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DOUTORAMENTO

CIÊNCIAS BIOMÉDICAS

Periodontal disease and its risk factors in a Portuguese adult population

Vanessa de Almeida Machado

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INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR

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Periodontal disease and its risk factors in a Portuguese adult 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 o João Botelho,

A minha Mãe

e o meu Pai

"Never limit yourself because of others' limited imagination;
never limit others because of your own limited imagination."
Mae Jemison

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Declaration of Honor (English and Portuguese)

In compliance with the current 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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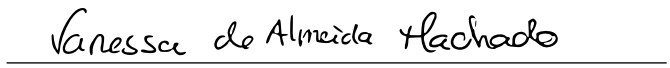
Em respeito do atual Código Ético de Conduta Académica da Universidade do Porto, aprovado em dezembro de 2017, e em particular consideração pelo seu Artigo 14º: Declaro que a presente tese é de minha autoria e não foi utilizada previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referenciação. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.



(Vanessa de Almeida Machado)

Scientific Output

According to "*Artigo 31º, nº.2 do Decreto-lei nº. 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 (listed by the date of publication) and author contribution per paper:

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Author contribution: Vanessa Machado 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.

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Author contribution: Vanessa Machado conceived and designed the experiment, collected data, contributed with bioinformatics analysis and with statistic interpretation, prepared figures and tables, authored drafts and approved the final draft.

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Salivary Cortisol and Periodontitis: A Systematic Review and Meta-analysis of Observational Studies. *Archives of Oral Biology* **2018**, 96, 58-65.

Author contribution: Vanessa Machado 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.

4. 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**, 33, e081.

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5. 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.

Author contribution: Vanessa Machado has created the project design and the main conceptual ideas and technicalities, computed sample size calculation, executed data analysis and interpretation, and wrote and approved the final work.

6. **Vanessa Machado**, João Botelho, Luís Proença, Ricardo Alves, Maria Alzira Cavacas, Luís Amaro, José João Mendes. Psychometric properties of the Brief Illness Perception Questionnaire (Brief-IPQ) in Periodontal Diseases. *Journal of Clinical Periodontology* **2019**, 46(12), 1183-1191.

Author contribution: Vanessa Machado conceived, designed the experiments, and had an substantial contribution to acquisition of data. Furthermore, Vanessa Machado contributed for analysis and interpretation of data, drafted the article including the preparation of figures and table, and approved the final draft.

7. 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**, 91, 1049-1056.

Author contribution: Vanessa Machado designed the study, performed the questionnaire and the periodontal measurements. Also, Vanessa Machado processed the experimental data, were involved in bioinformatic analysis, assisted in the interpretation of results and was a major contributor in writing the manuscript.

8. **Vanessa Machado**, João Botelho, Luís Proença, Ricardo Alves, Maria João Oliveira, Luís Amaro, Artur Águas, José João Mendes. Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: a structural equation modelling analysis. *BMC Oral Health*, **2020**, 20(229), 1-11.

Author contribution: Vanessa Machado conceived, planned the experiments, and also carried out the experiments. Vanessa Machado implemented the analysis according to Andersen's behavioral model, interpreted the results, drafted the article including the preparation of figures and table, and approved the final draft.

Abbreviations List

AAP – American Academy of Periodontology

ACES – Almada-Seixal Group of Health Centres

BMI – Body Mass Index

BoP – Bleeding on Probing

CAL – Clinical Attachment Loss

CDC – Central for Disease Control and Prevention

CFA – Confirmatory Factor Analysis AAP – American Academy of Periodontology

ACES – Almada-Seixal Group of Health Centres

ABM - Andersen's behavioral modelling

ARSLVT - Administração Regional de Saúde de Lisboa e Vale do Tejo

AgP – Aggressive Periodontitis

BMI – Body Mass Index

BoP – Bleeding on Probing

CAL – Clinical Attachment Loss

CDC – Central for Disease Control and Prevention

CEJ - Cementoenamel Junction

CFA – Confirmatory Factor Analysis

CFI - Confirmatory Fit Index

CI – Confidence Interval

CiiEM – Centro de Investigação Interdisciplinar Egas Moniz

CP – Chronic Periodontitis

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

FHU – Family Heatlh Unit

FMP – Full-mouth protocol

FRP – Full-mouth Recording Protocols

GC - Gingivitis cases

GFI – Goodness of Fit Index

GWAS – Genome-Wide Association Studies

GIF - Goodness of Fit Index

HbA1c - Glycated hemoglobin

IPQ – Illness Perception Questionnaire

IPQ-R-OH - Illness Perception Questionnaire Revised for Oral Health

LNSC - Late-Night Salivary Cortisol

MCAL – Mean Clinical Attachment Loss

MLM – Multilevel Modelling

MMPs – Matrix Metallopeptidases

MPD – Mean Probing Depth

NHANES – National Health and Nutrition Examination Study

NSPT – Nonsurgical Periodontal Treatment

NUTS – Nomenclatura das Unidades Territoriais para Fins Estatísticos

OHIP - Oral Health Impact Profile

OHRQoL – Oral Health-Related Quality of Life

OHI – Oral Health Instructions

OR – Odds Ratio

PAROKRANK - Periodontitis and Its Relation to Coronary Artery Disease

PD – Probing Depth

PPD – Periodontal Probing Depth

PMPR - Professional Mechanical Plaque Removal

PPOHP - Portuguese Public Oral Health Programme

PRA - Periodontal Risk Assessment

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

PRP – Partial Recording Protocols

PSR – Periodontal Screening and Recording

QoL – Quality of Life

RCT – Randomized Clinical Trial

REC – Gingival recession

RMSA - Root Mean Square of Approximation

ROC/AUC - Receiver Operating Characteristic / Area Under the Curve

SHIP – Studies of Health in Pomerania

SoPHiAS – Study of Periodontal Health in Almada-Seixal

SR – Self-reported

STROBE - STrengthening the Reporting of OBservational studies in Epidemiology

TOHNN - Tromstannen-Oral Health in Northern Norway

USA – United States of America

WHO – World Health Organization

Abstract

Periodontal disease, including gingivitis and periodontitis, represent a heterogeneous multifactorial chronic, dysbiotic, inflammatory and infectious gum disease that targets the supporting structure of the teeth. Dental plaque build-up, periodontopathogenic microbial specificity and the host's immune response can collectively be considered periodontitis major etiology factors. Nevertheless, several risk factor, such as age, sex, health literacy, smoking habits and oral hygiene habits and systemic diseases, have also impact on periodontal disease.

Initially, periodontal disease begins with a reversible local gum inflammation as a response to dental plaque along the gingival margin. Notwithstanding, the persistence of an uncontrolled inflammatory gum reaction and poor oral health care lead to the onset and progression of periodontitis. Moreover, while gingivitis is a common gingival reaction, that can appear throughout life, typically the onset of periodontitis is more common at early adulthood. Furthermore, periodontitis is characterized by a cycle of active and inactive periods of the disease, which means that the pattern of periodontal loss is not continuous.

Beyond its clinical characteristics, predisposing and environmental risk factors, periodontitis negatively impacts the perceived quality of life. The individuals' perception is recognized as a valid criterion to subjectively assess patient healthcare. In this context, the impact on Oral Health-Related Quality of Life (OHRQoL) are more pronounced with greater extent and severity of periodontitis. Besides, psychological factors like stress and xerostomia have been associated with periodontitis and OHRQoL. Therefore, measuring other characteristics, for instance stress and xerostomia in adults may enhance the understanding of these potential confounding variables.

Periodontal disease is categorically one of the major global public health problems. Comprehensively, severe periodontitis affects 5 to 20%, while mild to moderate periodontitis affects the majority of the adult population worldwide. Still, given the scarcity of available epidemiological data on periodontal disease in the Portuguese population, our main purpose was to assess the prevalence and extent of periodontal disease using a population-based stratified sample of

adults from the southern region of the Lisbon Metropolitan Area. Furthermore, the second aim was to investigate potential periodontal disease risk factors.

Prior to the large-scale study, four preliminary studies based in a Portuguese subpopulation from the Egas Moniz Dental Clinic (EMDC) (Almada, Portugal) were published **(Chapter 3)**. These pilot studies were useful to develop the research question, to fitting questionnaires, clinical observation assessment tools and critically assess whether the crucial components of the main study were viable. In this sense, the first retrospective clinical study revealed that periodontitis was highly prevalent among the adult population and increased with age. Multivariate analysis demonstrated that patients with 45-64 years old presented 3.85 more risk towards periodontitis. Besides, smoking, obesity and lower educational level were the main risk factors (**Chapter 3, Section 3.2**). This particular adult group had higher prevalence of periodontitis than data from national data. Then, to explore this discrepancy, we compared the bias magnitudes, sensibility and specificity of full-mouth recoding protocol (FRP) with partial recording protocols (PRP). In fact, we concluded that the PRP used in a previous Portuguese periodontal survey had low sensitivity and specificity, and might help explaining this difference (**Chapter 3, Section 3.3**). Taking into account this crucial information, we decided to apply the standard full-mouth periodontal examination in our large-scale Almada-Seixal survey. Also, we systematically appraised whether periodontitis and stress were associated. The results suggested that patients with aggressive periodontitis have higher salivary cortisol levels than healthy periodontal patients or patients with chronic periodontitis. This finding supported the implementation of a questionnaire for self-perceived stress in our larger epidemiological survey (**Chapter 3, Section 3.4**). Lastly, we investigated the effect of known risk factors on nonsurgical periodontal treatment (NSPT) response using a periodontal pocket depth fine-tuning multilevel linear model (MLM). The results revealed that reduction of probing depth and clinical attachment loss levels after NSPT may depend on three different levels (site-, tooth- and patient-level) (**Chapter 3, Section 3.5**).

Subsequently to these pilot studies, we conducted the Study of Periodontal Health in Almada-Seixal (SoPHiAS). This cross-sectional study aimed to describe the prevalence and extent of periodontal disease among adults in the southern region of the Lisbon Metropolitan Area. The overall results showed a high prevalence of periodontitis, with age, education level, smoking habits and

diagnosed of diabetes mellitus (DM) as risk factors associated with periodontitis (**Chapter 4**). We then explored, through Andersen's behavioral modelling, several risk factors towards OHRQoL. We concluded that age, number of missing teeth, DM, interproximal cleaning and perceived stress were the most impactful risk factors for OHRQoL (**Chapter 5**).

Moreover, the psychometric properties of the SoPHiAS adult concerning the knowledge on their periodontal disease were relevant for this project given the poor periodontal health and oral hygiene habits. In this sense, we attempted the validation of a short tool of illness perception, the Brief Illness Perception Questionnaire (Brief-IPQ) (**Chapter 6**). The Brief-IPQ showed acceptable reliability, confirmatory factor analysis revealed good model fit and invariance across sex among patients with periodontitis. This result suggests that the Brief-IPQ adequately provides the patients' cognitive and emotional representations of their periodontal condition. Therefore, this short instrument may be used as an easily applicable and valuable tool to determine illness perception in patients with periodontitis during the dental appointments.

Lastly, we analysed the association between periodontitis and bruxism (measured through a self-reported strategy). Overall, bruxers exhibited lower prevalence of periodontitis. Furthermore, bruxers with periodontitis had lower levels of periodontal destruction than patients with periodontitis and without bruxism. Therefore, self-reported bruxism and periodontal status might be negatively associated, yet the clinical importance of bruxism for the periodontal status shall be clarified in the future (**Chapter 7**).

In conclusion, this thesis reported a high prevalence of periodontal disease among an adult Portuguese population of the Southern Lisbon Metropolitan Area. In this particular adult population, age, number of missing teeth, DM, interproximal cleaning and perceived stress were the most impactful risk factors for OHRQoL. Still, we validated a potentially deployable tool to assess the illness perception of periodontitis. This tool might be easily applied in the daily practice and in Public Health programs. Also, bruxism was seen as a potential factor associated with the risk of periodontitis.

Keywords: Periodontal Disease, Periodontitis, Epidemiology, Adults, Public Health.

Resumo

A doença periodontal, gengivite e periodontite, representa um grupo heterogéneo multifatorial de doenças crónicas, disbióticas, inflamatórias e infeciosas da gengiva, que afetam a estrutura de suporte dos dentes. A formação de placa dentária, a especificidade da microbiana periodontopatogénica e a resposta imune do hospedeiro podem ser coletivamente consideradas os principais fatores etiológicos da periodontite. No entanto, vários fatores de risco têm sido implicados na doença periodontal, nomeadamente a idade, género, literacia médica, doenças sistémicas, hábitos tabágicos e hábitos de higiene oral.

A doença periodontal inicia-se com uma inflamação local e reversível da gengiva como resposta à acumulação de placa dentária ao redor da margem gengival. Não obstante, a persistência da placa, pela falta de cuidados de saúde oral, causa uma reação inflamatória gengival descontrolada podendo levar ao aparecimento e progressão da periodontite. Além disso, embora a gengivite seja uma reação gengival comum, que pode aparecer ao longo da vida, o início do desenvolvimento da periodontite está normalmente relacionado com a idade adulta e a atividade da doença pode intercalar-se entre períodos ativos e inativos da doença periodontal ao longo do tempo.

Além das suas características clínicas, indicadores de risco predisponentes e ambientais, a periodontite afeta negativamente a Qualidade de Vida Relacionada com a Saúde Oral (QdVRSO) percebida pelo paciente. Neste contexto, os impactos na QdVRSO estão associados com a gravidade ou extensão da periodontite. Além disso, fatores psicológicos, como o *stress* e a xerostomia, têm sido associados com periodontite e a QdVRSO. Assim, a avaliação de outras características na população adulta, nomeadamente o *stress* e a xerostomia, podem ajudar a melhorar a compreensão destas possíveis variáveis confundentes.

A doença periodontal é decisivamente um dos principais problemas de Saúde Pública em todo o mundo. Na população mundial, a periodontite severa afeta 5% a 20% das pessoas, enquanto que o seu estadio leve a moderado está presente na maioria da população adulta. Ainda assim, dada a escassez de dados epidemiológicos disponíveis sobre doença periodontal na população portuguesa, o nosso principal objetivo foi avaliar a prevalência e extensão da doença periodontal usando uma amostra estratificada de adultos da região sul

da Área Metropolitana de Lisboa. Além disso, o segundo objetivo foi investigar possíveis fatores de risco associados com a doença periodontal.

Previamente ao estudo epidemiológico em larga escala, foram publicados quatro estudos preliminares (**Capítulo 3**) baseados numa subpopulação portuguesa da Clínica Dentária Egas Moniz (CDEM) (Almada, Portugal). Esses estudos-piloto foram úteis para desenvolver a pergunta de investigação, adequar o questionário e as ferramentas de avaliação da observação clínica e avaliar criticamente a viabilidade dos componentes do estudo principal. Como primeiro estudo, um estudo retrospetivo na CDEM revelou que a periodontite é altamente prevalente na população adulta e aumenta consoante a idade. A análise multivariada demonstrou que pacientes com 45 a 64 anos apresentaram 3.85 maior risco de ter periodontite. Além disso, os hábitos tabágicos, a obesidade e o nível educacional foram os principais fatores de risco associados (**Capítulo 3, Seção 3.2.**). Particularmente no grupo dos adultos, a prevalência foi muito superior do que a relatada previamente num estudo nacional. Por esta razão, explorámos as possíveis razões para esta discrepância comparando as magnitudes de viés, sensibilidade e especificidade do protocolo de registo total periodontal (PRTP) com protocolos de registro parcial periodontal (PRPP). Concluímos que o PRPP usado no estudo nacional da prevalência de doença periodontal, previamente realizado em Portugal, apresentou baixa sensibilidade e especificidade e, este facto pode ajudar a explicar esta discordância de dados (**Capítulo 3, Secção3.3.**). Tendo em conta estes resultados, decidimos aplicar o exame periodontal com registo total no estudo epidemiológico de larga escala na região de Almada-Seixal. Além disso, analisámos sistematicamente a associação entre a periodontite e *stress* através dos níveis salivares de cortisol. Os resultados sugerem que pacientes com periodontite agressiva têm níveis mais altos de cortisol salivar do que pacientes periodontais saudáveis ou pacientes com periodontite crónica (**Capítulo 3, Secção 3.4**). Este resultado suportou a implementação de um questionário de *stress* auto-percebido na investigação epidemiológica a concretizar. Por fim, explorámos o efeito de fatores de risco conhecidos na resposta do tratamento periodontal não-cirúrgico (TPNC) usando a profundidade de sondagem e o nível de inserção clínico a partir de um modelo linear multinível. Os resultados revelaram que a redução da profundidade de sondagem e da perda de inserção clínica após o TPNC pode depender de três níveis (local, dente e paciente) (**Capítulo 3, Sessão 3.5**).

Após os estudos piloto, realizámos o Estudo de Saúde Periodontal em Almada-Seixal (SoPHiAS). Este estudo teve como objetivo descrever a prevalência e extensão da doença periodontal em adultos na região sul da Área Metropolitana de Lisboa. Globalmente, verificou-se uma alta prevalência de periodontite na idade adulta, sendo que a idade, o nível de escolaridade, os hábitos tabágicos e o diagnóstico de diabetes mellitus (DM) foram fatores de risco mais significativamente associados com a periodontite (**Capítulo 4**). Numa análise mais específica para a população adulta, analisámos através da modelagem comportamental de Andersen, diversos fatores de risco associados com a QdVRSO. Concluímos que a idade, o número de dentes perdidos, o diagnóstico de DM, a limpeza interproximal e o *stress* auto-percebido pelo paciente foram os fatores de risco mais impactantes para a QdVRSO (**Capítulo 5**).

Neste sentido, avaliámos as propriedades psicométricas do conhecimento da população sobre a doença periodontal. Neste sentido, pretendemos validar uma ferramenta curta de perceção da doença, o *Brief Illness Perception Questionnaire* (Brief-IPQ) (**Capítulo 6**). O questionário Brief-IPQ revelou uma aceitável confiabilidade, a análise fatorial confirmatória teve bom ajuste do modelo e não existiu variância entre os sexos entre os pacientes com periodontite. Este resultado sugere que o questionário Brief-IPQ fornece representações cognitivas e emocionais dos pacientes sobre a sua condição periodontal. Portanto, este breve instrumento pode ser facilmente aplicável como uma ferramenta valiosa para determinar a perceção da doença em pacientes com periodontite durante as consultas médico-dentárias.

Além disso, explorámos a associação da periodontite com o bruxismo (medido através de uma estratégia auto-reportada). De acordo com os dados, os pacientes bruxómanos apresentaram menor prevalência de periodontite. Ainda, os pacientes com bruxismo com periodontite apresentaram menores níveis de destruição periodontal do que pacientes com periodontite e sem bruxismo. Portanto, o bruxismo relatado pelo paciente e o estado periodontal podem estar negativamente associados, contudo a importância clínica do bruxismo no estado periodontal deverá ser clarificado no futuro (**Capítulo 7**).

Em conclusão, esta tese confirma uma alta prevalência de doença periodontal na população adulta portuguesa da região sul da Área Metropolitana de Lisboa. Nesta população adulta, a idade, o número de dentes perdidos, o diagnóstico de DM, a limpeza interproximal e o *stress* percebido pelo paciente são os fatores

de risco mais impactantes para a QdVRSO. Paralelamente, o Brief-IPQ apresentou validade como instrumento de perceção da periodontite na população adulta. Este instrumento pode ser facilmente usado no contexto clínico médico-dentário e nos programas de Saúde Pública. Finalmente, verificámos o bruxismo como um potencial fator de associação com o risco de periodontite.

Palavras-chave: Doença Periodontal, Periodontite, Epidemiologia, Adultos, Saúde Pública

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CHAPTER

1

General Introduction

1.1. Periodontal Health

The periodontium is a collective term referring to a complex and dynamic apparatus that supports the tooth, including mineralized tissues (root cementum and alveolar bone proper) and soft connective tissues (gingiva and periodontal ligament) [1–3] (Figure 1.1). In healthy conditions, only gingiva is clinically visible.

The main function of the periodontium is to attach the tooth to the alveolar bone and, consequently, to support the integrity of the masticatory mucosa's surface of the oral cavity [3,4].

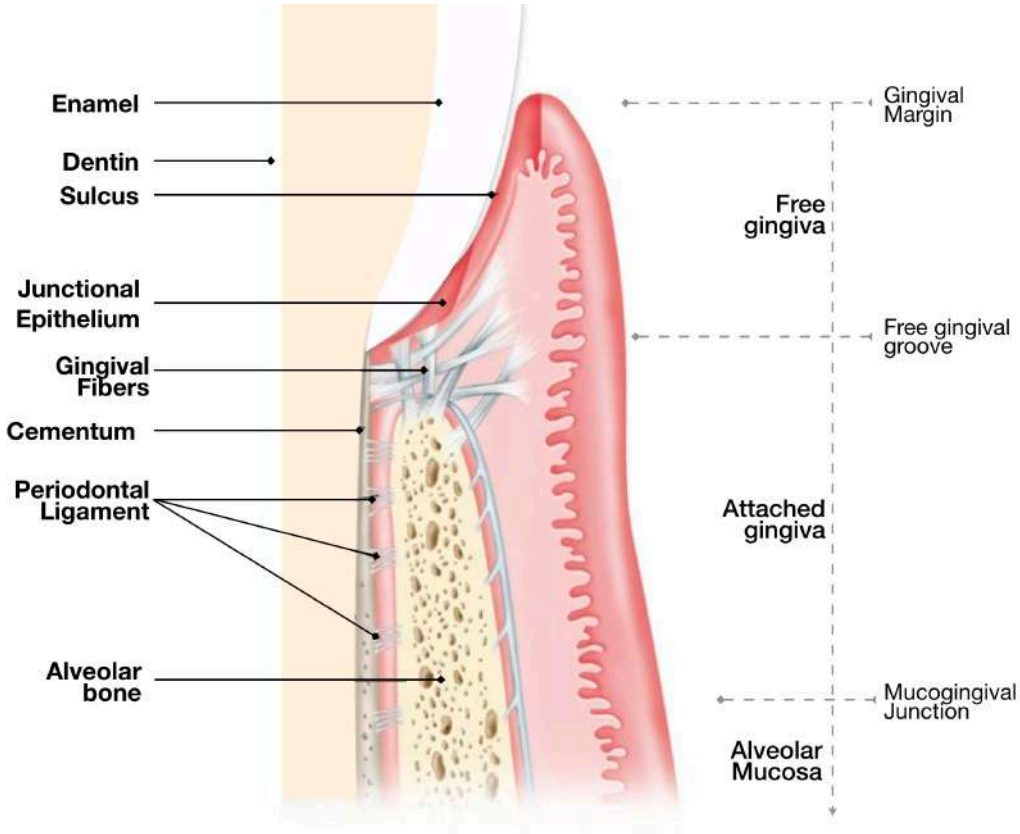

Figure 1.1. Diagram representing the longitudinal section through part of a tooth showing healthy periodontal tissues. (Original image).

In 1946, the World Health Organization (WHO) defined health as a "state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" [5]. Yet, this definition was considered problematic to achieve and, therefore, in 1977, Boorse et al. [6] proposed that a "person is

healthy only if all organs function within defined limits, given a statistically normal environment". Later, in 2007, Nordenfelt [7] suggested a holistic perspective, where "a person is healthy if he or she had the ability, given standard circumstances, to reach all of his or her vital goals". Recently, periodontal health was firstly defined in the 2017 European Federation of Periodontology (EFP)/ American Academy of Periodontology (AAP) World Workshop 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" [8]. This shift from a strictly biological and medical model to a holistic concept of health (embracing patients' perceptions, function and social well-being) is remarkable and key to define two main clinical situations of health in Periodontology [8,9]: 1) intact periodontium, whether is pristine or clinically well-preserved; and 2) reduced periodontium, with stable periodontal disease or in remission/control. Therefore, periodontal health shall be seen as the absence or very significant reduction of clinical signs of periodontal inflammation [8].

The major parameter to assess gum health or inflammation is bleeding on probing (BoP) after light pressure probing (0.25 Newtons) [8]. This clinical characteristic is measured as bleeding during or after periodontal probing into the bottom of a periodontal sulcus/pocket [8]. In an uneventful probing, that is without bleeding, it may be considered as clinically healthy and/or periodontal stable stage [8].

As any tissue in the human body, the periodontium experiences changes as the result of intrinsic and extrinsic factors. Therefore, it is crucial to recognize that, following the 2017 EFP/AAP classification, both shallow and deep pockets may exist as so-called relative healthy pockets [8]. Although debatable, deep pockets may endure stable and non-inflamed, particularly if the appropriate supportive periodontal care is provided. This highlights that periodontal probing depth (PPD), clinical attachment loss (CAL) and bone height must be considered and interpreted in association with BoP, modifying and predisposing factors [8] during periodontal diagnosis [10].

1.2. Periodontal Disease

Periodontal disease, including gingivitis and periodontitis, represents a heterogeneous non-communicable chronic inflammatory condition caused by dysbiotic plaque [10,11]. It is a microbially driven and host-mediated inflammation process that targets the periodontium [12].

Conceptually, periodontal disease begins with local gum inflammation as a response to dental plaque along the gingival margin [13]. Clinically, gingivitis is painless and characterized by edema, bleeding, and no periodontal loss [14–16]. Furthermore, it is a reversible condition if the biofilm is removed, however, if untreated, gingivitis can progress to periodontitis [10,11,15,17,18]. While gingivitis is a common gingival response throughout life, typically the onset of periodontitis occurs in the early adulthood or later [16,19,20].

The persistence of uncontrolled gingivitis and poor oral health care lead to the depeening of existing crevices between the gum and tooth root, the so-called periodontal pockets [11,17]. Because these pockets are difficult to resolve, periodontitis is defined as a chronic, nonreversible and inflammatory disease that impacts the periodontium [12]. As a consequence, the periodontium is progressively damaged due to a host immune response to a complex polymicrobial-driven infection [11,21,22]. If untreated, periodontitis can evolve to severe states with bleeding on toothbrushing, persistent malodor, painless tooth mobility and, ultimately, tooth loss [10,11,13,22–26]. Periodontitis is considered a "silent" non-communicable disease because its symptoms remain unnoticed by the patients for several decades [27], highlighting the importance of awareness programs towards periodontal disease.

1.3. Risk factors of periodontitis

Conceptually, a risk factor is an exposure or attribute that increases the likelihood of developing an injury or disease [28]. As far as periodontitis is concerned, dental plaque is the major etiologic factor implicated in the onset of periodontitis, although its progression and severity is (directly or indirectly) linked to: a) non-modifiable background factors; b) hereditary or acquired conditions; c) environmental and behavioral factors [13,29,30], summarized in Figure 1.2.

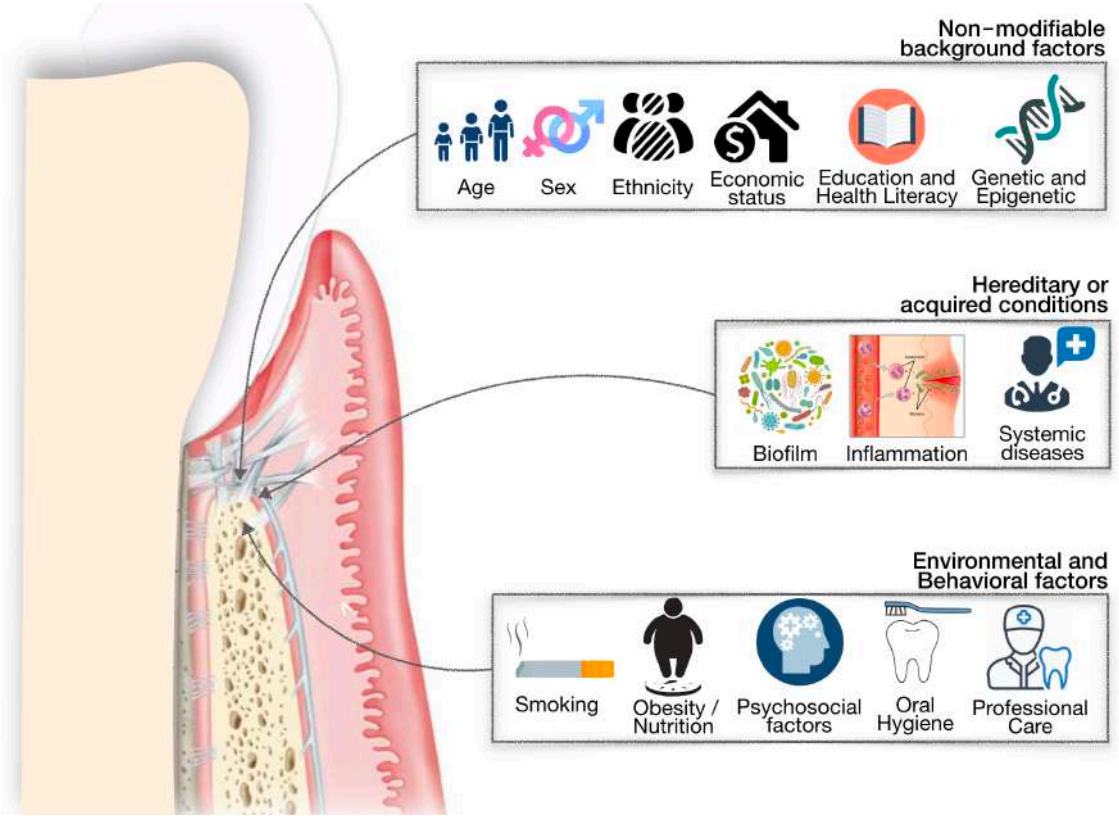

Figure 1.2. The interaction between non-modifiable background factors, hereditary or acquired condition, and environmental and behaviors factors. Periodontal disease develops at the intersection of predisposing factors, dysbiosis of the gum microbiota and environmental influences. None of the risk factors alone are sufficient for development of disease and complex interactions between each factor occurs, leading to development of periodontal disease. (Original image).

1.3.1 Non-modifiable background factors

Age, sex and ethnicity/race

The influence of age on periodontitis has been highly discussed among the known confounding factors of periodontitis. Considering the inflammatory pattern and chronic pattern of periodontitis, some studies have confirmed through stratification and thresholds approaches that age is associated with the severity of periodontitis [31–35]. Furthermore, age is a consistent risk factor and predictor of tooth loss due to periodontitis [36–38]. Nevertheless it is important to highlight that in periodontally healthy adults, age does not lead *per se* to worse periodontal clinical characteristics [39–41].

Another important risk factor towards periodontal disease is being male (i.e., sex-related), and has been raising importance in the periodontitis risk equation [22]. Men have significantly higher prevalence, extent and severity of periodontitis than women [34,35,42–45]. This sexual dimorphism as a risk factor towards periodontitis can be attributed to higher plaque accumulation and poorer oral hygiene habits [13,36]. Genetic variations and environmental mechanisms have been also correlated [36,44] with higher extent and severity of periodontitis in men [13,36]. Even though, the current notion of this difference is mainly due to behavioral and lifestyle factors rather than the existence of specific sex-based biological mechanisms [46].

Also, racial and ethnic characteristics have been recognized as a risk factor for periodontitis, but the findings are controversial. Data from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) estimated that Non-Hispanic white subjects had less percentage of severe periodontitis (6.3%) than Non-Hispanic black participants (13.3%) [47], and this may help explaining disparities in periodontitis prevalence [48,49]. However, the main explanation is heavily related to sociodemographic characteristics rather than purely racial/ethnic origins (for instance, genetic polymorphisms). Hence, race/ethnicity may be an important confounder factor for periodontal epidemiologic surveys [49].

Economic status

Socioeconomic status inequality is also a central issue affecting health [9]. Theoretically, higher socioeconomic standards are associated with better health, less disability, and longer life time [50]. According to Andersen's healthcare model, individuals more socioeconomically fragile are more likely to seek health and dental services [51]. However, there are no established evidence that supports the real impact of socioeconomic gradients in periodontal disease [52–54], particularly in Portuguese settings, and we might hypothesize that is due to the multifactorial nature of periodontal disease.

Education and Health Literacy

The association between educational level and oral health has been reported [55–60], where people with less schooling (in other words, with) are more prone

to have poor periodontal health [48,60]. Nevertheless, the routes through which low schooling affects periodontal health outcomes are not fully understood and one possible explanation is health literacy.

Health literacy is collectively seen as knowledge, motivation and competences to get, comprehend, judge, and employ health information in order to prevent and manage diseases based on correct decisions taken in a daily-basis [61]. The societal and environmental determinants (e.g. demographic situation, culture, language, political forces, societal systems), the personal determinants (e.g., age, sex, race, socioeconomic status, education, occupation, employment, income, literacy) and the situational determinants (e.g. social support, family influences, media usage and physical environment) [61] are the factors frequently impacting on health literacy.

The relationship between health literacy and health was recently established [62]. Besides, lower levels of health literacy are strongly associated with lower levels of education [56,58,59,61], as well as with frequent use of emergency health care, poor medication adherence, higher morbidity and mortality, and less attitudes of health promotion and preventive care [62].

Specifically, oral health literacy was associated with individual's ability to make decisions and judgments concerning their own oral health [58]. Likewise, lower oral health literacy was associated with poorer oral health knowledge [63–65], less self-reported oral health [55,64], irregular dental follow-up [55] and less demand for oral health information [55,66]. In contrast, people with suitable oral health literacy are more likely to present periodontal health, even considering factors such as age, sex or education [60].

Leventhal's common-sense model highlights the individuals' perception of illness as an important psychological factor [67] for a patient to adopt coping behaviors with his/her disease [68]. In this sense, the Illness Perception Questionnaire Revised for Oral Health (IPQ-R-OH) demonstrated an adequate reliability and construct factorial validity to evaluate the psychometric properties in periodontal patients [69,70]. Nevertheless, the usability of this extensive questionnaire in a clinical setting is debatable. Hence, seeking shorter self-perception instruments might be more reasonable considering the particular setting of the daily-practice in Periodontology and Dentistry, in general.

Genetics and epigenetics

The role of genetics in periodontitis was firstly investigated in twins to identify genes that could be associated with higher susceptibility to periodontal disease [71]. These studies demonstrated that the heritability for periodontal disease was estimated at 39% in women and 33% in men [71]. Addtionaly, 50% for of CAL processes in periodontitis patients were estimated to depend on genetic liability [72]. More recently, human genome studies identified genes loci that have associations with periodontitis [73], clinical measurement of periodontal disease [74], and severity of periodontitis [75]. Nevertheless, these investigations provided inconsistent evidence and more genome-wide association studies are needed to clarify this matter.

On the other hand, epigenetics refers to "causal mechanisms" by which genes give rise to specific phenotypes [76]. Currently, epigenetics has a prominent role in Periodontology since the environment can modify gene expression [77]. However, epigenetic pathways use modulation of inflammatory and anti-inflammatory genes that are still poorly understood [77,78]. Epigenetics is a new concept in Periodontology research and may improve the understanding of the susceptibility and allow new horizons on the association between genetics, periodontal disease phenotypes and environment. Moreover, within this information, in the future we may deliver personalized therapeutic approaches for periodontal patients [77,79–81]. Nevertheless, the research of epigenetics may only occur whenever the prevalence of periodontal disease and the impact in health is well known, and for this reason epidemiological studies are important to establish a strong knowledge basis.

1.3.2. Hereditary or acquired conditions

Biofilm and inflammation

The oral biofilm organization is a complex and specialized dynamic ecosystem [82] with diverse communities of microorganisms, namely bacteria, viruses, mycoplasmas, fungi and protozoa [83]. Overall, the oral microbiome exists in a symbiotic relationship with the host that ensures periodontal health and tissue homeostasis [84]. However, this healthy relationship can be affected by the pathogenic potential and/or host susceptibility, progressing to a dysbiotic microbiota [12,85].

Dental plaque accumulation is widely accepted as the main etiological factor for the onset and progression of periodontal disease [14]. Plaque dysbiosis and their subproducts induce a local inflammatory reaction that can progress systemically, causing a leukocytosis state with increasing white blood cells and segmented neutrophil counts [86–89]. These cells are in the frontline against periodontal infection [90–92] and, if persists, tissues produce a higher number of inflammatory cytokines [93–95] and the periodontal epithelium turns ulcerated (Figure 1.3). Thereby, periodontal pathogens may invade the organism and trigger a systemic response to neutralize any harmful consequences [91]. Additionally, the increase of proinflammatory levels (C-reactive protein, tumor necrosis factor alpha or interleukins) incite the host immune to react against the periodontal infection exacerbating the periodontal destruction [11,96].

Even with the evolution of immunological and molecular diagnostic tests, the specific pathogen-host interactions behind the pathogenesis or the immunopathology of the periodontal disease are not fully characterized and understand [97–99].

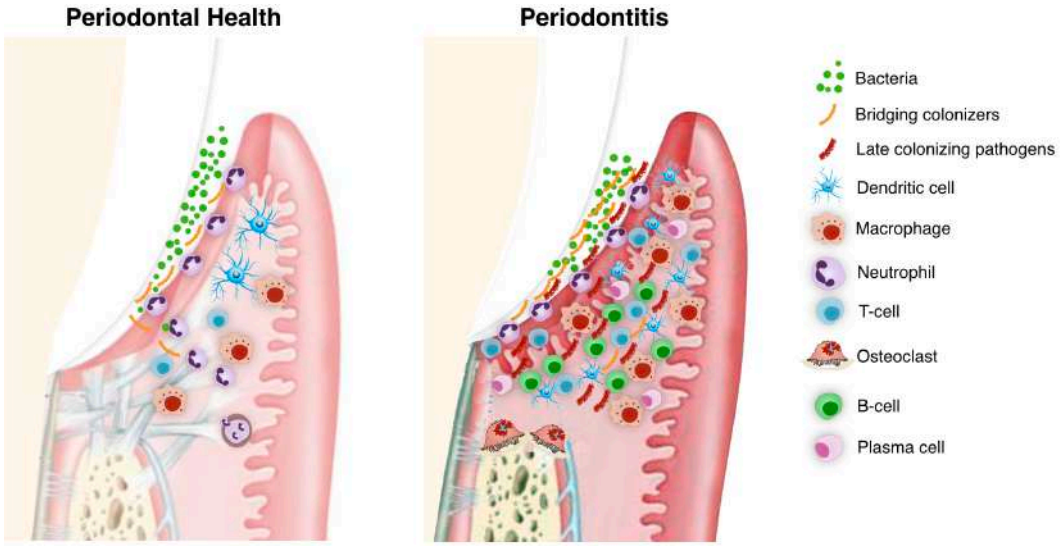

Figure 1.3. Immune responses in chronic periodontitis. In periodontal homeostasis, the host-pathogen interactions occur at the gingival crevice and periodontal sulcus site are characterized by controlled neutrophil and granulocyte infiltration. In consequence of plaque accumulation, evolving into a dysbiotic microbiota, chemotactic gradients created by the bacteria and the inflammatory response and lymphocyte infiltration that follow dendritic cell antigen presentation. (Original image).

Systemic diseases

The interest in the oral health-systemic health axis has grown in recent years. Periodontitis has been strongly associated (directly and/or indirectly) with a number of systemic diseases and medications [100].

Comprehensively, periodontal bacteria and/or their pathogenic subproducts can spread into the bloodstream through the ulcerated periodontal tissue [91] and, thereby, can increase the local and systemic inflammatory levels [24,96]. Among the chronic systemic diseases associated with periodontitis are diabetes mellitus [101], cardiovascular disease [102–105], rheumatoid arthritis [106,107] and adverse pregnancy outcomes [108,109]. In most of these associations, a bidirectional way has been extensively reported, that is, periodontitis affects the clinical status of a particular condition and vice-versa [106,110–113]. Furthermore, in some conditions, such as diabetes mellitus or cardiovascular disease, evidence shows that nonsurgical periodontal treatment is effective in the status improvement of that particular systemic illness [114].

1.3.3. Environmental and behavioral factors

Smoking

Smoking is a major modifiable risk factor for periodontitis [115,116], particularly alveolar bone loss [117–120], development of periodontal pockets [121–123] and, consequently, tooth loss [124–127]. The effect of smoking in periodontitis is cumulative, which means that higher smoking dose-exposure will contribute to worse periodontal destruction [128–131]. Also, active smokers have 2-14 times more risk to develop periodontitis compared with non-smokers [132].

Although this relationship is well established, the underlying mechanisms of its negative effects are not fully elucidated [133]. On the one hand, smoking impacts on the inflammatory response compromising the innate and adaptive immune systems and decreasing angiogenesis [133,134]. Blood flow in the healthy periodontium of smokers appears unaffected [135]. On the other hand, smoking impairs gingival vasculature worsening the local reaction against periodontal infections, supporting its role as a modifying factor rather than a causative one [133].

Moreover, smoking increases local vasoconstriction that reduces gingival blood flow and, consequently, contributes to lowered perfusion onto the periodontal sulcus/pocket [134]. Therefore, waste products are weekly turned-over and gingival crevicular fluid production decreases significantly [135–137]. As a consequence, the crevice cleanse by the gingival crevicular fluid is impaired and biofilm sediments may remain in subgingival sulcus [134,138]. Additionally, smoking derivates contribute to alter the microbiota content in Gram-positive bacterial microbiota, including periodontal pathogens [139]. Overall, less gingival redness [140] and reduced BoP in smokers are common clinical findings in gingivitis and periodontitis [141]. This suggests a suppression of the vascular response against plaque, and fewer blood vessels were found in the inflamed gums of smokers compared to non-smokers patients [142,143].

Importantly, smoking cessation significantly benefits the periodontal health by decreasing bone loss processes [117,122,128,144–148]. Furthermore, bleeding on probing increases over a 4- to 6-week period after smoking cessation [149], which also supports the notion of smoking as a true modifying factor.

The Epidemiology Department from the National Institute of Health Doctor Ricardo Jorge assessed the sociodemographic characteristics of smokers in Portugal between 1987-2014. Overall, the prevalence of smokers slightly increased from 19.9% to 20.2%. However, active smoking decreased in men (from 35.2% to 26.7%) and increased in women (from 6.0% to 14.6%) [150]. Despite the complete information about these habits in the Portuguese population, the impact on the periodontal status of those patients remains unclear.

Obesity

Obesity and overweight are non-communicable diseases that affect 39% of the adults population worldwide [151]. Obesity via body mass index (BMI) has been consistently associated with periodontitis, in both pre-clinical models [152] and clinical studies [102,153–156]. Consistently, subjects with normal weight and regular physical activity were associated with low prevalence of periodontitis [157–160]. The link between obesity and periodontal disease is established under multiple proposed pathways: 1) visceral adipose tissue secretes inflammatory mediators that negatively mediate endotoxin-induced injury in the periodontium [161–166]; 2) increased periodontal pathogens counts in obese

subjects [167]; 3) diabetic and obese patients have reduced insulin resistance and increased production and accumulation of advanced glycation end-products in the periodontium, promoting periodontal tissue destruction [168–170]; 4) the secretion of reactive oxygen species from adipose tissue and liver can contribute to the progression of periodontitis [171–173]; or 5) the impact of leptin (secreted from the adipose tissue) impairs immune functions of obese patients, and consequently modulates the pathogenesis of chronic inflammation [174–176]. Figure 1.4 is a proposed model that describes the signaling pathways known so far that may support the relationship between obesity and periodontitis.

Despite several explored theories, this link is not fully comprehensive, though inflammation, oxidative stress, endocrine impairment and bacterial dissemination may have decisive roles.

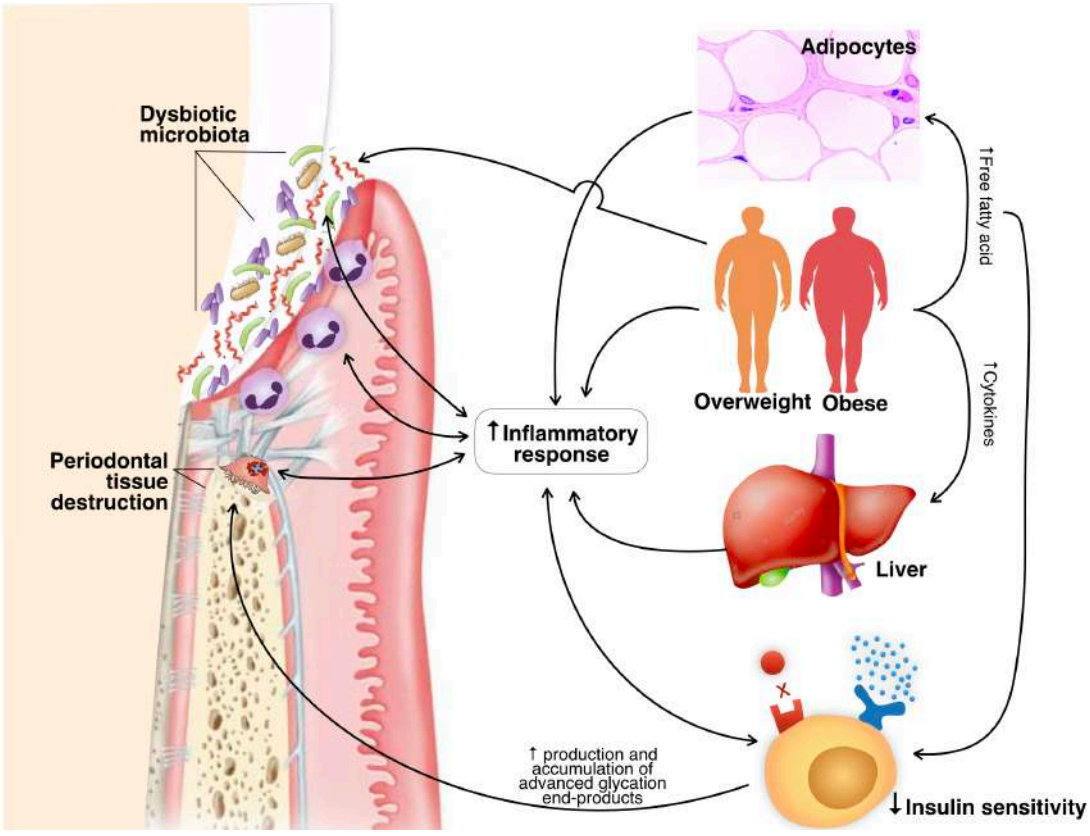

Figure 1.4. Possible mechanism of signaling pathway depicting possible links among obesity, inflammation and periodontitis. (Original image).

Psychosocial factors

Under the rationale that health depends upon physical, mental and social well-being [5], numerous studies had demonstrated that different psychosocial

factors, such as stress, anxiety, depression, negative life events, loneliness, occupational stress, daily strain, coping behaviors, and life satisfaction, were related with periodontitis [46,100,177,178].

The biological mechanism on the association between periodontitis and stress-related disorders is complex. On the one hand, neuroendocrine-derived peptides and hormones can modulate the immune system [177], by reducing serum circulating lymphocytes [179], increasing levels of systemic inflammatory burden [180,181] and cortisol levels [182]. All these factors make the patient to be more prone to periodontitis and its progression [182]. On the other hand, chronic stress and depression can modify health-related behaviors, such as oral hygiene, smoking habits and diet, increasing the susceptibility to and worsening of periodontitis [183,184].

Professional Care and Oral Hygiene

A proper professional care and appropriate oral hygiene habits are key to prevent periodontal disease. The primary prevention of periodontitis is achieved through the removal of biofilm deposits via thorough toothbrushing and interdental cleaning [18,185,186]. Also, a continuous dental care is helpful to preventing more localized plaque accumulation and inflammatory processes responsible for destroying periodontal tissue [18]. Secondary, the prevention aims at avoiding the periodontal disease recurrence in patients with reduced periodontium [18,185,186].

Despite professional care is important to secure a healthy periodontal status [99,187], home dental care alone is as effective as combining it with professional plaque removal [188]. In particular, Professional Mechanical Plaque Removal (PMPR) combined with oral health instructions (OHI) versus OHI alone resulted in equal plaque and gum bleeding control [188]. Therefore, the patients compliance with appropriate OHI is a key element for periodontal health [18,185,189–191], yet PMPR with concomitant OHI shoes benefits [188,190,192–194].

1.4. Periodontitis impact on Oral-Health Related Quality of Life

The concept of Quality of Life (QoL) refers to individuals "perceptions of their position in life in the context of culture and value systems in which they live, and in relation to their goals, expectations, standards, and concerns" [195]. Nowadays, it is recognized as valid criteria to assess subjectively patient physical and mental healthcare. Specifically, to assess QoL in oral health, the Oral-Health Related Quality of Life (OHRQoL) was one such instrument that allows to measures the patients' self-perception about functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap [196].

The dimensions of oral health have become a major focus in Periodontology [197]. In other words, self-reported measures display patients' perspective about their periodontal status, and its utility as a complement to the clinical diagnosis has been debated [197,198]. Currently evidence shows a correlation between clinically diagnosed periodontitis and deterioration of self-perceived OHRQoL in adult individuals [27,199]. In addition, severe and extensive stages of periodontitis have a more pronounced impact in the OHRQoL [27,200]. Hence, periodontitis negatively impacts OHRQoL perceptions due to the deterioration and loss of periodontal tissues [201], tooth mobility and in ultimately tooth loss [27,202]. Furthermore, the nonsurgical periodontal treatment is effective in improving OHRQoL of adults patients in a short-term period of 3 months, in terms of function, pain and psychologically [203,204].

These findings emphasize that self-reported OHRQoL might be considered to investigate whether patients know that they suffer from periodontal disease and how it impacts on their life. Comprehensively, a better informed patient about its periodontal disease, risk factors, long-term consequences and therapy options contribute to a successful periodontal treatment and helps attaining patient's needs and expectations [201]. To the best of our knowledge, research in this area is scarce and has never been conducted in Portuguese patients suffering from periodontal disease.

1.5. Epidemiological periodontal data in adults

Periodontal disease is undoubtedly one of the major global public health problems [205,206]. Overall, severe periodontitis affects 5% to 20% of the

worldwide population, while mild to moderate periodontitis affects the majority of the adult population [35,47,206–211].

In the NHANES 2009-2012, in the United States of America (USA), 25.3% to 48.7% of the adult population (aged between 30 and 64 years) were diagnosed with periodontal disease [47].

In Europe, a number of studies have been conducted concerning the periodontal state of adult population. In the Norwegian Circumpolar Communities, 16.1%, to 75.9% of papulation aged 20-64 were diagnosed with periodontitis according to Central for Disease Control and Prevention (CDC)/AAP periodontal case definition.

In the Eastern region of Germany, the prevalence of moderate periodontitis ranged between 11.5% to 42,7% in adults aged 20-59 years old [35]. Women were diagnosed with less cases of moderate periodontitis until 50-59 years old, and at that age women had more prevalence of moderate periodontitis than men (46.5% and 38.8%, respectively) [35]. Nevertheless, men presented higher percentage of severe periodontitis than women in all adult ages [35].

A cross-sectional study in Turin (Italy) based on the CDC/AAP case definition, also revealed a disturbing prevalence of moderate periodontitis from 34.2 to 35.0% in patients with 20 to 59 years old. Interestingly, more than 50% of participants aged 50-59 years were diagnosed with severe periodontitis [207]. Moreover, a national study in France, with participants aged 35-64 years, the prevalence of mild, moderate and severe periodontitis were 78%, 18% and 4%, respectively [212].

Nowadays, there is a scarcity of periodontal epidemiological data for the Portuguese adult population [213,214]. A single national epidemiological study was conducted by the Portuguese Health General Directorate in 2015 [214]. The prevalence of periodontal disease prevalence was 10.8% in adults (18-64 years old) [214]. Despite the clearly optimistic, this result is unlikely because in others European developed countries the prevalence ranged between 34.2 to 75.9% [207,208,215,216]. In this epidemiological survey, the Community Periodontal Index of Treatment Needs (CPITN) was used to evaluate the periodontal clinical characteristics of subjects aged 18, 35-44 and 65-74 years old. This partial recording protocol may explain the low prevalence observed, however reassessing the prevalence of periodontitis in a Portuguese adult population using a full-mouth recording protocol would be of interest. Furthermore,

studying the bias magnitude, sensibility and specificity of the partial-mouth recording protocols [217–219] used in this national study must aid us understanding the accuracy of these results.

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CHAPTER

2

Aims

In the Introduction section, a literature review highlighted the main risk factors and indicators for the onset and progression of periodontal disease. It was also emphasized the lack of epidemiological surveys on periodontal disease and its link with sociodemographic characteristics, habits and systemic health in the Portuguese adult population. This epidemiological characterization will aid specific preventive measures, diagnostic and therapeutic strategies for this specific population.

Therefore, the main aim of this thesis was to assess the prevalence, severity and extent of periodontal disease using a large-based epidemiologic survey of adults from the southern region of the Lisbon Metropolitan Area. A second aim was to appraise the impact of socioeconomic factors, behavioral factors, demands of dental care and the periodontal condition on OHRQoL.

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

STAGE 1. Studies prior to the large epidemiological survey

1.1. To assess the prevalence and extent of chronic periodontitis and its risk factors in patients from the Egas Moniz Dental Clinic forwarded to periodontal examination and the bias effect of partial recordings protocols in periodontal epidemiological surveys (Papers 1 and 2);

1.2. To perform a systematic review exploring the association between salivary cortisol levels and periodontitis (Paper 3);

1.3. To evaluate the influence of defined risk factors that may affect the efficacy of nonsurgical periodontal treatment (Paper 4).

STAGE 2. Large epidemiologic survey and associated factors

2.1. To conduct a large-based epidemiologic survey assessing the periodontal status of adults from the health centers of the Almada-Seixal Group of Health Centers (ACES) and its association with sociodemographic and medical conditions (Paper 5);

2.2. To employ Andersen's behavioral model to examine the direct and indirect factors associated with periodontal condition (sociodemographic characteristics, oral health behaviors and oral health) that interfere with OHRQoL (Paper 6);

2.3. To analyse the psychometric properties of the Portuguese version of the Brief-Illness Perception Questionnaire (Brief-IPQ) in patients with gingivitis

and periodontitis that were surveyed in a population-based epidemiologic study (Paper 7).

2.4. To investigate the association of self-reported bruxism and the periodontal status (Paper 8).

CHAPTER

3

An initial cross-sectional study on the prevalence and extent of periodontal diseases, and potential bias of partial recorded protocols

This chapter was based from the published work:

Paper I - 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. *Peer J* **2018** 6:e5258.

Paper II - 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(3), 145-153.

Paper III - 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.

Paper IV - 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**, 33, e081.

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3.1. The importance of pilot studies

Pilot studies or feasibility studies are small-scale and preliminary investigations which aim to assess whether crucial components of the main study will be feasible [1]. Furthermore, a pilot study can also be a development, pre-testing and/or "trying out" of a particular research instrument [2]. They may be useful in an attempt to predict issues, appropriate research protocols and facilitate the use of assessment tools. Consequently, if necessary, appropriate, realistic and workable methods can be added or removed to enrich and improve upon the various aspect before the main survey is conducted. In the words of De Vaus [3] "Do not take the risk. Pilot test first.".

Well-designed and well-conducted pilot studies can be "time-consuming, frustrating, and fraught with unanticipated problems, but it is better to ... deal with them before investing a great deal of time, money, and effort in the full study" [4]. Therefore, prior to the large-scale study, we conducted and published four preliminary studies in a Portuguese subpopulation of the Egas Moniz Dental Clinic (EMDC) [5–8] that allowed us to develop the research question, the research plan, and the questionnaire and clinical observation assessment tools.

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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 [26], 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 [27]: 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)

Variable		n (%)
	1 time/daily	75 (18.5)
	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

3. An initial cross-sectional study on the prevalence and extent of periodontal diseases, and potential bias of partial recorded protocols

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

Predictor variables		OR (95% CI)	p
Dental floss use	Yes	1	-
	No	1.66 (0.97-2.82)	0.063
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

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.

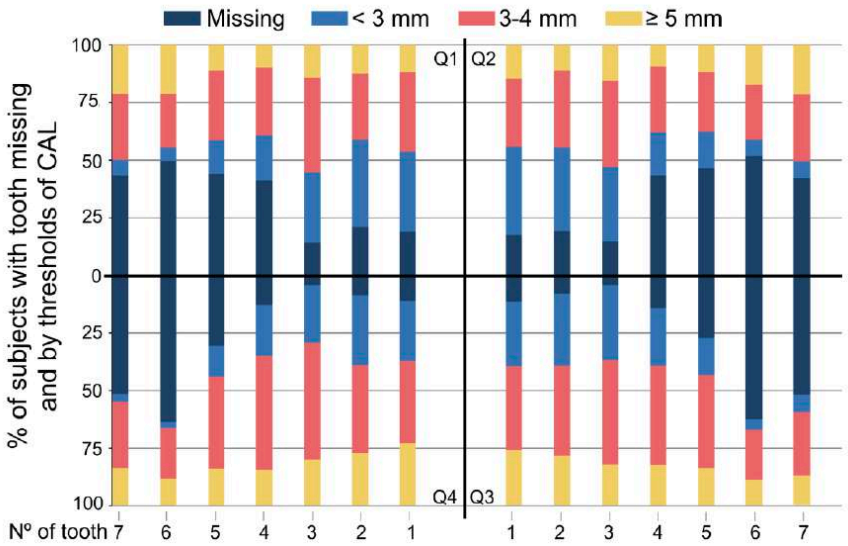

Figure 3.2.1. Percentage of subjects with the respective tooth present and by thresholds of CAL (mm), at each specific position, for all teeth in all quadrants. The black lines indicate the separation by each quadrant. Dark blue, percentage of missing teeth; Blue, percentage of teeth with than <3 mm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

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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).

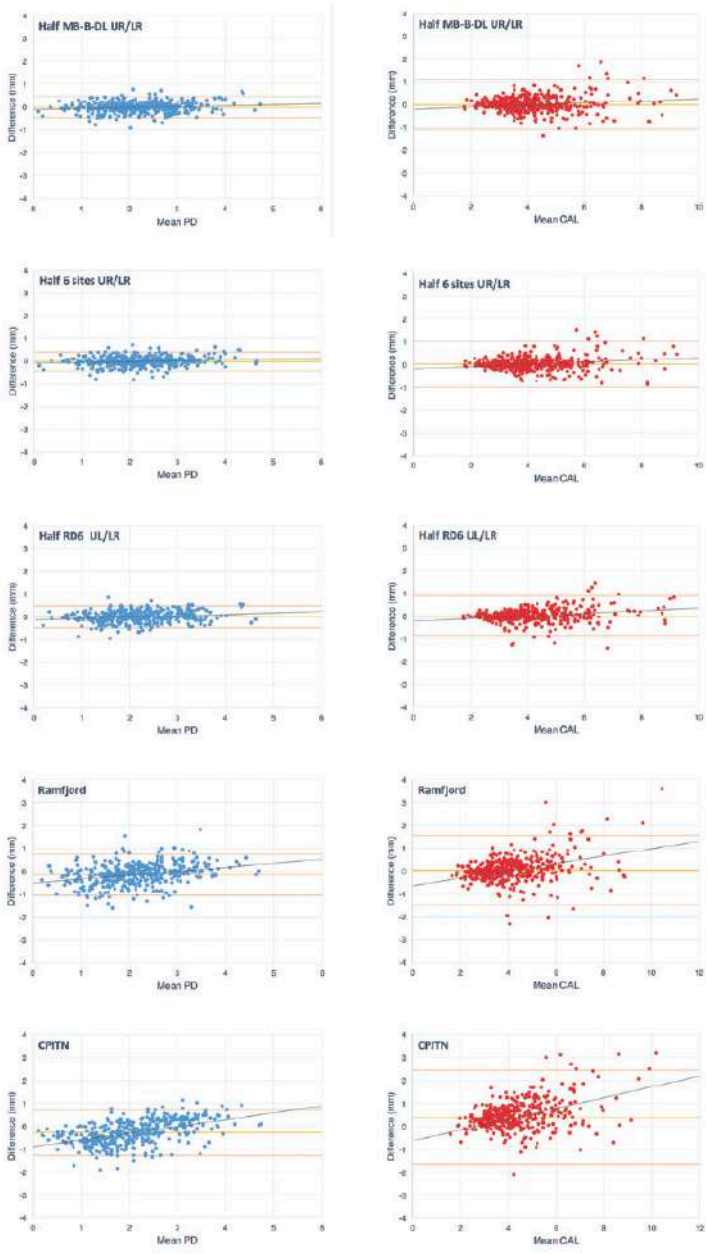

Figure 3.3.1. Bland-Altman plots to evaluate bias between the Mean PD (MPD) and Mean CAL (MCAL) differences for Half MB-B-DL UR/LR, Half 6 sites UR/LR, Half RD6 UL/LR, Ramfjord and CPITN. The area within the upper and lower orange lines sets 95% confidence interval and the yellow line the mean value.

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

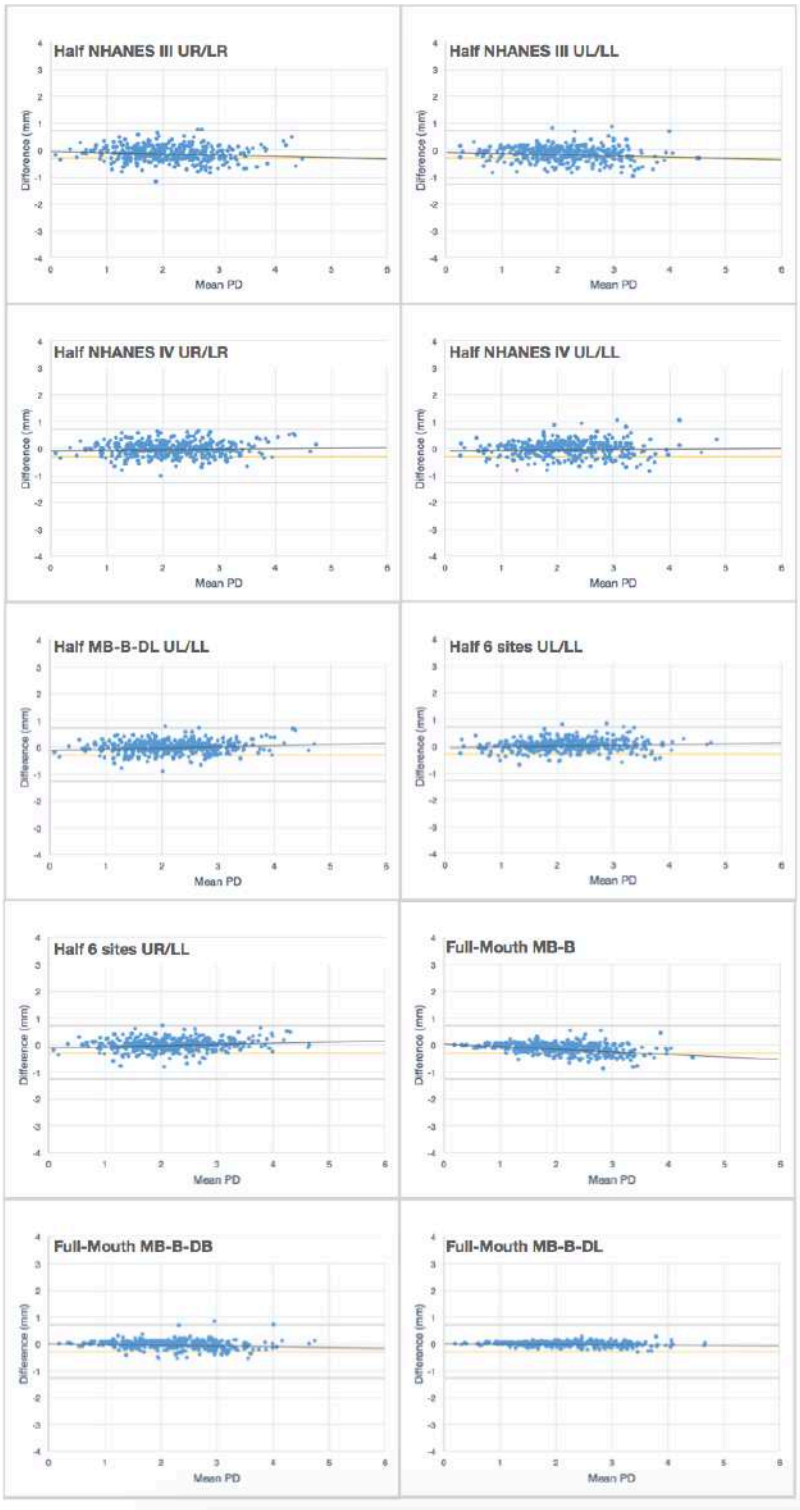

Figure S3.3.9. Bland-Altman plots to evaluate bias between the Mean PD (MPD) differences for Half NHANES III UR/LR, Half NHANES III UL/LL, Half NHANES IV UR/LR, Half NHANES IV UL/LL, Half MB-B-DL UL/LL, Half 6 sites UL/LL, Half RD6 UR/LL, Full-Mouth MB, Full-Mouth MB-B-DB and Full-Mouth MB-B-DL. The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

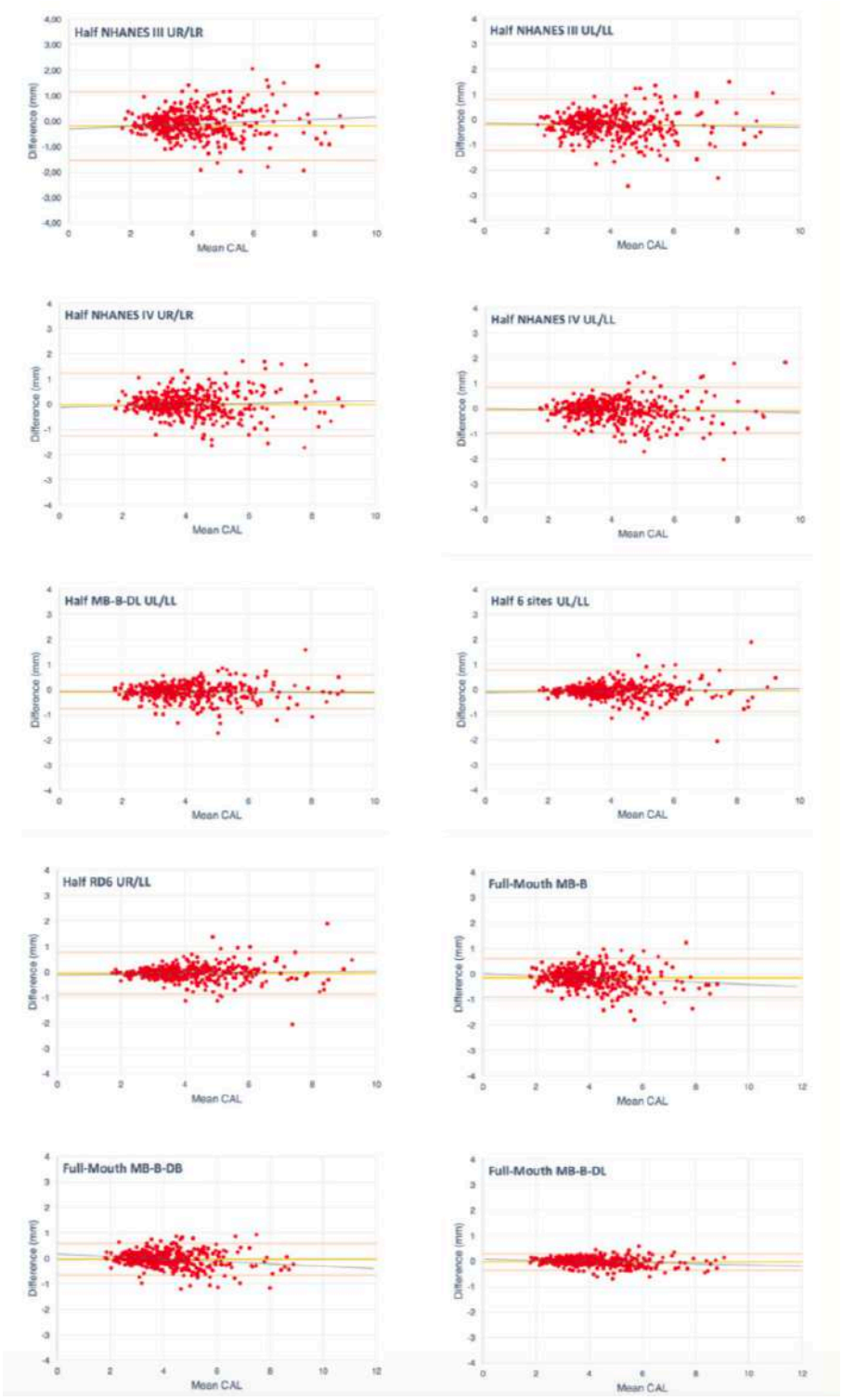

Figure S3.3.10. Bland-Altman plots to evaluate bias between the Mean CAL (MCAL) differences for Half NHANES III UR/LR, Half NHANES III UL/LL, Half NHANES IV UR/LR, Half NHANES IV UL/LL, Half MB-B-DL UL/LL, Half 6 sites UL/LL, Half RD6 UR/LL, Full-Mouth MB, Full-Mouth MB-B-DB and Full-Mouth MB-B-DL. The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

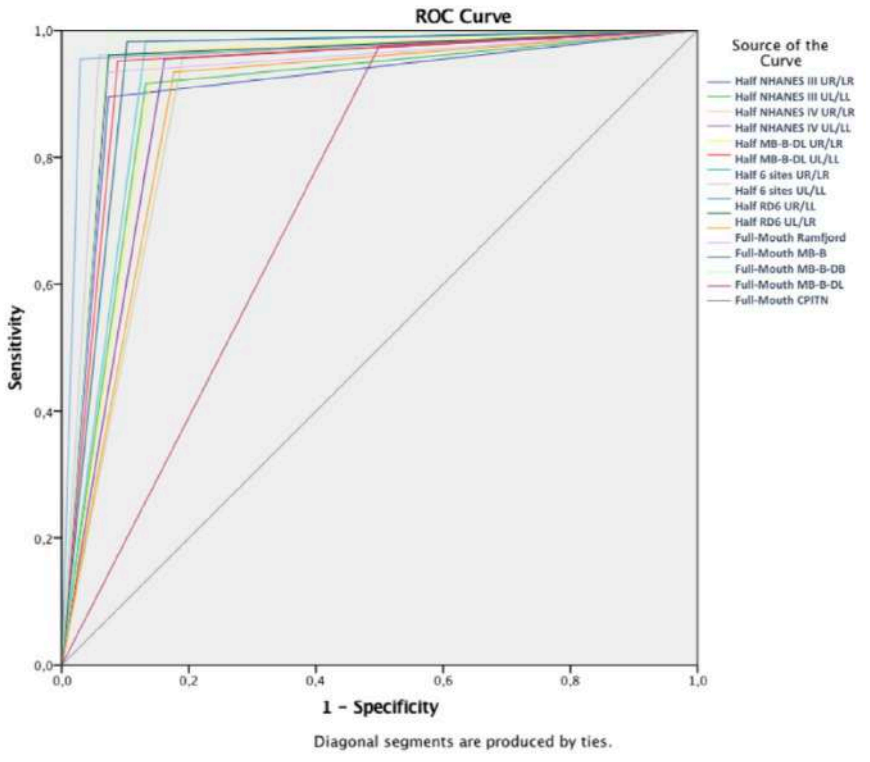

Figure S3.3.11. ROC curves for markers PRPs in discriminating patients with periodontal disease from healthy subject.

3.4. 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.

3.4.1. 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.

3.4.2. 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).

3.4.3. Results

Study selection

A total of 3677 records were identified through the electronic and manual searches, respectively (Fig. 3.4.1). 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.

Study characteristics

The characteristics of the included studies are shown in Table 3.4.1. 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 [299] to 171 participants [304]. 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.

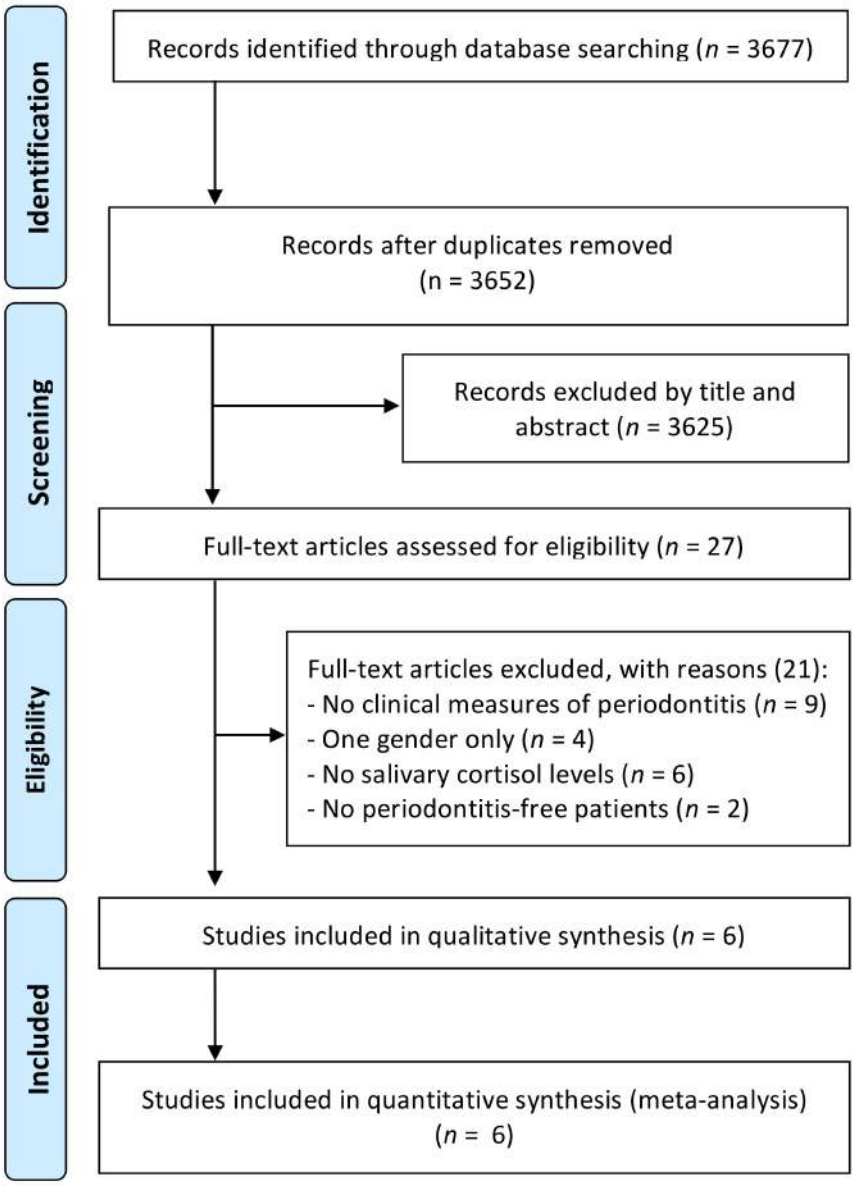

Figure 3.4.1. PRISMA flow-chart that depicts the results of the workflow to identify eligible studies.

Table 3.4.1. 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 3.4.2 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 3.4.2. 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%) [299–301], limited adequate adjustment for its analysis (n = 4, 66.67%) [299–301,304], 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 3.4.2, Fig. 3.4.2). All those studies provided data for the CP salivary cortisol response group assessment, while three [301–303] 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. 3.4.3) 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 S3.4.2). 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. 3.4.4).

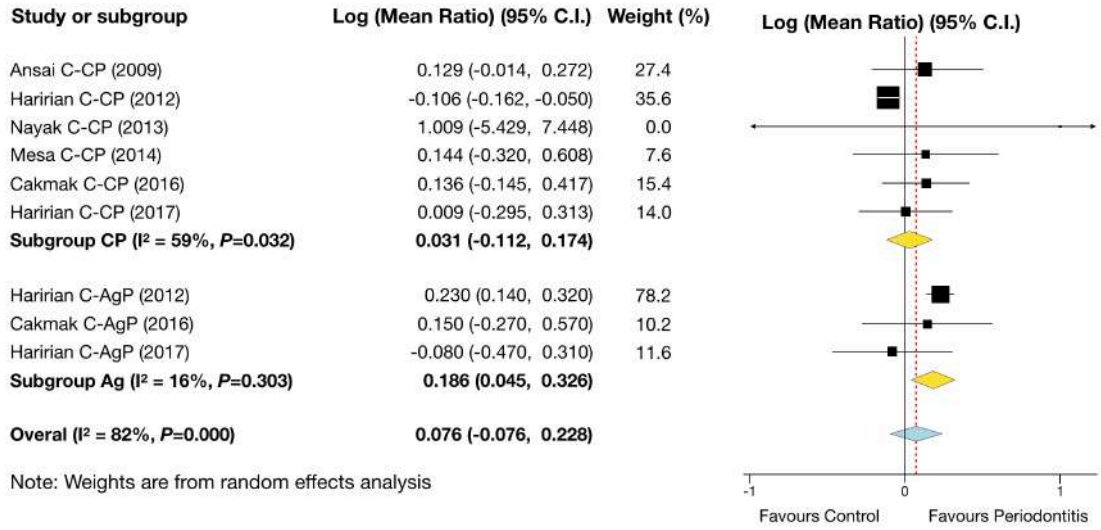

Figure 3.4.2. Subgroup forest plot of studies measuring chronic or aggressive periodontitis effect on salivary cortisol. Studies have been grouped according to the periodontitis type: chronic or aggressive. Logarithm of mean ratio (Log Mean Ratio) effect size estimates have been calculated with 95% confidence 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.

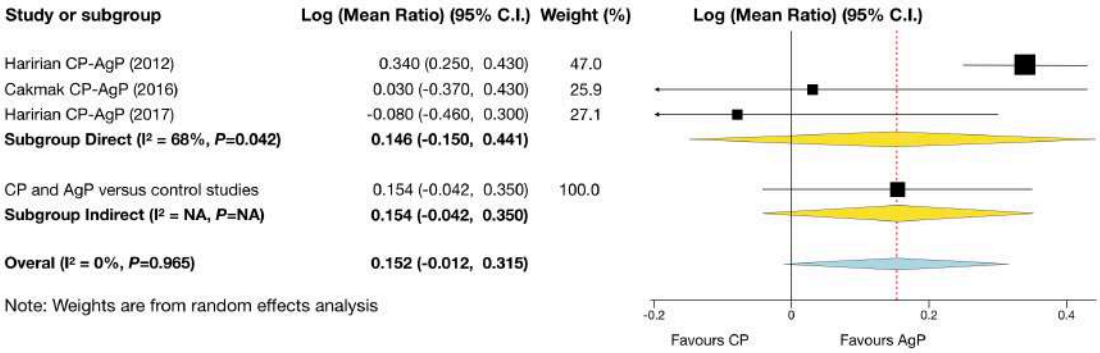

Fig. 3.4.3. Subgroup forest plot of studies comparing between chronic and aggressive periodontitis effect on salivary cortisol. Studies have been grouped according to the estimation type: direct or indirect, in a network adjusted meta-

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.

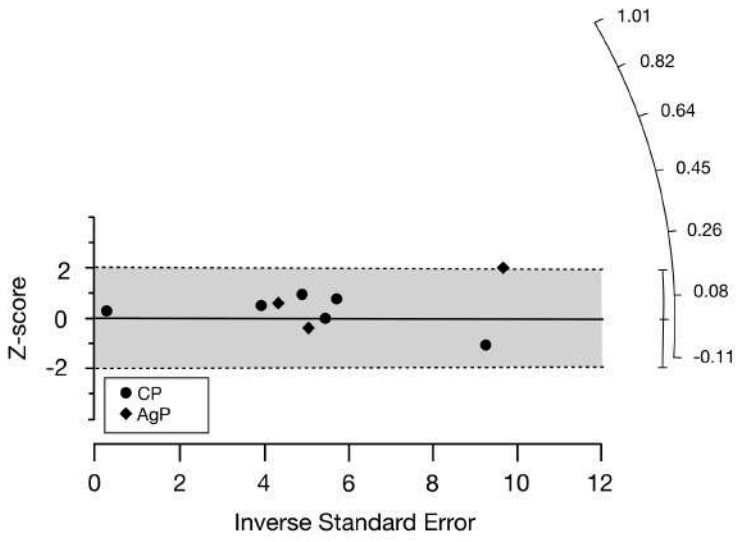

Fig. 3.4.4. Radial (Galbraith) plot for exploring the sources of heterogeneity on salivary cortisol levels according to periodontal loss levels. The slope of the central line represents the overall effect. 95% of studies are expected to lie within the area between the upper and lower lines.

3.4.4. 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.

3.4.5. 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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3.4.7. Supplementary material

Table S3.4.1. 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 S3.4.2. 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).

3.5. Fine-tuning multilevel modeling of risk factors 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.

3.5.1. 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 3.5.1). 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.

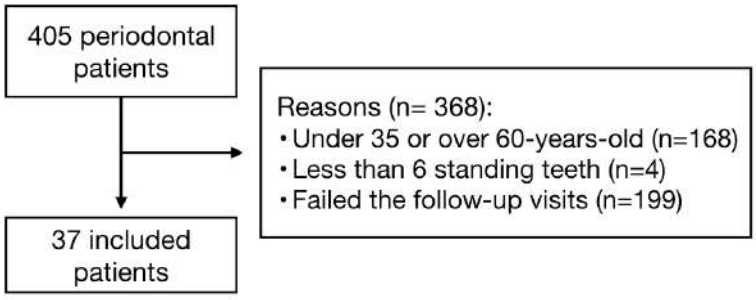

Figure 3.5.1. Flowchart of the included patients and reasons for exclusion.

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 3.5.1).

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 3.5.1. 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.

3.5.3. Results

This clinical study investigated a total of 37 patients. The baseline clinical and periodontal parameters are shown in Table 3.5.2. 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 3.5.2).

Table 3.5.2. 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)
Age (years)	57.92 (10.87)
BMI (kg/m²)	26.69 (3.97)
Number of missing teeth (N)	7.24 (5.00)
% of sites with plaque at baseline	31.64 (20.43)
% of sites with plaque at 3-month follow-up	21.20 (15.11)
% of sites with plaque at 6-month follow-up	21.02 (13.75)
%of sites with plaque at 12-month Follow-up	20.60 (10.82)
% of sites with BOP at baseline	10.56 (13.03)
% of sites with BOP at 3-month follow-up	4.04 (5.81)
% of sites with BOP at 6-month follow-up	4.94 (5.70)
% of sites with BOP at 12-month follow-up	4.10 (5.48)
% of sites with PD ≥ 5 mm at baseline	8.18 (9.25)
Patient level (n = 37)	N (%)
Sex	
Male	24 (64.86%)
Female	13 (35.14%)
Education	
Elementary School	21 (56.76%)
High School	8 (21.62%)
Higher	8 (21.62%)
Hypertension	
Yes	17 (45.95%)
No	20 (54.05%)
Diabetes	
Yes	11 (29.73%)
No	26 (70.27%)
Smokers	
Yes	7 (18.92%)
Former smokers	0 (0.00%)
No	30 (81.08%)
Tooth level (N = 758)	
Tooth position	
Anterior	366 (48.28%)
Premolar	221 (29.16%)
Molar	171 (22.56%)
Mobility	
No mobility	574 (75.73%)
Mobility ≤ 1 mm	114 (15.04%)
1 mm < mobility ≤ 2 mm	64 (8.44%)
Mobility > 2 mm	6 (0.79%)
FI (first premolars and molars) (n = 122)	
No involvement	209 (91.14%)
Degree I	9 (4.05%)
Degree II	2 (0.90%)
Degree III	2 (0.90%)
Site level (N = 1416)	
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 3.5.3). 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 3.5.3. 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	*< 0.001	
Tooth (level 2)	0.280 (14.0%)	0.047		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	*< 0.001	
Tooth (level 2)	0.276 (14.6%)	0.046		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 3.5.1). 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 3.5.4.

Table 3.5.4. 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 3.5.4). 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 3.5.4). 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 ($p = 0.034$).

3.5.4. 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.

3.5.5. 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.

3.5.6. References

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CHAPTER

4

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

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Study of Periodontal Health in Almada-Seixal (SoPHiAS): a cross-sectional study 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)

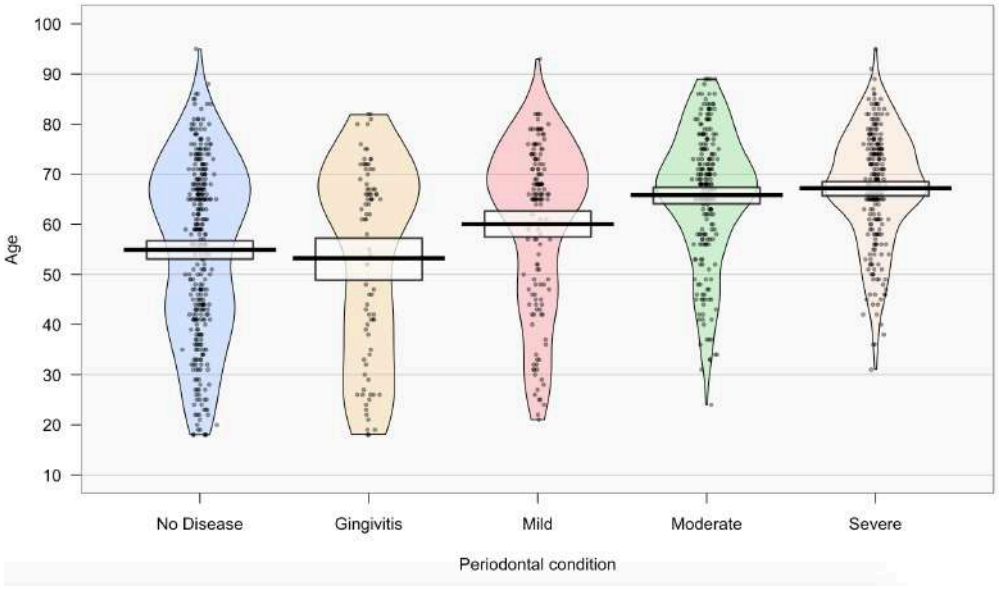

Figure 4.1. Density plot exhibiting the distribution of periodontal conditions over the age range.

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).

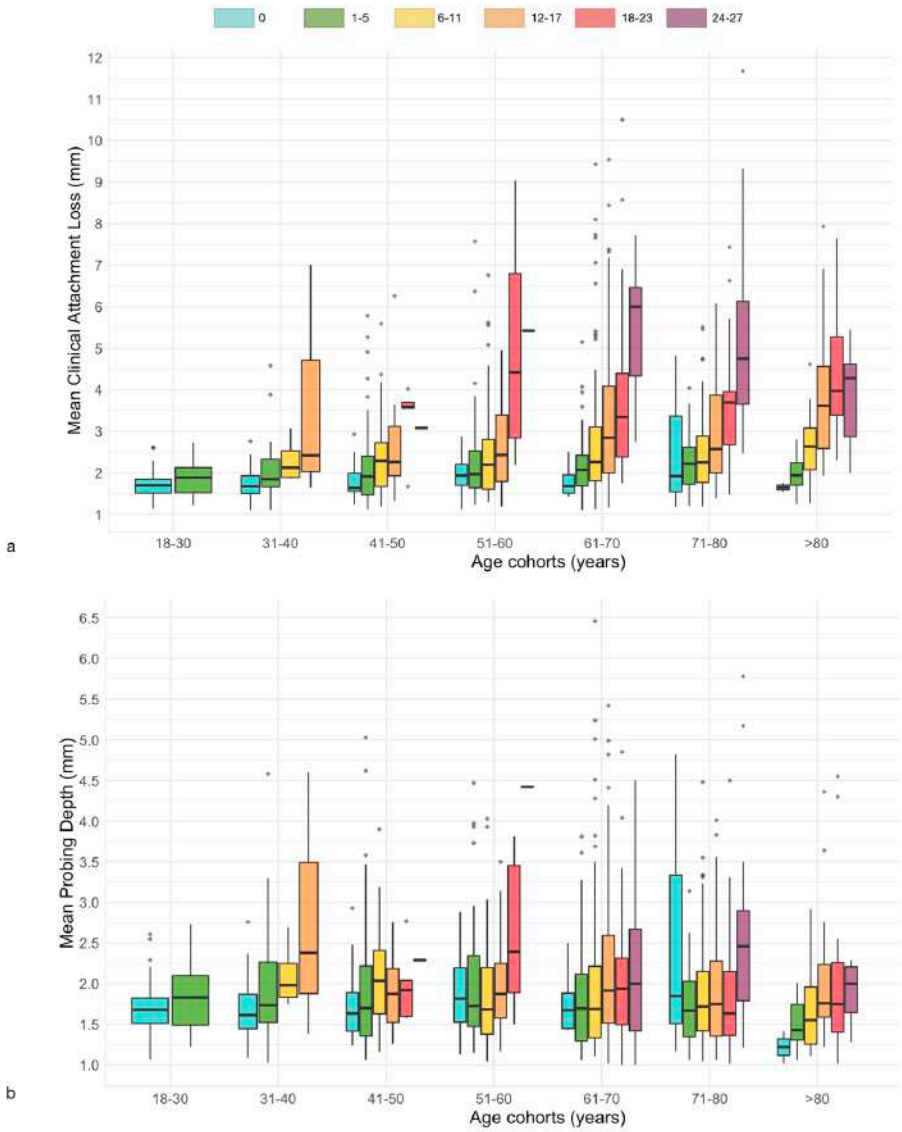

Figure 4.2. Distribution of tooth loss (coloring) as a function of mean clinical attachment loss (a) or mean probing depth (b), according to age cohorts (x-axis).

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 ($p<0.05$).

CHAPTER

5 Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: a structural equation modelling analysis

This chapter was based from the published work:

Paper VI - Vanessa Machado, João Botelho, Luís Proença, Ricardo Alves, Maria João Oliveira, Luís Amaro, Artur Águas, José João Mendes. Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: a structural equation modelling analysis. *BMC Oral Health* **2020** 20(229), 1-11.

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Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: a structural equation modelling analysis

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Abstract

Background**.** To determine if periodontal risk assessment (PRA), the number of missing teeth, diabetes mellitus (DM), perceived stress and interproximal cleaning are associated with oral health-related quality of life (OHRQoL), using Andersen's behavioral modelling (ABM).

Material and methods. Data derived from 472 adults derived from a representative population of the Study of Periodontal Health in Almada-Seixal (SoPHiAS) was used. Socioeconomic status, perceived stress scale (PSS-10), oral health behaviors and oral health impact profile (OHIP-14) were collected through questionnaire. Periodontal conditions were assessed with a full-mouth periodontal examination. PRA was computed through behavioral and clinical information. Variables were grouped into Predisposing Factors, Enabling, Need, Oral Health Behaviors and Perceived Health Outcome latent variables. Confirmatory factor analysis, structural ABM and model fitness were conducted.

Results. ABM applied to OHIP-14 showed acceptable model fit (χ^2=2.75, CFI=0.92, TLI=0.90, RMSEA=0.05, CI 90% [0.04- 0.07]). The average of OHRQoL was 9.5±11.3. Patient with periodontitis and with a high number of missing teeth experienced worse OHRQoL. Uncontrolled DM participants had more periodontal treatment necessity and poorer OHRQoL. Characteristic like aging and lower levels of education were directly associated with better OHRQoL, but in indirect path the OHRQoL was diminishes. Good oral hygiene and preventative measures were associated to lower periodontal treatment necessity. Lower periodontal treatment necessity was associated to higher OHRQoL. Age, tooth loss and interproximal cleaning were the most associated items to Predisposing, Need and Oral Health Behaviors, respectively.

Conclusion. ABM confirmed age, number of missing teeth, DM, interproximal cleaning and perceived stress as associated factors for OHRQoL. Uncontrolled DM was associated to higher Need and poorer OHRQoL. Good oral hygiene habits promote a healthy periodontium and, consequently, increases OHRQoL.

5.1. Introduction

Periodontal diseases (PD) are one of the major global public health problems [1]. Globally, adult populations suffer from mild to moderate periodontitis, while severe periodontitis prevalence range from 5-20 % [2–9]. Consequently, the economic burden of PDs was estimated to be profoundly impactful globally, with over fifty billion dollars in indirect costs due to severe periodontitis [10,11].

Over the past decades, several risk factors have been implicated in the onset and progression of PD such as age, gender, socioeconomic status, low education levels [12–15], diabetes mellitus (DM) [16], smoking and oral hygiene habits [17–20] and psychosocial factors, in particular stress [21,22]. Thereupon, the impact of PD on oral health-related quality of life (OHRQoL) became an important research matter. Many lines of evidence have proven that the worsening and extent of PD is very deleterious towards OHRQoL [23–27], though the treatment of PD can restore good OHRQoL levels [28]. Also, lifestyle habits and awareness towards periodontitis are strongly related to oral health behaviors [29]. Therefore, and considering the complexity of factors related to PD, the implementation of holistic periodontal risk network analyses has been gaining preponderance.

Currently, structural equation modelling (SEM) is a very popular strategy to investigate direct and indirect associations between several contributing factors [30]. Previously, SEM has been employed to assess the relationship of PD with anxiety and depression [31], fear of pain, dental fear and OHRQoL [32,33], and chronic systemic diseases [34–36].

One of the best known SEM approaches is Andersen's behavioral modelling (ABM), used to investigate the factors that interfere with the access to medical care [37] (Figure 5.1). In detail, ABM was initially developed to offer a scientific understanding under a complex structure including health outcomes and their social, behavioural and attitudinal determinants towards the use of health services [37]. In a subsequent investigations, ABM has been employed in dental care and oral health outcomes using the cost of treatment and key psychosocial factors [33,38,39], revealing a particular importance for OHRQoL [33,38]. Nevertheless, no study has introduced other relevant variables in an ABM approach in adults, such as the number of missing teeth, Periodontal Risk Assessment (PRA), periodontal diagnosis according to American Academy of Periodontology (AAP)/European Federation of Periodontology (EFP), DM, interproximal cleaning and self-perceived stress.

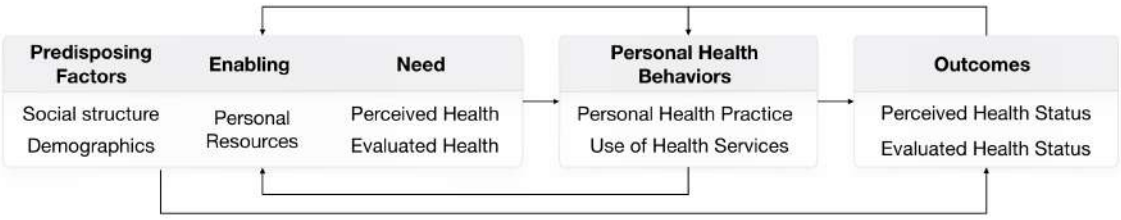

Figure 5.1. Model of health services' use and health outcomes based on Andersen's behavioural model (1995).

Therefore, we aimed to investigate whether the number of missing teeth, PRA, DM, interproximal cleaning and self-perceived stress are relevant factors towards OHRQoL through ABM, in the adult population of the Study of Periodontal Health in Almada-Seixal (SoPHiAS) survey.

5.2. Materials and Methods

Ethics and Study Design

The SoPHiAS is a cross-sectional representative study in the municipalities of Almada-Seixal, Portugal [12]. This study was approved by the Research Ethics Committee of the Regional Health Administration of Lisbon and Tagus Valley, IP (Portugal) (Approval numbers: Process 3525/CES/2018 and 8696/CES/2018) [12]. Informed consent was written obtained from all participants prior to commencement. This survey followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [40].

Setting

Sample size estimation and Measurement reproducibility

The sampling strategy and measurement reproducibility is available in Botelho and Machado el al. [12]. The estimated minimum sample size for the periodontitis prevalence in the Portuguese adult population, with a margin of error of 3.0%, for a 95% confidence level, was 412 individuals, based on the previously reported national prevalence data of 10.8% [41]. The required sample was stratified according to the number of adult (age group from 18–64 years) subjects assigned to each Family Health Units (FHU).

For the periodontal diagnosis, measures were performed by two trained and calibrated examiners (V.M. and J.B.). The inter-examiner correlation coefficients were 0.98 and 0.99, for clinical attachment loss (CAL) and periodontal pocket depth (PPD), respectively. The intra-examiner ICC ranged from 0.97 to 0.99, for both PD and CAL.

Periodontal Examination

We performed a full-mouth circumferential periodontal inspection with a manual periodontal North Carolina probe (Hu-Friedy® Manufacturing Inc.) at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual). Thrid molars and implants were excluded from the analysis. PPD was measured as the distance from the free gingival margin to the bottom of the pocket and gingival recession (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 PPD measurements for each site. Bleeding on probing (BoP) was used to evaluated the clinical periodontal inflammation and stability [42]. No radiographic examination was performed.

Gingivitis cases were defined according to Trombelli et al. [43] and periodontitis disease severity and extent according to Tonetti et al. [44]. At the end of the examination, participants were informed about their periodontal status. Patients diagnosed with periodontal disease were referred to the Egas Moniz Dental Clinic (EMDC) for its treatment without additional costs.

Participants

The participants of this study derive from SoPHiAS study. The exclusion criteria were participants: edentulous and 65 years old or older. From a total of 1,064 subjects, a subset of 472 adults were included.

Selection of variables

The five proposed latent variables were selected according to ABM [37] and we take into consideration three previous studies [33,38,39]. We included in the analysis: 1) Predisposing Factors; 2) Enabling; 3) Need; 4) Oral Health Behaviors; and 5) Perceived Health Outcome.

Predisposing Factors

Among the predisposing factors, age educational level, occupation, and marital status constituted the social structure elements. Age was evaluated as a continuous variable. Education was categorized according to the 2011 International Standard Classification of Education (ISCED-2011) [45], and were coded as: Elementary (ISCED 0-1 levels) = 1, Lower secondary education to Doctoral or equivalent level (ISCED 2–8 levels) = 0. Occupation of each participant was classified as: student (code = 0), employed (code = 1), unemployed (code = 2) or retired (code = 3). Marital status was defined as: single (code = 0), married/union of fact (code = 1), divorced (code = 2) or widowed (code = 3).

Enabling

We included household monthly income (in euros), and the Portuguese version of the Perceived Stress Scale (PSS) as two items: positive factor and negative factor [46]. The PSS-10 was a 10-item tool that assesses self-perceived stress [46]. Each item was rated on a 5-point Likert scale (coded never = 0, almost ever = 1, sometimes = 2, fairly often = 3 and very often = 4). The PSS-10 was divided in two domains: six positive (items 1, 2, 3, 6, 9 and 10) and four negative (items 4, 5, 7 and 8, that require reversion) worded items.

Need

Need were represented by the number of missing teeth; PRA (coded low risk = 0; moderate risk = 1; higher risk =2) [47]; periodontitis extent (coded non-periodontitis = 0; localized periodontitis [<30% of teeth involved] = 1; generalized periodontitis [≥30% of teeth involved] = 2) [44]; periodontitis staging (coded no-periodontitis = 0; gingivitis = 1; mild [Stage 1] = 2, moderate [Stage 2] = 3, and severe [Stage 3 and Stage 4] = 4) [43,44]; BoP [42]; denture stability (coded no denture = 0; stable denture = 1; unstable denture = 2); and DM was confirmed using medical records and through the hemoglobin A1c (HbA1c) (coded according to WHO criteria [48]: non-DM = 0; controlled DM (HbA1c < 6.5) = 1; uncontrolled DM (HbA1c ≥ 6.5) = 2).

Oral Health Behaviors

The participants' oral health behavior determinants and use of dental services were measured with the frequency of toothbrushing, used of interproximal cleaning and last dental attendance. For toothbrushing habits, we questioned "How often do you clean your teeth a day?" (coded one or less a day = 0, twice a day = 1, and more than twice a day = 2). For interproximal cleaning, we questioned "Do you regularly perform flossing or interdental brushing?" (coded no = 0, occasionally = 1, yes = 2). Dental attendance orientation was assessed in response to "When was your last visit to the dentist?" (coded more than 12 months = 0, 6 to 12 months = 1, less than 6 months = 2).

Perceived Health Outcome

OHRQoL was measured using the short-form oral health impact profile (OHIP-14) validated for Portuguese [49]. OHIP-14 assess fourteen items, each of the items

rated on a 5-point ordinal scale (never = 0, hardly ever = 1, occasionally = 2, fairly often = 3 and very often = 4) [50]. As previously divided for SEM analysis [33,38], OHRQoL was set in three major indicators – physical (items 1, 2, 3, 4, 5 and 10 were summed), psychological (items 6, 7, 8 and 9 were summed) and social impacts (items 11, 12, 13 and 14 were summed).

Data Analysis

Data were analysed using the IBM® SPSS® Statistics, v. 24 and AMOS 24. We started by performing an exploratory factor analysis (EFA) to reveal the underlying structure of the variables. Second, we performed a Confirmatory Factor Analysis (CFA) to identify the acceptability of the indicators within each latent construct [30]. CFA confirmed the scale items (indicators) representing each of the five constructs (Table 5.1 and Figure 5.3).

Next, we employed a SEM analysis following an ABM procedure. In accordance with the model and following [33,38], it was hypothesized that: 'predisposing factors' would predict 'enabling' and 'oral health behaviors'; both 'predisposing' and 'enabling' resources would predict 'need' and 'oral health behaviors'; 'predisposing factors', 'enabling' and 'oral health behaviors' would predict 'need' which would, in turn, predict 'perceived health outcome'. In addition, 'predisposing factors', 'enabling' and 'oral health behaviors' would predict 'perceived health outcome'. AMOS estimates the total effects, which are made up of both the direct effects (a path direct from one variable to another, e.g. predisposing factors → enabling) and indirect effects (a path mediated through other variables, e.g. predisposing factors → need via enabling). Given the presence of both non-normal and categorical data, the model was estimated using bootstrapping (n = 900+) [38]. The ML bootstrap estimates and standard errors (together with bias-corrected 90% confidence intervals [CI]) were then compared with the results from the original sample to assess the stability of parameters and test statistics [51].

As recommended [51,52], model fit was evaluated using a range of indices from three fit classes: absolute, parsimony adjusted and comparative. We considered as an acceptable model fit if: $\chi 2$/degrees of freedom (df) ratio < 3.0; Root Mean Square of Approximation (RMSEA) value < 0.06; Confirmatory Fit Index (CFI) and

Tucker Lewis index (TLI) ≥ 0.9; and a Standardized Root Mean Square Residual (SRMR) < 0.08 [52–54].

Table 5.1. Characteristics of the study variables (n=472).

	Value
Predisposing Factors	
Age, mean (SD)	46.1 (12.5)
Gender, n (%)	
Male	175 (37.1)
Female	297 (62.9)
Social structure	
Education, n (%)	
Primary school	78 (16.5)
Middle	308 (65.3)
Higher	86 (18.2)
Occupation, n (%)	
Student	19 (4.0)
Employed	284 (60.2)
Unemployed	127 (26.9)
Retired	42 (8.9)
Marital status, n (%)	
Single	145 (30.7)
Married / Union of fact	262 (55.5)
Divorced	56 (11.9)
Widowed	9 (1.9)
Enabling	
Household monthly income, mean (SD) (€)	1110.3 (790.6)
PSS 10 positive factor, mean (SD)	9.2 (6.0)
PSS 10 negative factor, mean (SD)	5.9 (3.3)
Treatment Need	
Missing teeth, mean (SD)	5.6 (5.5)
Periodontal risk assessment, n (%)	
Low	284 (60.2)

Moderate	42 (8.9)
Higher	146 (30.9)
Stages of periodontitis, n (%)	
No-periodontal disease	207 (43.9)
Gingivitis	48 (10.2)
Mild (Stage 1)	62 (13.1)
Moderate (Stage 2)	80 (16.9)
Severe (Stage 3 and 4)	75 (15.9)
Periodontitis extent, n (%)	
Localized Periodontitis	105 (22.2)
Generalized Periodontitis	112 (23.7)
Bleeding on probing (%), mean (SD)	14.0 (19.0)
Diabetes Mellitus, n (%)	
No	431 (91.3)
Yes and Hbc1A < 6.5	9 (1.9)
Yes and Hbc1A ≥ 6.5	32 (6.8)
Denture stability, n (%)	
Subjects without denture	373 (79.4)
Subjects with stable denture	87 (18.4)
Subjects with unstable denture	10 (2.1)
Personal health practice / use of dental services	
Tooth brushing, n (%)	
One or less a day	114 (24.2)
Twice a day	274 (58.1)
More than twice a day	84 (17.8)
Interproximal cleaning, n (%)	
Yes	106 (22.5)
Occasionally	64 (13.6)
No	302 (64.0)
Last dental attendance, n (%)	
< 6 months	140 (29.7)
6-12 months	67 (14.2)
> 12 months	265 (56.1)

Perceived oral outcome	
Oral health impact profile (self-reported), mean (SD)	
OHIP-14	9.5 (11.3)
OHIP -14 Physical	5.7 (5.8)
OHIP -14 Psychological	2.6 (4.0)
OHIP -14 Social	1.2 (2.8)

5.3. Results

Study Sample

All participants were recruited between December 2018 and April 2019 data. Overall, 472 participants from 18 to 64 years old were included, being mainly females (62.9% vs 37.1%), middle age (46.1 ± 12.5), presenting middle education levels (65.3%), and with low prevalence of DM (8.7%). The prevalence of periodontitis was 45.9%, of which 23.7% had generalized periodontitis and 15.9% had severe periodontitis. Indeed, the mean number of missing teeth was 5.6, and 30.9% of subjects showed a high-PRA risk. Indeed, only 20.5% had denture, of which 2.1% were unstable. Mean ± SD of OHIP-14 measured were 9.5 ± 11.3. Scale items representing each of the five constructs are detailed in Table 5.1.

Confirmatory factor analysis

The measurement model was an acceptable fit on three of the *a priori* indices (Table 2, Model 1). The correlation values within five latent variables ranged −0.43 and 0.75, exhibiting acceptable discriminant validity (i.e.<0.85) [353]. The bootstrapped standardized estimates for this five-factor measurement model can be seen in Figure 5.2.

Table 5.2. Fit indices for the measurement and structural models.

Model	χ^2/d.f.	p	RMSEA (90% CI	CFI	TLI	SRMR
1	2.77	0.00	0.06 (0.05-0.07)	0.91	0.89	0.070
2	2.75	0.00	0.05 (0.04-0.07)	0.92	0.90	0.065

Model 1 = measurement model; Model 2 = structural model; χ^2 = chi-square; d.f. = degrees of freedom; CFI = Comparative Fit Index; TLI = Tucker-Lewis Index; RMSEA = Root-Mean-Square Error of Approximation; CI = Confidence Interval; SRMR = Standardized Root Mean Square Residual. Figures in bold are those that meet the *a priori* model fitting criteria.

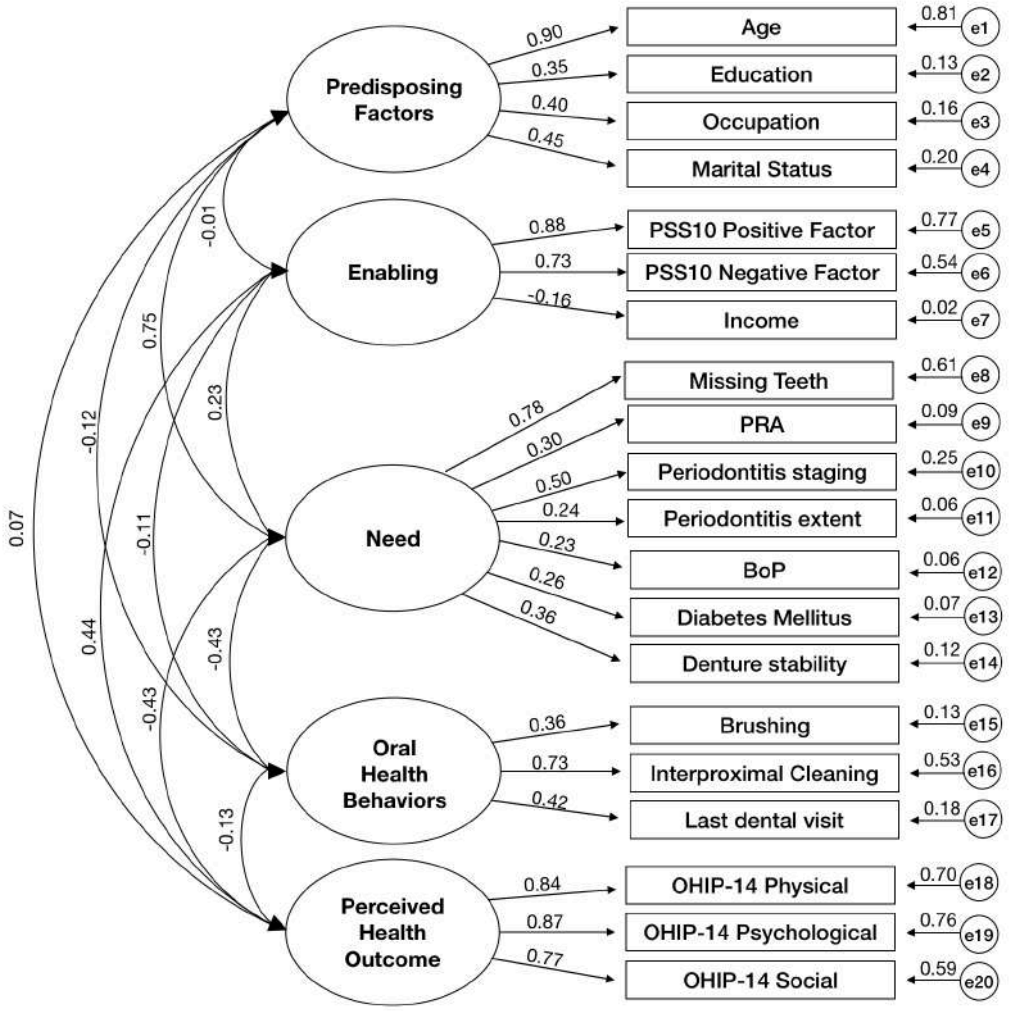

Figure 5.2. Bootstrapped ML standardized estimates for the Confirmatory Factor Analysis (CFA). All obtained effects were significant ($p < 0.001$). Factors (latent variables) are in ellipses, items (indicator variables) in rectangles and residual error terms in circles.

All item loadings were significant (<0.001) and with the expected direction. Aging, less qualifications, unemployed status and widowhood were associated with more of the 'predisposing factors'. Of these, age had the highest factor loading (0.90). Having less household income, and higher stress positive and negative factors were associated with more of the 'enabling' factors. A greater number of missing teeth, higher score of PRA, greater periodontitis severity and extent, having unstable denture and having uncontrolled DM were associated with more of 'need' factor. The most frequent brushing and flossing, and more regular visits to the the dentist were associated with higher levels of 'oral health behaviors'. The best indicator of evaluated 'need' was the missing teeth (0.78),

whilst the interproximal cleaning was the best indicator in 'oral health behaviors' (0.73). More physical, psychological and social impacts of oral health were associated with more of the 'perceived oral outcome' factor.

ABM outcomes

The model had acceptable fit to the data meeting all five of the latent variables (see Table 5.2, Model 2). Within this final model, ten paths were significant (Figure 5.3), and two hypothesized paths had no significance: 'predisposing factors' → 'enabling'; and 'predisposing factors' → 'oral health behaviors'. This ABM model revealed 69.1%, 2.7%, and 40.6% of variance for 'need', 'oral health behaviors' and 'perceived health outcome', respectively (Figure 5.3).

Direct effects

Accounting for the direct effects, six of the ten pathways hypothesized in Model 2 were significant (Table 5.3 and Figure 5.3). Less 'predisposing factors' (elder, less educated, be retired and widowed) was significantly linked to negative 'perceived health outcome' and higher 'need' (ß = -0.47 $p < 0.05$, and ß = 0.66 $p < 0.001$, respectively). Greater 'enabling' resources was associated with higher 'need' (ß = 0.18 $p < 0.05$). Greater 'oral health behaviors' was associated with lower 'need' (ß = -0.38 $p < 0.01$). A greater 'need' was associated with higher 'perceived health outcome' (ß = 0.80 $p < 0.001$). Contrary to prediction, greater 'enabling' resources was linked to higher 'oral health behaviors' (ß = -0.11 $p < 0.05$).

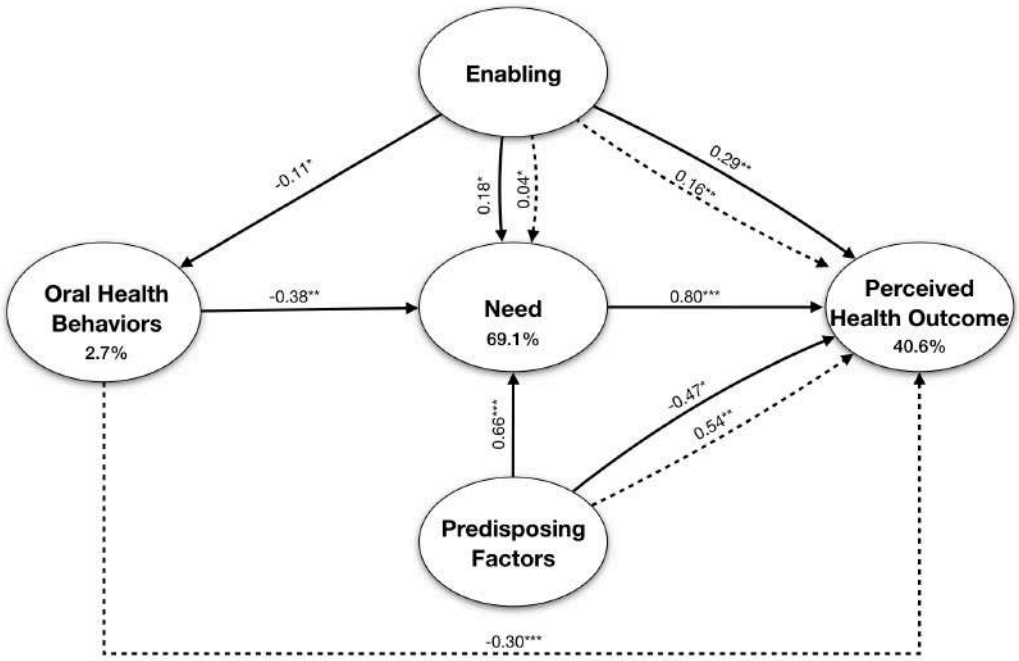

Figure 5.3. Bootstrapped ML standardized estimates for the Andersen model. *p < 0.05, ** p < 0.01, *** p < 0.001. Solid lines = direct effect; dashed lines = indirect effect.

Table 5.3. Direct effects for the Andersen model.

Effect	ß	Bootstrap SE	Bias-corrected 95% CI	% of total effect
Predisposing Factors - Enabling	-0.01	0.06	-0.11 / 0.09	100
Predisposing Factors - Oral Health Behaviors	-0.12	0.08	-0.26 / -0.01	_[a]
Predisposing Factors - Need	0.66	0.13	0.36 / 0.77 ***	94
Predisposing Factors - Perceived Oral Outcome	-0.47	0.31	-1.01 / -0.02 *	_[a]
Enabling - Need	0.18	0.01	0.04 / 0.30 *	82
Enabling - Oral Health Behaviors	-0.11	0.07	-0.22 / -0.01*	100
Enabling - Perceived Oral Outcome	0.29	0.10	0.11 / 0.42**	64
Need - Perceived Oral Outcome	0.80	0.39	0.18 / 1.40 ***	100
Oral Health Behaviors - Need	-0.38	0.08	-0.50 / -0.26 **	100
Oral Health Behaviors - Perceived Oral Outcome	0.22	0.18	-0.01 / 0.58	_[a]

ß = bootstrapped standardized estimate; SE = Standard Error; CI = Confidence Interval.
*P < 0.05, **P < 0.01. _[a]Could not be calculated because of suppression effect.

Indirect effects

There were three significant indirect paths (Table 5.4 and Figure 5.3). The path between the 'oral health behaviors' and 'perceived health outcome' was 100% indirect. In comparison, the impact of 'enabling' resources on evaluated 'need', 'enabling' resources on 'perceived health outcome', and 'predisposing factors' on 'need' were 18%, 36% and 6%, respectively.

Table 5.4. Indirect effects for the Andersen model

Effect	ß	Bootstrap SE	Bias-corrected 95% CI	% of total effect
Predisposing Factors - Oral Health Behaviors	0.01	0.01	-0.01 / 0.02	_[a]
Predisposing Factors - Need	0.04	0.03	-0.01 / 0.10	6
Predisposing Factors - Perceived Oral Outcome	0.54	0.31	0.07 / 1.01**	_[a]
Enabling - Need	0.04	0.03	0.01 / 0.10*	18
Enabling - Perceived Oral Outcome	0.16	0.09	0.02 / 0.33**	36
Oral Health Behaviors - Perceived Oral Outcome	-0.30	0.17	-0.67/ -0.09***	100

ß = bootstrapped standardized estimate; SE = standard error; CI = confidence interval.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. _[a]Could not be calculated because of suppression effect.

5.4. Discussion

The results of this study confirmed our initial hypothesis, namely the number of missing teeth, PRA, the 2018 PD case definition, DM, interproximal cleaning and self-perceived stress were significant for perceived health outcome within an ABM [37]. Therefore, we highlight new factors that may be relevant in the self-perception of oral health by adult populations. Also, we observed in this population a reduced average OHQRoL (9.5 ± 11.3), though a similar decrease was previously demonstrated in a British population [38] and also worse levels in the Tromstannen - Oral Health in Northern Norway (TOHNN) study [33].

In this context, our investigation supports the notion that oral health self-perception and their factors (both direct and indirect effects) must be analyzed in a holistic way, given the existing complex interrelationships. Comprehensively, the present findings emphasize that worse levels in the "need" latent variable (periodontitis, number of missing teeth, uncontrolled DM and

unstable denture) was linked to poorer perceived oral health outcomes. In other words, as an example, a participant with severe periodontitis and with a high number of missing teeth experienced worse OHRQoL. This influence on perceived oral health outcomes was very substantial (69.1%), and while for periodontitis and tooth loss our results are in agreement with previous evidence [25,55,56], for the remaining factors the results present novelty.

Overwhelming evidence has recognized DM as an important risk factor for PD [16,57,58]. In fact, our data showed a significant association between the DM status with periodontal health [57,58]. However, DM has never been included in ABM approaches for the purpose of studying its impact on OHRQoL, and our results highlight the role of uncontrolled DM (patients with HbA1c ≥ 6.5) for these complex interactions. Hence, further studies may consider this medical condition in future investigations.

Explaining human behavior in all its complexity is a difficult task [59], and the decision-making process is influenced by social and environmental conditions [60,61]. Onwards, our results recognize that 'predisposing factors' (age, education levels, marital status and occupation) have a profound direct influence on OHRQoL. Interestingly, characteristics like aging, lower levels of education, being retired or widower were directly associated with better perceived OHRQoL. Nevertheless, this association is considerably mediated by the 'need' latent variable, in other words, when the analysis takes into account the indirect effect of evaluated periodontal status, denture stability and DM, perception of OHRQoL by participants is affected and diminishes. This is particularly important in participants with chronic illnesses such as periodontitis because understanding and recognizing their illness is key to successful long-term periodontal maintenance and stability [62].

The majority of the elements within ABM are broadly established and overlapping [37]. Nonetheless, we added other factors into the ABM which might increase its explanatory power for OHRQoL, in particular, perceived stress into 'enabling' factor. Our results support an important role of perceived stress in perceived oral health outcomes. In other words, individuals with higher levels of perceived stress experienced worse OHRQoL, being in accordance with previous studies [22,63,64]. Furthermore, our findings suggest a negative link between 'enabling' factors (stress and income) and 'oral health behaviors'. Therefore, individuals may undergo unhealthy oral behaviors (such as poor oral hygiene

and avoiding dental appointments) because they might not be able to cope with stressful situations or they lack economic resources to do so, though this should be further developed in the future.

PD is an inflammatory condition caused mostly by the accumulation of polymicrobial biofilms and it is well established that periodontal health depends on the plaque control through appropriate toothbrushing techniques and careful interproximal cleaning [43,44,65–69]. Our results highlighted the link between oral health behaviors and periodontal status, and so, individuals with good oral hygiene and preventative measures will have better periodontal health and, consequently, better perceived OHRQoL. In the 'oral health behaviors' latent variable, we introduced interproximal cleaning to the ABM showing markedly impact. Our study is the first to introduce interproximal hygiene, and the results support the thesis that should be considered in future ABM studies since it strongly impacts on OHRQoL.

Although social status, economic resources, and individual health beliefs have been repeatedly profiled in an attempt to predict participant behaviors [59,70,71], previous efforts have focused on personal and professional bacterial removal for the treatment and control of PD [44,65,66]. The present study is one of the first to attempt to "unpack" likely key determinants of socioeconomic status and stress levels, personal oral health behaviors, periodontal extent, severity and inflammation, and oral health outcomes on OHRQoL and their interrelationships. We have demonstrated that OHRQoL related to periodontal status should not only consider plaque level but should undoubtedly encompass a holistic approach and consideration of all factors that may influence disease onset and extension [44,72].

Our results indicate that four out of ten adults had some type of PD. Furthermore, almost sixteen percent of the adult population exhibited severe periodontitis, which is a disturbingly elevated number when compared with other European countries, that range from 6.2 to 39.9% [2,5,33]. On the other hand, few periodontal epidemiological surveys provided extensive and comparable information in Europe, and this is one of the first to use the new AAP/EFP consensus.

The results provided by our investigation have some notable strengths but also limitations. The cross-sectional study design applied in this study cannot identify cause and effect relationships, but rather an exploratory analysis aimed at

examining the complex relationship between various contributing factors for OHRQoL. Toothbrush frequency and interproximal cleaning were self-reported items which may have introduced measurement bias. Also, HbA1c data was only available in DM patients and not to the entire population, and possibly we might have disregarded pre-diabetic patients. Another point is the low prevalence of DM (8.7%), though this prevalence is in line with recent national Portuguese evidence [73]. Additionally, OHRQoL was analysed in three different dimensions, though recent evidence suggested a four-dimensional OHRQoL mode [74] and its impact must be confirmed in future studies.

Notwithstanding, this survey has numerous strengths, including being the first study to employ ABM with a comprehensive clinical assessment of periodontal parameters as a "Need" factor, and to incorporate important variables such as diabetic status with HbA1c levels, interproximal cleaning, tooth loss, denture stability, PRA and self-perceived stress. In addition, the strengths include the representativeness and global geographic coverage based on the FHU where the study was carried out, the sample size calculation stratified for each FHU [12], the strict followed and the employment of the new AAP/EFP case definition enabling future comparability across studies [44,75,76].

In addition, the results validate previous findings that have evaluated items separately for periodontitis and OHRQoL [25,77]. Thereby, including multiple items through complex statistical methods allow direct estimates, indirect estimates and information on which and how variables are related.

5.5. Conclusion

Our findings confirm the number of missing teeth, uncontrolled diabetes mellitus, interproximal cleaning and perceived stress as important elements towards OHRQoL through ABM methodology. Periodontal Risk Assessment had low impact. Participants with a greater periodontal disease extent and severity, especially diabetic participants, have greater treatment necessity and worse OHRQoL. The number of missing teeth is highly related to increased need. Missing teeth, age, stress levels and interproximal cleaning were the items with the highest weight in their respective latent variables.

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CHAPTER

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

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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 (χ^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.

Clinical relevance

Scientific rationale for the study: to investigate the psychometric properties of the Brief Illness Perception Questionnaire (Brief-IPQ) in patients with gingivitis and periodontitis.

Principal findings: Brief-IPQ has acceptable reliability, with good model fit and construct factorial validity to periodontal diseases. Overall, Periodontal patients downgrade its illness and impact.

Practical implications: This short instrument may be used as an easily applicable and valuable tool to determine illness perception in patients with PDs. This questionnaire can help dental professionals in realize patient's awareness of their periodontal status and aid during periodontal care.

6.1. 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 an 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 a 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.

6.2. 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 6.1. 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 (χ^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 χ^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.).

6.3. 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 6.2. 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 6.2. Sociodemographic characteristics of the included participants (N = 571).

	Value
Age (years) n (%)	
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 6.3. '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 6.4).

Table 6.3. 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 6.4. 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 6.4). 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 6.5). These three items were not excluded regarding empirical and theoretical rationale.

Table 6.5. 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 6.4).

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 6.6).

Table 6.6 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).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

6.4. 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.

6.5. 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.

6.6. References

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6.7. Supplementary material

Figure S6.7.1. 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.

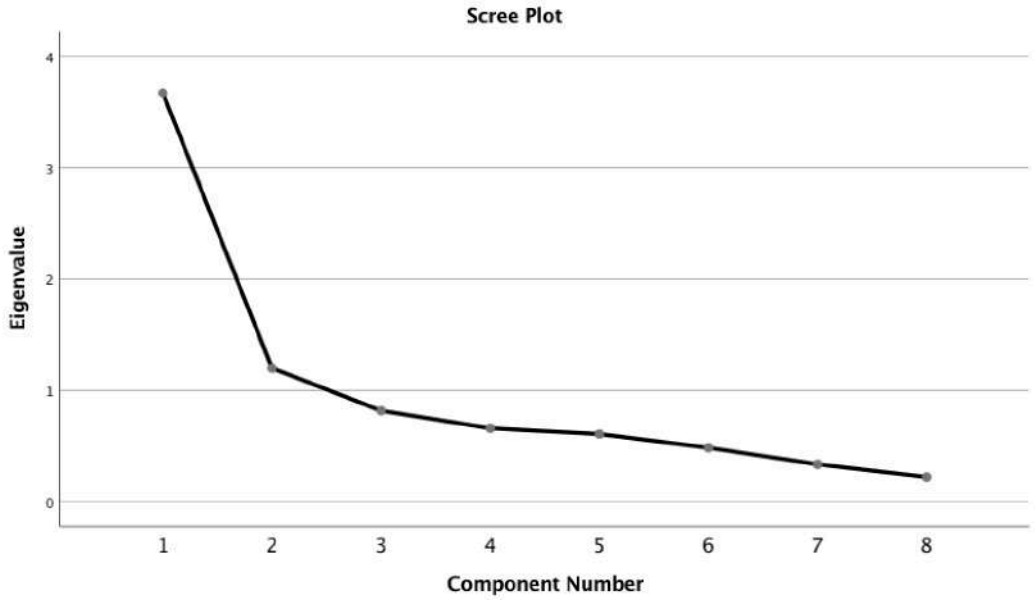

Table S6.7.2. Confirmatory Factor Analysis for the three factor structures.

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).

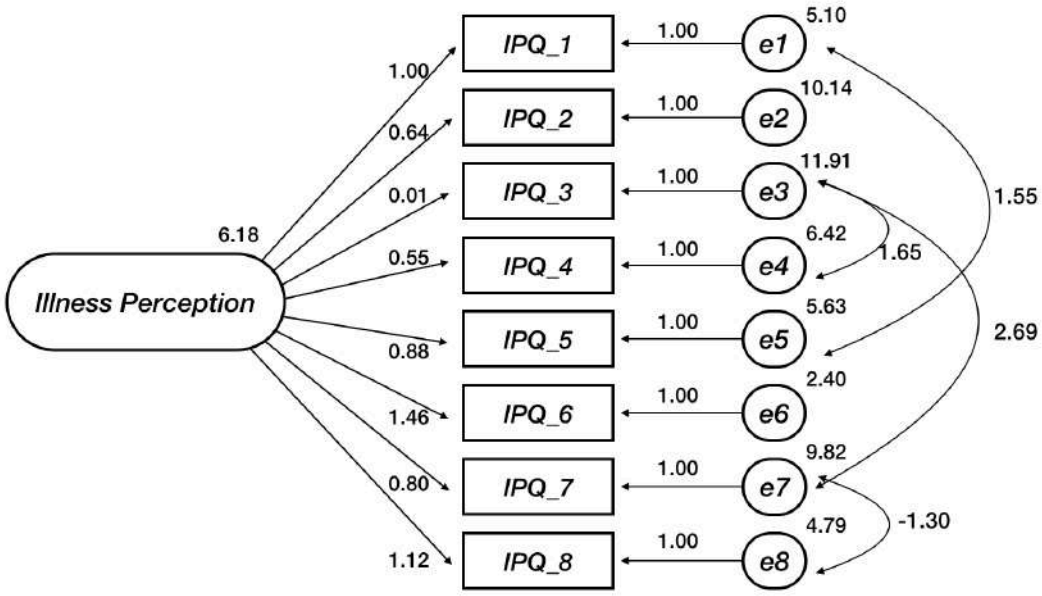

Figure S6.7.3. Confirmatory Factor Analysis (CFA) with standardized regression weights for model 3 (IPQ_1 to IPQ_8 represent Brief-IPQ items 1 to 8).

CHAPTER

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

This chapter was based from the published work:

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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 bruxism (SRB) 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 SB 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 SB towards periodontitis.

Results. Self-reported sleep bruxers exhibited lower prevalence of periodontitis. Additionally, self-reported bruxers with periodontitis had PD and CAL significantly lower than patients with only periodontitis. Multivariate analysis

suggests that SB 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 self-reported bruxers. When assessing the type of SB, significant differences among mean PD, CAL and BoP levels were also identified.

Conclusion. SB and periodontal status are negatively associated. Self-reported bruxers exhibit lower odds towards periodontitis and better periodontal clinical characteristics. Further studies are mandatory to clarify these findings.

7.1. 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.

7.2. 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 [7,21], 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].

SB 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 (Table S7.1.). 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.

7.3. Results

Sample description

The characteristics of the 1,064 participants are shown in Table 7.1. The mean age of participants with bruxism and without were 60.1 (± 13.0) and 62.8 (± 15.8) years, respectively.

Table 7.1. Sociodemographic characteristics of the participants (N = 1,064).

Variable	Total (N=1.064)
Gender	
Male	447 (42.0)
Female	617 (58.0)
Age (years)	
18-30	62 (5.8)
31-40	75 (7.0)
41-50	136 (12.8)
51-60	137 (12.9)
61-70	328 (30.8)
71-80	244 (22.9)
> 80	82 (7.7)
Educational level	
No education	42 (3.9)
Elementary	410 (38.5)
Middle	496 (46.6)
Higher	116 (10.9)
Marital status	
Single	170 (16.0)
Married / Union of fact	684 (64.3)
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)
Monthly family income (€)	
<= 600	337 (31.7)
601-1500	545 (51.2)
> 1500	182 (17.1)
Smoking status	
Non-smoker	626 (58.8)
Former smoker	293 (27.5)
Current smoker	145 (13.6)
Diabetes Mellitus	
No	860 (80.8)
Yes	204 (19.2)
Toothbrushing per day	
0	31 (2.9)
1	302 (28.4)
2+	731 (68.7)
Interproximal Cleaning	
No	718 (67.5)
Occasionally	161 (15.1)
Yes	185 (17.4)

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

The participants' distribution, considering their periodontal status is presented in Table 7.2. The prevalence of SB 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 SB as an important factor towards periodontitis simultaneously to other known risk factors (Table 7.3). Individuals with SB exhibited a lower risk towards periodontitis of 58% (OR = 0.42, 95% CI: 0.32-0.56) (Table 7.3).

Table 7.4 shows the clinical periodontal characteristics of the participants according to their periodontal and SB status. PD mean values are significantly different among all groups, with individuals from SB group having the overall lower scores and SB-P group having a significant difference from P group. Regarding CAL, the mean values are also significantly lower for individuals from SB-P group, when comparing to P group. Additionally, SB-P group has meaningful lower mean recession levels than P group.

Table 7.5 presents the clinical periodontal characteristics based on the SB questionnaire. Overall, individuals with awake/sleep SB pattern have the lowest values of PD, CAL and BoP. Further, patients with the awake SB form have significantly lower PD levels compared to probable no SB 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 7.2. Distribution of the participants according to their periodontal condition with SB status and sociodemographic variables (N=1,064).

	Non-Periodontitis (n=427)	Periodontitis (n=637)	P-value
Self-Reported 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)	

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

Table 7.3. 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
Self-reported 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.
*The model was statistically significant, χ^2 = 213.736, p < 0.001, explained 24.6% (Nagelkerke R^2) of the variance and correctly classified 68.7% of cases.

Table 7.4. Periodontal clinical characteristics (mean CAL, PD, REC and BoP) of the participants as a function of their periodontal condition and SB status (N=1,064).

Clinical Characteristic	None (n=199)	SB (n=228)	Periodontitis (P) (n=370)	SB-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, SB - Self-reported Bruxism. 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 7.5. Clinical periodontal parameters (mean CAL, PD, REC and BoP) of the participants as a function of SB type (N=1,064).

Clinical Characteristic	No SB (n=569)	Sleep SB (n=367)	Awake SB (n=114)	Awake/Sleep SB (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, SB – Self-Reported Bruxism, 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).

7.4. 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.

7.5. Conclusions

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

7.6. References

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7.7. Supplementary material

Table S7.1. 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
Self-reported 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.

CHAPTER

8

General discussion, Concluding Remarks and Future Directions

8.1. General Discussion

The overall aim of this thesis was to assess the prevalence, severity and extent of periodontal disease using a population-based epidemiologic survey of adults from the southern region of the Lisbon Metropolitan Area. We further identified associated risk factors, among which the socioeconomic and behavioral were the most impactful. Also, we have assessed the impact of periodontal status on OHRQoL in this adult population of the southern Lisbon Metropolitan Area.

To achieve this purpose, we divided our main goal into two stages. Firstly, we conducted three small-scale and preliminary pilot studies with a Portuguese subpopulation of the EMDC (Papers I, II and IV). In other words, we explored the prevalence and the risk factors of a subpopulation located in the same target region where the epidemiological study would be carried out. Interestingly, these pilot studies were relevant to improve and adapt the research protocols and tools and were in accordance with literature [1]. Furthermore, the systematic review on the association between periodontitis and salivary cortisol levels (Paper III) guided us to including a stress questionnaire on the epidemiological survey.

Comprehensively, the results of the retrospective cross-sectional study carried out at the EMDC demonstrated a 81.2% prevalence of periodontitis in the adult population (65.6% and 87.6% among the 20-44 and 45-64 age group, respectively) referred to periodontal care after a triage screening, and using the CDC/AAP full-mouth periodontal examination [2] (Paper I). These results revealed a significantly different prevalence from that reported in the single national epidemiological study conducted by the Portuguese Directorate-General of Health [3]. However, these findings cannot be compared with previous investigations performed in Portugal. One main reason is the fact this observational study has looked over patients seeking periodontal care and not an epidemiological survey *per se*. Secondly, the applied periodontal diagnosis differed from the one used in the study of the Portuguese Directorate-General of Health [3], which was a PRP. In this sense, the prevalence and severity of periodontitis reported by the Directorate-General of Health could be underestimated and this should be investigated for the sake of future periodontal research.

Those discrepancies guide us to explore the bias magnitudes, sensibility and specificity of PRP to estimate periodontal clinical measurements and periodontal status. Our estimates (Paper II) showed the CPITN was the protocol that demonstrated the highest bias results among all PRP [4]. Consequently, carrying out a large-based epidemiological survey became essential to allow a more comprehensive understanding of the current periodontal status in the area where the EMDC is located (Almada-Seixal) and the assessment of possible related risk factors.

On the other hand, we explored the possible influence of recognized risk factors in the response to NSPT using a PPD fine-tuning MLM. The results showed that the reduction of PPD and CAL was associated with patients', tooth's and site's characteristics after NSPT (Paper IV) [5]. These findings supported the health promotion message in patients diagnosed with periodontal disease in the epidemiological study that was carried out.

All three studies from stage 1 of this thesis are retrospective in nature (Paper I, II and IV). A retrospective methodology presents important limitations that can bias the results and damage internal validity [6,7]. For instance, data originally collected may have not all relevant information, there can be unknown potential confounders or medical charts may have inherent registrations errors increasing the number of excluded patients [6,7]. For these reasons, an epidemiological study could improve the estimation of periodontal disease in the region, overcoming these set of disadvantages and allowing more accurate estimates.

Hence, in the SoPHiAS study (Paper V), the first periodontal population-based representative study conducted on Almada-Seixal Health Centers (Portugal), 6 out of 10 participants were diagnosed with periodontitis [8]. Furthermore, specifically in the adult target population, 4.6 out of 10 were diagnosed with periodontitis [9] (Paper VI). In fact, the SoPHiAS results showed that age was the main factor towards the prevalence of periodontitis in the adult population, supporting the results of EMDC study. In other words, the prevalence of moderate and severe periodontitis increases with age [8] being in agreement with other European epidemiological studies [10–12].

Considering the prevalence of periodontitis in the Portuguese population according to Portuguese Directorate-General of Health data [3], our expectations were that the prevalence would be low. Then, we confirmed our concerns that the prevalence could be underestimated since the prevalence of periodontitis in

the SoPHiAS's adult population was 45.9% [9] (Paper IV). In fact, these results referring to the adult population living in the Almada-Seixal region are in agreement with EMDC results (Paper I) [13], global estimates from the Global Burden of Disease [14], WHO [15], and with other epidemiological studies of periodontitis prevalence in several European regions [10–12].

Regarding the economic status in the EMDC study, it was not feasible to realize the effect of income on periodontitis because over 70% of participants refused to provide this information [13] (Paper I). Furthermore, in the SoPHiAS study, socioeconomic status was not a relevant risk factor for the prevalence and severity of periodontitis [8] (Paper V), although in the adult subgroup structure equation modelling showed a negative link between income and oral health behaviors [9] (Paper VI). In other words, adult participants may undergo unhealthy oral behaviors due to the lack of economic resources to do so (Paper VI). Nevertheless, the true impact of economic status in periodontal disease is still uncertain [16,17] and, therefore, might be a co-factor linked to patient behaviors factors.

Another non-modifiable background factor, firstly recognized in the EMDC study and further identified in the SoPHiAS survey (Paper V), was the education level. Interestingly, when the SoPHiAS's adult participants were analyzed for the impact of periodontitis in OHRQoL, lower levels of education were directly associated with better perceived OHRQoL and simultaneously with greater treatment needs. In other words, people with less schooling have a higher prevalence and extension of periodontitis, more missing teeth and greater instability of the prosthesis, and indirectly have a lower OHRQoL (Paper VI). Conversely, lower levels of education are directly associated with a higher perception of OHRQoL. This fact may be explained by the relationship between levels of education and medical literacy [18–21]. Accordingly, future studies collecting information about the patient's level of education and applying a questionnaire to measure medical literacy are essential to explore this possible association.

Individual's illness perception is a cognitive and emotional representation of the illness, and recently was investigated in patients with periodontitis [22]. Nevertheless, although the IPQ-R-OH adopted in this previous study revealed an interesting tool for periodontal patients screening, the suitability of this extent questionnaire in the clinical periodontal setting is debatable. Therefore, in our

study, we validated the Portuguese version of the Brief-IPQ in patients with periodontal disease [23] (Paper VII). Comprehensively, this short instrument had good overall validity and reliability, being in agreement with the original Brief-IPQ study [24]. Also, in our point of view, this tool can discriminate patients who had an unreal or negative perception regarding the periodontal state and, consequently, can lead to a more neglected behavior from patients (not seeking dental treatment or refusing to undergo advised periodontal treatments). One the other hand, it is important to highlight that 69% of patients from the EMDC study failed the periodontal visit despite triage referral [13] (Paper I). Therefore, in the future, it is important to assess whether the perception of periodontal disease using the Brief-IPQ conditions the demand and acceptance of periodontal treatment.

Regarding DM, previously studies demonstrated a possible bidirectional association with periodontal disease [25–27]. In the overall population, patients diagnosed with DM had an increased risk of having periodontitis [8]. Specifically in the adult population, the results showed that patients with uncontrolled DM had more periodontal treatment needs and poorer OHRQoL [9] (Paper VI). Moreover, it is essential to emphasize that information regarding the DM patients was sourced from a self-reported questionnaire, combined with medication registration and levels of glycated hemoglobin. Despite this method is highly accepted as the gold standard, we were not able to gather glycated hemoglobin data from self-reported non-diabetic patients, and therefore, we may have lacked some pre-diabetic or non-controlled diabetic participants. In the future, we shall incorporate serum laboratory data, though this depends on logistic and financial availability.

Smoking habits is undoubtedly an important risk factor towards periodontitis [28–30]. Nevertheless, it is important to highlight a possible weakness of self-reported questionnaire used to collect this information, where participants were asked whether they are current smoker at the time of examination. There may be an underestimation specially if smoking is perceived as socially undesirable, although one study showed that participants are truthful about their smoking habits [31]. In both EMDC and SoPHiAS studies (Paper I and V, respectively), smoking was a relevant risk factor on the prevalence of periodontitis in the overall population, however, the exploratory factory analysis in the structural equation modelling (Paper VI) confirmed that smoking habits are have no

underlying relationships between measured variables, though the observational nature of the study may explain this result.

Others, the removal of biofilm deposits through tooth brushing and interproximal cleaning was another risk factor described in literature [32–34]. In the EMDC study [13] toothbrushing and dental floss frequencies had no association with periodontal disease. However, this controversy results may have occurred because, as a retrospective study, this information was collected in a limited way (Paper I). In the SoPHiAS' subset of adults, interproximal cleaning was the most important factor associated with a healthy periodontium and less periodontal treatment needs, and consequently linked to higher OHRQoL [9] (Paper VI). As discussed, this is a very good example on how retrospective studies might provide underestimated results, and why epidemiological studies must be conducted.

In addition, stress-related disorders have been related with periodontitis [35–39] through a plausible change in immune response which can increase periodontitis susceptibility [40–42]. Nevertheless, although some articles highlighted a possible association between psychological stress and the increase risk for worse periodontitis, the biological mechanism upon this association is not well explained. Thus, we conducted a systematic review confirming on the association of aggressive periodontitis with salivary cortisol levels, a stress-related hormone [43] (Paper III). These findings highlighted the importance of including an evaluation of stress via a validated questionnaire (Perceived Stress Scale-10) in our epidemiological survey. Remarkably, in the adult group, individuals with higher levels of perceived stress reported worse OHRQoL, being in accordance with previous studies [41,44,45] (Paper VI). Furthermore, the results suggested that individuals may endure more negligent oral behaviors because they might not be able to cope with stressful situations, though this should be explored in the future.

Associated with the potential role of stress in the SoPHiAS survey, we also centered our efforts in bruxism, an oral condition also associated with stress [46–48]. Despite the etiology of bruxism is complex, studies have demonstrated that bruxers experience higher perceived stress through the Perceived Stress Scale-10 [49], although this association is controversial [50]. Also, as a condition characterized by an overload into the periodontium, bruxism has been linked with the teeth-supporting tissues [51–53], although the notion until recently was

that bruxism cannot damage the periodontal tissue [54]. Given this uncertainty, we assessed the association between self-reported bruxism and periodontal status (Paper VIII). Overall, self-reported bruxers were associated with less prevalence of periodontitis, lower periodontal clinical measures and lower risk towards periodontitis [55]. Furthermore, self-reported bruxers with periodontitis had lower average of periodontal clinical characteristics than patients diagnosed with healthy periodontium [55]. Despite the novelty of these results, further studies are warranted to ascertain the cause-effect of this possible association.

8.2. Concluding Remarks

Paper I – The subpopulation of patients who were forwarded to a periodontology appointment in a university dental clinic of the Lisbon region demonstrate a high prevalence and severe extent of periodontal destruction. Nevertheless, a disturbing percentage of patients who were referred for periodontal consultations in the first medical screening, missed or cancelled the periodontal appointment. Age and smoking habits were identified as an important risk factors towards periodontitis in patients with 45-64 years old. Within the limitations of this study, the results highlight the importance of developing appropriate public health programs to educate the Portuguese population about the burden of periodontal disease. In this sense, this study was fundamental to support the risk factors assessed in the epidemiological research carried out in Paper V.

Paper II – Half-mouth three sites and two half-mouth six sites protocols can be applied to access periodontal clinical measurements in Portuguese patients with limited bias, high sensitivity, specificity, and concordance. Despite 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. Therefore, when comparing the full-mouth periodontal evaluations with the partial ones, it was possible to conclude that the best methodological approach for the epidemiological study would be the full-mouth periodontal assessment.

Paper III – The systematic synthesis revealed that patients 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. Nevertheless, it is important to highlight the complex nature of periodontitis and there are many confounders factors that may have contributed to this outcome. In the future, more robust research should be gathered with larger samples and well-designed longitudinal designs to endorse this possible association and to elucidate explain the pathological mechanism beyond. Also, this systematic review allowed us to concluded that it would be important to apply a stress questionnaire in the epidemiological study.

Paper IV – All three hierarchical levels (patient, tooth, and site levels) included risk factors influencing the degree of pocket depth and clinical attachment loss reduction after nonsurgical periodontal therapy. Specifically, the tooth type, surface involved and tooth mobility site-level risk factors had the strongest impact on the reduction of periodontal clinical recovery. Further, the periodontal clinical measures (probing depth and clinical attachment loss) showed major recovery in the first three months after nonsurgical periodontal treatment.

Paper V – The epidemiological research in the adult population of the southern region of the Lisbon Metropolitan Area (Portugal) indicates that seven out of ten adults had some type of periodontal disease, and six out of ten had periodontitis. Further, almost half of the population was diagnosed with moderate or severe periodontitis. These findings provide a comprehensive understanding about the requirement of appropriate national public oral health programmes and population-based preventive actions. Age, education level, smoking habits and diabetes mellitus were important risk factors towards periodontal disease, in accordance with paper I regarding age and smoking habits.

Paper VI – The adult population of the SoPHiAS study demonstrated that the number of missing teeth, diabetes mellitus, interproximal cleaning and perceived stress are important factors towards oral health-related quality of life (OHRQoL), using Andresen's Behavioral Modelling. Further, diabetes mellitus was associated to higher treatment need and poorer OHRQoL. In contrast, good oral hygiene habits promote a healthy periodontium and, consequently, increases OHRQoL. The confirmation that periodontal disease is associated with risk factors such as diabetes mellitus, smoking habits and stress endorses the results published in papers I and III.

Paper VII – The Brief-Illness Perception Questionnaire is a valid instrument with acceptable reliability and construct factorial validity. Further, it is an easily applicable questionnaire to assess illness perception in patients with periodontal diseases. This tool can help professionals in realizing patient's awareness of their periodontal status and aid during periodontal care. The validation of this questionnaire for periodontal disease can be important to answer the needs of public health strategies based on the results of Articles I and V.

Paper VIII – Self-reported bruxism revealed to be an important factor towards periodontitis, particularly in adult participants. Self-reported bruxism and periodontal status were negatively associated, that is self-reported bruxers had

lower odds of periodontitis and better periodontal clinical characteristics. Furthermore, bruxism as a stress dependent disease highly affects adult patients but may have positive relationship with the periodontal status, and may explaining the prevalence results of articles III and VI. Nevertheless, more studies are needed to clarify the mechanism upon such relationship.

Overall, with this Thesis, we concluded that:

- This group of adults presented a high prevalence of periodontal disease.
- Careful interproximal cleaning was a key oral health behavior contributing to decrease the need for periodontal treatment and for the presence of better OHRQoL in adults.
- Patients with uncontrolled diabetes mellitus also presented higher periodontal treatment needs and worse OHRQoL.
- Stress was confirmed to be associated with periodontitis in a systematic review and, it was further demonstrated that self-perceived stress is an important confounding factor towards OHRQoL and periodontal treatment needs.
- Self-reported bruxism, a stress related condition, was found to be negatively linked to periodontitis.
- As result of the poor periodontal status and neglected behaviours, we were able to validate an illness perception instrument in periodontal disease patients. This tool might be straightforwardly implemented in the clinical daily practice and in future public health programmes.

8.3. Future Directions

Periodontal disease is a public health concern for several reasons but namely due to the high prevalence worldwide. While the oral biofilm shift is known to play a significant role in onset and progression of periodontal disease, the non-modifiable background factor such as age, genetic, behavioral, the hereditary and acquired diseases, and the environmental and the patient's habits are further risk factors that contribute to periodontal disease. In fact, it is the interrelation between all factors that triggers the state of periodontal disease. Although some of such factors were previously measured in specific population, the holistic periodontal risk network had never before been assessed in a Portuguese population, and therefore the SoPHiAS survey demonstrated to be a successful epidemiological programme in this specific population. In the future, well-designed cohort and longitudinal studies should be carried out with added systemic assessments in existing health programmes, at regional and national levels.

Mindful of the worrying findings in the SoPHiAS regarding the adult Portuguese population, a national periodontal prevention and treatment programme is warranted. In this sense, we must bear in mind the age range of the periodontitis onset and the repercussions to quality of life. Also, periodontal medical literacy is key to motivate patients for prevention, or periodontal treatment and maintenance when the disease is present.

Also, there will be no “magic bullet” in periodontal treatment, as the underlying cause of periodontal disease may differ in each patient. In light of this, for a successful prevention or treatment, it is imperative that the underlying cause is successfully identified and addressed. Furthermore, the complexity of periodontitis stresses the need for developing a personalized approach highly adapted to the patient's particular needs.

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CHAPTER

9

Annexes

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

Exma. Senhora
Dr.ª Vanessa Machado
vanessamachado558@gmail.com

C/C:

Sua Referência	Sua Comunicação de	Nossa Referência	Data
		3525/CES/2018	09.04.2018

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

A Comissão de Ética para a Saúde da ARSLVT, apreciou o projecto mencionado em epígrafe, na reunião da secção de investigação do dia 6 de abril, tendo sido emitido um parecer favorável ao estudo.

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 deste estudo.

Com os melhores cumprimentos,

O Conselho Directivo

LUÍS PISCO
Presidente do Conselho Directivo da ARSLVT, I.P.

Av. Estados Unidos da América nº75-77, 1749-096 Lisboa
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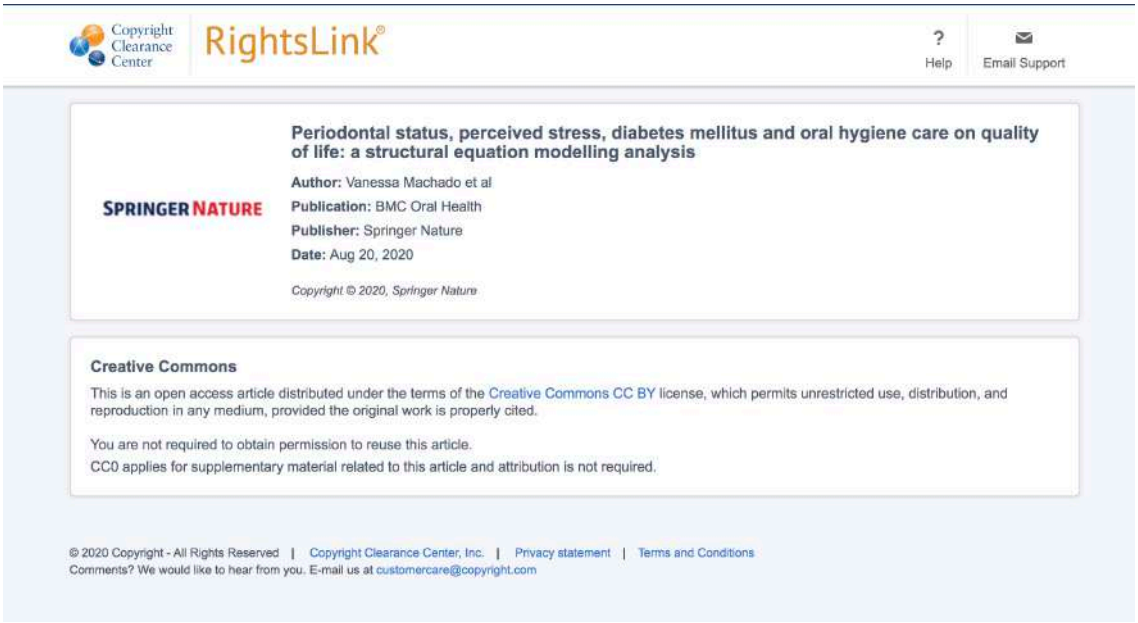

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Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: a structural equation modelling analysis

Author: Vanessa Machado et al
Publication: BMC Oral Health
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