig. 4.2). Mean REC, missing teeth and teeth with mobility also increased with age increase.

Table 4.3. PD, CAL, REC, missing teeth and teeth with mobility (presented as mean, standard deviation and 95% CI for mean), stratified by CAL and PD thresholds (%) (≥ 4 and ≥ 6 mm) and age group.

Measures	18-30 Mean (SD) [95% CI]	31-40 Mean (SD) [95% CI]	41-50 Mean (SD) [95% CI]	51-60 Mean (SD) [95% CI]	61-70 Mean (SD) [95% CI]	71-80 Mean (SD) [95% CI]	>80 Mean (SD) [95% CI]	*rho* (p-value)*	Total Mean (SD) [95% CI]
Mean PD (mm)	1.8 (0.4) [1.7-1.9]	1.9 (0.6) [1.7-2.0]	1.9 (0.7) [1.8-2.1]	2.0 (0.8) [1.9-2.1]	2.0 (0.9) [1.9-2.1]	1.9 (0.7) [1.8-2.0]	1.9 (0.7) [1.7-2.0]	-0.027 (0.382)	1.9 (0.8) [1.9-2.0]
PD ≥ 4 mm (%)	3.8 (5.4) [1.4-4.1]	6.3 (13.6) [3.2-9.5]	7.9 (13.8) [5.5-10.2]	9.5 (15.5) [6.9-12.1]	9.5 (17.4) [7.7-11.4]	7.6 (14.3) [5.8-9.4]	6.4 (13.6) [3.4-9.4]	0.015 (0.614)	8.0 (15.0) [7.1-8.9]
PD ≥ 6 mm (%)	0.0 (0.2) [0.0-0.1]	1.0 (4.2) [0.1-2.0]	1.3 (4.4) [0.5-2.0]	1.6 (3.9) [1.0-2.3]	2.2 (6.5) [1.5-2.9]	1.8 (6.1) [1.1-2.6]	0.9 (3.1) [0.3-1.6]	0.047 (0.126)	1.6 (5.3) [1.3-1.9]
Mean CAL (mm)	1.8 (0.4) [1.7-1.9]	2.0 (0.8) [1.8-2.2]	2.2 (1.0) [2.1-2.4]	2.6 (1.5) [2.4-2.9]	2.9 (1.6) [2.8-3.1]	2.9 (1.4) [2.8-3.1]	3.4 (1.5) [3.1-3.7]	0.349 (<0.001)	2.7 (1.4) [2.6-2.8]
CAL ≥ 4 mm (%)	3.1 (5.5) [1.7-4.5]	7.9 (15.9) [4.2-11.5]	14.8 (21.0) [11.2-18.4]	22.4 (26.7) [17.9-26.9]	27.9 (28.7) [24.8-31.0]	30.1 (27.3) [26.6-33.5]	40.4 (30.8) [33.7-47.2]	0.416 (<0.001)	24.1 (27.4) [22.5-25.8]
CAL ≥ 6 mm (%)	0.1 (0.2) [0.0-0.1]	2.1 (9.5) [0.0-4.2]	4.0 (10.7) [2.2-5.8]	8.9 (18.0) [5.9-12.0]	12.1 (22.0) [9.7-13.5]	10.9 (18.4) [8.6-13.2]	15.0 (20.7) [10.4-19.5]	0.336 (<0.001)	9.2 (18.4) [8.1-10.3]
Mean REC (mm)	0.0 (0.0) [0.0-0.0]	0.1 (0.3) [0.0-0.2]	0.3 (0.5) [0.2-0.4]	0.7 (0.9) [0.5-0.8]	1.0 (1.1) [0.8-1.1]	1.1 (1.1) [0.9-1.2]	1.6 (1.2) [1.3-1.8]	0.562 (<0.001)	0.8 (1.0) [0.7.0.8]
Missing Teeth (n)	0.9 (1.2) [0.6-1.2]	2.3 (3.3) [1.5-3.0]	5.1 (5.1) [4.2-6.0]	8.2 (5.5) [7.2-9.1]	10.8 (6.5) [10.0-11.5]	12.0 (6.6) [11.2-12.8]	14.0 (7.5) [12.3-15.6]	0.544 (<0.001)	9.1 (7.0) [8.6-9.5]
Teeth with mobility (n)	0.1 (0.4) [0.0-0.2]	0.3 (1.4) [0.0-0.6]	0.6 (1.5) [0.3-0.8]	1.3 (2.5) [0.9-1.8]	1.4 (2.6) [1.1-1.7]	1.1 (1.9) [0.9-1.4]	1.3 (2.2) [0.8-1.8]	0.197 (<0.001)	1.1 (2.2) [0.9-1.2]

* Overall trend across age groups assessed by Spearman's rank correlation coefficient (rho). Significant correlations identified in bold (p<0.05).

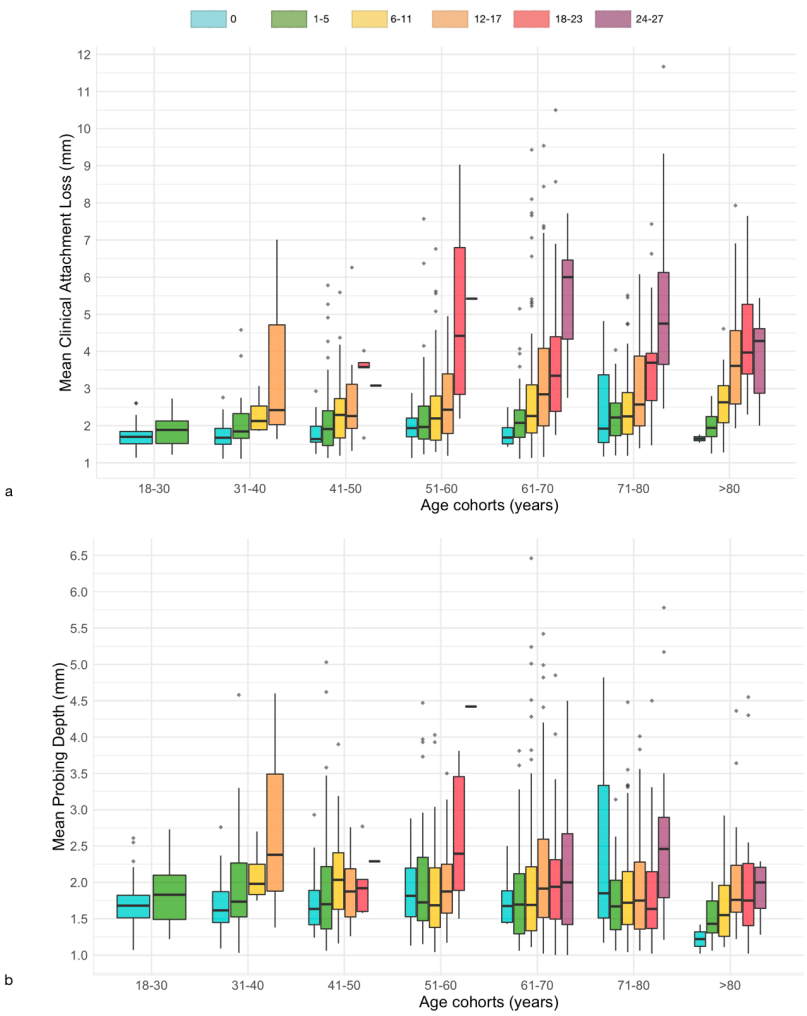

Figure 4.2. Distribution of tooth loss (coloring) as a function of mean clinical attachment loss (a) or mean probing depth (b), according to age cohorts (x-axis).

Bleeding on Probing (BoP) and Plaque Index (PI)

The mean values of BoP and PI, stratified by periodontitis severity and age group, are presented in Table 4.4. BoP was equally distributed in the population for all age groups, and increased with level of severity of periodontitis, with a mean of 5.7% for persons with no periodontitis, 15.9% for persons with non-severe periodontitis and 28.5% for persons with severe periodontitis. Similarly, the average PI was 23.2%, and increased with the severity of periodontitis and age.

Table 4.4. Mean Bleeding on Probing (BoP) and Plaque Index (PI) (%) (presented as mean, standard deviation and 95% CI for mean), stratified by periodontitis severity and age group.

Measures	18-30 Mean (SD) [95% CI]	31-40 Mean (SD) [95% CI]	41-50 Mean (SD) [95% CI]	51-60 Mean (SD) [95% CI]	61-70 Mean (SD) [95% CI]	71-80 Mean (SD) [95% CI]	>80 Mean (SD) [95% CI]	*rho** (*p*-value)	Total Mean (SD) [95% CI]
Mean BoP (%)	13.6 (17.1) [9.3-18.9]	12.5 (16.8) [8.7-16.4]	15.1 (20.0) [11.7-18.5]	15.7 (22.5) [11.9-19.5]	15.2 (21.3) [12.9-17.5]	14.9 (20.9) [12.2-17.5]	14.3 (20.1) [9.9-18.8]	-0.021 (0.502)	14.8 (20.6) [13.6.16.1]
No Periodontitis	8.3 (9.8) [5.6-11.1]	5.6 (8.3) [3.2-8.0]	5.4 (9.6) [3.1-7.6]	2.9 (4.5) [1.7-4.1]	6.1 (10.8) [4.0-8.1]	5.5 (8.3) [3.6-7.5]	5.8 (13.5) [-1.2-12.7]	-0.096 (0.048)	5.7 (9.3) [4.8-6.5]
Non-severe Periodontitis	38.1 (22.4) [23.1-53.1]	23.5 (19.7) [14.5-32.5]	20.2 (16.8) [15.1-25.3]	17.8 (17.9) [12.6-23.1]	13.5 (18.8) [10.1-16.8]	13.8 (20.9) [9.6-18.1]	11.3 (17.5) [5.5-17.0]	-0.287 (<0.001)	15.9 (19.6) [13.9-17.9]
Severe Periodontitis	-	34.2 (25.0) [3.1-65.3]	40.5 (28.5) [26.8-54.3]	33.1 (31.4) [22.3-43.9]	27.9 (26.7) [22.4-33.4]	25.4 (24.8) [19.7-31.1]	24.0 (23.5) [14.7-33.3]	0.127 (0.043)	28.5 (26.8) [25.2-31.9]
Total Periodontitis	38.1 (22.4) [23.1-53.1]	25.6 (20.7) [17.2-33.9]	26.3 (22.8) [20.6-32.1]	24.3 (25.5) [18.7-30.0]	19.6 (23.6) [16.5-22.8]	18.9 (23.4) [15.4-22.5]	16.6 (13.5) [11.4-21.8]	-0.181 (<0.001)	21.0 (23.5) [19.1-22.8]
Mean PI (%)	11.0 (15.9) [7.0-15.0]	10.6 (21.0) [5.7-15.4]	11.4 (19.8) [8.1-14.8]	20.3 (24.4) [15.3-25.3]	23.7 (29.9) [20.4-26.9]	31.1 (32.9) [27.0-35.3]	42.6 (37.6) [34.3-50.8]	0.296 (<0.001)	23.2 (30.3) [21.4-25.0]
No Periodontitis	9.5 (14.8) [5.4-13.7]	7.3 (15.0) [3.0-11.6]	6.4 (14.2) [3.0-9.7]	7.7 (13.9) [3.9-11.4]	13.6 (22.7) [9.3-17.9]	20.9 (26.2) [14.9-27.0]	29.3 (36.7) [10.4-48.2]	0.220 (<0.001)	12.3 (21.1) [10.3-14.3]
Non-severe Periodontitis	17.9 (20.0) [4.5-31.3]	17.7 (31.3) [3.4-32.0]	16.4 (23.1) [9.3-23.4]	17.0 (22.5) [10.4-23.5]	24.2 (29.1) [19.1-29.3]	33.5 (34.8) [26.4-40.5]	42.1 (38.0) [29.6-54.6]	0.231 (<0.001)	26.0 (31.1) [22.8-29.1]
Severe Periodontitis	-	12.7 (13.8) [-4.4-29.7]	19.3 (25.2) [7.2-31.5]	44.7 (39.8) [31.0-58.4]	34.6 (34.2) [27.6-41.6]	38.2 (34.2) [30.3-46.0]	51.7 (36.1) [37.4-65.9]	0.191 (0.002)	37.3 (35.1) [33.0-41.6]
Total Periodontitis	17.9 (20.0) [4.5-31.3]	16.7 (28.6) [5.2-28.3]	17.3 (23.6) [11.4-23.2]	28.8 (33.8) [21.4-36.2]	28.7 (31.7) [24.4-32.9]	35.5 (34.5) [30.3-40.8]	46.1 (37.3) [36.8-55.3]	0.231 (<0.001)	30.5 (33.2) [27.9-33.1]

BoP – Bleeding on Probing, PI – Plaque Index, SD – Standard Deviation

* Overall trend across age groups assessed by Spearman's rank correlation coefficient (rho). Significant correlations identified in bold (p<0.05).

Risk factors for periodontitis

Crude and adjusted Odds Ratio (OR) for putative risk factors towards periodontitis were determined and are presented in Table S4.2 (Supplementary material) and Table 4.5, respectively. Within the final reduced model obtained by a multivariate logistic regression procedure, age (OR = 1.05 , 95% CI: 1.04-1.06), educational level (OR = 2.08, 95% CI: 1.32-3.27, OR = 1.86, 95% CI: 1.13-3.05, for middle and elementary education, respectively), smoking status (OR = 3.76, 95% CI: 2.44-5.80 and OR = 2.11, 95% CI: 1.52-2.91, for current smoker and former smoker, respectively) and diabetes mellitus (OR = 1.53, 95% CI: 1.06–2.21) were the significantly risk indicators that were identified towards periodontitis.

Table 4.5. Multivariate logistic regression analysis (final reduced model) (*) on potential risk factors towards periodontitis.

Predictor variables	Odds Ratio (OR)	OR (95% CI)	*p*-value
Age	1.05	1.04-1.06	<0.001
Education			
Higher	1	-	-
Middle	2.08	1.32-3.27	0.002
Elementary	1.86	1.13-3.05	0.015
No education	2.08	0.88-4.90	0.095
Smoking status	-	-	<0.001
Non-smoker	1	-	-
Current Smoker	3.76	2.44-5.80	<0.001
Former smoker	2.11	1.52-2.91	<0.001
Diabetes Mellitus			
No	1	-	-
Yes	1.53	1.06-2.21	0.023

* The model was statistically significant, $\chi 2(7) = 174.786$, $p < 0.001$, explained 20.5% (Nagelkerke R^2) of the variance and correctly classified 68.7% of cases.

4.4. Discussion

This is the first periodontal population-based representative study carried out in Portugal and one of the very first to use the new periodontitis and gingivitis case definitions [7,18]. The results of this epidemiological study indicate that seven out of ten adults in the target population had some type of periodontal disease, and six out of ten had periodontitis. Moreover, almost half of the population exhibited moderate and severe periodontitis. In particular, this study sample was low educated, with the majority being below secondary education, and a largest share were under a situation of work inactivity. Also, they self-reported good brushing frequency, poor interproximal cleaning habits and low periodontal disease awareness, being equivalent to the national average [21]. Regarding the systemic state, a very high percentage presented comorbidities. The prevalence of smokers and former smokers were

13.6% and 27.5%, respectively. The DM prevalence was slightly above the national average, however it is explained by the greater percentage of elderly among the included sample [22]. In Portugal, to date, there is only one national epidemiological study on the prevalence of periodontal disease. The results estimated a prevalence of 10.8% and 15.3% in adults and elderly, respectively. Nonetheless, it can not be compared with the present study because it used CPITN methodology [9]. Oppositely, the present findings indicated a higher severity of periodontal destruction. In fact, the use of partial recording protocols underestimate the periodontal prevalence and extent by almost 50% [16,23].

Few periodontal epidemiological studies provide comprehensive and comparable information in Europe. Furthermore, due to the novelty of the AAP/EFP consensus, the number of studies using this case definition is still scarce. When compared to other European population-based representative studies, the Tromstannen–Oral Health in Northern Norway (TOHNN) study reported an overall prevalence of 9.1% of severe periodontitis [15], and in the Periodontitis and Its Relation to Coronary Artery Disease (PAROKRANK) in Sweden the prevalence of severe periodontitis was of 6.2% [24]. The Study of Health in Pomerania (SHIP) revealed a prevalence of 17.6% of severe periodontitis and 25.3% of moderate periodontitis [25], while for the Turim regional survey these prevalence were of 39.9% and 40.8% for severe and moderate periodontitis

respectively [12]. In the USA, the National Health and Nutrition Examination Survey (NHANES) 2009-2012 estimates of severe and moderate periodontitis were of 8.9% and 30.9%, respectively [6].

Drawing parallels with the findings of this investigation, the prevalence of severe periodontitis was only surpassed by the Turim study, whereas for moderate periodontitis the estimates ranked lower [12]. Notwithstanding, the aforementioned studies used the CDC/AAP case definition as already mentioned, and it is not known what is the difference magnitude between these two classifications.

Undoubtedly, the prevalence of moderate and severe periodontitis peaked in the age of 61-70 years old (34.3% and 36.9%, respectively), having subsequently reduced. Another important aspect to be addressed is the relevantly high prevalence of both localized (34.4%) and generalized (34.6%) periodontitis in the same age interval. Similar results have been found in other articles [12,15,25].

Further, the multivariate logistic regression analysis performed in this study revealed age, education, smoking status and diabetes mellitus as significantly potential risk factors towards periodontitis. Similarly to previous literature, periodontal complications was linked to aging within this population [6,12,15,26,27]. Moreover, the clinical periodontal hallmarks (CAL and PD), tooth loss and teeth with mobility were age-related. However, previous data and this survey suggest that intact supporting periodontal tissues prevail in patients of all age ranges, suggesting pathological CAL is not an aging consequence per se [27,28].

Concerning the smoking status, being an active smoker was strongly associated with periodontitis (adjusted OR=3.76), while past smoking history revealed a lower but also significant association (adjusted OR=2.11). These results are in accordance with previous studies whose OR ranged between 2 and 6 [8,12,29-31] and is widely accepted that smoking has a harmful effect on the onset and progression of periodontitis along with other risk factors for periodontitis [7,32,33]. Likewise, it is also very important to highlight the influence of a past history of smoking activity and the repercussions of bad behaviours on the periodontal status and on tooth loss in the long-term [34].

Regarding the education level, low educated people had a higher risk of having periodontitis being in line with previous reports [6,13,15,26,35]. In this

population, the number of low educated participants is substantial and can be explained by the high number of elderly population and represent a generation that had little educational access. As a risk factor, low educational attainment has been linked to a greater loss of periodontal support and is more prominent when evaluated together with other sociocultural determinants [1,36,37].

As well, DM was a risk factor towards periodontitis in this population in the same way as established in the literature [38-40]. Diabetes increases the risk for periodontitis (particularly if poorly controlled) and evidence suggests that advanced periodontitis also compromises glycaemic control. The new consensus has established DM as a grading modifier for the progression of periodontitis through the glycated hemoglobin (HbA1c) levels. Though, we have recorded HbA1c of our DM patients, as part of the standard clinical follow-up in FHUs, most non-DM patients have never been analyzed for, and to prevent bias in the multivariate analysis we will address this in a future focused study.

Strengths and Limitations

This survey has numerous strengths, including the representativeness and global geographic coverage based on the FHUs where the study was carried out, the sample size calculation stratified for each FHU, the strict methodology followed and the employment of the new AAP/EFP case definition enabling future comparability across studies.

Nevertheless, there are some shortcomings to mention. Due to the peculiarly low periodontitis prevalence previously reported and that based sample size calculation, more than half of the participants had $\geq$ 61 years old, which might have overestimated the prevalence of periodontitis. Also, the target population's sociodemographic characteristics and oral hygiene behaviours must be carefully considered when extrapolating the present findings to other European populations, particularly the elderly subset that had low education and economic constraints. Lastly, people were directly invited to participate in the study, which can bias the population coverage for sampling, however also increases the probability of having a more accurate representation of the participant's oral situation.

4.5. Conclusions

This study reveals a high burden of periodontitis in the adult population of the southern region of the Lisbon Metropolitan Area, in Portugal. Age, education level, smoking status and diabetes mellitus were identified as significantly potential risk factors towards periodontitis. These findings provide new knowledge that will empower appropriate public oral health programmes and population-based preventive actions.

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4.7. Supplementary material

Table S4.1. STROBE checklist of items that should be included in reports of *case-control studies*.

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	6-7
		(*c*) Explain how missing data were addressed	6-7
		(*d*) If applicable, explain how matching of cases and controls was addressed	6-7
		(*e*) Describe any sensitivity analyses	6-7
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	NA

Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	7
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8
		(*b*) Report category boundaries when continuous variables were categorized	7-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	7-8
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			8-10
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Table S4.2. Univariate (crude) analysis on potential risk factors towards periodontitis.

Predictor variables (*)	Odds Ratio (OR)	OR (95% CI)	*p*-value
Gender			
Female	1	-	-
Male	1.91	1.48-2.46	<0.001
Age	1.04	1.03-1.05	<0.001
Education			
Higher	1	-	-
Middle	2.33	1.54-3.54	<0.001
Elementary	3.50	2.28-5.37	<0.001
No education	4.78	2.18-10.48	<0.001
Marital status			
Married / Union of fact	1	-	-
Single	0.39	0.28-0.56	<0.001
Divorced	1.32	0.85-2.05	0.222
Widowed	1.75	1.11-2.77	0.016
Occupation			
Employed	1	-	-
Student	0.06	0.01-0.43	0.005
Unemployed	1.08	0.74-1.58	0.678
Retired	2.41	1.81-3.20	<0.001
Smoking status			
Non-smoker	1	-	-
Current Smoker	1.93	1.32-2.83	0.001
Former smoker	2.20	1.63-2.95	<0.001
Family income (monthly, €)			
> 1500	1	-	-
601-1500	1.45	1.03-2.03	0.032
<= 600	1.71	1.18-2.46	0.004
Diabetes Mellitus			
No	1	-	-
Yes	2.21	1.56-3.08	<0.001

Comorbidity (by Aimetti 2015			
No	1	-	-
Yes	2.21	1.60-3.05	<0.001
Brushing frequency (daily)			
3+	1	-	-
2	1.27	0.90-1.80	0.170
1	1.64	1.12-2.40	0.011
0	3.09	1.26-7.54	0.013
Interproximal cleaning			
Yes	1	-	-
Occasionally	0.98	0.64-1.49	0.914
No	1.81	1.30-2.50	<0.001
Periodontal diseases awareness			
Yes	1	-	-
No	1.28	0.94-1.76	0.122

* Odds Ratio (OR) determined within univariate logistic regression models. Significant predictor variable categories identified in bold ($p<0.05$).

5 Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: findings from a cross-sectional survey

This chapter was based from the published work:

Paper VI – Botelho J, Machado V, Proença L, Oliveira MJ, Cavacas MA, Amaro L, Águas A, Mendes JJ. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: findings from a cross-sectional survey. *BMC Oral Health* **2020**, 20, 199. Doi: 10.1186/s12903-020-01183-7

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5. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: findings from a cross-sectional survey

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Abstract

Background: To investigate if self-perceived xerostomia and stress are significant variables on the Oral-Health Related Quality of Life (OHRQoL) of elderly patients, considering the periodontal status, oral hygiene habits and sociodemographic characteristics simultaneously.

Methods: The study cohort included 592 participants (320 females/272 Males), aged 65 years or older, representing the elder inhabitants of the Study of Periodontal Health in Almada-Seixal (SoPHiAS). Patients answered a socio-demographic and oral hygiene habits questionnaire. The Oral Health Impact Profile-14 (OHIP-14), Summated Xerostomia Inventory-5 (SXI-5) and Perceived Stress Scale-10 (PSS-10) were used. Full-mouth circumferential periodontal inspection was carried out. Multivariable regression analyses were used considering the level of periodontitis, clinical characteristics, the number of teeth, SXI, PSS-10, age, gender and oral hygiene habits.

Results: Self-perceived xerostomia and stress showed a positive significant correlation with OHRQoL and each of its domains. Multiple linear regression analysis demonstrated the significant impact of SXI-5 (B=1.20, p<0.001) and PSS-10 (B=0.35, p<0.001) on the OHRQoL. SXI-5 (Odds Ratio (OR)=1.28, p<0.001) and PSS-10 (OR=1.03, p=0.022) were associated with a more frequently affected OHRQoL. The number of missing teeth, being male, mean probing depth and mean clinical attachment loss were also significant towards a frequently affected OHRQoL. Conversely, age was negatively associated with a lower OHRQoL.

Conclusion: Self-perceived xerostomia and stress are significant variables towards OHRQoL in elderly patients. Future studies should consider these self-perceived xerostomia and stress when investigating the impact of periodontitis and missing teeth on quality of life of older adults.

5.1. Background

Patient-reported outcomes (PROs) are emerging key measures to aid oral health care decision-making policies [1]. In a disease or treatment situation, patients' perspective is often the most significant result over clinical outcomes [2]. Besides, in a world with a population ageing so fast, doing so healthy and with good quality of life has become an exciting matter to study [3].

Periodontal diseases are highly prevalent among older people and remain a substantial epidemiological challenge [4-6]. Periodontal diseases negatively impact on oral health-related quality of life (OHRQoL), especially with the worsening and extent of disease, and with special relevance in the elderly populations [7-11]. Notwithstanding, the lost quality of life can be recovered after nonsurgical periodontal therapy [12].

The impact of periodontitis on the OHRQoL, in elder populations, is poorly studied. Nevertheless, though periodontitis leads to poorer quality of life, its clinical consequences as missing teeth and denture use have apparently a higher impact [13,14]. Nevertheless, it is important to investigate whether other confounders could influence the quality of life perception. Stress has been linked to both periodontitis and OHRQoL [15,16], and xerostomia has been associated to poorer quality of life [17,18]. Self-reported objective and subjective dry mouth complaints impact OHRQoL in senior men and women, because the majority has

medical conditions and medications that might cause xerostomia [19,20]. Besides, perceived stress has also been linked to worse OHRQoL in older people [21]. However, the impact of self-perceived xerostomia and stress together with periodontitis on the OHRQoL of elderly individuals has never been explored, and may be potential influential variables.

Therefore, we aimed to evaluate whether self-perceived xerostomia and stress could influence OHRQoL in a representative elderly population, considering also the extent of periodontitis, the number of missing teeth, clinical variables and oral hygiene habits.

5.2. Methods

Ethics and Study design

The Study of Periodontal Health in Almada-Seixal (SoPHiAS) is a population-based representative study, with a target population living in the municipalities of Almada and Seixal (Portugal) [22]. This study was approved by the Research Ethics Committee of the Regional Health Administration of Lisbon and Tagus Valley, IP (Portugal) (8696/CES/2018) and in accordance with the Declaration of Helsinki, as revised in 2013. Participants were informed about their periodontal status after examination. Patients diagnosed with periodontal disease were referred to the Egas Moniz Dental Clinic (EMDC) for treatment without additional costs [22]. The study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [23].

Sample size and Measurement reproducibility

A fully detailed report on elderly sampling strategy and measurement reproducibility are mentioned in [22]. A total of 1,064 participants, aged 18 to 95 years, gave their consent and were examined [22]. For the purpose of this study, a subset of 592 participants, 320 women and 272 men, with 65 years old or over were studied.

Periodontal examination and clinical variables

Each clinical examination was performed using proper lightening with the individuals seated on an adjustable stretcher in the FHU's medical office. Periodontal examination was made as described in Botelho et al. [22]. Periodontitis case definitions were defined according to the new AAP/EFP consensus [24].

Questionnaires

Information on sociodemographic characteristics and behaviours was collected by a self-reported questionnaire. The questionnaire covered questions on the following items: 1) gender, age, marital status, educational level, occupation; 2) monthly family gross income; 3) smoking habits; 4) oral hygiene-related behaviours (tooth brushing frequency, interproximal cleaning); 5) attitudes and awareness towards oral health.

Participants completed the Portuguese versions of the Oral Health Impact Profile-14 (OHIP-14-PT) [25] to assess OHRQoL, Summated Xerostomia Inventory-5 (SXI-5-PT) [26] to quantify xerostomia and Perceived Stress Scale-10 (PSS-10-PT) to estimate recognised stress [27].

The OHIP-14 consists of 14 questions representing seven domains (functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap) of OHRQoL. Each question is scored by 0 (never,) 1 (hardly ever), 2 (occasionally), 3 (fairly often) and 4 (very often). Thus, a higher score indicates poorer OHRQoL. Each pair of questions represents one of seven domains of the OHIP-14. The sum of the scores of the 14 questions ranges from 0 to 56 and the sum of each domain ranges from 0 to 8 (Slade et al. 1997). Further, individuals were categorised as frequently affected individual with respect to OHRQoL (answering with 3 or 4 to at least one of the questions in the OHIP-14) or with less affected individuals (responding with 0, 1 or 2 on all the items) [13].

The SXI-5 is a 5-questions tool where each question is scored by 0 = never, 1 = occasionally 2 = frequently. The scores from the five questions are summed, with the result representing the degree of xerostomia the subject feels [26].

The PSS-10 is 10-items instrument indicated to assess self-perceived stress. Each of the items on the PSS-10 is rated on a 5-point Likert scale, and each question is scored 0 = never, 1 = almost ever, 2 = sometimes, 3 = fairly often and 4 = very often. The PSS-10 consists of two domains: six positively (items 1, 2, 3, 6, 9 and 10) and four negatively (items 4, 5, 7 and 8, that require reversion) worded items. Total scores range from 0 to 40, with higher scores indicating higher levels of perceived stress [28].

Assessment of confounders

Furthermore, the participants have also been categorised into three groups according to the extent of periodontitis: no disease, localized periodontitis and generalized periodontitis [24]. Concerning oral hygiene, patients were categorized for their interproximal hygiene (no = 0, occasionally = 1, and yes = 2) and for frequency of toothbrushing per day (less than one time per day = 0, one time per day = 1, and two or more times per day = 2). Also, patients were questioned to the use of dentures and registered as a dichotomous variable (no = 0, or yes =1).

Statistical Methods

The total scores of OHIP-14, PSS-10 and SXI-5 were calculated and their correspondent descriptive measures (mean and standard deviation (SD)) were computed. For analysis purposes, these scores were considered as continuous variables. The data analyses were conducted for all participants and for sample subsets, according to gender and periodontitis extent. Mann-Whitney and Kruskal-Wallis tests were used to compare OHRQoL scores as a function of gender and periodontitis extent. For categorical variables, the analyses were performed using Chi-square test. Spearman's rank correlation coefficient (rho) was used to analyse the correlation of OHIP-14 scores with PSS-10 and SXI-5 total scores, number of missing teeth and periodontal clinical variables. The effect size of correlations was analysed according to Cohen's standard. Further, a multiple forward stepwise linear regression analysis was carried out in order to evaluate the impact of those variables on the OHIP-14 total score. Next, a multivariable forward stepwise logistic regression was applied using the

dichotomised dependent OHIP-14 variable "frequently affected" vs "less affected" OHRQoL as in [13]. Odds ratio (OR) and correspondent 95% confidence level intervals (95% CI) were calculated. Data were analysed using IBM SPSS Statistics, v. 25, (NY, USA). A level of significance of 5% was considered in all inferential analyses.

5.3. Results

Sample description

In general, the average total OHIP-14 score indicates that these participants perceived their OHRQoL as modest, although men perceived better OHRQoL than women (Table 5.1). Regarding periodontitis extent, 70.9% had periodontitis with 24.0% and 47.0% being localized and generalized forms, respectively. Men had more prevalence of localized and generalized periodontitis than women. Women had significantly more missing teeth, although self-reporting better oral hygiene habits than men, on interproximal cleaning and toothbrushing. Almost half of the population were denture wearers, with the majority using partial and removable acrylic types. Besides, this population self-reported moderate signs of dry-mouth and stress with significant differences between gender, with men experiencing more stress and women more signs of xerostomia.

Table 5.1. Oral Health Impact Profile (OHIP-14), Summated Xerostomia Inventory-5 (SXI-5) and Perceived Stress Scale-10 (PSS-10) scores, age, number of missing teeth, periodontitis extent, oral hygiene variables and denture wearers, according to gender and for the overall participants (N = 592).

	Women (n = 320)	Men (n = 272)	P-value	Overall (N = 592)
OHIP-14 Total, mean (SD)	9.57 (11.70)	5.83 (8.54)	< **0.001***	7.85 (10.53)
OHIP-14 domains, mean (SD)				
Functional limitation	1.23 (2.01)	0.85 (1.58)	**0.032***	1.05 (1.83)
Physical pain	2.54 (2.70)	1.93 (2.40)	**0.008***	2.26 (2.58)
Psychological discomfort	1.67 (2.58)	0.86 (1.94)	< **0.001***	1.30 (2.34)
Physical disability	1.58 (2.47)	0.97 (1.91)	**0.003***	1.30 (2.25)
Psychological disability	1.28 (2.20)	0.58 (1.54)	< **0.001***	0.96 (1.95)

Social disability	0.41 (1.27)	0.17 (0.74)	**0.029***	0.30 (1.07)
Handicap	0.87 (1.87)	0.47 (1.34)	**0.008***	0.68 (1.66)
SXI-5, mean (SD)	7.1 (2.1)	6.3 (1.5)	**0.001***	6.7 (1.9)
PSS-10, mean (SD)	14.2 (8.2)	16.1 (7.6)	**0.002***	15.1 (8.0)
Age, mean (SD)	71.9 (6.2)	73.4 (6.6)	**0.007***	72.6 (6.4)
Mean PD (mm), mean (SD)	1.88 (0.70)	2.00 (0.88)	0.372*	1.94 (0.79)
Mean CAL (mm), mean (SD)	2.86 (1.28)	3.22 (1.76)	0.259*	3.02 (1.53)
Mean Rec (mm), mean (SD)	0.98 (0.94)	1.24 (1.26)	**0.047***	1.10 (1.10)
Mean BoP (%), mean (SD)	15.1 (20.7)	14.9 (21.5)	**0.026***	15.0 (21.1)
Missing teeth, mean (SD)	10.9 (6.7)	11.9 (6.8)	**< 0.001***	12,72 (6.77)
Periodontitis, n (%)				
Severity				
Healthy	104 (32.5)	68 (25.0)	**0.032#**	172 (29.1)
Stage I (Mild)	48 (15.0)	36 (13.2)		84 (14.2)
Stage II (Moderate)	79 (24.7)	77 (28.3)		156 (26.4)
Stage III (Severe/Advanced)	89 (27.8)	91 (33.5)		180 (30.4)
Extent				
No	104 (32.5)	68 (25.0)	0.135#	172 (29.1)
Localized	73 (22.8)	69 (25.4)		142 (24.0)
Generalized	143 (44.7)	135 (49.6)		278 (47.0)
Interproximal cleaning, n (%)				
No	227 (70.9)	233 (85.7)	**< 0.001#**	460 (77.7)
Occasionally	35 (10.9)	18 (6.6)		53 (9.0)
Yes	58 (18.1)	21 (7.7)		79 (13.3)
Toothbrushing frequency per day, n (%)				
0	8 (2.5)	18 (6.6)	**< 0.001#**	26 (4.4)
1	79 (24.7)	114 (41.9)		193 (32.6)
2+	233 (72.8)	140 (51.5)		373 (63.0)
Denture wearer, n (%)				
No	137 (42.8)	170 (62.5)		307 (51.9)

Yes	183 (57.2)	102 (37.5)	< **0.001**#	285 (48.1)
Denture extent, n (%) (n= 285)				
Partial	128 (69.9)	77 (75.5)	-	205 (71.9)
Full	12 (6.6)	5 (4.9)		17 (6.0)
Both	43 (23.5)	20 (19.6)		63 (22.1)
Type of denture, n (%) (n= 285)				
Removable Acrylic	124 (67.8)	73 (71.6)	-	197 (69.1)
Removable Metallic	58 (31.7)	24 (23.5)		82 (28.8)
Removable Acrylic and Metallic	1 (0.5)	0 (0)		1 (0.4)
Fixed	1 (0.5)	5 (4.9)		6 (2.1)

*Mann-Whitney test. #Chi-square test. Significant differences identified in bold (p<0.05). Mean BoP – Mean bleeding of probing; Mean CAL – Mean clinical attachment loss; Mean PD – Mean probing depth; Mean Rec – Mean gingival recession; OHIP-14 - Oral Health Impact Profile-14; PSS-10 - Perceived Stress Scale-10; SD – standard deviation; SXI-5 - Summated Xerostomia Inventory-5.

Furthermore, in the overall sample, patients with generalized periodontitis stated average similar OHRQoL than patients with no periodontitis and localized (Table 5.2). Only the handicap domain demonstrated notable differences in men, between no disease patients and both localized and generalized periodontitis inmates. Periodontitis severity showed to influence OHRQoL levels, with more severe cases presenting worse OHRQoL parameters (Table 5.3). Also, in this analysis, only the functional limitation domain had differences among women between no disease and the different types of periodontitis.

Table 5.2. Oral Health Impact Profile (OHIP-14) scores, total and for each domain, presented as mean and standard deviation (SD), according to gender and periodontitis extent.

	Women (n = 320)				Men (n = 272)				Overall (N = 592)			
	ND	L	G	P-value #	ND	L	G	P-value #	ND	L	G	P-value #
OHIP-14, mean (SD)	9.02 (11.90)	9.29 (10.45)	10.11 (12.23)	0.430	4.28 (6.46)	5.64 (8.78)	6.71 (9.24)	0.165	7.15 (10.35)	7.51 (9.81)	8.42 (10.98)	0.178
OHIP-14 domain, mean (SD)												

Functional limitation	1.19 (2.23)	1.25 (1.70)	1.25 (2.00)	0.435	0.71 (1.39)	0.77 1.37)	0.96 (1.76)	0.732	1.00 (1.95)	1.01 (1.56)	1.11 (1.89)	0.466
Physical pain	2.44 (2.68)	2.56 (2.45)	2.61 (2.85)	0.897	1.81 (2.32)	1.77 (2.44)	2.07 (2.43)	0.501	2.19 (2.56)	2.18 (2.47)	2.35 (2.66)	0.866
Psychological discomfort	1.56 (2.60)	1.75 (2.45)	1.66 (2.61)	0.405	0.53 (1.69)	0.84 (1.91)	1.04 (2.07)	0.079	1.15 (2.33)	1.31 (2.24)	1.36 (2.38)	0.178
Physical disability	1.57 (2.47)	1.36 (2.18)	1.68 (2.62)	0.936	0.81 (1.70)	0.87 (3.88)	1.11 (1.97)	0.395	1.27 (2.23)	1.12 (2.09)	1.40 (2.34)	0.601
Psychological disability	1.13 (2.09)	1.36 (2.19)	1.35 (2.28)	0.443	0.25 (1.01)	0.58 (1.45)	0.74 (1.77)	0.199	0.78 (1.80)	0.98 (1.90)	1.05 (2.06)	0.223
Social disability	0.49 (1.29)	0.23 (1.01)	0.44 (1.39)	0.348	0.04 (0.27)	0.2 (0.85)	0.21 (0.83)	0.267	0.31 (1.04)	0.22 (0.93)	0.33 (1.15)	0.708
Handicap	0.64 (1.59)	0.78 (1.45)	1.07 (2.22)	0.387	**0.13 (0.54)[a]**	**0.61 (2.15)[b]**	**0.57 (1.52)[b]**	**0.043***	0.44 (1.31)	0.7 (1.45)	0.83 1.93)	0.060

G – Generalized; L – Localized; ND – No Disease. # Kruskal-Wallis test. Significant differences identified in bold (*p<0.05). OHIP-14 - Oral Health Impact Profile-14; SD – standard deviation.

Table 5.3. Oral Health Impact Profile (OHIP-14) scores, total and for each domain, presented as mean and standard deviation (SD), according to gender and periodontitis severity.

	Women (n = 320)					Men (n = 272)					Overall (N = 592)				
Stage	**0**	**1**	**2**	**3**	**P-value #**	**0**	**1**	**2**	**3**	**P-value #**	**0**	**1**	**2**	**3**	**P-value #**
OHIP-14. mean (SD)	9.0 (11.9)	6.3 (8.9)	8.6 (10.4)	12.8 (13.2)	0.101	4.3 (6.5)	6.3 (9.0)	5.6 (7.4)	7.0 (10.3)	0.502	7.2 (10.3)	6.3 (8.9)	7.1 (9.2)	9.9 (12.2)	0.225
OHIP-14 domain. mean (SD)															
Functional limitation	1.2 (2.2)	0.8 (1.6)	1.4 (1.9)	1.3 (2.1)	**0.027**	0.7 (1.4)	1.3 (2.1)	0.6 (1.2)	1.0 (1.7)	0.216	1.0 (2.0)	1.0 (1.9)	1.0 (1.6)	1.2 (1.9)	0.466

Physical pain	2.4 (2.7)	1.9 (2.5)	2.2 (2.5)	3.4 (2.9)	0.410	1.8 (2.3)	1.7 (2.3)	2.0 (2.5)	2.0 (2.5)	0.704	2.2 (2.6)	1.8 (2.4)	2.1 (2.5)	2.7 (2.7)	0.397
Psychological discomfort	1.6 (2.6)	1.1 (2.2)	1.5 (2.3)	2.3 (2.9)	0.293	0.5 (1.7)	1.0 (2.3)	1.0 (1.8)	1.0 (2.1)	0.126	1.2 (2.3)	1.0 (2.2)	1.2 (2.1)	1.6 (2.6)	0.190
Physical disability	1.6 (2.5)	1.0 (1.9)	1. 4 (2.7)	1.1 (2.1)	0.404	0.8 (1.7)	1.0 (1.8)	0.9 (1.8)	1.1 (2.2)	0.835	1.3 (2.2)	1.0 (1.9)	1.2 (2.2)	1.6 (2.5)	0.715
Psychological disability	1.1 (2.1)	1.0 (2.0)	1.1 (2.0)	1.8 (2.5)	0.765	0.3 (1.0)	0.7 (1.4)	0.5 (1.3)	0.9 (2.0)	0.266	0.8 (1.8)	0.9 (1.8)	0.8 (1.7)	1.3 (2.3)	0.725
Social disability	0.5 (1.3)	0.0 (0.2)	0.4 (1.2)	0.6 (1.6)	0.112	0.0 (0.3)	0.2 (0.8)	0.2 (0.5)	0.3 (1.0)	0.300	0.3 (1.0)	0.1 (0.6)	0.3 (1.0)	0.4 (1.3)	0.261
Handicap	0.6 (1.6)	0.5 (1.4)	0.7 (1.8)	1.4 (2.3)	0.946	0.1 (0.5)	0.5 (1.1)	0.4 (1.1)	0.8 (1.9)	0.101	0.4 (1.3)	0.3 (1.3)	0.5 (1.5)	1.1 (2.1)	0.493

0 – Healthy; 1 – Stage I, Mild Periodontitis; 2 – Stage II, Moderate Periodontitis; 3 – Stage III, Severe/Advanced Periodontitis; OHIP-14 - Oral Health Impact Profile-14; SD – standard deviation.

Kruskal-Wallis test. Significant differences identified in bold (*p<0.05).

OHIP-14 and covariates impact

Spearman's rank-order correlation coefficient was used to investigate the possible correlation between total and each domain of OHIP-14 with SXI-5 total, PSS-10 total, number of missing teeth, mean probing depth (PD), mean clinical attachment loss (CAL), mean gingival recession (Rec) and mean bleeding on probing (BoP) (Table 5.4). SXI-5 total and the number of missing teeth had small positive significant correlations with OHIP-14 and each domain scores.

To investigate which variables impacted the OHIP-14 total score, a multiple linear regression analysis was conducted (Table 5.5). Afterwards, age, the SXI-5 total, the PSS-10 total, missing teeth and mean PD significantly contributed to the OHIP-14 score. Notably, the factors that most contributed for the OHIP-14 score were mean PD (B = 1.56, 95% CI: 0.62-2.51) and SXI-5 (B = 1.20, 95% CI: 0.77-1.63). Conversely, age was a negative contributor (B = -0.24, 95% CI: -0.36, -0.97).

A multivariate logistic regression, with Oral Health Impact Profile (OHIP-14) dichotomised into less affected vs frequently affected, was also carried out (Table 5.6). Overall, age, SXI-5 total, PSS-10 total, gender and mean CAL were significant for the model. While age has a negative effect (OR = 0.96, 95% CI: 0.93-0.99), being male (OR = 1.44, 95% CI: 1.00-2.06), SXI-5 total (OR = 1.28,

95% CI: 1.14-1.43) and mean CAL (OR = 1.25, 95% CI: 1.10-1.41) increase the risk towards a poorer quality of life.

In both analyses, the SXI-5 and PSS-10 significantly influenced the OHRQoL perception (Table 5.4 and 5.5).

Table 5.4. Correlation of OHIP-14 total and domain scores with Summated Xerostomia Inventory-5 (SXI-5), Perceived Stress Scale-10 (PSS-10) scores, number of missing teeth, mean PD, mean CAL, mean Rec and Mean BoP.

	OHIP-14							
	Total	Functional limitation	Physical pain	Psychological discomfort	Physical disability	Psychological disability	Social disability	Handicap
SXI-5 Total	**0.281****	**0.246****	**0.213****	**0.185****	**0.207****	**0.218****	**0.143****	**0.192****
PSS-10 Total	**0.083***	0.008	0.019	**0.093***	0.017	**0.114*****	0.053	**0.115****
Number of missing teeth	**0.184****	**0.165****	**0.105****	**0.158****	**0.182****	**0.135****	**0.091****	**0.125****
Mean PD	**0.126****	0.043	**0.097***	**0.118****	**0.092***	**0.095***	0.068	**0.084***
Mean CAL	**0.162****	**0.089***	**0.090***	**0.140****	**0.131****	**0.117****	0.077	**0.124****
Mean Rec	**0.136****	**0.085***	0.066	**0.115****	**0.106****	**0.095***	0.054	**0.115****
Mean BoP	**0.110****	-0.015	0.105*	**0.109****	0.071	0.071	0.060	**0.090***

Overall trend across OHIP-14 scores, total and for each domain, assessed by Spearman's rank correlation coefficient (rho). Significant correlations identified in bold (*p<0.05, **p<0.01). Mean BoP – Mean bleeding of probing; Mean CAL – Mean clinical attachment loss; Mean PD – Mean probing depth; Mean Rec – Mean gingival recession; OHIP-14 - Oral Health Impact Profile-14; PSS-10 - Perceived Stress Scale-10; SXI-5 - Summated Xerostomia Inventory-5.

Table 5.5. Multiple linear regression describing the influence of continuous variables on the total OHIP-14 score, with regression coefficients (B) and correspondent 95% confidence intervals (95% CI).

	OHIP-14 Total Score	
	B	p-value
Age	-0.24 (-0.36, -0.97)	<0.001***
SXI-5 Total	1.20 (0.77-1.63)	<0.001***
PSS-10 Total	0.35 (0.26-0.45)	<0.001***
Missing teeth	0.24 (0.13-0.35)	<0.001***
Mean PD	1.56 (0.62-2.51)	0.001**

**p < 0.01 and ***p < 0.001. R2= 0.51. OHIP-14 - Oral Health Impact Profile-14; PSS-10 - Perceived Stress Scale-10; SXI-5 - Summated Xerostomia Inventory-5.

Table 5.6. Multivariate stepwise logistic regression, with Oral Health Impact Profile (OHIP-14) dichotomised into less affected vs frequently affected, with Odds ratio (OR) and correspondent 95% CI.

	OHIP-14 Total Score	
	OR (95% CI)	p-value
Age	**0.96 (0.93-0.99)**	**0.003****
SXI-5 Total	**1.28 (1.14-1.43)**	**<0.001*****
PSS-10 Total	**1.03 (1.00-1.05)**	**0.022***
Gender		
Female	**1**	-
Male	**1.44 (1.00-2.06)**	**0.048***
Mean CAL	**1.25 (1.10-1.41)**	**<0.001*****

*The model was statistically significant, $\chi 2(5) = 71.041$, $p < 0.001$, explained 15.1% (Nagelkerke R2) of the variance and correctly classified 63.3% of cases. Mean CAL – Mean clinical attachment loss; OHIP-14 - Oral Health Impact Profile-14; PSS-10 - Perceived Stress Scale-10; SXI-5 - Summated Xerostomia Inventory-5.

5.4. Discussion

In this study, we hypothesized that self-perceived xerostomia and stress can change OHRQoL perception, analysing simultaneously the periodontal status, number of missing teeth, clinical characteristics and other variables. To test this hypothesis, through a significant dataset from a representative elderly

population, we developed multivariable regression analyses accounting for these variables. Our results confirmed that self-perceived xerostomia and stress are associated with OHRQoL. The number of missing teeth, gender, and mean PD and CAL had a meaningful association to predict OHRQoL.

These findings have wide implications. (1) Self-perceived xerostomia revealed to be influential on OHRQoL in a population of elders, with a similar magnitude to the extent of periodontitis. (2) Self-perceived stress has a mild influence on OHRQoL. (3) The number of missing teeth and age are important variables to the OHRQoL variation. (4) The periodontal status does not influence the OHRQoL, rather some clinical features do. (5) As a result, self-perceived xerostomia and stress are important variables towards the OHRQoL in elderly patients.

As previously debated [22], the results of this epidemiological study indicate a disturbing prevalence of periodontitis among this elderly population and affecting more men. Further, this population reported faulty oral hygiene habits in agreement with a previous national report [29]. These characteristics may explain the high number of missing teeth and, as a consequence, more than half of the population were denture wearers. Besides, this population reported high levels of xerostomia and stress with significant differences between gender.

In terms of potential variables on the OHRQoL perception, SXI-5 exhibited a meaningful effect, while the perception of stress was meaningful but with mild impact. Comprehensively, the higher the perception of xerostomia and stress factors the higher odds of poorer OHRQoL. These results are in line with recent literature where perceived chronic stress impacted the perception of dry mouth and quality of life [18,21,30].

Two recent systematic reviews asserted that periodontal diseases might have an impact on OHRQoL [9,31]. Though none of them had centred exclusively on elder populations, higher disease severity leads to a greater negative impact on OHRQoL. The results of this study agree with the latter since greater PD and CAL values led to worse OHRQoL, although neither the periodontal condition nor the extent of the disease had an influence on the quality of life perception. Besides, the number of missing teeth was a significant factor and complies with a recent systematic review in which retention of teeth is associated with better OHRQoL

[32]. Apparently, patients esteem much more the number of missing teeth for their quality of life than having periodontitis or its extension and confirms what has already been reported [13,14,33]. The number of missing is a characteristic that patients often recognize, though with periodontitis this perception is scarce [22,34]. Recently, we have validated a brief perception questionnaire for periodontal diseases [34], and it will be interesting to associate the perception levels towards periodontitis with the OHRQoL impact.

The association between OHRQoL and gender has been addressed in many investigations. In our report, women perceived poorer OHRQoL than men similarly to what has been found before [18,33,35,36], although there are contrary results showing that men report poorer OHRQoL [37], or no difference whatsoever in the perception between men and women [13]. Still, we demonstrate that men have a higher risk of a frequently affected quality of life than women, possibly because of the overall poorer periodontal condition reported.

Strengths and Limitations

The results provided by our investigation have some notable strengths, as previously proposed [9]. We employed a full-mouth protocol with circumferential inspection, ensuring precise estimation of the prevalence and extent of periodontitis [38]. Also, we used the new AAP/EFP joint case definition with an up-to-date diagnosis with PD and CAL combination analysis. Besides, these results are representative and with adequate sample size calculation, stratified for each health centre. Further, we have included a periodontally healthy control group and possible factors.

However, there are limitations worth to mention in our study. Although we have included the number of missing teeth we could not account for occlusal pairs that have proven impact on OHRQoL [32]. The lack of information related to dentinal sensitivity as a result of gingival recession was also a limitation and shall be considered in the future. Further, the control group was derived from the same sample which can be seen as a possible shortcoming. Too, we employed the OHIP-14 which is more focused on the impact of pain on the patient's psychological and behavioural traits, while the Geriatric Oral Health Assessment Index (GOHAI) tool is more suitable to examine functional

limitations in relation to pain [39,40][139, 140][139,140][139, 140] and ought to be considered in further investigations.

5.5. Conclusions

We demonstrate that self-perceived xerostomia and stress are significant variables for the total score of OHRQoL in elderly patients, that is, they worsen the quality of life. Also, the significance was maintained even when analyzed simultaneously with the periodontal status, the number of missing teeth, periodontal clinical measures and oral hygiene habits. Future studies should consider these parameters when investigating the impact of periodontitis in quality of life.

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6 Relationship between bruxism and periodontal status: findings from a cross-sectional Study

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6. Relationship between self-reported bruxism and periodontal status: findings from a cross-sectional study

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Abstract

Background: Several studies seek to prove the relationship between bruxism and periodontal status although it remains unclear and debatable. We aimed to assess the association between self-reported (SR) bruxism with the periodontal status in a large-scale survey.

Material and Methods: A total of 1,064 individuals from the southern region of the Lisbon Metropolitan Area (Portugal) were enrolled. Patients were interviewed for the SR bruxism assessment through a self-report questionnaire. Full-mouth periodontal status was assessed with Probing Depth (PD), Clinical Attachment Loss (CAL), Gingival Recession (REC) and Bleeding on Probing (BoP) being measured. The American Association of Periodontology/European Federation of Periodontology 2018 case definitions was used. Logistic regression analyses provided information on the influence of SR bruxism towards periodontitis.

Results: SR bruxers exhibited lower prevalence of periodontitis. Additionally, SR bruxers with periodontitis had PD and CAL significantly lower than patients with

only periodontitis. Multivariate analysis suggests that SR bruxism was significantly associated with a lower risk of periodontitis (Odds Ratio [OR] = 0.42 95%CI: 0.32-0.56). Mean PD and CAL were significantly lower in SR bruxers. When assessing the type of SR bruxism, significant differences among mean PD, CAL and BoP levels were also identified.

Conclusion: SR bruxism and periodontal status are negatively associated. SR bruxers exhibit lower odds towards periodontitis and better periodontal clinical characteristics. Further studies are mandatory to clarify these findings.

6.1. Introduction

Bruxism is a multifaceted phenomenon that has been associated with several factors mediated by the central nervous system [1]. According to an updated international consensus in 2018, bruxism is a repetitive masticatory muscle activity that is not necessarily a disorder in healthy individuals [2]. There are two clearly different entities within the umbrella of bruxism, namely: awake bruxism and sleep bruxism [2]. Awake bruxism is defined as masticatory muscle activity during wakefulness that is characterized by repetitive or sustained tooth contact (such as clenching and grinding) and/or by bracing or thrusting of the mandible and is not dyskinetic in otherwise healthy individuals [2]. Sleep bruxism is a masticatory muscle activity during sleep that is characterized as rhythmic (phasic) or non-rhythmic (tonic) and is not a movement or sleep disorder in otherwise healthy individuals [2].

Periodontal disease is one of the most prevalent diseases in the world and is undoubtedly a serious public health problem that has a large socioeconomic impact [3,4]. Periodontal disease is characterized by a chronic non-communicable inflammatory condition which results in the progressive destruction of the tooth-supporting tissues due to host's immune response to a complex polymicrobial-driven infection [5–12].

Approaches to assess bruxism can be distinguished as non-instrumental (notably self-report) or instrumental (clinical assessment) [2]. Given the difficulty and inaccuracy on bruxism diagnose patented in literature, a grading system was suggested in 2013 [13] and reviewed in the 2018 consensus [2]. In these, possible sleep/awake bruxism is based on a positive self-report only, probable sleep/awake bruxism is based on a positive clinical examination, with or without

a positive self-report and definite sleep/awake bruxism is based on a positive instrumental assessment, with or without a positive self-report and/or a positive clinical inspection. Although this revised grading system seems to point out that self-report is not the ideal way to assess bruxism in the clinical setting, the consensus paper also states that it may be useful [2].

Mastication is the major function of the dentition and, the periodontium is the tooth support mechanism that allows the teeth to fulfill this basic function. Over the past years, the potential deleterious effects of bruxism on the temporomandibular joints, masticatory muscles, and natural teeth have been continually addressed [14–17]. Notwithstanding, the relationship between excessive occlusal force and periodontium remain a complex and controversial issue [18], and only one systematic review [17] investigated the effect of bruxism as a potential risk factor for the teeth-supporting tissues. Despite the limitations, bruxism apparently "cannot cause periodontal damage per se" and the authors underline the need for more research on the association of bruxism and its types on periodontal patients [17].

Given the weak literature references available and considering the hypothesis that bruxism and the periodontium might be linked, this study aimed to assess the association between self-reported bruxism (SB) and periodontal status in a large-scale survey.

6.2. Materials and Methods

Study design and inclusion criteria

This study was designed as a population-based cross-sectional representative study, geographically stratified, with a target population of inhabitants over 18 years of age (adults and elderly). It was carried out at the public health centres of Almada and Seixal municipalities, located in the Lisbon Metropolitan Area, in Portugal. Only one health centre had dental treatment facilities, although they do not provide periodontal treatments. The exclusion criteria were: age under 18 years, edentulous patients, unable to participate in the survey and answer questionnaires or if they refuse to reply to the questionnaire. A total of 1,064 participants were enrolled in the study. Data were collected between December

2018 and April 2019. This survey followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [19] (Supplement).

Sample size estimation

In September 2018, 386,168 inhabitants in the selected age groups lived in the two municipalities (institutional data provided). We based our estimation in a reported national prevalence data of 10.8% and 15.3%, for adults and elderly, respectively (DGS 2015). To achieve an estimate of the periodontitis prevalence in the population, with a margin of error of 3.0%, for a 95% confidence level, a minimum of 962 individuals were required to be examined. We stratified the required sample according to the number of subjects assigned to each health centre (institutional data provided). The invitation to participate in the survey was made by direct contact at the waiting room of the FHU, explaining the purpose of the study and including a description of the clinical examination.

Participants

The participants were recruited during an epidemiologic study carried out in the southern region of the Lisbon Metropolitan Area, in Portugal - Study of Periodontal Health in Almada-Seixal (SoPHiAS) [20]. Previously, SoPHiAS project was approved by a state-recognized Ethics Committee: the Research Ethics Committee of the Regional Health Administration of Lisbon and Tagus Valley, IP (Registration numbers: 3525/CES/2018 and 8696/CES/2018). All participants gave their previous written informed consent.

Periodontal examination and diagnosis

Two trained and calibrated examiners (V.M. and J.B.) performed the periodontal diagnosis. The inter-examiner correlation coefficients ranged from 0.98 and 0.99 and between 0.93 and 0.99, for mean Probing Depth (PD) and mean Clinical Attachment Loss (CAL), respectively. Gingivitis and Periodontitis cases were defined according to the AAP/EFP 2017 consensus [8,21], with a patient being a periodontitis case if interdental CAL is detectable at ≥2 non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth. At the end of the examination, participants were informed about their periodontal

status. Individuals diagnosed with Periodontal Diseases were referred to the Egas Moniz Dental Clinic (EMDC) for its treatment without additional costs.

A full-mouth periodontal examination was performed with a periodontal probe ‖. Third molars, implants and retained roots, were excluded from the examination. Plaque index (PI) [22], gingival recession (REC), probing depth (PD), and bleeding on probing (BoP) were circumferentially recorded at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). PD was measured as the distance from the free gingival margin to the bottom of the pocket and REC as the distance from the cementoenamel junction (CEJ) to the free gingival margin, and this assessment was assigned a negative sign if the gingival margin was located coronally to the CEJ. CAL was calculated as the algebraic sum of REC and PD measurements for each site. The measurements were rounded to the lowest whole millimeter. Furcation involvement (FI) was assessed using a Naber probe® [23]. Tooth mobility was further appraised [24].

SR Bruxism assessment

Based on the 2018 consensus, patients with positive SB were acknowledged as possible bruxers [2]. The questionnaire comprised five previously detailed questions [25]:

1. Sleep grinding item: Are you aware of the fact that you grind your teeth during sleep?
2. Sleep grinding referral item: Has anyone ever told you that you grind your teeth during sleep?
3. Sleep clenching item: Upon awakening in the morning or awakening during the night, do you have your jaws thrust or braced?
4. Awake clenching item: Do you clench your teeth while awake?
5. Awake grinding item: Do you grind your teeth whilst awake?

All questions had a dichotomous yes/no answer [25]. Positive answers for Questions 1 and/or 2 and/or 3 indicated Sleep SB, and a positive answer to Questions 4 and 5 indicated that the participant had Awake SB.

Sociodemographic variables

Sociodemographic data comprised gender, age, educational level (no education, elementary, middle or higher), occupation status (student, employed, unemployed or retired), marital status (single, married / union of fact, divorced or widowed), smoking habits (no smoker, former smoker or current smoker) and average family monthly income (in euros). In the medical questionnaire, patients reported the presence of systemic diseases and medications, in particular, diabetes mellitus (DM).

Statistical Analysis

Data analysis was performed using SPSS for Windows [#]. Descriptive and inferential statistics methodologies were applied. All patients completed the questionnaires and missing data was not required. Chi-square test was used to evaluate the association between periodontal condition and sociodemographic variables. Clinical periodontal data were compared among periodontal condition and bruxism status groups by using ANOVA with Brown-Forsythe correction followed by Games-Howell post-hoc test. Odds Ratios (OR) towards periodontitis were calculated, both for univariate and multivariate analyses, through logistic regression procedures. Preliminary analyses were performed using univariate models (Supplementary File). Next, a multivariate model was constructed for the outcome presence of periodontitis. Only variables showing a significance $p \leq 0.25$ in the univariate model were included in the multivariate forward stepwise procedure. The contribution of each variable to the model was evaluated by Wald statistics. Interactions were also tested for the considered variables. A level of significance of 5% was set in all inferential analyses.

6.3. Results

Sample description

The characteristics of the 1,064 participants are shown in Table 6.1. The mean age of participants with bruxism and without were 60.1 (± 13.0) and 62.8 (± 15.8) years, respectively.

The participants' distribution, considering their periodontal status is presented in Table 6.2. The prevalence of SR bruxism was found to be higher in individuals without periodontitis when compared to periodontitis participants. Moreover,

there were significant differences in the sociodemographic data between non-periodontitis and periodontitis individuals.

After univariate analysis (Supplementary 1), multivariate stepwise procedure confirmed SR bruxism as an important factor towards periodontitis simultaneously to other known risk factors (Table 6.3). Individuals with SR bruxism exhibited a lower risk towards periodontitis of 58% (OR = 0.42, 95% CI: 0.32-0.56) (Table 6.3).

Table 6.4 shows the clinical periodontal characteristics of the participants according to their periodontal and SR bruxism status. PD mean values are significantly different among all groups, with individuals from SR bruxism group having the overall lower scores and SR bruxism -P group having a significant difference from P group. Regarding CAL, the mean values are also significantly lower for individuals from SR bruxism-P group, when comparing to P group. Additionally, SR bruxism-P group has meaningful lower mean recession levels than P group.

Table 6.5 presents the clinical periodontal characteristics based on the SR bruxism questionnaire. Overall, individuals with awake/sleep SR bruxism pattern have the lowest values of PD, CAL and BoP. Further, patients with the awake SR bruxism form have significantly lower PD levels compared to probable no SR bruxism and sleep bruxism patients. In terms of recession, no significant differences were identified among the SB groups, although they differ from the no SB group.

Table 6.1. Sociodemographic characteristics of the participants (N=1,064).

Variable	Total (N=1.064)	(table prolongation)	
Gender		**Monthly family income (€)**	
Male	447 (42.0)	<= 600	337 (31.7)
Female	617 (58.0)	601-1500	545 (51.2)
Age (years)		> 1500	182 (17.1)
18-30	62 (5.8)	**Smoking status**	
31-40	75 (7.0)	Non-smoker	626 (58.8)
41-50	136 (12.8)	Former smoker	293 (27.5)
51-60	137 (12.9)	Current smoker	145 (13.6)

61-70	328 (30.8)	**Diabetes Mellitus**	
71-80	244 (22.9)	No	860 (80.8)
> 80	82 (7.7)	Yes	204 (19.2)
Educational level		**Toothbrushing per day**	
No education	42 (3.9)	0	31 (2.9)
Elementary	410 (38.5)	1	302 (28.4)
Middle	496 (46.6)	2+	731 (68.7)
Higher	116 (10.9)	**Interproximal Cleaning**	
Marital status		No	718 (67.5)
Single	170 (16.0)	Occasionally	161 (15.1)
Married / Union of fact	684 (64.3)	Yes	185 (17.4)
Divorced	103 (9.7)		
Widowed	107 (10.1)		
Occupation			
Student	19 (1.8)		
Employed	327 (30.7)		
Unemployed	163 (15.3)		
Retired	555 (52.2)		

Values expressed as n (%, according to the variables).

Table 6.2. Distribution of the participants according to their periodontal condition with SR bruxism status and sociodemographic variables (N=1,064).

	Non-Periodontitis (n=427)	Periodontitis (n=637)	P-value
SR Bruxism			
Yes	228 (53.4)	267 (41.9)	<0.001
No	199 (46.6)	370 (58.1)	
Gender			
Male	140 (32.8)	307 (48.2)	<0.001
Female	287 (67.2)	330 (51.8)	
Age (years)			
18-30	51 (11.9)	11 (1.7)	<0.001
31-40	49 (11.5)	26 (4.1)	

41-50	73 (17.1)	63 (9.9)	
51-60	55 (12.9)	82 (12.9)	
61-70	108 (25.3)	220 (34.5)	
71-80	74 (17.3)	170 (26.7)	
> 80	17 (4.0)	65 (10.2)	
Educational level			
No education	11 (2.6)	31 (4.9)	<0.001
Elementary	134 (31.4)	276 (43.3)	
Middle	209 (48.9)	287 (45.1)	
Higher	73 (17.1)	43 (6.8)	
Marital status			
Single	104 (24.4)	66 (10.4)	<0.001
Married / Union of fact	262 (61.4)	422 (66.2)	
Divorced	33 (7.7)	70 (11.0)	
Widowed	28 (6.6)	79 (12.4)	
Occupation			
Student	18 (4.2)	1 (0.2)	<0.001
Employed	165 (38.6)	162 (25.4)	
Unemployed	79 (18.5)	84 (13.2)	
Retired	165 (38.6)	390 (61.2)	
Monthly family income (€)			
<= 600	121 (28.3)	216 (33.9)	0.015
601-1500	217 (50.8)	328 (51.5)	
> 1500	89 (20.8)	93 (14.6)	
Smoking status			
Non-smoker	296 (69.3)	330 (51.8)	<0.001
Former smoker	85 (19.9)	208 (32.7)	
Current smoker	46 (10.8)	99 (15.5)	
Diabetes Mellitus			
No	374 (87.6)	486 (76.2)	<0.001
Yes	53 (12.4)	151 (23.7)	

Toothbrushing per day			
0	7 (1.6)	24 (3.8)	
1	107 (25.1)	195 (30.6)	0.011
2+	313 (73.3)	418 (65.6)	
Interproximal Cleaning			
No	254 (59.5)	464 (72.8)	
Occasionally	81 (19.0)	80 (12.6)	<0.001
Yes	92 (21.5)	93 (14.6)	

SR – Self-reported. Values expressed as n (%, within each periodontal condition category). #Chi-square test, with significant differences identified in bold ($p<0.05$)

Table 6.3. Adjusted model (*) with Odds Ratios (OR) and correspondent 95% confidence intervals (95% CI) on potential risk factors towards periodontitis. OR obtained within multivariate logistic regression analysis.

	OR (95% CI) towards Periodontitis	p-value
SR Bruxism	0.42 (0.32-0.56)	<0.001
Gender		
Male	1	-
Female	0.66 (0.49-0.90)	0.009
Age	1.05 (1.04-1.06)	<0.001
Educational level		
Higher	1	-
Middle	2.22 (1.39-3.54)	0.001
Elementary	2.01 (1.21-3.36)	0.007
No Education	2.11 (0.88-5.06)	0.094
Smoking status		
Non-smoker	1	-
Former smoker	3.52 (2.23-5.54)	<0.001
Current smoker	1.90 (1.33-2.70)	<0.001
Diabetes Mellitus		
No	1	-
Yes	1.55 (1.06-2.26)	0.023

OR - Odds Ratio, SR – Self-reported. *The model was statistically significant, $\chi^2 = 213.736$, $p < 0.001$, explained 24.6% (Nagelkerke R^2) of the variance and correctly classified 68.7% of cases.

Table 6.4. Periodontal clinical characteristics (mean CAL, PD, REC and BoP) of the participants as a function of their periodontal condition and SR bruxism status (N=1,064).

Clinical Characteristic	None (n=199)	SR bruxism (n=228)	Periodontitis (P) (n=370)	SR Bruxism-P (n=267)	p-value (a)
PD (mm)	1.59 (± 0.30) [a] [1.55-1.64]	1.44 (± 0.28) [b] [1.40-1.47]	2.34 (± 0.85) [c] [2.25-2.43]	2.06 (± 0.76) [d] [1.97-2.15]	<0.001
CAL (mm)	1.78 (± 0.38) [a] [1.73-1.84]	1.66 (± 0.31) [b] [1.62-1.70]	3.61 (± 1.61) [c] [3.44-3.77]	3.08 (± 1.32) [d] [2.92-3.24]	<0.001
REC (mm)	0.20 (±0.30) [a] [0.15-0.24]	0.23 (± 0.24) [a] [0.20-0.26]	1.28 (± 1.27) [b] [1.15-1.41]	1.02 (± 0.95) [c] [0.91-1.14]	<0.001
BoP (%)	7.8 (± 9.7) [a] [6.5-9.2]	6.2 (± 8.7) [a] [5.1-7.4]	12.5 (± 15.8) [b] [10.8-14.1]	11.8 (± 15.2) [b] [10.0-13.6]	<0.001

CAL - Clinical Attachment Loss, PD - Probing Depth, REC - Recession, BoP - Bleeding on Probing, SR - Self-reported. Values expressed as mean (± standard deviation) and [95% confidence interval for mean]. (a) One-way ANOVA with Brown-Forsythe correction followed by Games-Howell post-hoc test. Different letters indicate significant differences between means (p<0.05).

Table 6.5. Clinical periodontal parameters (mean CAL, PD, REC and BoP) of the participants as a function of SB type (N=1,064).

Clinical Characteristic	No SR bruxism (n=569)	Sleep SR bruxism (n=367)	Awake SR bruxism (n=114)	Awake/Sleep SR bruxism (n=14)	p-value (a)
PD (mm)	2.08 (± 0.79) [a] [2.01-2.14]	1.85 (± 0.69) [b] [1.78-1.92]	1.56 (± 0.52) [c] [1.46-1.66]	1.45 (± 0.68) [bc] [1.06-1.84]	<0.001
CAL (mm)	2.97 (± 1.58) [a] [2.84-3.10]	2.51(± 1.24) [b] [2.38-2.64]	2.20 (± 1.15) [b] [1.98-2.41]	2.09 (± 1.10) [b] [1.45-2.73]	<0.001
REC (mm)	0.90 (±1.16) [a] [0.80-0.99]	0.67 (±0.81) [b] [0.58-0.75]	0.64 (± 0.87) [b] [0.48-0.80]	0.64 (±0.78) [b] [0.19-1.09]	<0.001
BoP (%)	10.8 (± 14.1) [a] [9.7-12.0]	9.8 (± 13.8) [b] [8.4-11.2]	8.2 (± 10.1) [c] [6.3-10.0]	2.5 (± 5.1) [c] [0.0-5.5]	<0.001

CAL - Clinical Attachment Loss, SR – Self-Reported, PD - Probing Depth, REC - Recession, BoP - Bleeding on Probing. Values expressed as mean (± standard deviation) and [95% confidence interval for mean]. (a) One-way ANOVA with Brown-Forsythe correction followed by Games-Howell post-hoc test. Different letters indicate significant differences between means (p<0.05).

6.4. Discussion

In this cross-sectional study, we hypothesized that bruxism and the periodontal status might be linked. To test this hypothesis, we have assessed a representative population for periodontal status and SB along with other significant confounding variables. Hence, we have compared the periodontal clinical characteristics according to their SB status. Also, bruxism was appraised in a multivariate analysis with known risk factors towards periodontitis. Overall, we show that SB is associated with less prevalence of periodontitis, lower periodontal measures and is a relevant factor for periodontitis.

These findings have wide implications. (1) To the best of our knowledge, this study is the first to investigate the association between periodontal condition and SB. (2) Based on a previous recommendation [17], the prevalence of SB was assessed in a representative sample of patients to study the possible consequences of teeth clenching/grinding on the periodontium. (3) SB revealed to be a significant factor towards periodontitis, even in a multivariate analysis.

Moreover, self-reported bruxers had lower risk towards periodontitis (OR = 0.42, 95% CI: 0.32-0.56) even when adjusted for known risk factors. Beyond that, self-reported bruxers with periodontitis have significant lower average levels of all clinical characteristics (PD, CAL, REC and BoP) than no-periodontitis individuals. Yet, awake and awake/sleep SB types appear to be the patterns most associated with lower PD and CAL features. As expected, patients with periodontitis have a statistically higher percentage of BoP compared to non-periodontitis patients [26].

Comprehensively, self-reported bruxers were associated with shallower pocket depths and lower loss of attachment. Concerning the epidemiological nature of this study, the small differences observed are far from clinically significant and demand clinical confirmation. Furthermore, the novelty of these results is the probable effect on healthy periodontium, inasmuch as effect of occlusal discrepancies in active periodontitis lead to deeper pockets and higher risk of tooth loss [27,28]. Therefore, future studies are mandatory to ascertain the cause-effect of bruxism and periodontal status and its clinical implications.

The relationship between bruxism and the periodontium has been much investigated and debated [29-35]. It has been shown that mechanical stresses caused by occlusal overload initiate a cascade event in the periodontal tissues

[29]. Moreover, the periodontal ligament plays an important role in balancing and distributing stress into the alveolar bone [30-31], reacting with small teeth movements [32], which in turn leads to a biological cellular response [33-35].

Changes of periodontal tissues caused by occlusal trauma have been proved in animal models, mainly in the periodontal ligament and alveolar bone [36-37]. They conclude that periodontal pressure zones exhibit transient bleeding, edema, thrombosis, increased vascularization, disorganization of periodontal ligament bundles, and alveolar bone resorption [36-37]. However, all evaluations used single-tooth excessive forces models, and unable to inferred conclusions to bruxism contexts.

Furthermore, it is widely defined that excessive occlusal forces do not trigger periodontal diseases or loss of periodontal attachment, and there is no scientific rationale to prove that excessive occlusal forces cause abfraction or gingival recession [38]. Also, bruxism is unlikely to provoke periodontal damage per se [17]. Clinically, the results of this study meet what is consensually established, which is the absence of periodontal damage triggered by bruxism.

Strengths and Limitations

The main strengths of this study are the representativeness of the sample and potential generalisability, although it requires validation in other settings. Also, the use of up-to-date international case definitions to periodontitis and followed the recommendations of the 2018 bruxism consensus on self-reported assessment. And, to the best of our knowledge, there is novelty for being first large-based epidemiologic study to address both conditions.

However, there are some shortcomings to remark. The primary limitation is the fact that single-reporting time self-report of bruxism is not the most suitable approach to assess bruxism in the clinical setting, also as far as the discrimination between awake and sleep bruxism is concerned. On the other hand, it remains an inevitable approach to gather data for screening purpose in large-sample epidemiological studies [2,25]. Also, as an observational study, we cannot appraise causality, exposure timing, disease onset and its relation with known periodontitis' risk factors. Notwithstanding, when adjusting for known risk factors, SB risk towards periodontitis remained significant.

Therefore, future prospective randomized clinical trials using definite bruxism diagnosis are mandatory, as well as laboratory studies to understand the biological and biochemical differences in the periodontal tissues on different bruxism patterns.

6.5. Conclusions

Within the limitations of this epidemiological study, the results show an association between SR bruxism and periodontitis. SR bruxism was related with less periodontal tissues destruction and lower periodontitis prevalence. Further studies are mandatory to clarify these findings using definite bruxism diagnosis.

FOOTNOTES

[‖] PCP UNC 15, Hu-Friedy® Chicago, IL, USA

[¶] 2N, Hu-Friedy®, Chicago, IL, USA

[#] IBM SPSS Statistics version 25.0 for Windows, IBM Corporation, Armonk, NY, USA

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6.7. Supplementary material

Table S6.1. Crude (univariate) model with Odds Ratios (OR), and correspondent 95% confidence intervals, on potential risk factors towards periodontitis. OR obtained within logistic regression analyses procedures.

	OR (95% CI) towards Periodontitis	p-value
SR Bruxism	0.63 (0.49-0.81)	<0.001
Gender		
Female	1	-
Male	1.91 (1.48-2.46)	<0.001
Age	1.04 (1.03-10.5)	<0.001
Educational level		
Higher	1	-
Middle	2.33 (1.54-3.54)	<0.001
Elementary	3.50 (2.28-5.37)	<0.001
No Education	4.78 (2.18-10.48)	<0.001
Monthly family income (€)		
> 1500	1	-
601-1500	1.45 (1.03-2.03)	0.032
<= 600	1.71 (1.19-2.46)	0.004
Marital status		
Married / Union of fact	1	-
Single	0.39 (0.28-0.56)	<0.001
Divorced	1.32 (0.85-2.05)	0.222
Widowed	1.75 (1.11-2.77)	0.016
Occupation		
Employed	1	-
Retired	2.41 (1.81-3.20)	<0.001

Students	0.06 (0.01-0.43)	0.005
Unemployed	1.08 (0.74-1.58)	0.678
Smoking status		
Non-smoker	1	-
Former smoker	2.20 (1.63-2.95)	<0.001
Current smoker	1.93 (1.32-2.83)	0.001
Diabetes Mellitus		
No	1	-
Yes	2.19 (1.56-3.08)	<0.001
Toothbrushing per day		
2+	1	-
1	1.37 (1.03-1.80)	0.028
0	2.57 (1.09-6.03)	0.031
Interproximal Cleaning		
Yes	1	-
Occasionally	0.98 (0.64-1.49)	0.914
No	1.81 (1.30-2.50)	<0.001

OR - Odds Ratio, SR - Self-Reported.

7 Psychometric properties of the Brief Illness Perception Questionnaire (Brief-IPQ) in Periodontal Diseases

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7. Psychometric properties of the Brief Illness Perception Questionnaire (Brief-IPQ) in Periodontal Diseases

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Abstract

Aim: to investigate the psychometric properties of the validated Portuguese version of the Brief Illness Perception Questionnaire (Brief-IPQ) in patients with gingivitis and periodontitis.

Material & Methods: This study enrolled 571 patients with periodontal diseases (PDs) (67 gingivitis and 504 periodontitis cases), in a population-based epidemiologic survey conducted at the health centers in the South Lisbon Metropolitan Area. Brief-IPQ, a 9-item self-reported scale, reliability was evaluated using Cronbach's alpha. Confirmatory Factor Analysis (CFA) was used to construct validation. Multigroup analysis tested invariance across gender.

Results: The Brief-IPQ showed acceptable reliability (α=0.80). CFA revealed good model fit ($\chi 2$ (16) = 41.236, GFI=0.982, CFI=0.985, RMSEA=0.053). All factors loaded similarly to the original Brief-IPQ scale, with the exception of the 'personal control' domain. Periodontal patients downgrade its illness and likely impact. The 'consequences' domain showed significant positive correlations with all factors, except 'treatment control' and 'understanding' domains. The 'concern' and 'emotional response' domains had the highest significant correlation. Multigroup analysis findings supported factor invariance across the sex groups.

Conclusion: The Brief-IPQ revealed acceptable reliability, construct factorial validity and invariance across gender. This short instrument may be used as an easily applicable and valuable tool to determine illness perception in patients with PDs.

7.1. Introduction

Health is defined as a “complete physical, mental and social well-being and not merely the absence of disease or infirmity” [1] and the 2017 World Workshop have defined periodontal health as “a state free from inflammatory periodontal disease that allows an individual to function normally and not suffer any consequences (mental or physical) as a result of past disease” [2]. Furthermore, periodontal diseases (PDs), mostly with a silent symptomatological pattern, are an unmistakable multifactorial public health problem with serious consequences for the quality of life [3,4] and socio-economic activity [5,6].

In the most recent diseases case definition, PDs are described by having a multifactorial nature involving an intricate interplay between microbiota, the host immune, inflammatory responses, and environmental modifying factors [2,6,7]. Thus, the treatment and/or control of this disease should not consider only the plaque level but must encompass a holistic approach and consideration of all factors that may influence the onset of the disease [6,8].

Psychosocial factors such as emotional stress and depression have emerged as conditions that affect the periodontal attachment apparatus [8–12]. Moreover, it has been shown that PDs play an important role in the impact of oral health on the patient's quality of life, and this relation is more pronounced with greater severity or extent of the disease [3].

Individuals’ perception of illness is a psychological concept that evolved in the 1960s as a basic construction of Leventhal's Common-Sense Model [13]. According to this regulatory model, each patient creates an individual cognitive and emotional representation of the illness or health threat. Cognitive representation has five dimensions: identity (through the symptoms that are part of the disease), the timeline (duration of illness), cure / control (perceived controllability of the disease and treatment efficacy), and causality (factors believed to be the cause of the illness) [14].

These representations are processed in parallel through three stages. Firstly, the patient forms the representation of the illness or health threat (i.e. illness representations), secondly, they adopt behaviors to cope with this (i.e. coping strategies), and, lastly, they appraise the efficacy of these behaviors (i.e. appraisal) [15].

Recently, the properties of the Illness Perception Questionnaire Revised have been investigated for Oral Health (IPQ-R-OH) and in patients with self-perceived PDs [16,17]. In this study, IPQ-R-OH revealed to be an interesting tool for potential periodontal patients screening and education. However, the suitability of this questionnaire within the clinical setting is debatable due to its extensiveness, which is widely accepted that can affect the strain on the patient [18].

The Brief Illness Perception Questionnaire (Brief-IPQ) is a useful questionnaire that measures the patient's illness perception [15]. It is essential to assess the quality of the items' questionnaire and the reliability and validity of the construct for PDs. In addition, it is important to elucidate the pertinence of the theoretical model, the psychometric properties and the validity of the Brief-IPQ as a questionnaire to measure the perception of PDs. This shorter questionnaire is especially useful to assess the illness perceptions as one part of a large population-based study [15].

To the best of our knowledge, the evaluation of the illness perception by patients with PDs, with shorter validated surveys like Brief-IPQ, has not been studied. Therefore, this study aimed to analyze the psychometric properties of the Portuguese version of the Brief-IPQ in patients with gingivitis and periodontitis that were surveyed in a population-based epidemiologic study.

7.2. Materials and Methods

Participants

The participants were part of a large-scale epidemiologic study carried out in the southern region of the Lisbon Metropolitan Area - Study of Periodontal Health in Almada-Seixal (SoPHiAS). This study was designed as a population-based cross-sectional representative study, geographically stratified by each public health center of Almada and Seixal municipalities, in Portugal. The target population were dentate subjects over 18 years of age (adults and elderly) and complete edentulous patients were excluded from the study. Data were collected between December 2018 and April 2019. All participants previous gave their written informed consent. After periodontal diagnosis, all patients with PDs (gingivitis or periodontitis) were contacted in order to answer the questionnaire. From a

total of randomized 1,064 participants enrolled in the SoPHiAS study, 343 subjects with healthy periodontium (32.1%) were excluded, being 722 patients (67.9%) with PDs contacted. Further, the exclusion applied criteria were: subjects with no education [19] and unable to participate in the survey and answer questionnaires or if they refuse to reply to the questionnaire (n = 151). Thus, a final sample of 571 subjects was considered. This study was approved by a state recognized Ethics Committee: the Research Ethics Committee of the Regional Health Administration of Lisbon and Tagus Valley, IP (Registration numbers: Process: 3525/CES/2018 and 8696/CES/2018, respectively).

Periodontal examination and diagnosis

Two trained and calibrated examiners (V.M. and J.B.) performed the periodontal diagnosis. The inter-examiner correlation coefficients were 0.98 and 0.99, for CAL and PD, respectively. The intra-examiner ICC ranged from 0.97 to 0.99, for both PD and CAL. Full periodontal examination was performed with a manual periodontal North Carolina probe (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual). Gingivitis cases (GC) were defined according to [7] and periodontitis disease severity according to [6]. At the end of the examination, participants were informed about their periodontal status. Patients diagnosed with PDs were referred to the Egas Moniz Dental Clinic (EMDC) for its treatment without additional costs. Patients were not informed that they would be contacted to perform the Brief-IPQ.

Brief Illness Perception Questionnaire (Brief-IPQ)

The adapted Portuguese version of the Brief Illness Perception Questionnaire (Brief-IPQ) [19] was used. The validated Portuguese version of the Brief-IPQ is shown in Table 6.1. The Brief-IPQ [15] consists of one section containing nine items: five appraising cognitive illness through ‘consequences’ (Item 1), ‘timeline’ (Item 2), ‘personal control’ (Item 3), ‘treatment control’ (Item 4), and ‘identity’ (Item 5); two assessing emotional representations: ‘concern’ (Item 6) and ‘emotional response’ (Item 8); one assessing illness comprehensibility ‘understanding’ (Item 7). These eight items are rated on a response scale ranging from 0 (e.g. does not affect at all) to 10 (e.g. severely affects my life).

The last item is a causal open-response item, adapted from the IPQ-R [20], which asks patients to list the three main causal factors in their illness (Item 9). Responses to the causal item can be grouped into different categories allowing a subsequent categorical analysis. The total score generated by summing up the scores for the Brief-IPQ items with a reverse scoring of items 3, 4 and 7. A higher total score reflects a more threatening perception of illness.

Table 7.1. Original and Portuguese versions of the Brief-IPQ

Brief IPQ question	Original [a]	Portuguese [b]
Consequences (item 1)	How much does your illness affect your life?	Qual o grau em que a sua doença afecta a sua vida?
Timeline (item 2)	How long do you think your illness will continue?	Quanto tempo pensa que vai durar a sua doença?
Personal control (item 3)	How much control do you feel you have over your illness?	Qual o grau de controlo que sente sobre a sua doença?
Treatment control (item 4)	How much do you think your treatment can help your illness?	Até que ponto pensa que o seu tratamento pode ajudar a sua doença?
Identity (item 5)	How much do you experience symptoms from your illness?	Qual o grau em que sente sintomas da sua doença?
Concern (item 6)	How concerned are you about your illness?	Qual o grau de preocupação com a sua doença?
Understanding (item 7)	How well do you feel you understand your illness?	Até que ponto sente que compreende a sua doença?
Emotional response (item 8)	How much does your illness affect you emotionally? (e.g. does it make you angry, scared, upset or depressed?)	Até que ponto a sua doença o (a) afecta emocionalmente? (ex. fá-lo sentir se zangado, assustado)
Three main causal factors in their illness (item 9)	Please list in rank-order the three most important factors that you believe caused your illness. The most important causes for me:	Por favor coloque por ordem de importância os factores que considera que causaram a sua doença. As causas mais importantes para mim são:

[a] Broadbent et al. (2006); [b] Figueiras et al. (2010).

Each interviewer (V.M. and J.B.) received detailed instructions on the Brief-IPQ from an experienced health psychologist (C.R.). The questionnaires were administered through telephone interviews. To minimize bias, the interviewers

applied the questionnaire by reading each question consecutively and word for word. The interviewers were blinded to the detailed periodontal diagnosis. Responses were immediately recorded in an online platform using a Google® Form.

Sociodemographic variables

Sociodemographic data comprised age, gender, educational level (elementary, middle or higher), occupation status (student, employed, unemployed or retired) marital status (single, married / union of fact, divorced or widowed) and average family monthly income (in euros).

Statistical Analysis

First, a descriptive analysis of the Brief-IPQ was performed. The Brief-IPQ was validated in several populations including the Portuguese population [19,21]. However, this questionnaire was not validated in periodontal samples, and for that reason, we started by testing the factorability, followed by an Exploratory Factor Analysis (EFA), in order to explore the factor structure of Brief-IPQ in the Portuguese periodontal sample (Supplementary material 6.7.1-6.7.3). The factorability of the questionnaire was confirmed through the Kaiser-Meyer-Olkin (KMO) and was required to exceed 0.60 [22]. KMO was 0.84, revealing sufficient evidence of at least one common factor underlying the observed variables.

It was followed by a Confirmatory Factor Analysis (CFA) to verify the best model fit of the three factorial structures. The maximum likelihood method was used to estimate the model and chi-square ($\chi 2$) was used to evaluate the differences between models, utilizing a likelihood ratio test. Multiple fit indices were used to assess the CFA model fit, including the $\chi 2$/df ratio (good adjustment with values < 2), the Root Mean Square of Approximation (RMSEA) (good model adjustment considered for values between 0.05-0.10, 90% confidence interval), the confirmatory fit index (CFI) and the goodness of fit index (GFI). Appropriateness of the model was considered achieved when, for the four indices, values were higher than 0.90 [23,24].

The psychometric properties of the Brief-IPQ were also calculated. The subscales were also tested with multiple linear regression modelling. Factorial validity was

considered verified when factorial weight values were ≥ 0.5. Reliability was evaluated using Cronbach's alpha, with values higher than 0.80 considered as satisfactory. The composite reliability was not performed since Brief-IPQ exhibited a unidimensional scale in the studied sample. Chi-square tests were used to compare the clinical data as a function of gender. Spearman's rank correlation coefficient (rho) was used to assess intra-subclass correlations.

Then, the best-fitting model was tested on a multi-group CFAs through a stepwise procedure [25] to establish the invariance of the Brief-IPQ across gender. Subsequently, we estimated four successive models, one unconstrained, one with factor loadings constrained (M1), one with factor loadings and structural covariances constrained (M2) and other with factor loadings, structural covariances and measurement residuals constrained (M3). To measure the invariance, the CFI delta values (ΔCFI) were used, with a cut-off point less than 0.01, which indicated invariance [25,26]. Finally, the chi- chi-square delta values ($\Delta\chi 2$) were also used and a value lower than standardized $\Delta\chi 2$ for $1-\alpha$ = .095 indicated the invariance between the models [27,28].

All statistical tests were two-tailed, and a p-value < 0.05 was considered significant. Statistical analyses were carried out using IBM SPSS Statistics, Version 24.0 (Armonk, NY, USA: IBM Corp.) and IBM SPSS AMOS - Analysis of Moment Structures, Version 24.0 (Armonk, NY, USA: IBM Corp.).

7.3. Results

Sample description

The study group PDs prevalence distributions was as follows: gingivitis (n=67), mild periodontitis (n=116), moderate periodontitis (n=185) and severe periodontitis (n=212). The sociodemographic characteristics of this sample are presented in Table 7.2. The age of participants ranged from 18 to 95 years (mean 64.9 ± 15.3 years), and the majority were female (52.2%). Regarding education, 7.5% had completed higher education, while the majority (71.6%) declared having elementary education. Overall, most of the participants were married or cohabitating (65.5%) and retired (56.4%) with 14.5% of the individuals reportedly in an unemployment status.

In the studied group, 11.7% (n = 67, 20 Males [M]/ 47 Females [F]) had gingivitis and 20.3% (n = 116, 51 M / 65 F), 32.7% (n = 187, 89 M / 98 F) and 35.2% (n = 201, 113 M / 88 F) had mild, moderate and severe periodontitis, respectively. A significant difference between gender's diseases prevalence was found (p = 0.002, Chi-square test).

Table 7.2. Sociodemographic characteristics of the included participants (N = 571).

Age (years) n (%)	Value
18-30	22 (3.9)
31-40	23 (4.0)
41-50	67 (11.7)
51-60	76 (13.3)
61-70	198 (34.7)
71-80	138 (24.2)
> 80	47 (8.2)
Education n (%)	
Elementary	409 (71.6)
Middle	119 (20.8)
Higher	43 (7.5)
Marital Status n (%)	
Single	73 (12.8)
Married	374 (65.5)
Divorced	67 (11.7)
Widowed	57 (10.0)
Occupation n (%)	
Student	7 (1.2)
Employed	159 (27.8)
Unemployed	83 (14.5)
Retired	322 (56.4)
Average Monthly Family Income (€) Mean (SD)	1,074.1 (772.9)

Descriptive Analyses

Descriptive data of Brief-IPQ are displayed in Table 7.3. 'Treatment control' had the highest mean score, 7.3 (± 2.9) while 'emotional response' and 'identity' had the lowest mean scores, 2.5 (± 3.5) and 2.5 (± 3.2), respectively. Patients

reported poor mean score of 'understanding', 3.4 (±3.7), of their illness and the majority do not know the cause factor (78%). Concerning the factor believed to cause periodontal disease (item 9), the majority answered to not know the cause (78%), although dental factors (8%), environmental factors (7%), systemic disease and/or medication (3%), age (2%) and genetic predisposition (2%) have been mentioned. Multivariate normal distribution was confirmed for all items (skewness coefficient < |2| and kurtosis coefficient < |2|). A CFA was performed to confirm Brief-IPQ unifactorial structure (Table 7.4).

Table 7.3. Descriptive statistics of Brief-IPQ scores (mean, standard deviation (SD), median, and interquartile range (IQR), minimum and maximum), of important factor that patients consider the cause of periodontal disease.

Item	Mean (SD)	Median (IQR)	Min.	Max.
Consequences (item 1)	3.0 (3.4)	1 (6)	0	10
Timeline (item 2)	5.3 (3.6)	5 (6)	0	10
Personal control (item 3)	4.7 (3.5)	5 (7)	0	10
Treatment control (item 4)	7.3 (2.9)	8 (4)	0	10
Identity (item 5)	2.5 (3.2)	0 (5)	0	10
Concern (item 6)	3.9 (3.9)	3 (8)	0	10
Understanding (item 7)	3.4 (3.7)	3 (7)	0	10
Emotional response (item 8)	2.5 (3.5)	0 (5)	0	10

Table 7.4. Model fit indices in the unifactorial model and configurational invariance by sex

Description	χ^2	*d.f.*	CFI	GFI	RMSEA [90% CI]	Δ CFI	Δ χ^2	d.f.
Unifactorial model	41.236*	16	0.985	0.982	0.053 [0.033-0.073]	-	-	-
Measurement invariance by gender								
Unconstrained	64.217*	32	0.981	0.974	0.042 [0.027-0.057]	-	-	-
Model 1	69.584*	39	0.982	0.972	0.037 [0.022-0.051]	0.001	5.367	7
Model 2	88.014*	48	0.976	0.967	0.038 [0.025-0.051]	0.001	5.390	8

Model 3	104.018 *	60	0.973	0.967	0.036 [0.024-0.047]	0.001	21.393	20

* p < 0.01; χ^2= chi-square; d.f. = degrees of freedom; GFI = Goodness of Fit Index; CFI = Comparative Fit Index; RMSEA = Root Mean Square Error of Approximation; CI = Confidence Interval; M1 = factor loadings constrained; M2 = factor loadings and structural covariances constrained; M3 = factor loadings, structural covariances and measurement residuals constrained

Construct Validity

Model fit indexes showed that the first order unifactorial model resulted from Confirmatory Factor Analysis (CFA) revealed a good model fit: χ^2 (16) = 41.236, GFI = 0.982, CFI = 0.985, RMSEA = 0.053, CI 90% (0.033- 0.073) (Table 7.4). Overall, regarding factor loadings, all items significantly loaded onto their subscale and the standardized regression weights were > 0.5 for all items, with the exception of item 3 (Personal Control) for total sample and both genders. The item 2 (Timeline) and item 4 (Treatment control) were significant but their regression weights were nearly 0.5, which may indicate that these values may be good with a larger sample (Table 7.5). These three items were not excluded regarding empirical and theoretical rationale.

Table 7.5. Standardized (β) regression weights for total and for groups.

Item	Total		Female		Male	
	β	*p*	β	*p*	β	*p*
Consequences (item 1)	0.740	<0.001*	0.712	<0.001*	0.779	<0.001*
Timeline (item 2)	0.446	<0.001*	0.454	<0.001*	0.434	<0.001*
Personal control (item 3)	0.005	0.916	-0.014	0.822	0.010	0.873
Treatment control (item 4)	0.473	<0.001*	0.461	<0.001*	0.493	<0.001*
Identity (item 5)	0.677	<0.001*	0.678	<0.001*	0.659	<0.001*
Concern (item 6)	0.919	<0.001*	0.942	<0.001*	0.893	<0.001*
Understanding (item 7)	0.534	<0.001*	0.516	<0.001*	0.556	<0.001*
Emotional response (item 8)	0.785	<0.001*	0.750	<0.001*	0.826	<0.001*

* $p < 0.001$

Psychometric Properties

The current Portuguese version of Brief-IPQ applied in a periodontal sample proved good psychometric properties. The obtained Cronbach's alpha value of 0.80 proves an acceptable reliability. The convergent and discriminant validities were not possible to calculate, due to the unifactorial nature of the Brief-IPQ.

Measurement invariance across gender

The multi-group CFA tested the configural invariance of Brief-IPQ across gender. Regarding the invariance for gender groups, when comparing the unconstrained model with M1, results confirmed the invariance of the factor loadings, ΔCFI = 0.001 and $\Delta\chi^2$= 5.367 is lower than standardized $\Delta\chi^2$. When comparing the unconstrained model with M2 the results confirmed the invariance of the structural covariances, ΔCFI = 0.001 and $\Delta\chi^2$ = 5.390 is lower than standardized $\Delta\chi^2$. When comparing the unconstrained model with M3, the results confirmed the invariance of measurement residuals, ΔCFI = 0.001 and $\Delta\chi^2$= 21.393 is lower than standardized $\Delta\chi^2$ (Table 7.4).

Relationships between components of illness perception

To further examine the psychometric properties of the Brief-IPQ, inter-subscale correlations were calculated. High number of significant correlations suggests a great degree of dependence between the subscale scores. Item 1 ('consequences') showed significant positive correlations with all other factors, except with item 4 (Treatment control) that had a negative correlation. The highest correlations were noted between item 6 (Concern) and item 8 (Emotional response) as well as between item 1 (Consequences) and items 5, 6 and 8 (Identity, Concern and Emotional response, respectively) (Table 7.6).

Table 7.6. Correlation between Brief-IPQ item scores.

	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8
Consequences (item 1)	**0.389*****	0.007	**-0.311*****	**0.640*****	**0.677*****	**-0.345*****	**0.600*****

Timeline (item 2)	1.000	0.019	**-0.285*****	**0.339*****	**0.391*****	**-0.172*****	**0.341*****
Personal control (item 3)	-	1.000	**0.182*****	-0.038	-0.015	**0.224*****	0.021
Treatment control (item 4)	-	-	1.000	**-0.302*****	**-0.445*****	**0.319*****	**-0.316*****
Identity (item 5)	-	-	-	1.000	**0.615*****	**-0.385*****	**0.530*****
Concern (item 6)	-	-	-	-	1.000	**-0.503*****	**0.722*****
Understanding (item 7)	-	-	-	-	-	1.000	**-0.320****

Values are the Spearman's rank correlation coefficient (rho).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

7.4. Discussion

To the best of our knowledge, this is the first time that illness representation was assessed in a large group of patients with PDs using Brief-IPQ [15]. The results suggest that the Brief-IPQ provided patients' cognitive and emotional representations of their condition. Despite its short dimension, Brief-IPQ showed an adequate internal consistency, which is considered adequate [29].

These results demonstrate that Brief-IPQ has good overall validity and reliability for use among adult patients with PDs (Cronbach's alpha value of 0.80), being in agreement with the original Brief-IPQ study [15] and within the range of similar chronic diseases researches [30,31].The factorial invariance concerning gender was also confirmed showing, therefore, that the Brief-IPQ is a reliable instrument to both genders, similarly to previous reports that have employed the IPQ-Revised questionnaire [16,17].

Importantly, the findings of this questionnaire items evidence meaningful differences for other chronic diseases. Considering that a higher score reflects a more threatening view of the illness, the overall results across items show that periodontal patients present a devaluing view of their illness comparing with other chronic diseases such as hypertension, gout, obesity, chronic obstructive pulmonary disease (COPD), asthma, allergies or diabetes [15,19,32,33]. Furthermore, understanding domain (item 7) reveals a strict interaction with all the remaining domains, which connote a disturbing lack of knowledge of the disease and its impact on the quality of life. In other words, the poor understanding levels are causing a lack of awareness of the consequences of

this disease, its chronic nature, little alertness to the symptoms, minor concern and low emotional impact. Also, higher levels of understanding lead to positive beliefs in treatment efficiency.

Evidence shows an association between PDs and quality of life, and this effect is more pronounced with greater severity and extent [3]. Notwithstanding, our results proof that the expected effects and outcomes of these conditions on physical, social and psychological well-being (item 1) are positively observed by participants. However, in the future such association needs to be further explored with other health measures, such as health-related quality of life.

In the cognitive illness representation, beyond beliefs in low consequences for their lives (item 1), subjects report low levels of symptoms (item 5). Moreover, treatment control (item 4) had higher scores than personal control (item 3), suggesting that the majority believe that clinical therapy is the main mean of managing their PDs. In view of this, it is considered that personal control (item 3) reflects the belief of an internal locus of control, while treatment control (item 4) is an external one, and this control interaction should be addressed in further research. On the other hand, timeline domain (item 2) revealed a medium score which means that most patients expect a moderate duration of illness and full recovery from their injury. Likewise, the items of the emotional (item 6 and item 8) representation showed low levels which allows us to conclude that periodontal disease has a poor impact. All in all, the cognitive domain displays a lack of knowledge of patients and unrealistic perceptions about these conditions and their causes. A possible explanation relies on the low oral health literacy previously described [34] or the low levels of education within this population, though this matter should be addressed in the future.

Health literacy is a strong predictor of individuals' health, health behaviors, and health outcomes. Lack of patient oral health literacy can be a hidden barrier to healthcare, being associated with lack of preventive behaviors, delayed diagnoses, more invasive treatments, poor adherence to treatment and medical instructions, and consequently poor health outcomes [34–37]. Especially in patients with chronic illnesses such as periodontitis, despite the periodontal treatment crucialness, it is essential to find ways to reduce the effects of low health literacy, in order to enhance a proactive and effective involvement of daily self-care.

Clinically, gingival bleeding, gingival itching, discomfort, pain and teeth mobility may be the expected signs and symptoms in periodontal patients. In this particular population, though clinical signs and symptoms were of moderate levels and all participants have been informed about the diagnosis, the included participants revealed poor levels of PDs identity (item 5). For this reason, it is of the utmost importance to increase the patient's ability to recognize PDs' symptomatology and guarantee their diagnosis understanding. This recognition capacity and the consequent valorization of the disease are of particular importance for a positive outcome in the control and treatment of the disease [38,39].

In the light of these results, in our opinion, this tool has also potential to be used in a clinical setting and daily basis practice. Patients who have an unrealistic or negative perception about PDs may benefit from a brief intervention such as counselling. A multidimensional approach, combining clinical diagnosis with psychosocial and systemic dimensions, is essential to improve global understanding of PDs [5,34,40].

Strengths and limitations

The length of questionnaires has a considerable response rate burden resulting in considerable non-response rates [41]. Consequently, long questionnaires may have poor interest in the daily clinical practice, and for this purpose this short-form may be more suitable for patients with this condition [17].

Nevertheless, test-retest reliability is essential to assess if a test has the same score for the equal population on different periods. The meaning and implications of this evaluation can confirm the veracity of the data and reduce the risk of dubious interpretation [29]. Besides that, mixed methods should be used ideally to broaden the dimensions and provide a more complete picture of human behavior and experience, and hence the power of the research [42]. The studied population was interviewed before the periodontal treatment scheduled as part of the ethical and epidemiologic duties. However, the retest interview window timeline would certainly overlap the planned therapy. In this way, it became unfeasible to make the retest since the subjects' knowledge would automatically bias the results [43].

Another limitation may be the mode of questionnaire administration. Telephone interview has several advantages such as more complete population coverage, increases survey response and completion of the questionnaire, decreases recall dropouts, and is preferred by the respondents [44]. However, there are important potential biases to highlight, for instance, high response-choice order effects, and high social desirability and interviewer biases [44]. To minimize them each question was read slowly and carefully, word by word.

Despite the limitations of the present study, it provides information on the nature of illness representations in these patients and grounds a basis for further longitudinal research.

7.5. Conclusions

The results of this study validate the Brief-IPQ as an illness perception tool in patients with periodontal diseases. These findings are relevant because this is a short and easily applicable questionnaire in both academic and clinical contexts. This questionnaire can help dental professionals in realize patients' awareness of their periodontal status and aid during periodontal care.

7.6. References

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7.7. Supplementary material

Table S7.1. Scree plot from the EFA with no forced factors. The exploratory analysis of the imputed dataset, with no forced factors, resulted in three factors exceeding an eigenvalue of one, and the scree plot showed a change in the curve after five factors.

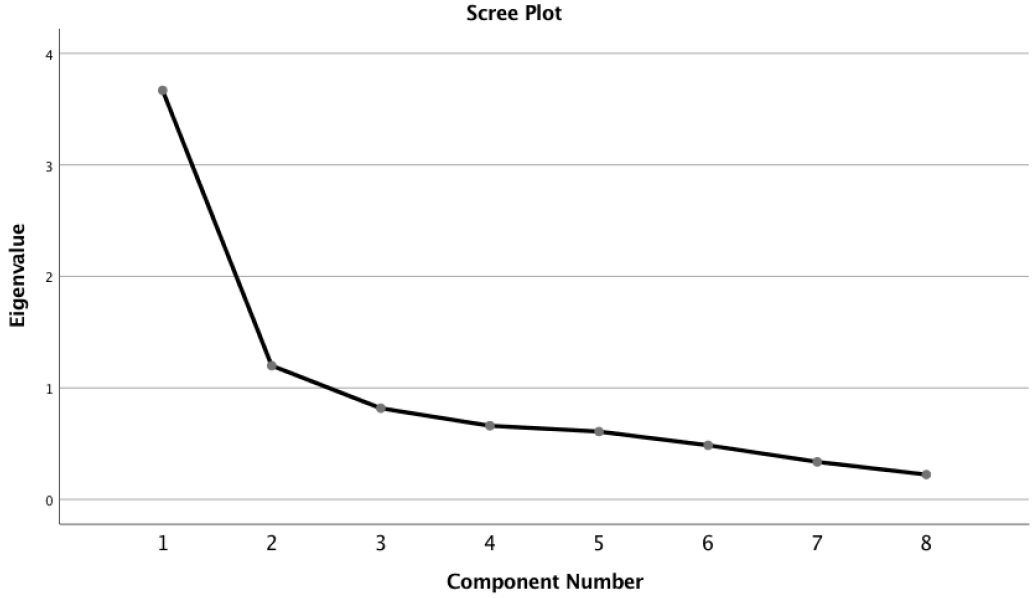
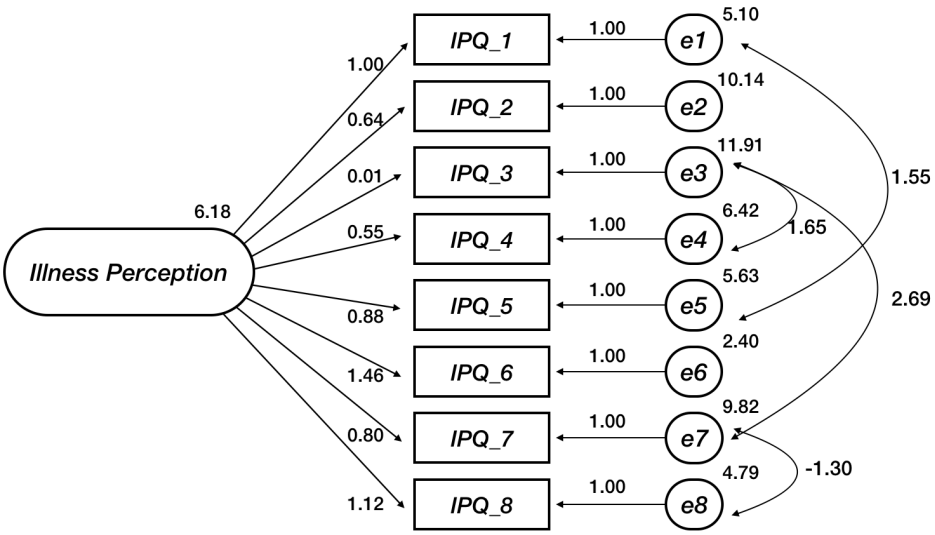


Table S7.2. Confirmatory Factor Analysis for the three factor structures.

Model	χ2	d.f.	CFI	GFI	RMSEA [90% CI]
Model 1- Two factor model	148.099*	19	0.922	0.937	0.109 [0.093-0.126]
Model 2- Three factor model	70.074*	16	0.967	0.969	0.077 [0.059-0.096]
Model 3- Unifactorial model	41.236*	16	0.985	0.982	0.530 [033-0.073]

* $p < 0.001$; Model 1 – two factor model with unfixed factors, Factor 1 – Items 1, 4, 5, 6, 8; Factor 2 – Items 2, 3, 7; Model 2 – three factor model with 3 fixed factors; Factor 1 - items 1, 5, 6, 8; Factor 2 – Items 2,4,; Factor 3 – Items 3, 7; Model 3 – Unifactorial model (Broadbent et al., 2006); Regarding Exploratory Factor Analysis, the assumptions were confirmed with Bartlet's Test ($p < 0,05$) and Kaiser-Meyer-Olkin (KMO) ≥ 0.50. We used Principal Component Analysis for factors extraction and Varimax rotation for factors rotation. The number of factors (Model 1 and Model 2) were selected according to the % of explained variance and eigenvalue (> 1).

Figure S7.3. Confirmatory Factor Analysis (CFA) with standardized regression weights for model 3 (IPQ_1 to IPQ_8 represent Brief-IPQ items 1 to 8).



General Discussion, Concluding Remarks and Future Directions

8.1. General Discussion

Our central objective was to explore the prevalence of periodontal disease and the associated risk factors in Portuguese elders from the Southern Lisbon Metropolitan Area. Overall, the prevalence of periodontal disease in elderly from this region was very substantial and the main risk factors were age, smoking habits, education level and self-reported diabetes mellitus.

The decision to divide our primary goal in two milestones was significant and successful. The first milestone composed of three pilot studies and one systematic review revealed to be important in framing strategies in our epidemiologic survey. Although the interest in pilot studies has decreased [1], conducting pilot studies contributes to research consistency and validity [2-3] and they are mostly useful to consider the validity of certain procedures, instruments, or strategies [4]. In other words, studying a Portuguese subpopulation of the EMDC, located in the same region as the target area, was relevant to prioritize study variables and to unveil a first outlook on important risk factors.

In the study carried out at the CDEM (**Paper I**), age was a main risk factor for periodontitis in patients aged 65 years old or older as they presented 770% more risk of having periodontitis. In the SoPHiAS survey (**Paper V**), age endured a main risk factor for periodontitis with a 5% increased risk for each year of age. Comprehensively, the detected association of age with periodontitis is in agreement with other European epidemiologic studies [5-9]. As debated before, a conceivable reason may be the immunosenescence of the periodontium that increases the risk for more severe states of periodontitis and an impaired response of the immune system [10]. Furthermore, upon a certain age, systemic diseases might act as deteriorating factors for the periodontal status, such as diabetes mellitus [11] that was a significant associated risk factor. Regarding smoking habits, a recognized risk factor towards periodontitis [12], was observed to be significant in the overall population (both in the CDEM and the SoPHiAS studies), however, it was not observed as a relevant factor in the elderly subset because the prevalence of smokers was low.

Others, the level of education was another risk factor firstly identified in the preliminary study conducted in the CDEM and further confirmed in the SoPHiAS study. When the SoPHiAS subset of elderly participants was analyzed for the impact of periodontal disease in OHRQoL, the education level was a non-

significant variable. In our view, a possible explanation for this result may be the fact we focused on schooling itself and not on education for oral health. On the other hand, regardless of education level, it was possible to validate a perception questionnaire for periodontal disease, which seems to corroborate this notion of education for oral health itself and not education in general. However, the design of these studies prevents any definitive conclusion and may be seen challenging, since the educational background has been evolving in Portugal for the past decades.

Oral health education in this elderly population was in fact neglected and explains the reported faulty oral hygiene habits in our studies, being in line with national data [13]. This may help elucidating the long-term consequences for oral health, with higher number of missing teeth and, as a consequence, a high number of denture wearers. On the one hand, the existence of a periodontal disease, along with a lack of knowledge about the disease, may lead to the progression of the disease that, in the long term, leads to tooth loss. On the other hand, with the scarcity of oral health services in the Portuguese national health system and the lack of demand for treatments, patients seek treatments more often when an irreversible clinical status, which explains the large number of users of dental prostheses, particularly in this group of elders. Nevertheless, this population showed to be highly adapted to dentures as this was not a contributing factor to the deterioration of OHRQoL.

Originally, the expected prevalence for the target population in Almada-Seixal would be low, considering the numbers from the *DGS* [14]. Nevertheless, since the CDEM paper (**Paper I**), the results for this age group were very inconsistent. Thus, we hypothesized three reasons for such discrepancy: the location of the dental clinic might be overestimating the results; the retrospective nature of this study with patients who were forwarded to a periodontal care; or, the periodontal diagnostic strategy could be contributing to its underestimation because it was partial and not full-mouth periodontal examination. Upon the assessment of the bias magnitudes, sensibility, and specificity of particular PRPs we concluded that full-mouth partial protocols failed to estimate PD and CAL means, presenting high underestimation potential (**Paper II**). These results supported the decision to undergo a standard full-mouth circumferential examination in our epidemiologic study and also confirmed the discrepancy to the estimates provided by *DGS*. Lastly, we further confirmed the accuracy of our

preliminary forecasts at the CDEM report, with the disturbing prevalence in the elderly population of the SoPHiAS study. Therefore, the SoPHiAS survey, as an epidemiological regional study, was very important to conclude that this type of studies is warranted in both regional and national levels to accurately determine the prevalence of periodontal disease. Furthermore, our results are in line with WHO global estimates [15] and the Global Burden of Disease [16].

Consequently, our third study (**Paper III**) aimed to investigate the influence of known risk factors on NSPT response using a PD fine-tuning MLM. This study hypothesized that PD and CAL reduction were affected by patient, tooth, and site-level factors after NSPT. This study generated evidence that treatment of periodontitis is effective and contingent on many levels (patient, tooth and site). Furthermore, these results were very relevant to empower health-promoting messages during the epidemiologic study and to motivate participants to endure periodontal treatment if a periodontal disease was diagnosed.

In parallel, stress was as a risk factor of interest in the association with periodontitis due to the existence of prior conceptions on this link that have been greatly relinquished [17-19]. Under this rationale we produced a systematic review confirming that salivary cortisol levels were associated with periodontitis and severity (**Paper IV**). These results guided us to use the planned epidemiological study to introduce the stress variable. However, due to financial and methodological constraints, the laboratory assessment of cortisol levels (whether salivary or serum) was impossible to accomplish and, for this reason, we chose to include a validated self-reported questionnaire (PSS-10) to understand if stressful self-perceived statuses might influence the perception of OHRQoL. Remarkably, in this elderly sample, stress was a factor with mild impact in the perception of OHRQoL (**Paper VI**), along with self-perceived xerostomia. The higher the perception of xerostomia and stress factors the higher odds of poorer OHRQoL. These results are in agreement with previous studies, as perceived chronic stress impacted the perception of dry mouth and quality of life in adults [20-22].

Also, the number of missing teeth was a significant factor and conforms with a recent systematic review in which the higher the number of missing teeth the worse OHRQoL [23]. There were disturbing values of tooth loss and periodontal parameters, which revealed elevated periodontal destruction. Evidently, patients esteem much more the number of missing teeth for their quality of life than

having periodontitis or its extension and confirms what has already been reported [24-26]. Additionally, missing teeth was a characteristic that patients often recognized, contrary to periodontitis where its perception is limited, and this notion urges the need for a holistic oral health education in all domains.

Another factor studied in this project was bruxism. Despite the still unknown physiology and pathology, bruxism has been highly associated with stress and anxiety through validated methods [27,28], though there are some contradictory evidence [29,30]. Our study in elders from the SoPHiAS study showed that stress was a relevant confounding factor for OHRQoL in this elderly population (**Paper III)**, grounded on the results of the systematic review previously carried out (**Paper IV**). Yet, to the best of our knowledge, the association of the periodontal status with bruxism had never been studied in an epidemiological setting. The fact that a systematic review concluded bruxism would be unlikely to cause damage to the periodontium [31], also revealed that these conclusions were supported by limited data and studies with methodologic limitations. Extraordinarily, our results showed self-reported bruxers exhibiting lower prevalence of periodontitis (**Paper VII**). Additionally, bruxers with periodontitis had PD and CAL significantly lower than patients with only periodontitis. Multivariate analysis also confirmed the role of bruxism even in adjusted models. Overall, self-reported bruxers and periodontal status showed to be negatively associated, with self-reported bruxers exhibiting lower odds towards periodontitis and better periodontal clinical characteristics.

From another point of view, milestone 1 revealed the first evidence that this population could present a poor oral health education and important to be studied. In paper I, 69% of patients failed the periodontal visit despite triage referral to seek periodontal care, and therefore the perception of periodontal disease would have to take into account in our epidemiological study. Coupled to the high prevalence of periodontal disease in **Papers I, V and VI**, we intended to study the individuals' perception of illness towards periodontal disease based on the Diefenbach & Leventhal regulatory model [32]. To this end, we seek to validate a preferably short instrument to be easily applicable and to determine illness perception in patients with. Therefore, the Brief-IPQ [33] was successfully validated in patients with periodontal diseases from the SoPHiAS studies, both adults and elders (**Paper VIII**). This validation is noteworthy as Brief-IPQ is a short and easily applicable questionnaire in both academic and clinical contexts and

might aid dental professionals in realize patients' awareness of their periodontal status and help during periodontal care.

Generally, there are notable strengthens among the studies in both milestones. Regarding the first milestone, we chose to retrospectively study a group of patients referred to the Department of Periodontology at CDEM. This decision was based on the already discussed advantage of carrying out pilot studies, for example on samples already collected. This dataset allowed us to immediately initiate a thorough analysis of the periodontal characteristics of referred patients, risk factors for periodontitis and the patient's attitudes towards referral after diagnosis of periodontitis.

Additionally, the SoPHiAS study employed a full-mouth protocol with circumferential examination, certifying an accurate estimation of the prevalence and extent of periodontitis because the new AAP/EFP joint case definition with an up-to-date diagnosis with PD and CAL combination analysis was used [34]. Besides, these results are representative and with adequate sample size calculation, stratified for each health centre.

However, there are fallouts worth mentioning along the different papers that compose this thesis. Firstly, the first four outputs (**Papers I-IV**) considered the 2012 CDC/AAP case definition [35], until that date the gold-standard classification. Soon after, in 2018, the AAP/EFP joint [34] divulged a new case definition that was promptly applied in the following outputs of milestone 2. This point may be seen as a limitation, since comparability between two milestones became more problematic.

Secondly, milestone 1, and previously debated, comprise a series of preliminary retrospective studies and one evidence-based review. While the systematic review on stress (**Paper IV**) was very relevant to determine stress as an attention-grabbing confounding variable, the retrospective studies are conceived to analyse pre-existing data, and therefore might be subject to bias potential [36]. Particularly, retrospective trials have a control group recruited from a convenience sample without representativeness, prevent a causal and temporal relationships determination, preclude the realization of other confounding factors and probable inadequate chart review for the study question [37].

Another limitation was the fact that, in the bruxism study (**Paper VII**), single-reporting time self-report of bruxism is not the most suitable approach to assess

bruxism in the clinical setting, also as far as the discrimination between awake and sleep bruxism is concerned. On the other hand, it remains an inevitable approach to gather data for screening purpose in large-sample epidemiological studies [28]. Also, the observational design precluded the inference of causality, though when adjusting for known risk factors, the association remained significant.

The confirmation of systemic conditions, previously introduced as possible risk factors towards periodontitis in elders, was based on self-report and through the medication usage. Despite this is not the most suitable and precise method, we believe that it has more advantages than merely self-reported information. In the future, we shall look for more accurate methods, based in gold-standard case definitions and within the financial and methodological restricts present.

Although we have included the number of missing teeth in the assessment of OHRQoL in elders (**Paper VI**), we could not account for occlusal pairs that previously been proven to influence OHRQoL [23]. The lack of information related to dentinal sensitivity as a result of gingival recession was also a limitation and shall be considered in the future. Further, the control group was derived from the same sample which can be seen as a possible shortcoming. Too, we employed the OHIP-14 which is more focused on the impact of pain on the patient's psychological and behavioural traits, while the Geriatric Oral Health Assessment Index (GOHAI) tool is more suitable to examine functional limitations in relation to pain [38,39] and ought to be considered in further investigations.

Regarding the validation of Brief-IPQ, as discussed, the main limitation was the studied population has been interviewed prior to periodontal treatment given ethical and epidemiologic duties. However, the retest interview timeline would certainly overlap the planned therapy. In this way, it became unfeasible to make the retest since the subjects' knowledge would automatically bias the results [40]. Also, the questionnaire administration mode was the telephone interview that that provides and wider population coverage, increases survey response and completion of the questionnaire, decreases recall dropouts, and is preferred by the respondents [41]. However, this type of interview has high response-choice order effects, and high social desirability and interviewer biases [41].

8.2. Concluding Remarks

- Paper I – This specific subpopulation of individuals referred to periodontal examination in a university dental clinic of the Lisbon region presented high prevalence and severe extent of chronic periodontitis. Age and smoking were identified as risk indicators for chronic periodontitis in this referred subpopulation. Within the limitations of this study, these results highlight the importance of developing appropriate public health programs to educate the Portuguese population about the burden of periodontal diseases.
- Paper II – Half-mouth three sites and two half-mouth six sites protocols can be used to estimate periodontal clinical measurements and status in Portuguese patients with limited bias. Also, these protocols showed high sensitivity, specificity, and concordance. Nevertheless, although all full-mouth partial protocols had high sensitivity levels, they all failed to estimate pocket depth and clinical attachment loss means, presenting less ability then half-mouth partial protocols. These results supported the use of a full-mouth protocol in a large-based survey as in Paper V.
- Paper III – In the present study, pocket depth fine-tuning multilevel modelling (MLM) showed that NSPT had a significant healing effect for moderate-to-severe CP with considerable reductions in PD and CAL. PD and CAL showed major recovery in the first 3 months after NSPT. The PD fine-tuning MLM analysis found that all 3 levels influenced the reduction of PD and CAL levels. The largest effect on PD and CAL reductions was seen at the site level.
- Paper IV – Subjects with aggressive periodontitis have higher salivary cortisol levels than healthy ones or patients with chronic periodontitis. Such salivary cortisol response difference may have a negative impact on the periodontium, contributing to worsening the burden of aggressive periodontitis disease. However, although the analysis suggested this relationship, periodontitis has a complex nature, and many confounders factors may have contributed to this outcome. Thus, in the future, more robust evidence about this topic should be gathered through the implementation of larger, well-designed longitudinal studies, to confirm this possible association and to elucidate the pathological mechanism beyond. Also, we strongly recommend to perform initial cortisol

screening, retrospective analysis of total exposure and baseline assessment, and take into consideration menstrual cycle-phase, oral contraceptives and physical exercise in exclusion criteria.

- Paper V – This study reveals a high burden of periodontitis in the adult population of the southern region of the Lisbon Metropolitan Area, in Portugal. Age, education level, smoking status and diabetes mellitus were identified as significantly potential risk factors towards periodontitis, confirming the results produced in Paper I.
- Paper VI – We demonstrate that self-perceived xerostomia and stress are significant variables for the total score of OHRQoL in elderly patients, that is, they worsen the quality of life. Also, the significance was maintained even when analysed simultaneously with the periodontal status, the number of missing teeth, periodontal clinical measures and oral hygiene habits. The confirmation of stress as a relevant factor in periodontitis endorses the results published in paper IV. Future studies should consider these parameters when investigating the impact of periodontitis in quality of life.
- Paper VII – The results point to the association between SR bruxism and periodontitis with ageing being a contributor factor. SR bruxism was related with less periodontal tissues' destruction and lower periodontitis prevalence. Overall, bruxism is a condition related with stress, and its apparent association with the periodontal status may help explaining the results in papers IV and VI.
- Paper VIII – The Brief-IPQ is a valid tool to the perception of periodontal diseases. These findings are relevant because this is a short and easily applicable questionnaire, in particular in the elderly populations where illness perception is challenging. This questionnaire may assist the clinician to educate patients' awareness towards its periodontal status and during periodontal care. This validation may be important to respond to the needs of public health strategies based on the results of the Papers I and V.

Overall, with this Thesis, we concluded that:

- This representative population of elders presented a disturbing prevalence of periodontal disease.
- Stress was initially disclosed as associated with periodontitis and, further, we demonstrated that self-perceived stress is an important stressor to OHRQoL in elders.
- Self-perceived xerostomia was also a key contributing factor to decrease OHRQoL in elders.
- Suffering from periodontal disease showed to be less impelling to OHRQoL revealing lack of awareness, education for oral health, and the ignorance with the potential consequences to oral and systemic health.
- Self-reported bruxism was found to be linked with periodontitis, and ageing was a major key contributor to this association.
- This project resulted in the validation of a prospective useful tool to be implemented in the clinical daily practice and in public health programmes.

8.3. Future Directions

The results of the present Thesis bring new insights to the prevalence of periodontal disease among a representative sample of elders among the Lisbon Metropolitan Area. The SoPHiAS showed to be a successful surveillance programme to explore the overall periodontal status of this particular population. Hence, this type of survey should be expanded nationally and in a more frequently fashion manner. Considering the foundations of our National Health Service, periodontal health is nothing less than a key point for systemic health, and everything must be done to maintain it, as disease levels are very distressing.

Mindful of these worrisome results, a national program on periodontal health focusing patients with 65 years old or older is warranted. Due to the fact that periodontitis is learned to be an age-dependent disease and consistently associated with conditions that affect mainly elders (such as diabetes mellitus, cardiovascular disease, rheumatic conditions, and others), promoting periodontal health is fundamental to uphold systemic health. We anticipate that elders with healthier gums are likely to have better controlled systemic conditions.

Furthermore, these results support the notion that elders are unaware of the existence of periodontal disease nor the potential negative impact into the oral health. In this respect, the validation of a self-perceived questionnaires towards periodontal disease is of paramount importance to identify patients ignoring the fact they have a periodontal disease and they must treat it. Future studies should investigate if such instruments might have predictive value concerning adherence to periodontal treatment, compliance during periodontal treatment and the effect of modelling these domains on therapeutic success and public health strategies.

Still, concerning the self-perception of periodontal diseases, the national program already suggested should also endeavor a strategy of oral education, the risks of having a periodontal disease to both the oral and general health, and the treatment of periodontitis has high success rates. Efforts should be carried out to expand the communication in particular with elders where these are, often, an obstacle. Therefore, future investigations should focus what

strategies work better and are more prone to be accepted by elders, and to improve communication skills.

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Annexes

Annex 1. Ethical clearance from ARS-LVT Ethical Committee.

Exma. Senhor
Dr. João Botelho
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C/C:

Sua Referência	Sua Comunicação de	Nossa Referência	Data
		8696/CES/2018	18.09.2018

Assunto: Prevalência, severidade e fatores de risco da doença periodontal numa população idosa Portuguesa.

A Comissão de Ética para a Saúde da ARSLVT, apreciou uma adenda ao protocolo deste estudo, submetida pelo investigador, na reunião da secção de investigação, do dia 14.09.2018, e emitiu um parecer favorável.

Declaração de conflito de interesses: Nada a declarar

O Conselho Directivo, atento ao teor do parecer emitido, entende estarem reunidas as condições para a concretização do solicitado.

Com os melhores cumprimentos,

O Conselho Directivo

LUÍS PISCO
Presidente do Conselho Diretivo da ARS LVT, I.P.

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Para: joaobotelho09@gmail.com

JB

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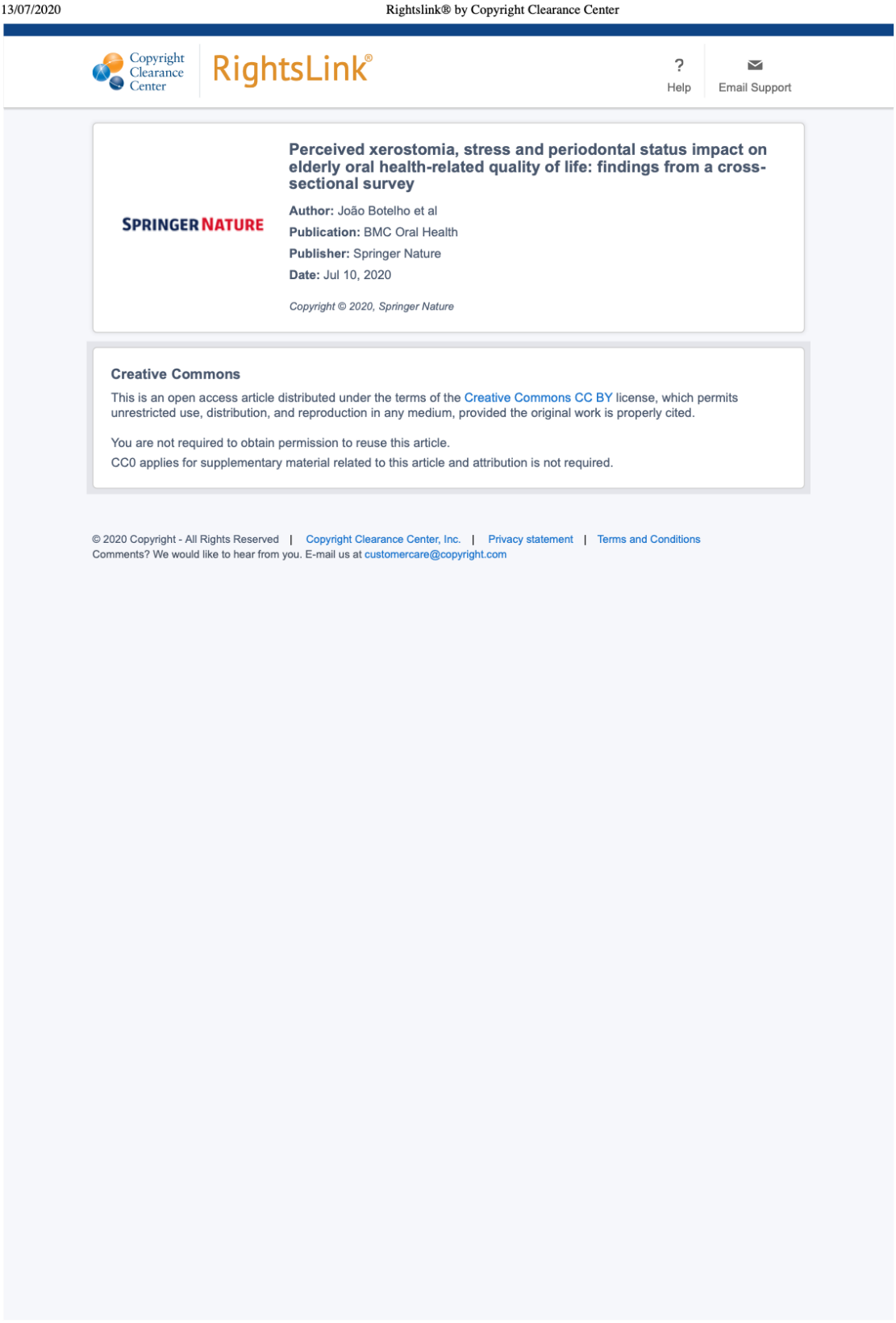

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