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CIÊNCIAS BIOMÉDICAS

Periodontal treatment impact on halitosis and quality of life

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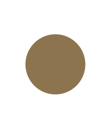
INSTITUTO DE CIÊNCIAS BIOMÉDICAS ABEL SALAZAR



Catarina Pequito Izidoro de Sousa Pinto

Periodontal treatment impact on halitosis and quality of life

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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Aos meus filhos, Ana Maria e João Maria.

"When we learn how to become resilient, we learn how to embrace the beautifully broad spectrum of the human experience."

Jaeda Dewalt

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Declaration of Honor (English and Portuguese)

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(Catarina Pequito Izidoro de Sousa Pinto)

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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 that integrate this thesis.

Articles published in journals indexed in ISI Web of Knowledge and author contribution per paper:

1. **Catarina Izidoro**, João Botelho, Vanessa Machado, Ana Mafalda Reis, Luis Proença, Ricardo Alves and José João Mendes. Revisiting Standard and Novel Therapeutic Approaches in Halitosis: A Review. *International Journal of Environmental Research and Public Health*. **2022**, 19(18):11303.

Author Contribution:

Catarina Izidoro selected the articles, extracted the data from each article, conducted the review, contributed to analysis and interpretation of data, drafted the article including the preparation of figures and tables, and approved the final work.

2. **Catarina Izidoro**, João Botelho, Vanessa Machado, Ana Mafalda Reis, Luis Proença, Ricardo Alves and José João Mendes. Periodontitis, Halitosis and Oral-Health-Related Quality of Life - A Cross-Sectional Study. *Journal of Clinical Medicine*. **2021**, 10(19):4415.

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Catarina Izidoro designed the study, performed the questionnaire, the periodontal measurements and halitosis assessment. Also, Catarina Izidoro collected and processed the experimental data, contributed to analysis, was involved in bioinformatic analysis, and interpretation of data, drafted the article including the preparation of figures and tables, and was a major contributor in writing the manuscript.

3. **Catarina Izidoro**; João Botelho; Vanessa Machado; Ana Mafalda Reis; Luis Proença; Helena Barroso; Ricardo Alves; José João Mendes. Non-Surgical Periodontal Treatment Impact on Subgingival Microbiome and Intra-Oral Halitosis. *International Journal of Molecular Sciences*. **2023**, 24, 2518.

Author Contribution:

Catarina Izidoro designed the study, performed the questionnaire, the periodontal measurements, halitosis assessment, collected the samples, did periodontal treatment and participated in the extraction of DNA from the samples. Also, Catarina Izidoro collected and processed the experimental data, was involved in bioinformatic analysis, and interpretation of data, drafted the article including the preparation of figures and tables, and was a major contributor in writing the manuscript.

Acronyms and Abbreviations

BANA - benzoyl-DL-arginine-2 naphthylamide.

BoP - Bleeding on Probing.

CAL - Clinical attachment loss.

CEJ - Cemento enamel junction.

CH_3SH - Methylmercaptan.

CHX - Clorohexidine.

CRP - C-reactive protein.

EMDC - Egas Moniz Dental Clinic.

EOH - Extra-oral halitosis.

FI - Furcation involvement.

FMD - Full-mouth-disinfection.

GI - Gingival index.

H_2S - Hydrogen sulfide.

HbA1c - Glycosylated hemoglobin.

IL-1 - Interleukin 1.

IL-8 - Interleukin 8.

IOH - Intra-oral halitosis.

ISBOR - International Society for Breath Odor Research.

LPS - Lipopolysaccharide.

MMPs - Matrix metalloproteinases.

NHANES - National Health and Nutrition Examination Survey.

NSPT - Non-surgical periodontal treatment.

OHRQoL - Oral health related quality of life.

PCR - Polymerase Chain Reaction.

PD - Probing Depth.

PESA - Periodontal epithelial surface area.

PI - Plaque index.

PISA - Periodontal inflamed surface area.

ppb - Parts per billion.

PST - Periodontal Supportive therapy.

SPH - Self-perceived halitosis.

SRP - Scaling and root planing.

TNF-α - Tumor necrosis factor alpha.

VSC/VSCs - Volatile sulphur compound(s).

Abstract

Periodontitis is an inflammatory disease that leads to the destruction of periodontal supporting tissues and is characterized by impaired homeostasis due to polymicrobial aggression. In a representative Portuguese study, the prevalence of periodontitis was estimated at 59.9%, with 24.0% and 22.2% of the participants exhibiting severe and moderate periodontitis, respectively. In more advanced stages may imply limitations such as, chewing difficulty, halitosis, tooth mobility, loss of interproximal papillae, pain and even lead to tooth loss.

Periodontal disease activity and severity are assessed by clinical parameters such as probing depth, bleeding on probing, and clinical attachment level. On the other hand, there are other clinical parameters of periodontitis, such as gum color change, bleeding during brushing, tooth migration, chewing difficulty and bad breath, which are consequences of chronic inflammation and periodontal destruction. From a clinical point of view, these symptoms are often not documented, however, they are highly relevant from the patient's point of view and often have a considerable negative impact on their quality of life.

It is essential to demonstrate the importance that oral diseases have on the physical and psychological well-being of individuals and, consequently, on the Oral health-related quality of life. By assessing the subjective experiences of individuals to determine the impact of oral health conditions on well-being and self-esteem, it is possible to improve clinical interventions and thus quality of life.

The consequences of periodontitis such as halitosis, gingival bleeding, tooth mobility, tooth migration, and tooth loss, compromises patient daily quality of life. However, few studies have evaluated the impact of periodontal disease and its treatment on the quality of life. This is an area not yet studied in a Portuguese population, and it deserves further study.

Halitosis, also known as bad breath, is a term used to define the unpleasant odor that emanates from the oral cavity and is one of the main symptoms of periodontitis, often leading patients to seek treatment. Patients with intraoral halitosis have higher concentrations of volatile sulphur compounds (VSC), namely Hydrogen Sulfide and Methylmercaptan in the oral cavity.

Subgingival periodontal biofilm is mainly composed of Gram-negative anaerobic bacterial species, which are proteolytic in nature, capable of degrading sulphur-containing substrates in different oral cavity surfaces, including periodontal pockets, releasing volatile sulphur compounds. Despite its clinical and social relevance, there are few epidemiological studies evaluating the prevalence of halitosis and the impact of its treatment on quality of life.

Subgingival microbiome is thought to play a crucial role in this condition, however, there are few studies that evaluate subgingival microbiome before and after periodontal treatment.

The subgingival microbiome is decisively related to the initiation, maintenance, and progression of the disease. The subgingival niche offers ecological conditions with available nutrients, which favor the growth of a diverse microbiota. The subgingival abundance of specific periodontal pathogens such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* is considered a major risk factor for periodontitis, which is why the search for these bacteria in subgingival plaque samples may be relevant in clinical trials.

To these ends, our main purpose was to investigate the impact of periodontal treatment on halitosis, quality of life and subgingival microbiome.

Prior to designing the study, we carried out a literature review on halitosis and its treatment (**Chapter 3**), which would allow us to design the clinical questionnaire that we would later use in the assessment of intra-oral halitosis. We conclude that halitosis is highly prevalent, with multifactorial origins, and high burden for social and self-esteem. This review emphasizes the importance of a multidisciplinary approach. Despite the current decision trees for the clinical management of halitosis, there are still some inconsistencies that require robust randomized clinical trials comparing standard and innovative therapies.

Next, we evaluated (**Chapter 4**), in a population of the Egas Moniz dental clinic, the intra-oral halitosis (IOH) in patients with periodontitis and the oral health-related quality of life of these patients. In this cross-sectional study, we sought to explore the relationship of PISA (periodontal inflamed surface area)

and PESA (periodontal epithelial surface area) with VSC. We hypothesize exploring such far-reaching measures would provide a more comprehensive

understanding on how periodontal destruction relates with VSCs. Our results confirmed VSC counts may be associated with the amount of PESA of the posterior-lower region. To the best of our knowledge, this study may be the first to demonstrate such association.

Finally, in **Chapter 5**, we intend to characterize and compare subgingival microbiome before and after periodontal treatment to learn if any changes of the subgingival microbiota were reflected in intra-oral halitosis. We tested the hypothesis that intra-oral halitosis (VSC levels) correlates with corresponding subgingival bacterial levels before and after periodontal treatment. Subgingival plaque samples, in twenty patients with generalized periodontitis, were collected at baseline and 6-8 weeks after nonsurgical periodontal therapy. Subgingival microbiome were characterized by sequencing on an Illumina platform. The correlation of bacterial variation with VSCs measured in the periodontal diagnosis and in the reassessment after treatment were evaluated. *Fusobacterium nucleatum*, *Capnocytophaga gingivalis* and *Campylobacter showaei* showed correlation with the reduction of VSC after periodontal treatment (p-value= 0.044; 0.047 and 0.004 respectively). We concluded that microbial diversity was high in the subgingival plaque on periodontitis and intra-oral halitosis participants of the study. Furthermore, there were correlations between subgingival plaque composition and VSC counting after periodontal treatment. The subgingival microbiome can offer important clues in the investigation of pathogenesis and treatment of halitosis.

With this thesis, we conclude that 1) the intra-oral and extra-oral causes of halitosis are well documented in the literature. The treatment approach of halitosis is multidisciplinary, however, there are still some gaps in the literature with a need for more randomized clinical trials comparing standard and innovative therapies; 2) VSC counts may be associated with the amount of PESA of the posterior-lower region, when other causes of extra-oral halitosis are excluded. Further intervention studies are mandatory to verify a possible causal association; 3) Emphasizing the promotion of increased public education and awareness about halitosis should be a priority. Patients should be informed and educated about the condition, and future generations of dental professionals should be trained to effectively address it; 4) Subgingival microbiome can offer important clues in the investigation of the pathogenesis and treatment of halitosis.

Keywords: Periodontitis, Halitosis, Oral Health- related Quality of Life, Subgingival microbiome.

Resumo

A periodontite é uma doença inflamatória que leva à destruição dos tecidos de suporte do dente, caracterizada por uma diminuição da homeostase devido à agressão polimicrobiana. Num estudo representativo realizado em Portugal, a prevalência da periodontite estimada foi de 59,9%, com 24,0% e 22,2% dos participantes apresentando casos graves e moderados de periodontite, respectivamente. Em estadios mais avançados, pode implicar limitações, como dificuldade de mastigação, halitose, mobilidade dentária, perda de papilas interproximais, dor e até mesmo perda dentária.

A atividade e a severidade da doença periodontal são avaliadas por parâmetros clínicos, como profundidade de sondagem, hemorragia à sondagem e nível de inserção clínico. Por outro lado, há outros sinais e sintomas da periodontite, como mudanças de cor nas gengivas, hemorragia à escovagem, migração patológica, dificuldade na mastigação e halitose, que são consequências da inflamação crónica e da destruição periodontal. Do ponto de vista clínico, esses sintomas não são frequentemente documentados, no entanto, são altamente relevantes do ponto de vista do paciente e frequentemente têm um impacto negativo considerável na sua qualidade de vida.

É fundamental demonstrar a importância que as doenças orais têm no bem-estar físico e psicológico do indivíduo e, consequentemente, na qualidade de vida relacionada com a saúde oral. Ao avaliar as experiências subjetivas dos doentes é possível determinar o impacto das condições de saúde oral no bem-estar e autoestima, e assim, é possível melhorar as intervenções clínicas e a qualidade de vida.

As consequências da periodontite, como halitose, hemorragia gengival, mobilidade dentária, migração patológica e perda dentária, comprometem a qualidade de vida do paciente diariamente. No entanto, poucos estudos avaliaram o impacto da doença periodontal e seu tratamento na qualidade de vida. Esta é uma área ainda não estudada numa população portuguesa e merece mais estudos.

A halitose, também conhecida como mau hálito, é um termo usado para definir o odor desagradável que provém da cavidade oral e é um dos principais sintomas da periodontite, levando frequentemente os pacientes a procurar tratamento.

Pacientes com halitose de causa intra-oral apresentam concentrações mais elevadas de compostos sulfurosos voláteis, como Sulfeto de Hidrogénio e Metilmercaptano, na cavidade oral.

O biofilme periodontal subgengival é composto principalmente por espécies bacterianas anaeróbias Gram-negativas, que são proteolíticas e capazes de degradar substratos que contém enxofre em diferentes superfícies da cavidade oral, incluindo bolsas periodontais, libertando compostos sulfurosos voláteis. Apesar da sua relevância clínica e social, há poucos estudos epidemiológicos que avaliam o impacto do seu tratamento e na qualidade de vida.

O microbioma subgengival é considerado crucial nesta condição, mas poucos estudos avaliam o microbioma subgengival antes e depois do tratamento periodontal.

O microbioma subgengival está relacionado decisivamente com a iniciação, manutenção e progressão da doença. O nicho subgengival oferece condições ecológicas com nutrientes disponíveis, que favorecem o crescimento de uma microbiota diversa. A presença abundante de microorganismos específicos, como *Porphyromonas gingivalis*, *Tannerella forsythia* e *Treponema denticola*, é considerada um fator de risco importante para a periodontite, o que torna relevante a pesquisa dessas bactérias em amostras de placa subgengival em ensaios clínicos.

Assim, o nosso principal objectivo foi investigar o impacto do tratamento periodontal na halitose, qualidade de vida e microbioma subgengival.

Antes de iniciar o estudo, realizámos uma revisão da literatura sobre halitose e o seu tratamento (**Capítulo 3**), o que nos permitiu desenhar um questionário clínico que usaríamos posteriormente na avaliação da halitose intra-oral. Concluímos que a halitose é altamente prevalente com origens multifatoriais e grande impacto social e na autoestima. Esta revisão enfatiza a importância de uma abordagem multidisciplinar. Apesar das atuais árvores de decisão para a abordagem clínica da halitose, ainda existem algumas incongruências que requerem ensaios clínicos randomizados robustos comparando terapias padrão e inovadoras.

De seguida, avaliámos (**Capítulo 4**), numa população da clínica dentária Egas Moniz, a halitose intra-oral em pacientes com periodontite e a qualidade de vida relacionada com a saúde oral destes pacientes. Neste estudo transversal,

procurámos explorar a relação da PISA (Área de superfície periodontal inflamada) e PESA (Área de superfície periodontal epitelial) com os compostos sulfurosos voláteis (CSV). Hipotetizamos que a exploração de medidas tão abrangentes forneceria uma compreensão mais profunda de como a destruição periodontal se relaciona com os CSVs. Os nossos resultados confirmaram que as contagens de CSVs podem estar associadas à quantidade de PESA da região posterior-inferior. À luz do nosso conhecimento actual, este estudo pode ser o primeiro a demonstrar tal associação.

Por fim, no **Capítulo 5**, pretendemos caracterizar e comparar o microbioma subgengival antes e após tratamento periodontal para verificar se houve mudanças no microbioma subgengival que se reflectissem na halitose intra-oral. Testámos a hipótese de que a halitose intra-oral (níveis de CSV) se correlaciona com os níveis bacterianos subgengivais correspondentes antes e depois do tratamento periodontal. Foram recolhidas amostras de placa subgengival, em vinte pacientes com periodontite generalizada, no diagóstico e 6-8 semanas após tratamento periodontal não-cirúrgico. O microbioma subgengival foi caracterizado por sequenciação numa plataforma Illumina. Foi avaliada a correlação da variação bacteriana com os CSVs medidos no diagnóstico periodontal e na reavaliação após o tratamento. *Fusobacterium nucleatum*, *Capnocytophaga gingivalis* e *Campylobacter showaei* apresentaram correlação com a redução de CSV após o tratamento periodontal (p-valor = 0,044; 0,047 e 0,004 respectivamente). Concluímos que a diversidade microbiana foi elevada na placa subgengival em participantes com periodontite e halitose intra-oral. Além disso, houve correlações entre a composição da placa subgengival e a contagem de CSV após o tratamento periodontal. O microbioma subgengival pode oferecer pistas importantes na investigação da patogénese e tratamento da halitose.

Com esta Tese, concluímos que 1) as causas intra-orais e extra-orais da halitose estão bem documentadas na literatura. A abordagem da halitose é multidisciplinar, no entanto, quando se trata de tratamento, ainda existem lacunas na literatura, havendo necessidade de mais ensaios clínicos randomizados comparando terapias padrão e inovadoras; 2) a concentração de CSV na cavidade oral pode estar associada à quantidade de PESA da região postero-inferior, quando outras causas de halitose extra-oral são excluídas. Estudos de intervenção são mandatórios para verificar esta possível associação causal; 3) Enfatizar a promoção de uma maior educação e consciencialização

sobre halitose deve ser uma prioridade. Os pacientes devem ser informados e educados sobre esta condição e futuras gerações de profissionais de saúde oral devem ser treinadas para abordá-la de forma efetiva; 4) O microbioma subgengival pode oferecer pistas importantes na investigação da patogénese e tratamento da halitose.

Palavras-chave: Periodontite, Halitose, Qualidade de vida relacionada com saúde oral, Microbioma subgengival.

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1

General Introdution

1. General Introdution

1.1. Periodontal disease

The term "periodontal disease" encompasses a wide variety of chronic inflammatory conditions of the gingiva (the soft tissue that surrounds the teeth), bone and ligament (the connective tissue collagen fibers that allow the tooth to attach to the alveolar bone) that support the teeth. Periodontal disease begins with gingivitis, a localized inflammation of the gums, initiated by bacteria from dental plaque, that is, the microbial biofilm that forms on the teeth and gums [1].

Gingivitis is an inflammatory condition of the soft tissues surrounding the teeth (the gums) and is a direct immune response to the dental microbial plaque that builds up on the teeth. Gingivitis is influenced by several factors such as smoking, certain medications and hormonal changes that occur during puberty and pregnancy [2].

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested by clinical attachment loss (CAL) and radiographic alveolar bone loss, the presence of periodontal pockets and gingival bleeding. Periodontitis is a major public health problem because of its high prevalence and because it can lead to tooth loss and disability. As a result, masticatory function and aesthetics are compromised contributing to social inequality and impaired quality of life. Periodontitis accounts for a significant proportion of edentulism and masticatory dysfunction, results in significant dental care costs and has a plausible negative impact on general health [3;4].

1.1.1 Epidemiology and risk factors

The mechanism underlying the destruction of periodontal tissues includes tissue damage caused by products of plaque and bacterial-inducible host immune responses [4].

Recent studies indicate that periodontal infection is a potential and constant source of infection and is associated with several systemic diseases, including

atherosclerosis, diabetes, cancer, rheumatoid arthritis, aspiration pneumonia and adverse pregnancy outcomes. Oral infections and systemic side effects can be linked by transient systemic bacteremia due to infections of the oral cavity, lesions caused by circulating oral microbial toxins, and systemic inflammation caused by immunological lesions induced by oral microorganisms [5].

It is believed that microorganisms in a dental biofilm are involved in the pathogenesis of periodontitis, and in particular, subgingival bacteria play an important role in its initiation and progression [6].

Prevalence and significance

According to data from the National Health and Nutrition Examination Survey (NHANES) 2009–2014, 42% of adults in the United States had periodontitis, with 7.8% having severe periodontitis [7]. This survey confirmed a high prevalence of periodontitis in the United States affecting almost 50% of the adult population (30 years old or older) [7]. Globally, approximately 11% of the world population may have severe periodontitis, affecting 743 million individuals [8–10]. In Portugal, a prevalence of periodontitis in adults of 59,9% was found, with 22.2% referring to a moderate stage and 24% to a severe stage. It is also concluded that localized periodontitis affects 23.2% and generalized 36.7% of the Portuguese population [11]. Furthermore, a previous study has identified potential associations between periodontitis and certain non-communicable chronic diseases.

In more advanced stages, it may imply limitations such as chewing difficulty, halitosis, tooth mobility, loss of interproximal papillae, pain and even lead to tooth loss. The activity and severity of periodontal disease are assessed by clinical parameters such as probing depth, bleeding on probing and clinical attachment level [5]. On the other hand, there are other clinical parameters of periodontitis, such as gingival color and appearance, bleeding during brushing, displacement of teeth, and bad breath, which are consequences of chronic inflammation and destruction of the periodontium. From a clinical point of view, these symptoms are often not documented [12]. However, they are highly relevant from the patients' point of view and often have a substantially negative effect on oral health related quality of life (OHRQoL), while successful management may improve patients' OHRQoL [13–15].

Risk Factors

For more than a century, the etiological agents of periodontal disease have been studied and sought. Research began in the "Golden Age of Microbiology" (approximately 1880–1920), when the etiologic agents of several clinically important infections were determined. Parallel investigations into the etiology of periodontal disease were initiated at this time [16].

Mixed bacterial colonization in oral tissue is the primary etiology of periodontal disease. However, there are other factors that act as secondary etiological factors, which alter the susceptibility or resistance of the host, accelerating the progression and development of periodontal disease. They are: calculus, overhanging restorations, anatomical characteristics such as short roots, non-carious cervical lesions, systemic factors, genetic factors, smoking and stress [17].

Knowing and controlling the risk factors for periodontal disease is essential for its prevention and treatment, and although certain risk factors are not modifiable, their identification is essential to determine the appropriate treatment plan [18,19].

A risk factor can be defined as an occurrence or characteristic that has been associated with the increased rate of a subsequently occurring disease. It is important to make the distinction that risk factors are associated with a disease but do not necessarily cause the disease [19–21].

Evidence used to identify risk factors usually is derived from the following types of studies in order of increasing strength of evidence: case reports, case series, case-control study, cross-sectional studies, longitudinal cohort studies, and controlled clinical trials, also known as interventional studies. All these studies can identify factors associated with a disease though they are not equal in strength. The longitudinal study may be capable of identifying a causal relationship. The interventional study gives the strongest evidence of a causal relationship and furthermore can provide evidence of the benefit of eliminating the risk factor. Associations identified through longitudinal and interventional studies are termed risk factors whereas associations, based on the observations of cross-sectional and case-controlled studies are termed risk indicators. Thus the term risk factor denotes a greater weight of evidence supporting an association than does the term risk indicator [21].

Risk factors for periodontitis can be subdivided into modifiable risk factors, such as poor oral hygiene, smoking, diabetes, and non-modifiable risk factors, which include age and heredity, including genetic disorders [20,22].

Modifiable risk factors

- **Insufficient oral hygiene**

Poor oral hygiene practices play a significant role in the initiation and progression of periodontitis [23–25]. Improper oral hygiene techniques can lead to the accumulation of plaque on the teeth, initiating gingivitis and potentially progressing to periodontitis. This relationship has been demonstrated in the literature, with the increasing accumulation of dental plaque being directly associated with the increase in the severity and prevalence of periodontal disease [26]. As a result of poor oral hygiene, the anaerobic organisms responsible for the progression of periodontal disease can colonize deeper areas of the periodontium, where they carry out their destructive actions. The main bacteria found in periodontitis include *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*. When penetrating deeply into the periodontium, these organisms produce inflammation triggering the release of inflammatory mediators and other host defensive products [27].

- **Smoking habits**

Smoking is the single greatest modifiable risk factor for periodontal disease, as demonstrated in association, progression, and intervention studies, with attributable risk estimates ranging between 2.5 and 7.0. Smokers have a worse periodontal condition and experience more severe tooth loss than non-smokers [1].

Compared to non-smokers or past smokers, smokers exhibited a significantly higher prevalence of red-complex periodontal pathogens in their subgingival biofilm [28–30]. Furthermore, a potential negative effect of smoking on host immune cells, especially neutrophils, was reported, making their host more susceptible to periodontitis [31–33].

The association between *P. gingivalis*, *Treponema denticola*, *T. forsythia* pathogens and smoking leads to an increased risk of developing and progressing periodontitis. Several studies report that, given a dose-response effect of tobacco consumption and periodontal disease, the rates of prevalence

and severity of the disease increased in relation to the number of cigarettes consumed and duration of consumption. The same is true of cigars, cigarillos, smokeless tobacco and chewing tobacco [34].

Consistent with these findings, light and heavy smokers are at a greater risk for developing alveolar bone loss with an odds ratio of 3.25 and 7.28, respectively, compared to non-smokers. Similarly, light and heavy smokers are at a greater risk for developing periodontal attachment loss with an odds ratio 2.05 and 4.07, respectively, compared to non-smokers [35].

Cigarette smoking creates a more favorable environment for the growth of periodontal pathogens, thereby promoting disease development [36].

Furthermore, smoking has a negative impact on the outcome of active periodontal therapy as well as on long-term maintenance periodontal therapy [37,38].

Prospective studies have demonstrated higher rates of progression of chronic periodontitis and tooth loss, and treatment studies have demonstrated inferior outcomes of surgical and non-surgical periodontal therapy in smokers compared to non-smokers. Notably, signs of gingival inflammation may be less pronounced in smokers than non-smokers due to vasoconstriction and increased keratinization of the gingival tissue [1].

Thus, patients should be continuously reminded of the importance of smoking cessation for successful management of periodontitis [18–20,22,39,40].

- **Diabetes *mellitus***

The prevalence and severity of periodontitis is increased in individuals who have long-standing diabetes m*ellitus*, and particullary in patients with poor glycemic control. In contrast, chronic periodontitis may have a negative effect on metabolic control of glucose in individuals with diabetes *mellitus*, as it contributes to increased inflammatory burden and insulin resistance [1].

Several studies show that type 1 and type 2 diabetes, undiagnosed or poorly controlled, increase susceptibility to the development of periodontal disease. On the other hand, patients with controlled diabetes can maintain a state of periodontal health and respond favorably to periodontal treatment. There is also scientific evidence that periodontal treatment can improve glycemic control in patients with type 2 diabetes, leading to a significant decrease in the

level of glycosylated hemoglobin (HbA1c) raging between 0.4% and 0.7% [19,39,41].

- **Obesity**

Overweight and obesity have several adverse effects on general health and are associated with the development of chronic diseases such as cardiovascular disease, diabetes, cancer, liver disease, kidney disease and periodontal disease.

Worldwide, it is estimated that the prevalence of obesity has tripled since the 1980s, currently affecting 36% of the adult population and about 18% of children and adolescents. The association between obesity and periodontal disease is due to the increased release of pro-inflammatory cytokines produced by adipose tissue. Visceral fat is associated with increased tumor necrosis factor (TNF-α) and interleukin-6, which has an effect on osteoclast formation and host response, inducing the destruction of alveolar bone and connective tissue [19,41].

- **Osteoporosis**

Osteoporosis is a systemic disorder characterized by loss of bone density. This pathology has a prevalence of 13 to 18% in women and 3 to 6% in men. This discrepancy is essentially due to menopause, which is associated with a reduction in estrogen production, resulting in increased bone resorption.

Several cross-sectional studies have shown that alveolar bone density is altered in osteoporotic individuals. Fewer studies have demonstrated a relationship with clinical attachment levels. However, these results have also been contradicted by several other studies. In longitudinal studies a relationship has been shown between osteoporosis and alveolar bone loss, but not between osteoporosis and clinical attachment levels [19,21].

- **HIV**

HIV is a disease that compromises the immune system and affects about 34 million people worldwide. This is the disease that causes the most inequalities in access to health care, economic opportunities and in the protection of human rights in the world. There are several forms of transmission of this disease, with emphasis on transmission through sexual intercourse and the

use and sharing of injectable drugs, hereditary transmission from mother to child, and also transmission via blood in healthcare settings [22].

The patient with HIV, being immunosuppressed, presents a greater susceptibility to the development of periodontal disease. For this reason, in these patients, there is a higher level of attachment loss of periodontal tissues and an increase in gingival recession [22,42].

- **Diet**

Most chronic diseases such as cardiovascular disease, diabetes, cancer, obesity, and dental disease are related to diet. A diet rich in saturated fatty acids and non-dairy extrinsic sugars, and low in polyunsaturated fats, fiber and vitamins A, C and E, increases the patient's propensity to develop periodontal disease. Severe vitamin C deficiency and malnutrition can also result in the development of periodontal disease. Missing teeth at advanced ages can also weaken the nutritional status of patients with pre-established periodontal disease [22,41].

- **Stress**

Psychosocial factors are related to cardiovascular disease, diabetes, and other chronic diseases. Stress causes the alteration of the host's defenses, having an immunosuppressive effect, due to the release of noradrenaline. It also promotes an increase in cortisol and a decrease in cytokines, which leads to the release of a neuropeptide, which is involved in several inflammatory processes. Also, because of stress, harmful behaviors can be triggered, such as poor oral hygiene, less frequent dental appointments and changes in eating habits.

Consequently, with the alteration of the immune response associated with risk behaviors, the onset and progression of periodontal disease can be promoted [19,22,39].

Non-modifiable risk factors

- **Age**

Age is a non-modifiable risk factor for periodontal disease, widely discussed in the literature. Older people have been shown to have a more severe inflammatory response to plaque build-up, a response that contains a greater

number of inflammatory cells. This aggregation of inflammatory cells places older individuals at greater risk of experiencing periodontium destruction.

Furthermore, because aging is associated with a loss of dexterity, older individuals tend to be less proficient in their oral hygiene practices. This results in higher levels of plaque, which is a known risk factor for developing periodontal disease. Additionally, research has shown greater clinical attachment loss (CAL) in individuals aged between 60 and 90 compared to those under 50 [36].

Age is directly related to the severity of periodontal disease, as well as the level of bone loss. Age, per se, is not a risk factor for periodontal disease, but rather the cumulative effect of exposure to established risk factors and periodontal damage over the years [18].

- **Sex**

It is widely described in the literature that the sex most affected and with greater severity is the male gender. After analyzing the data collected through the National Health and Nutrition Examination Survey (NHANES) of 2009/2010, it was observed that the male gender has a prevalence greater than 50%. Regarding the severity of the disease, men have 33%, 28% and 180% more chances of having mild periodontitis, moderate periodontitis and severe periodontitis, respectively, than women. It has also been shown that there is a difference between the two sexes that could explain the higher risk of periodontal disease in males, however, there seems to be no difference, thus being described as a result of lifestyle [19].

- **Race/ Ethnicity**

The risk of developing periodontitis is also influenced by different races and ethnicities. Let's take the American population as an example, where African American and Latin American individuals have a higher prevalence of this pathology. These differences can be explained by the low socioeconomic status and behaviors of these populations [18].

- **Genetis and epigenetics**

Genetic polymorphisms are modifications in genes that can trigger the alteration of a protein or its expression, resulting in alterations in the host's immune response. Genetic factors are extremely important for individual

susceptibility to the appearance and development of periodontal disease, since its transmission between members of the same family varies between 40% and 50% [19].

Genetic predispositions have been considered important for both the initiation and progression of periodontitis, with high heritability estimates reaching 50%. However, genome-wide association studies available to date have failed to consistently identify specific single nucleotide polymorphisms across populations. In contrast to Mendelian diseases, where the pathological phenotype is typically the result of an abnormality affecting a single gene, the genetic predisposition to periodontitis is likely conferred collectively by hundreds or thousands of genes, whereas the clinical phenotype is defined by the interaction between environmental, genetic, and epigenetic factors. Epigenetic factors have only recently gained attention, and further investigation into their role is awaited [1].

Epigenetic factors

- **Pregnancy**

Pregnancy is associated with fluctuations in hormone levels, changes that have been shown to promote an inflammatory response that is linked to gingivitis and periodontitis. Although not clearly understood, maternal hormones have been shown to be positively correlated with levels of *Porphyromonas gingivalis*, a key microorganism in the progression of periodontal disease. Both hypoestrogenism and hyperestrogenism have been shown to contribute to gingivitis [36].

Finally, several genetically related systemic diseases have been manifested as risk factors for periodontal disease. The etiology of the development of periodontal disease within these systemic diseases has also been documented in the literature. These diseases include Down syndrome, Ehlers-Danlos syndrome (types IV and VIII), and Crohn's disease [36].

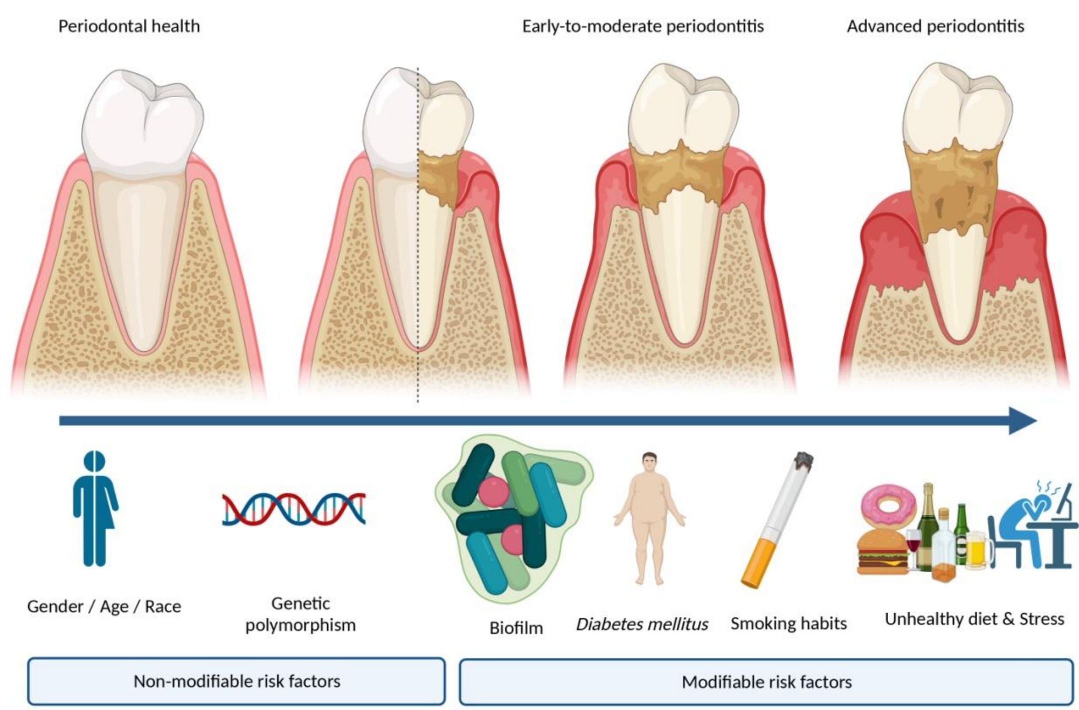

Figure 1.1: Representative diagram of the evolution of periodontitis, non-modifiable risk factors and modifiable risk factors.

1.1.2 Etiopathogenesis

To understand the etiopathogenesis of periodontal disease, it is essential to know the complex dental biofilm, as well as the immune response associated with the disease [17].

Bacterial plaque is a complex biofilm consisting of bacteria surrounded by a protective matrix. This matrix is composed of extracellular polysaccharides and glycoproteins, which provide a protective environment for microorganisms in the dental biofilm. This component of dental biofilm makes it 1000 to 1500 times more resistant to antimicrobial agents. The various circulatory channels present in the biofilm help in the distribution of many nutrients and in the excretion of metabolic waste generated [17].

Gingivitis and periodontitis are initiated and sustained by microorganisms in dental plaque. In fact, microbial biofilm has been widely studied and can comprise around 150 species in a single individual, and up to now, up to 800 different species have been identified in human dental plaque samples. The debate over which species are particularly virulent and which can lead to localized disease has lasted for decades and remains unresolved. Putative

pathogens include Gram-negative anaerobic bacteria, spirochetes and even viruses, but it is likely that no single pathogen is the cause, rather that dysbiosis itself (an imbalance of the microbial biofilm) is the pathogenic "driver". If periodontal disease was caused by one or a few specific pathogens, the preferred therapeutic strategy would be a targeted alteration of the plaque microbiota rather than total biofilm removal [1]. The biofilm, due to different concentrations of pH and metabolites, has several microenvironments. These microenvironments make the ecosystem suitable for a variety of microbes that inhabit the same dental plaque [17]. The initial layer deposited on the surface of the teeth in the formation of dental plaque is the acquired pellicle. This layer is formed within seconds of exposure of tooth surfaces, followed by initial attachment of the first biofilm colonizers [17].

Streptococcus and *Actinomyces* species are primary colonizing, facultative Gram-positive bacteria. "Adhesion receptors" on the surface of primary colonizers bind to the pellicle's proline-rich proteins. This binding leads to the disclosure of receptor sites known as a "cryptope", and further to coaggregation. Gradually, there is a deposition of layers of dental plaque that leads to a deficiency of oxygen in the environment, which can eventually lead to the colonization of anaerobic bacteria. The linking microbe between the primary and secondary colonizers is the *Fusobacterium* species. The gradual shift from these aerobic to anaerobic conditions marks the progression of gingivitis to periodontitis [17].

The host response to bacterial infection is gradual through the classic innate immune response. This response includes signs of acute inflammation, including increased gingival redness, bleeding and swollen gums, as well as migration of neutrophils to the site of inflammation [17].

For a susceptible host, microbial infection of the subgingival dental biofilm by periodontal pathogens, in particular a group of specific Gram-negative anaerobic species referred to as the red complex, results in chronic inflammation [23,24]. These red-complex bacteria include *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, which are predominantly found in deep periodontal pockets of patients with periodontitis [16,23,24,43]. Lipopolysaccharide (LPS) along with other virulence factors from these periodontal pathogens stimulate the host macrophages, and other inflammatory and constituent cells, leading to the production of a range of

pro-inflammatory cytokines such as tumour necrosis factor (TNF)-a, interleukin (IL)-1b and prostaglandin E2 (PGE2). The presence of these pro-inflammatory cytokines and virulence factors stimulates the production of matrix metalloproteinases (MMPs) by macrophages, fibroblasts, junctional epithelial cells, and neutrophils [44,45]. The resulting MMPs then mediate the destruction of collagen fibres in periodontal tissues, especially periodontal ligament [44]. In addition, the pro-inflammatory cytokines induce the expression of receptor activator of nuclear factor kB ligand (RANK-L) on the osteoblasts and T helper cells. The resulting RANK-L on the osteoblasts and the T helper cells then interacts with receptor activator of nuclear factor kB (RANK) on osteoclast precursors, and this interaction results in the genesis of osteoclasts and their maturation. The mature osteoclasts mediate alveolar bone destruction [46,47]. Periodontitis was previously believed to progress at a constant rate until treatment or tooth loss [48].

Innate immunity (Figure 1.2) also activates the body's primary host cells, preparing the body to defend against bacterial infections, as well as triggering adaptive immunity. Innate immunity also triggers the host cell to differentiate into more specific cells, in turn increasing pro-inflammatory mediators such as Interleukin 1-beta, prostaglandins and tumor necrosis factor. The cascade is activated, resulting in adaptive immunity activation (Figure 1.2), activating specific T and B lymphocytes. There is documentation on B and T cell involvement in the activation of RANK (nuclear factor-kB) which results in bone loss due to activation of osteoclasts [17].

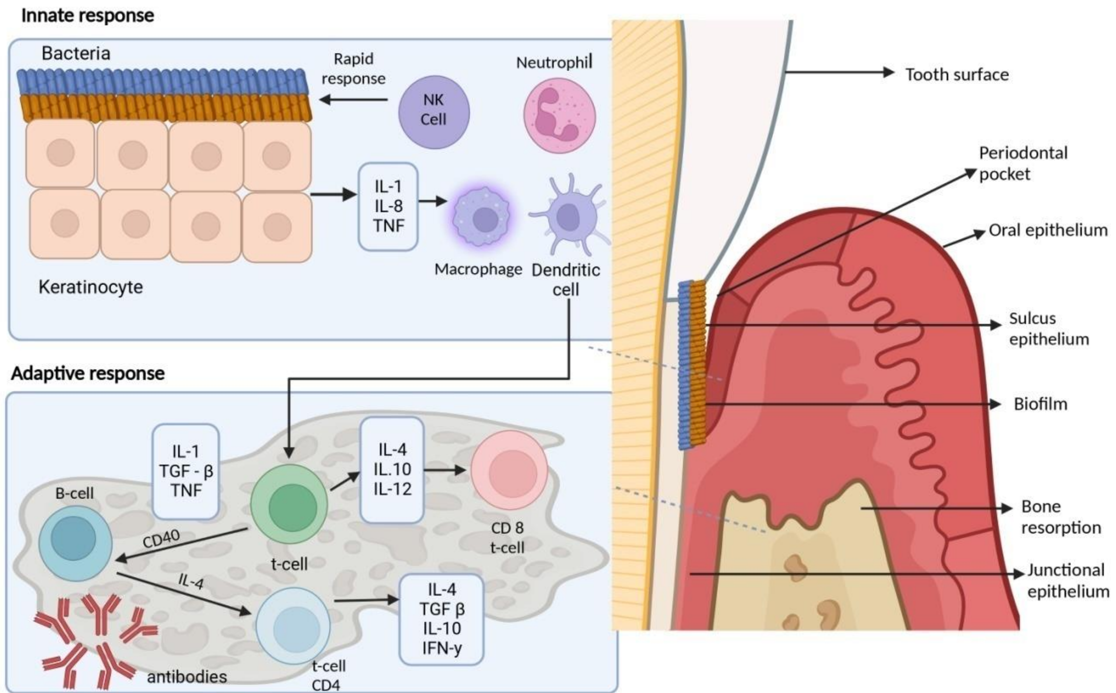

Figure 1.2: Immune responses in periodontitis (left side) - Effect of periodontitis. Tissue affected by periodontitis, accumulation of bacterial plaque on the surface of the tooth and root, causing destruction of periodontal tissue and alveolar bone (right side).

Currently, based on longitudinal observations from human and animal studies, periodontitis is now believed to progress by recurrent acute episodes instead [48,49]. During their lifetime, patients with periodontitis exhibit a cycle of bursts of destruction at individual sites over short periods of time, followed by longer periods of remission [48,50]. As periodontal disease progresses from gingivitis to periodontitis, greater numbers of anaerobic organisms colonize deeper periodontal pockets, such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, which trigger the inflammatory response in the host. This response includes the production and dissemination of C-reactive protein (CRP), a biomarker of inflammation, as well as various neutrophil and macrophage compounds such as tumor necrosis factor alpha (TNF-a), matrix metalloproteinases (MMPs), and interleukins. (IL-1 and IL-8). An elevated serum CRP level suggests that inflammation resulting from periodontitis may be correlated with cardiovascular pathology [36].

Periodontal disease are multifactorial infections elicited by a complex of bacterial species that interact with host tissues and cells, causing the release

of a wide range of inflammatory cytokines, chemokines and mediators, some of which lead to the destruction of periodontal structures, including the supporting tissues of teeth, alveolar bone and periodontal ligament [16].

The bacteria associated with periodontal disease trigger inflammatory responses in cells of the immune system, which in more advanced stages of the disease, lead to loss of soft tissue structures, such as the hard tissue that supports the teeth [6,16].

Periodontitis is an inflammatory disease induced by biofilm and characterized by loss of bone around the teeth. Microbial plaque is necessary to induce the inflammatory response, but it is not sufficient to induce periodontitis. However, a complex balance is responsible for maintaining health [51].

1.1.3 Microbiota in periodontitis

The subgingival microbiota is decisively related to the initiation, maintenance, and progression of the disease. The subgingival niche offers ecological conditions with available nutrients, which favor the growth of a diverse microbiota [52].

The oral cavity is home to a natural and diverse microbiota that persists on oral surfaces as well as on the tooth structure, and multi-species biofilms that are functionally organized have a symbiotic relationship with the host. The host provides a warm and nutritious habitat, while the resident oral microbiota provide important health benefits (e.g. exclusion of pathogens, immune modulation, reduction of the entero-salivary nitrate cycle [53,54].

The oral microbiota is composed of a combination of viruses, protozoa, fungi, archaea and bacteria. Historically, the composition of subgingival microflora has been characterized by culture methods. However, culture techniques have important drawbacks. For example, only viable bacteria can grow in specific culture media and strict sampling and transport conditions are essential. Furthermore, if non-selective media are used, the sensitivity of the bacterial culture can be particularly low, with detection limits averaging 10^3-10^4 bacterial cells. Thus, high numbers of a specific bacteria in a sample are required to allow its detection [55].

The bacteria that colonize the hard and soft tissues of the oral cavity profoundly influences oral health and disease. Microbiological studies identified more than 700 species of microorganisms in the oral cavity, including a large number of non-culturable species (~50%), more than 400 of which were detected in the sulcus or periodontal pocket, the remaining species were identified from other oral locations such as the tongue, oral mucous membranes and carious lesions [51].

There is recognized evidence of the role of the oral microbiota in the etiology of periodontal disease and some specificity between certain species or groups of bacterial species and the various forms of periodontal disease. However, although the complexity and diversity of the periodontal microbiota have been confirmed by numerous studies, until now, only three bacterial species have been recognized as true periodontal pathogens - namely, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia* [56].

However, since then, the study of the subgingival microbiota has expanded considerably. The composition, complexity, and diversity of the oral microbiome have been studied in greater depth in recent years. These advances in knowledge were mainly a consequence of technological progress in molecular methods, which allowed for an open analysis of the local microbiome and/or the study of the non-cultivated segment of the microbiota [56].

Subgingival microbiome changes

Microorganisms live symbiotically with humans and play an important role in health and disease. Changes in the microbiome contribute to the pathogenesis of many diseases and reflect the health or disease state of the host. Thus, monitoring changes in the microbiome is a potentially promising new application in disease diagnosis and prognosis [57,58].

The subgingival microbiome, that is, the community of microorganisms that inhabit the subgingival environment, has been the subject of investigation over several decades. Research compares the subgingival microbiota under different periodontal conditions and has been conducted using various techniques. However, the techniques available at the time did not allow a global view of the

microbial composition of a given sample, in conjunction with high-resolution taxonomy and high-throughput sample processing [59].

Although some periodontal pathogens have occasionally been detected and at low levels in specimens from individuals whose disease has not been diagnosed, many of the organisms that have recently been implicated in disease have only been detected at sites of inflammation. The factors that lead to changes in the microbiota in periodontal disease are not fully understood. Several theories have been postulated to explain the change from a symbiotic to a dysbiotic relationship with the host. These theories range from exogenous infections, co-infection with viruses, proliferation of minor species within the biofilm following changes in the local environment, to the low abundance of key pathogens that compose commensal species to provoke a destructive inflammatory response. However, experimental evidence to prove these concepts is sparse [53].

According to Diaz *et al.* 2016, studies revealed a large proportion of subgingival microbial diversity and community dynamics, which are crucial to the current understanding of the etiopathology of periodontitis. All these studies agree that periodontal disease is associated with changes in the composition of the community of microorganisms in the periodontal sulcus or pocket, compared to periodontal health [59].

Subgingival microbial complexes

Socransky *et al.* 1998, who studied the bacterial complexity in subgingival plaques using whole genomic DNA probes and checkerboard DNA-DNA hybridization, reported the association between the oral bacterial community and periodontal disease. The results identified the presence of 40 different taxa in subgingival plaque and suggested that 3 bacterial species, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, are the main pathogenic periodontal bacteria, which have been associated with red complex bacteria [23].

Orange complex bacteria, including *Fusobacterium nucleatum*, *Prevotella nigrescens*, and *Prevotella intermedia*, are known as bridge bacteria between early colonizing strains and red complex bacteria [17,60].

Periodontitis is a dysbiotic disease resulting from subgingival variation, from Gram-positive bacteria to Gram-negative bacteria. The development of periodontal dysbiosis occurs over an extended period, which slowly transforms the symbiotic association of host and microorganism to a pathogenic form. Among the microbial complexes, the first one that has been related to the disease is the orange complex, which consists of anaerobic Gram-negative species, such as *Prevotella intermedia*, *Prevotella nigrescens*, *Prevotella micros* and *Fusobacterium nucleatum*, which during disease progression changes to the red complex, which consists of *Tannerella forsythia*, *Tannerella denticola* and *Porphyromonas gingivalis* [27,61,62].

According to Mohanty *et al.* 2019, cluster analysis and community ranking techniques of more than 13,000 subgingival plaque samples from 185 adult individuals were used to demonstrate the presence of specific microbial groups within dental plaque. Six closely associated groups of bacterial species were recognized which included *Actinomyces*, a yellow complex consisting of members of the genus *Streptococcus*, a green complex consisting of *Capnocytophaga* species, *A.actinomycetemcomitans* serotype a, *E. corrodens* and *Campylobacter*, and a purple complex consisting of *Veillonella parvula* and *Actinomyces odontolyticus*. These groups of species are the primary colonizers of the tooth surface, whose growth generally precedes the multiplication of the predominantly Gram-negative orange and red complexes [16].

The orange and red complexes are constituted by the species considered the main etiological agents of periodontal disease. Red complex bacteria are known to appear together in plaque samples, often adjacent to the epithelial tissue of the periodontal pocket, in deeper areas. This is mainly due to the cross-species interaction, co-aggregation and metabolic interdependence between these three bacterial species [61,63]. Among the red complex bacteria, the amount of *P. gingivalis* showed the strongest association with the severity of the periodontal condition, and the co-occurrence of *P. gingivalis* with *T. denticola* and/or *T. forsythia* showed a marked progression of periodontitis [64].

Red complex members are rarely found in the absence of orange complex members. With increasing colonization by the orange complex, more sites are colonized by increasing numbers of the red complex (Figure 1.3) [16].

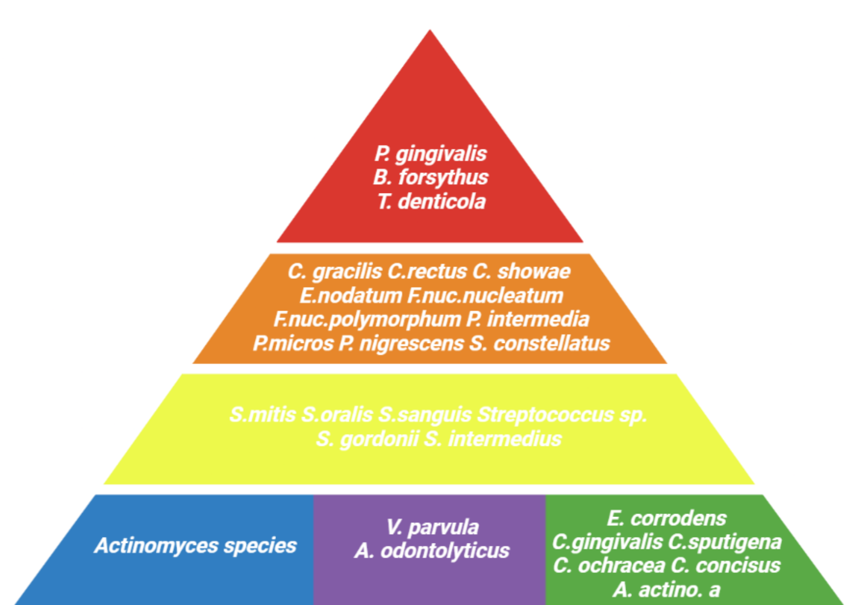

Figure 1.3: Different subgingival microbial complexes.

P. gingivalis has long been considered an important member of the periodontopathogenic microbiota involved in the progression of periodontal disease and the destruction of bone and tissue [16,65].

Interactions between *P. gingivalis* and other members of the oral microbiota, including *Streptococcus spp*. and *F. nucleatum* result in specific co-aggregation, which contributes to the ability of microorganisms to effectively colonize the subgingival sulcus. The initial event in the pathogenicity of *P. gingivalis* is its interaction (adhesion) to the oral cavity. To achieve this, *P. gingivalis* employs several bacterial components: fimbriae, proteases, hemagglutinins and lipopolysaccharides [16].

1.1.4 Diagnosis

Diagnosis of periodontal disease requires comparison of results against healthy periodontium. This comparison utilizes clinical visual examination, periodontal probing, and assessment of radiographically observed bone levels [36].

A healthy periodontium consists of stippled, pale pink gingiva that is well-adapted to the underlying bone. Between the gingiva and the tooth, there is a

physiological sulcus of 1 to 3 mm that normally does not show signs of hemorrhage. Clinical features of periodontitis include redness, change in texture and swelling of the gingival margin, bleeding from the gingival pocket area during probing, increased depth of the periodontal pocket detected with periodontal probing, clinical attachment loss, destruction of supporting structures of the teeth (alveolar ligament and bone), recession of the gingival margin (which exposes the root), increased tooth mobility, bad taste/odor, radiographic bone loss, and eventually tooth loss. Pain may arise with acute exacerbations due to abscesses or tooth displacement caused by weakened tooth support. However, typical periodontal disease is painless, and it is common for it to reach advanced degrees of severity before it is detected and treatment begins [1,3,41,66].

The first challenge in the treatment of periodontal disease is a timely and accurate diagnosis, since the loss of bone and soft tissue is progressive and largely irreversible, but it is particularly difficult since early periodontal disease is painless, and patients rarely seek care in the early stages of the disease. In fact, the initial symptom of gingivitis is bleeding during brushing; pain is rarely reported [36].

A patient's medical history should be obtained prior to periodontal assessment. This will provide identification of any systemic or environmental risk factor for periodontitis, such as diabetes and smoking. In the clinical history, the following should be considered: what is the main complaint and expectations of the patient, medical and medication history, family history, dental history, oral hygiene habits and other habits, such as smoking (amount and duration of the habit) and consumption of alcohol and/or drugs.

The diagnosis of periodontitis is based on a series of clinical measurements and their recording on the periodontal chart, which include the plaque index (PI), gingival index (GI), clinical attachment loss (CAL), bleeding on probing (BoP), probing depth (PD), suppuration, gingival margin, furcation lesions, tooth mobility, mucogingival defects, oclusal trauma, overhanging/over-contoured restorations, tooth migration and food impaction [5,41,67]. Accurate diagnosis requires the recording of these multiple parameters in six locations per tooth (affected or not), which results in a laborious diagnostic process that depends on the level of experience of the evaluator. This procedure needs to be repeated at regular visits to monitor the course of the

disease and compare treatment results. These clinical parameters are the best measures currently available for diagnosis; however, they can only assess the current extent and severity of the disease. No information can be extrapolated about future disease activity due to the low sensitivity and low positive predictive value of this test [1].

Clinically, periodontitis is characterized by:

1. Interdental clinical attachment loss (CAL) is detectable at ≥ 2 non-adjacent teeth; or,

2. Attachment loss of 3 mm or more, on the buccal or lingual/palatal surface in at least 2 teeth, with the exception of the following situations: gingival recession of traumatic origin; dental caries reaching the cervical area of the tooth; attachment loss on the distal surface of a second molar and associated with poor positioning or extraction of a third molar; endo-periodontal lesion with drainage through the marginal periodontium; vertical root fracture [3,5].

A comprehensive radiographic evaluation is part of the initial periodontal evaluation to determine the extent of horizontal and vertical alveolar bone loss. According to the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [3], a new periodontitis classification categorises the disease based on a multi-dimensional staging and grading system. Staging is determined by the severity of the disease at initial presentation and the complexity of disease management [3]. Grading is used as an indicator of the rate of periodontitis progression, which is determined by the history as well as the presence of risk factors for periodontitis [3].

Depending on the severity and degree of progression, periodontitis is classified into different stages (I, II, III and IV) and degrees (A – slow progression, B – moderate progression, C – rapid progression), respectively. The definition of the stage, which refers to the severity of the disease, presupposes the evaluation of several parameters such as the clinical attachment loss, the amount and percentage of bone loss, the probing depth, the extent of angular bone defects and furcation involvement, tooth mobility and tooth loss. For all stages, the extent of disease should be classified. It can be classified as localized (<30% of the teeth involved) or generalized (≥ 30 of the teeth involved) or according to Molar-Incisor distribution. The degree, in addition to assessing aspects related to the risk of periodontitis progression

and general health, associates other factors that modify the degree, such as smoking and the level of metabolic control of diabetes [3,5].

Radiographs can be used to aid diagnosis and help determine the likely prognosis of specific teeth when taken together with a comprehensive clinical examination and patient history. The gold standard radiograph for periodontal assessment is a periapical radiograph taken using a long-cone paralleling technique. Correctly positioning this radiograph will give an accurate, non-distorted two-dimensional picture of bone levels in relation to both cemento enamel junction (CEJ) and total root length. This technique involves the use of a beam aiming device which helps achieve better and more consistent results [68].

Once the diagnosis is made, the etiological factors must be removed (the microbial biofilm on the surfaces of the teeth and gums) and the patient must be informed and motivated to change modifiable risk factors (for example, poor oral hygiene, smoking and uncontrolled diabetes *mellitus*). Due to the fact that modifiable risk factors are predominantly within the patient's control, the successful management of chronic and severe periodontitis strongly depends on patient motivation and behavior changes and is therefore a clinical challenge [1].

1.1.5 Periodontal treatment

Periodontal treatment aims to restore periodontal health through the elimination of bacterial plaque, periodontopathogenic organisms and reduction of risk factors that may potentiate the disease, therefore, the patient has a fundamental role in the success of the treatment [67,69].

The goals of periodontal treatment are:

1. Reduction or resolution of gingival inflammation: obtain a percentage of bleeding on probing ≤25%;

2. Probing depth reduction: absence of periodontal pockets ≥ 5mm;

3. Elimination of furcations in multirooted teeth (furcation involvement should not exceed 2-3 mm in the horizontal direction);

4. Absence of pain;

5. Satisfactory aesthetics and function [67].

Periodontal treatment has four distinct phases:

1. Systemic Phase: its main objective is to eliminate or reduce the influence of systemic conditions on treatment results and protect the patient and health professionals against infectious risks. At this stage, a consultation should also be carried out with the aim of promoting a healthy lifestyle, which should include a smoking cessation consultation;

2. Initial/Causal/Hygienic Phase: aims to eliminate and prevent the recurrence of bacterial plaque deposits on the supra and subgingival dental surfaces and any retention factor. Clinicians should educate patients about the importance of effectively removing dental biofilm at home, especially prior to proceeding with active periodontal therapy [70]. The importance of adequate home care should be reinforced frequently during the initial and subsequent phases of periodontal treatment. Achieving adequate home care is an essential component of prevention of periodontal disease, successful periodontal therapy and long-term retention of the dentition [71,72].

It is in this phase that the Non-Surgical Periodontal Treatment (NSPT) is carried out, which includes:

- Oral hygiene instructions: the patient should be instructed about the most appropriate brushing technique for his/her case. Interdental hygiene should also be promoted using dental floss, brushes and interdental brushes. In certain situations, the use of an electric toothbrush may be recommended;

- Chemical control: it can be carried out through mouthwashes with chlorhexidine, cetylpyridinium chloride or through toothpastes containing fluoride, triclosan or chlorhexidine;

- Removal of bacterial plaque and calculus deposits by performing scaling and root planing (SRP) [1,73].

After adequate home care or biofilm control is achieved, scaling and root planing should be performed at the sites with periodontal probing depths of 5 mm or greater. This phase of treatment should be delivered in conjunction with correction of local contributing factors, removal of local plaque retention factors, extraction of hopeless teeth and treatment of active carious lesions. During scaling and root planing, adequate local anaesthesia should be

administered prior to initiating the procedure to ensure patient comfort. Automated instruments, such as piezoelectric or ultrasonic scalers, may be used in combination with manual instruments [74]. For areas where access is difficult, automated instruments may be superior to curettes for removal of subgingival biofilm and calculus [75]. Occlusal adjustment should be considered to relieve fremitus, severe mobility, or excessive central and lateral excursive contact [76].

Root planing aims to remove infragingival calculus and infected cementum, to restore periodontal biological compatibility on the surface of the roots. This therapeutic approach, considered the gold standard in periodontal therapy, can be performed using different types of instruments: manual (curettes), ultrasonic (scaler). Curettes are sharp instruments with one or two cutting edges used for the removal of calculus and plaque, both supragingival and subgingival, which is crucial in periodontal disease. Ultrasonic versions of these instruments vibrate in the ultrasonic range (approximately 25,000-30,000 cycles per second) and can be used, along with a water jet, to remove deposits adhering to teeth. Both manual and ultrasonic instruments are effective in removing subgingival calculus and altering the subgingival microbiota and their use is complementary in SRP [1,77].

This procedure allows for improvements in clinical parameters: reduction of probing depth, increased clinical attachment level and reduction of bleeding on probing [1,77].

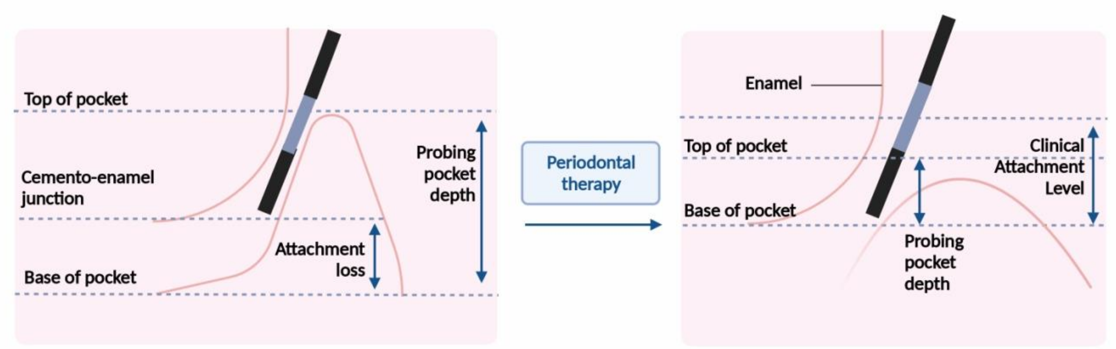

Figure 1.4: Representative scheme of the effect of periodontal therapy: reduction of probing depth, increased clinical attachment level.

This treatment can be performed using two techniques: the conventional, with the treatment of only one quadrant per session/week, making a total of 4 sessions, and the full-mouth-disinfection (FMD), performed in one or two sections within a period of 24 hours. The existence of very deep pockets, fissures and root concavities and overhanging/over-contoured restorations can impair healing and the effectiveness of non-surgical periodontal treatment [17,67].

Once SRP is completed, a period of 6-8 weeks is required for proper connective tissue healing before reassessment.

During the reassessment visit, clinical measurements are again recorded and response to initial treatment is assessed [1,77].

At this stage, the patient may present with:

· Periodontal pockets + PI and GI ≥ 20%, in which case it is necessary to perform oral hygiene motivation;

· Periodontal pockets > 5mm + BoP and/ or suppuration + PI and GI ≤ 15-20%, making it possible to perform periodontal surgery;

· Absence of periodontal pockets + PI and GI ≤ 15-20%, which allows the patient to move on to the supportive periodontal therapy.

If there are no teeth with residual inflammation and periodontal pockets, then the patient is placed on periodontal maintenance. However, if there is residual inflammation and active disease, further treatment is required, which can be localized or generalized, surgical or non-surgical, depending on the extent and severity of residual inflammation [1].

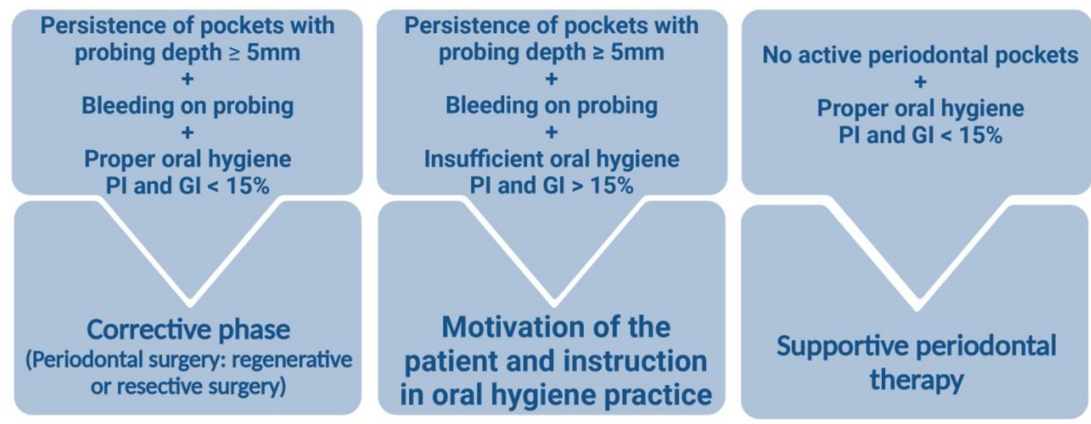

Figure 1.5: A decision tree on periodontal re-evaluation.

3. Corrective Phase: if, after non-surgical periodontal treatment, there are still deep pockets with bleeding and/or suppuration, it becomes necessary to resort to surgical periodontal treatment [25]. This has the objective to create a more effective access for the performance of root planing by the clinician, to establish a gingival morphology that facilitates the control of plaque by the patient and to regenerate lost periodontal tissues. As previously mentioned, surgical periodontal treatment should always be preceded by non-surgical periodontal treatment, with the aim of reducing tissue inflammation and facilitating surgery, as there will be less bleeding, greater predictability of the final position of the margins, less amount of calculus and presence of less friable tissues. The success of periodontal surgery will depend on the patient's habits, such as oral hygiene and smoking, and on the anatomy of the bone defects. These can be classified into intraosseous, supraosseous and furcation defects. There are several types of periodontal surgeries, which aim to treat periodontal pockets:

3.1. Resective Surgery: its main objectives are the reduction of periodontal pockets, the removal of residual calculus and the achievement of a positive bone architecture, which allows good control of bacterial plaque in the long term. It is based on the techniques of osteoplasty (alveolar bone remodeling), seeking to reconstitute the physiological anatomy, without removing the supporting bone, and osteotomy (eliminating the supporting bone), which aims to eliminate bone defects and remodel the alveolar bone. Gingivectomy, the apical repositioning flap and root surgery are also part of the resective techniques. The treatment of furcation defects is an example of

root surgery, where techniques such as amputation, tunneling or hemisection of roots are used [25];

3.2. Regenerative Surgery: mainly used in the regeneration of deep infraosseous defects, it aims for the regeneration of lost or injured tissues, so that their architecture and function are restored. The cementum, periodontal ligament and alveolar bone can be regenerated. Examples of this type of technique are grafts (autogenous, allogeneic, xenograft and alloplastic material) and guided tissue regeneration [25].

4. Periodontal Supportive therapy (PST): aims to prevent the recurrence of periodontal disease. It can be limited through optimal plaque control by the patient and through periodic maintenance appointments. This phase must be individually adapted to each patient and normally consists of remotivation for good oral hygiene, root planing of residual pockets and control and elimination of causal and risk factors [41,67,78].

1.2 Halitosis

Halitosis is a common problem that manifests itself as an unpleasant and disgusting odor emanating from the mouth [79]. Malodor is mainly caused by the putrefactive action of microorganisms on endogenous or exogenous proteins and peptides. Oral malodor is an embarrassing condition that affects a large percentage of the human population. This condition often results in nervousness, humiliation, and social difficulties, such as the inability to approach people and speak to them [80–84].

Halitosis can be divided into extra-oral halitosis (EOH) and intra-oral halitosis (IOH) [80,81,83]. The factors that increase the likelihood of halitosis include periodontal diseases, dry mouth, smoking, alcohol consumption, dietary habits, diabetes, and obesity. Halitosis can also be affected by the general hygiene of the body (i.e., dehydration, starvation, and high physical exertion), advanced age, bleeding gums, decreased brushing frequency, but also by stress [81,85–87].

More and more patients are struggling with bad breath and report this problem to their primary care practitioner for diagnosis and management [88,89]. However, many physicians and dentists have insufficient knowledge regarding the cause and biochemistry of this disease.

1.2.1 Prevalence

Epidemiological studies report that halitosis prevalence rates range from 2.4 to 78%. According to the American Dental Association (ADA), about 50% of American adults suffer from bad breath [90]. Based on a cross-sectional study, which aimed to measure self-perception, knowledge and awareness of halitosis among 392 volunteer university students in Saudi Arabia, the prevalence of halitosis was 21.4% [91]. Almost all participants (89.3%) of an online panel of over 1000 individuals considered representative of the Dutch population reported regularly encountering people with halitosis, with 40% occurring at least once a week [92]. On the other hand, a study in Pakistan reported a 75% prevalence of halitosis. This cross-sectional study was carried out with the aim of assessing the prevalence of halitosis and associated factors among 833 students and interns at seven Dental Schools in Lahore, Pakistan [93].

Some recent data from Asia reported high prevalence rates: 44.9% in Japan and 65.9% in China. In the study carried out in Japan, with the objective of investigating the prevalence and risk factors associated with halitosis, in 520 primary school students and 248 secondary school students, through a questionnaire, halitosis was measured using a method of organoleptic evaluation and oral clinical examination. As a result, 44.9% of the individuals had halitosis [94]. Regarding the study carried out in China, it included individuals with a complaint of halitosis who attended the clinics between 2014 and 2016. Questionnaires were used to obtain general information from the patients and to assess halitosis, organoleptic tests were performed and VSC was measured. In total, 205 samples were included in the data analysis, and the patients' ages ranged from 18 to 71 years. Among these individuals, 65.9% had halitosis [95].

Many factors can influence the great variability between studies, namely the method used for the assessment of halitosis, the geographic region where the study was carried out and the year of the study. Regarding the last factor, latest studies showed a higher prevalence of bad oral odor. A possible cause is the worldwide change in dietary patterns, with an increase in alcohol consumption [90]. While in developed countries the prevalence of halitosis was 29%, in developing countries it was 39.8%. The estimated prevalence in the general population was 31.8% [90]. The available epidemiological data are based on convenience samples and on self-perception of oral odor, which is

why there is little consensus in the literature on the prevalence of halitosis [90]. Probably due to the subjective nature of the information collected and the methodological differences between the studies [87].

1.2.2 Volatile Compounds

Halitosis mainly results from presence of odoriferous substances - named volatile sulfur compounds (VSC), which are produced mainly by bacteria in the oral cavity. In the oral cavity, nearly 700 different compounds have been detected [96]. To these volatile substances belong sulfur compounds, aromatic compounds, amines, short-chain fatty or organic acids, alcohols, aliphatic compounds, aldehydes, and ketones (Table 1.1) [97–100]. It is considered that hydrogen sulfide, methyl mercaptan, and dimethyl sulfide are the main volatile compounds in IOH [101–104]. In Table 1.1, values of odor thresholds are presented. Amid VSCs, which are the most often described compounds in IOH, methyl mercaptan has the lowest value of odor threshold, followed by hydrogen sulfide and dimethyl sulfide. This means that these substances are mainly responsible for the unpleasant smell in the mouth. Besides, methyl mercaptan is in much lower concentrations than the other compounds.

Categories	Compounds	Odor Threshold (ppm)[105,106].
Volatile sulphur compounds	Methyl marcaptan: CH_3SH	5.1×10^{-13}
	Hydogen sulphide: H_2S	0.00004
	Dimethyl sulphide: $(CH_3)_2S$	0.00012
Diamines	Putrescine: $NH_2(CH_2)_4NH_2$	No data
	Cadaverine: $NH_2(CH_2)_5NH_2$	No data
	Butyric acid: $CH_3CH_2CH_2COOH$	0.001
	Propionic acid: CH_3CH_2COOH	0.00099
	Valeric acid: $C_5H_{10}O_2$	0.000037
Phenyl compounds	Indole: C_8H_7N	0.0003
	Skatole: C_9H_9N	0.0000056
	Pyridine: C_5H_5N	0.01
Alcohols	Methanol: CH_4O	3.05
	Ethanol: C_2H_6O	0.09
	Propanol: C_3H_8O	0.031
Nitrogen-containing compounds	Ammonia: H_3N	0.043
	Urea: $(NH_2)_2CO$	No data
Aldehydes and ketones	Acetaldehyde: C_2H_4O	0.0015
	Acetone: C_3H_6O	0.4
	Acetophenone: C_8H_8O	0.00024
	Benzophenone: $C_{13}H_{10}O$	No data

Table 1.1: Volatile molecules contributing to oral malodour and their thresholds **[103–106].**

Studies have shown that volatile sulfur compounds are the major contributors to bad breath. These are produced mostly by anaerobic bacteria. The increased production of malodorous gases occurs mainly in tongue coating, and in diseases such as gingivitis and periodontitis and, to a less extent, in pericoronitis, oral ulcers, periodontal abscesses, and herpetic gingivitis [107–109].

Other molecules involved in this bacterial degradation process are diamines (indole and skatole) or polyamines (cadaverin and putrescin). They seem to play a less important role in the expression of bad breath. Most of these components are produced in the proteolytic degradation process of peptides. The most predominant substrates in this VSC production are cysteine, cystine and methionine [110]. The main substrate for skatole and indole production is tryptophan, whereas lysine and ornithine are the basis for the putrescin/cadaverin production.

Volatile sulfur compounds can be toxic for human cells even at low concentrations. They contain thiols (-SH groups) that interact with other proteins and support the negative interaction of bacterial antigens and enzymes. The result of this effect is chronic inflammation, periodontal gingivitis, and periodontitis [111]. In human gingival fibroblasts, H_2S activates the mitochondrial pathway of apoptosis [112]. The H_2S is a known genotoxic agent, which has an impact on genomic instability and cumulative mutations [113]. In studies on rats, it was demonstrated that hydrogen sulfide leads to ultrastructural changes in epithelial cells and periodontal destruction [114]. Increased amounts of H_2S by the activation of proliferation, migration, and invasion can also lead to carcinogenesis [115,116]. *Fusobacterium nucleatum* and *Porphyromonas gingivalis* belong to the most essential carcinogenic oral bacteria producing VSCs [115,117]. Acetaldehyde is also cancerogenic and is produced from ethanol by mucosal epithelial cells or oral microflora, e.g., *Candida albicans*, *Candida* non-albicans, *Neisseria sp*., and *Streptococcus sp*. Acetaldehyde binds to DNA and leads to the formation of DNA adducts, point mutations, and DNA cross-linking [118,119]. Other important substances causing IOH are diamines, such as putrescine and cadaverine. Both compounds are produced from amino acids, putrescine from arginine, and cadaverine from L-lysine [120,121] (Figure 1.6). Both diamines are associated with the putrefaction of food by bacteria occurring in the dental plaque and severe

periodontitis [122]. Gram-negative bacteria, mostly *Enterobacteriaceae*, which can colonize the oral cavity and dentures, produce urease that hydrolyzes urea into carbon dioxide and ammonia [123]. *Escherichia coli* can form ammonia from cysteine using cysteine desulfhydrase [124] or reduce nitrates to ammonia [125]. Major contributors to trimethylamine production are gut bacteria, which can be inhabitants of the oral cavity, such genus *Anaerococcus*, *Clostridium, Collinsella*, *Desulfovibrio*, *Lactobacillus*, *Escherichiai*, *Citrobacter*, *Edwardsiella*, *Providencia*, and *Proteus* [126–129]. Indole and skatole are produced in high amounts by intra-oral, Gram-positive *Streptococcus milleri*, and anaerobic Gram-negative bacteria such as *Porphyromonas intermedia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. Small amounts of both aromatic compounds can be produced by *Aggregatibacter aphrophilus*, *Staphylococcus epidermidis*, and *Streptococcus sanguis* [130].

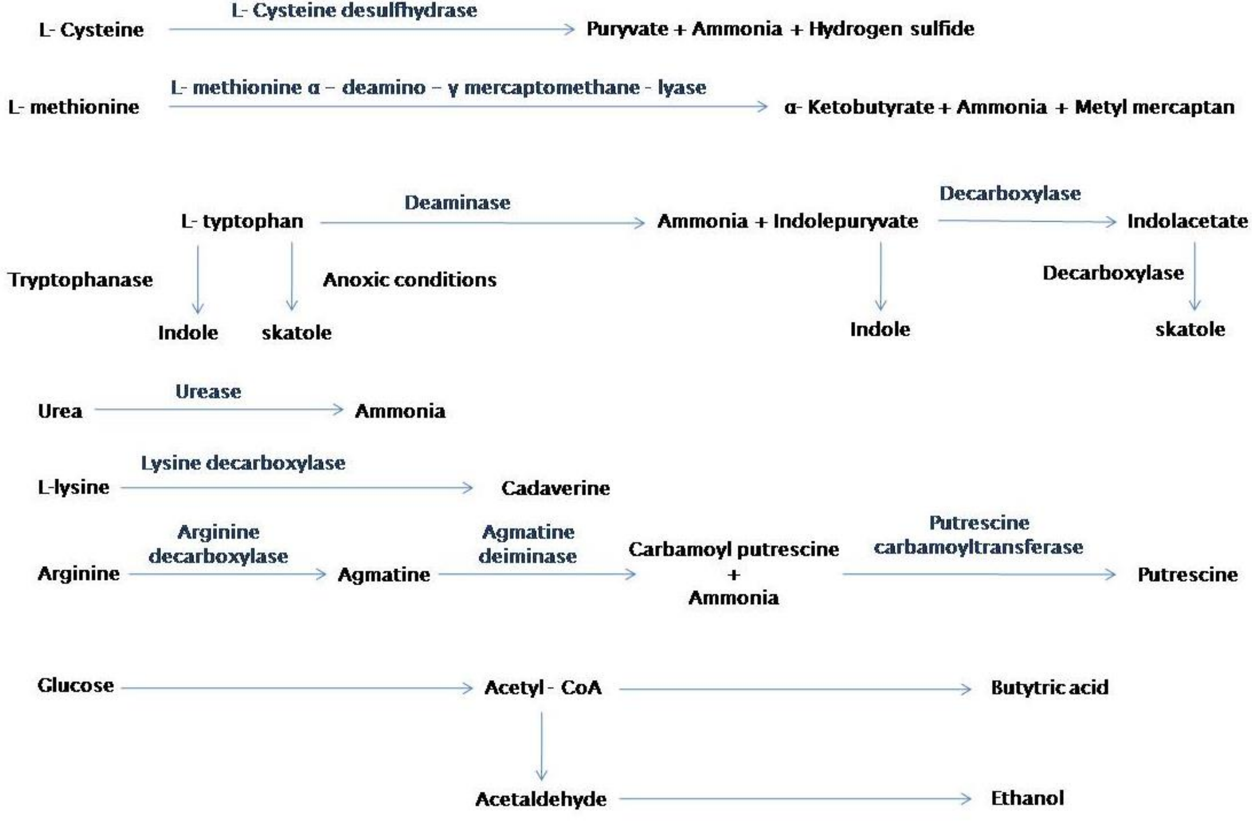

Figure 1.6: Simplified ways of selected odorous compounds production [93,131–133].

1.2.3 Microbiota Responsible for Intra-Oral Halitosis

The human oral cavity microbiota is an ecosystem consisting of various symbiotic microbes. There is a relationship between the global composition of indigenous bacterial populations and human health [131,132]. The oral microbiota is truly diverse and consists of 50–100 billion bacteria. There are about 700 taxa, of which one-third cannot be grown *in vitro* [133]. A vast range of microorganisms inhabit the human oral cavity, including bacteria, fungi, viruses, and protozoa [134,135]. The basic oral microbiota consists of phyla, such as *Firmicutes*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, and *Actinobacteria*. The most dominant genera are *Streptococcus*, *Veillonella*, *Gemella*, *Granulicatella*, *Neisseria*, *Haemophilus*, *Selenomonas*, *Fusobacterium*, *Leptotrichia*, *Prevotella*, *Porphyromonas*, and *Lachnoanaerobaculum*. Current findings reported that oral bacteria can be biomarkers that differentiate healthy and pathological conditions within the oral cavity. The oral microbiota research is used as a diagnostic and prognostic tool in the aspect of human health. In the human body, the oral cavity is the second site, after the colon, containing the largest diversity of microbial populations [136]. Simultaneously, changes in the gut microbiota are reflected in the oral microbiota, and the microbial communities of the oral cavity and gastrointestinal tract are predictive of each other [137–139].

The oral bacteria that are most likely to produce hydrogen sulfide from L-cysteine or serum are *Bacteroides* spp., *Eubacterium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., *Porphyromonas* spp., *Selenomonas* spp., *Tannerella forsythia*, and *Veillonella* spp. Another essential component of VSC is methyl mercaptan produced from L-methionine or serum. It is a metabolic product mainly derived from *Bacteroides* spp., *Eubacterium* spp., *Fusobacterium* spp., *Porphyromonas* spp., and *Treponema denticola* [97,140] (Table 1.2).

The most active producers of hydrogen sulfide are Gram-negative anaerobes *Prophyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (red complex). Furthermore, the red complex microorganisms are associated with periodontal disease. Hydrogen sulfide and methyl mercaptan are produced in large quantities in periodontal inflammation [141–143]. During periodontitis, *Porphyromonas* spp., *Prevotella* spp., and *Treponema denticola* may play the most crucial role in providing aminoacids to other anaerobic bacteria. Through

this process, anaerobes acquire the opportunity to produce H_2S and CH_4S [144] (Figure 1.6).

In the studies of Takeshita et al., the producers of hydrogen sulfide in saliva were bacteria from the genera *Neisseria*, *Fusobacterium*, *Porphyromonas*, and SR1. In contrast, producers of the methylmercaptan are representatives of the genera *Prevotella*, *Veillonella*, *Atopobium*, *Megasphaera*, and *Selenomonas* [145]. Significant contributors to methylmercaptan production are also gut bacteria, which can be inhabitants of the oral cavity, such as *E.coli*, *Citrobacter spp*., and *Proteus spp*. [146].

Volatile Sulphur compounds	Bacteria
H_2S from cysteine	*Peptosteptococcus anaerobius* *Micros prevotii* *Eubacterium limosum* *Bacteroides spp.* *Centipedia periodontotii*
H_2S from serum	*Prevotella intermedia* *Prevotella loescheii* *Prophyromonas gingivalis* *Treponema denticola* *Selenomonas artemidis*
CH_3SH from methionine	*Fusobacterium nucleatum* *Fusobacterium periodonticum* *Eubacterium spp.* *Bacteroides spp.*
CH_3SH from serum	*Treponema denticola* *Porphyromonas gingivalis* *Porphyromonas endodontalis*
Other	*Prevotella melaninogenica* *Tanerella forsythensis* *Eikenella corrodens* *Solobacterium moorei* *Treponema forsythensis* *Centipeda periodontii* *Atopobium parvulum*

Table 1.2: : Bacteria responsible for VSC production.

Many studies showed that bacterial diversity in the group of patients with IOH is much higher than in the control group. Furthermore, many publications draw attention to the correlation between halitosis and individual microorganisms. The relationship between tongue coating and VSC gases is also mentioned by many authors [81,147]. Veloso et al. mentioned that in 85% of the patients IOH is caused by Gram-negative bacteria [84]. According to Wei et al., the oral microbiota responsible for IOH includes a wide range of microbial communities, including 13 phyla, 23 classes, 37 orders, 134 genera, 266 species, and 349 operational taxonomic units. The largest percentage amongst the oral cavity microorganisms are genera, like *Prevotella*, *Alloprevotella*, *Leptotrichia*, *Peptostreptococcus*, and *Stomatobaculum*. These bacteria present a higher percentage of occurrence in the sample of patients with IOH than in the control samples from healthy patients [148]. In turn, the presence of bacteria, such as *Firmicutes, Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Fusobacteria*, was demonstrated in both samples from examined and control groups. *Firmicutes* was the most abundant phylum in saliva samples from both groups [149,150]. The composition of the tongue microbiota has an essential influence on IOH. The most common molecular technique for testing and evaluating an oral cavity microbiome is DNA sequencing [151–154]. Seerangaiyan et al. published a review in 2017, in which they showed the composition of the microbiome by *Aggregatibacter*, *Campylobacter*, *Capnocytophaga*, *Clostridiales*, *Leptotrichia*, *Parvimonas*, *Peptostreptococcus*, *Peptococcus*, *Prevotella*, *Selenomonas*, *Dialister*, *Tannerella*, and *Treponema* in the group of patients with IOH. Using the amplification and sequencing of the 16S rRNA encoding gene, there searchers also demonstrated a high prevalence of *Solobacterium moorei* strains in the IOH group. By testing the control group, significant differences were found in both groups of healthy and sick people. Furthermore, using polymerase chain reactions (PCRs), Seerangaiyan et al. showed the positive correlation of *Leptotrichia spp.* and *Prevotella spp.* to oral malodor severity, contrary to *Haemophilus spp.*, *Gemella spp.* and *Rothia spp.* [151]. Patients with IOH have a specific biofilm on the dorsal part of the tongue. Bernardi et al. stated that this biofilm consists of a significant proportion of *Fusobacterium nucleatum* and *Streptococcus spp.*. The occurrence of these two types of bacteria in patients with IOH was completely related. According to the authors, these microorganisms contribute significantly to IOH and can be used as treatment

targets [155]. In other research, Bernardi and partners showed that *Actinomyces graevenitzii* and *Veillonella rogosae* were closely related to the occurrence of IOH in a group of volunteers. Also, *Streptococcus mitis/oralis*, *S. pseudopneumoniae*, and *S. infantis*, as well as *Prevotella spp*. were detected often in malodor patients. Moreover, following the earlier findings, the researchers' results revealed the presence of *Actinomyces odontolyticus*, *Solobacterium moorei*, *Prevotella melaninogenica*, *Fusobacterium periodonticum*, and *Tannerella forsythia* in IOH patients. Furthermore, microorganisms such as *Streptococcus parasanguinis*, *S. salivarius*, *Veillonella spp*., and *Rothia mucilaginosa* dominated the oral microbiota of healthy people [153].

Yitzhaki et al. noticed the connection between IOH and wearing dentures. The unpleasant odor was organoleptically assessed and the oral microbiome was analyzed using 16S rRNA gene sequencing technology. Researchers have identified bacterial taxa, including nine phyla, 29 genera, and 117 species. The samples taken from patients with IOH showed the dominance of the phyla Firmicutes and Fusobacteria and the genera *Leptotrichia*, *Atopobium*, *Megasphaera*, *Oribacterium*, and *Campylobacter*. The analyses revealed a significant diversity of the oral microbiota among samples from IOH patients wearing dentures and significant differences in comparison to the control group [154].

The use of tobacco also has a massive impact on the oral microbiota diversity. After examining a group of smokers and non-smokers, researchers reported that in both groups, most of the oral microbiota were Gram-negative bacterial strains. Simultaneously, *Klebsiella pneumoniae* dominated in smokers'saliva and *Pseudomonas aeruginosa* in non-smokers'saliva samples. An essential finding of the research was also that the *Candida* species accounted for the largest percentage of microbes amongst smokers with halitosis [156]. Al-Zyound et al. performed tests showing an increased level of three bacterial genera in smokers: *Streptococcus*, *Prevotella*, and *Veillonella*. Researchers provided evidence that tobacco smoking has a direct effect on the oral microbiota. They also suggested that after smoking cessation, it is possible to return to the standard composition of the oral cavity microbiota [157]. Wu et al. noticed significant changes in the oral microbiota that occurred amongst obese people suffering from malodor. The *Prevotella*, *Granulicatella*,

Peptostreptococcus, *Solobacterium*, *Catonella*, and *Mogibacterium* were more abundant genera in the obesity group than in healthy people [158]. Halitosis has often been reported amongst the symptoms related to *Helicobacter pylori* infection and gastroesophageal reflux disease. Anbari et al. made the observations that the incidence of malodor amongst *Helicobacter pylori*-positive patients was 74% [108]. However, Tagerman et al. disagreed about a possible relationship between *Helicobacter pylori* infection and objective halitosis [159]. In Table 1.3, results of studies concerning microbiota associated with IOH are presented. Summarizing the table, the oral bacteria that are most related to IOH are *Actinomyces spp.*, *Bacteroides spp.*, *Dialister spp.*, *Eubacterium spp.*, *Fusobacterium spp.*, *Leptotrichia spp.*, *Peptostreptococcus spp.*, *Porphyromonas spp.*, *Prevotella spp.*, *Selenomonas spp.*, *Solobacterium spp.*, *Tannerella forsythia*, and *Veillonella spp.*

Author / year of publication	Bacteria related to Intra-Oral Halitosis	Studied Population	Study Method
Persson et al, 1990. **[140]**	*Bacteroides gracilis, B. intermedius, B. loescheii, B. oralis, Capnocytophaga ochracea, Centipeda periodontii, Eikenella corrodens, Eubacterium brachy, E. lentum, E. limosum, Fusobacterium alocis, F. nucleatum, F. periodonticum, F. sulei, Mitsuokella dentalis, Peptostreptococcus anaerobius, P. magnus, P. micros, P. prevotii, Porphyromonas endodontalis, P. gingivalis, Propionibacterium propionicum, Selenomonas artemidis, S. dianae, S. flueggei, S. infelix, S. noxia, S. sputigena, Tannerella forsythia, Treponema denticola, Veillonella dispar, V. parvula*	9 participants	Bacterial culture
De Boever, Loeche, 1995. **[160]**	*Fusobacterium sp., P. gingivalis, Prevotella intermedia*	16 IOH adults or children	Bacterial culture
Roldán et al, 2003. **[161]**	*Campylobacter rectus, F. nucleatum, P. micros, P. gingivalis, P. intermedia, T. forsythia*	40 IOH patients	Anaerobic culture
Grover et al, 2015. **[162]**	*Fusobacterium sp., P. gingivalis, P. intermedia, T. forsythia*	20 IOH adults	Anaerobic culture
Faveri et al, 2006.	*P. gingivalis, P. intermedia, P. melaninogenica, P. nigrescens, Streptococcus*	10 adult persons	Checkerboard DNA-DNA

[163]	*constellatus, T. forsythia, T. denticola, V. parvula*		hybridization technique
Ademovski et al, 2013. **[164]**	*Actinomyces israelii, A. neuii, A. odontolyticus, Aggregatibacter actinomycetemcomitans (serotype a), Atopobium parvulum, Prevotella bivia, P. disiens, P. nigrescens, Pseudomonas aeruginosa, Staphylococcus epidermis, S. constellatus, Streptococcus mitis, T. forsythia, V. parvula*	21 IOH Adults	Checkerboard DNA-DNA hybridization technique
Kamaraj et al, 2011. **[165]**	*F. nucleatum, P. gingivalis, T. forsythia*	30 adults	PCR
Awano et al, 2002. **[166]**	*P. gingivalis, P. intermedia, T. forsythia*	101 IOH Adults	PCR
Tanaka et al 2004. **[167]**	*P. gingivalis, P. intermedia, P. nigrescens, T. forsythia, T. denticola*	29 IOH patients and 10 healthy adults	Real-time PCR
Nani et al, 2017. **[168]**	*F. nucleatum, Solobacterium moorei, T. forsythia*	78 adult males	Quantitative real-time PCR
Adedapo et al, 2020. **[169]**	*A. actinomycetemcomitans, F. nucleatum, P. gingivalis, P. intermedia, T. denticola*	31 IOH patients and 31 healthy adults	16S rDNA-directed PCR

Takeshita et al, 2012. **[145]**	*Atopobium sp., Dialister sp., Eubacterium sp., Fusobacterium nucleatum, Leptotrichia sp., Megasphaera sp., Neisseria sp., Parvimonas sp., Peptococcus sp., Peptostreptococcus sp., P. gingivalis, P. endodontalis, Prevotella sp., Selenomonas sp., Solobacterium sp., SR1 sp., Veillonella sp.*	30 IOH patients and 13 healthy persons	PCR and sequencing
Bernardi et al, 2020. **[153]**	*A. odontolyticus, F. periodonticum, Leptotrichia sp., Okadaella gastrococcus, Prevotella melaninogenica, S. moorei, T. forsythia*	6 IOH patients and 6 healthy adults	PCR and sequencing
Yitzhaki et al, 2018. **[170]**	*phyla Firmicutes and Fusobacteria, genera Atopobium, Campylobacter, Leptotrichia, Megasphaera, Oribacterium*	26 full dentures patients	PCR and sequencing
Riggio et al, 2008. **[171]**	*A. odontolyticus, Atopobium parvulum, Lysobacter-type species, Porphyromonas sp., P. melaninogenica, P. pallens, P. veroralis, Streptococcus salivarius, S. mitis, S. oralis, V. parvula*	20 IOH patients and 12 healthy adults	PCR and DNA sequencing
Haraszthy et al, 2007. **[172]**	*Eubacterium sp., Dialister sp., Granulicatella elegans, Porphyromonas sp., P. intermedia, Staphylococcus warneri, S. moorei*	8 IOH patients and 5 healthy adults	PCR and DNA sequencing
Seerangaiyan, et al 2017. **[83]**	*Aggregatibacter sp., A. segnis, Campylobacter sp., Capnocytophaga sp., Clostridiales, Dialister sp., Leptotrichia sp., Parvimonas sp., Peptostreptococcus sp., Peptococcus sp., Prevotella sp., Selenomonas sp., SR1, Tannerella sp., TM7-3, Treponema sp.*	16 IOH patients and 10 healthy adults	16S RNA Sequencing

Table 1.3: Results of studies concerning bacteria associated with intra-oral halitosis (IOH).

1.2.4 Halitosis Assessment

The diagnosis of halitosis results from a questionnaire and clinical examinations [173]. Most patients (93.9%) with a primary complaint of halitosis have objective evidence of halitosis, as supported by organoleptic methods [174]. However, there is still some constraint in approaching the need for halitosis treatment [175]. A study was carried out with a representative sample of the Dutch population, where 1006 participants answered an online questionnaire with four questions about how they would act when in contact with people with halitosis. It was concluded that the probability of someone drawing attention to a person regarding their halitosis would be lower, the greater the social distance between people. More specifically, it was found that 40% of the sample said that they would draw the attention of a colleague to halitosis and less than 6% indicated that they would do so with a person found accidentally [175].

Clinical Questionnaire

The first step towards a correct diagnosis includes taking a complete clinical history (medical history, history of dental treatments performed, medication habits, patient's main complaint, oral hygiene habits, nutritional and behavioral habits, self-perception of bad breath and confirmation by the others) [176,177]; assessment of oral health status (including soft tissue, dental and periodontal examination, oral hygiene indices, tongue coating); and finally halitosis measurement [177].

Regarding halitosis complaints, it is important to understand the frequency, duration, time when the halitosis complaint appear, and who identified the problem; as well as knowing the list of medications taken, habits (smoking, alcohol consumption) and other symptoms (nasal secretion, anosmia, cough, pyrexia and weight loss) [176].

It is also important to identify and record clinical manifestations of dry mouth, saliva pH, tooth decay, periodontal disease, presence of oral lesions and general oral health. Any associations with underlying systemic conditions should be considered. Patients may have multiple factors contributing to halitosis, which may be a combination of medications, foods, local, systemic, and psychological causes [177].

Halitosis assessment tests

The diagnostic tests currently used in halitosis examination can be divided into direct and indirect. Direct tests include self-perception of odor or evaluation by others and determination of odorous substances by halitometry or gas chromatography. Indirect tests determine the presence of some bacterial species, metabolic products produced by microorganisms *in vitro* or enzymes [178].

Cases lacking biological causes for objective halitosis, such as poor oral hygiene, tongue coating, overhanging restorations, infectious in the oral cavity, and systemic disorders including paranasal, gastric, hepatic, pulmonary, urinary, endocrine, metabolic, degenerative, autoimmune, respiratory, or intestinal illnesses, may require psychiatric consultation [179].

Direct halitosis diagnosis methods

Direct halitosis diagnosis methods include halitosis self-perception, the organoleptic method, gas detectors, for measuring VSC levels such as gas chromatography (e.g. OralChroma™) and portable sulphide monitor (e.g. Halimeter®) [177].

- Self-perception of halitosis as a diagnostic method

Patients seek treatment for halitosis usually due to self-perception or third-party feedback regarding their breath [174]. Self-perceived halitosis correlated positively with real halitosis only when subjects smelled their own saliva, isolated from the mouth [174].

One study compared 252 self-assessments of patients with halitosis, organoleptic and halitometric results and concluded that self-perception was positively correlated with clinical halitosis. The sensitivity and specificity of self-perceived halitosis was 47.2% and 59.2%, respectively [180].

Although an efficient gas measurement protocol for separating oral, nasal and alveolar odor and the ability to measure oral odor has been described, the diagnosis of halitosis should be based primarily on self-perception or the assessments of others [174]. Although organoleptic and VSC measurement tests are objective methods to assess oral malodor, they require a trained

examiner and specific equipment. On the other hand, self-perception reduces costs and delay in clinical examination [90].

- **Organoleptic method**

The organoleptic test is considered the *Gold Standard* test in the diagnosis of halitosis, since only the human sense of smell can detect different mixed odors, whether they are VSC or organic compounds. It is a practical and inexpensive exam, as it does not require any necessary material for its execution [174,181].

The organoleptic evaluation carried out by the clinician's sense of smell for odor perception is a direct measurement technique. In this method, trained human judges evaluate, by smell, the odor of the patient's breath [81]. It may, however, present the risk of disease transmission [182]. The examiner must be in good physical and health condition, must abstain from drinking coffee, tea or juice, from smoking and from using perfumed cosmetics before the evaluation process. For the evaluation to be effective, the patient must also follow instructions: 1) not to take antibiotics during the 3 weeks prior to evaluation; 2) avoid eating garlic, onions and spicy foods in the 48 hours prior to the assessment; 3) avoid using perfumed cosmetics in the previous 24 hours; 4) do not ingest any food or drink for at least 3-4h before the test; 5) not perform oral hygiene, at least 2 hours before; 6) avoid the use of mouthwashes; 7) avoid smoking for at least 12 hours before the assessment [178].

Below, several possible techniques for the organoleptic assessment of halitosis are described.

· Plastic tube method

A plastic tube is placed in the patient's mouth and the patient is asked to inhale through the nostrils and exhale slowly into the tube. During this period, the examiner assesses the odor on the other side of the tube, 20 cm away. The purpose of using the tube is to decrease the intensity of exhaled air [178].

· Count-to-twenty test

In this technique, the patient must hold his breath while the examiner positions the nose, 10 cm from the oral cavity. The judge sniffs the expired air

while the patient counts from 1 to 20, to promote dryness of the palate and tongue mucosa and thus facilitate the release of VSC [176].

· Saliva odor test

In the saliva odor test (wrist licking test), the patient licks the wrist and after 10 seconds the judge assigns a score [176].

· Olfactory test

The odor coming from the nasal breath is evaluated by the judge, while the patient exhales with the mouth closed [176].

· Spoon test

In the spoon test, the back of the tongue is scraped with a spoon, and the odor is further evaluated [176].

· Paper bag method

As an alternative and less uncomfortable method, the patient exhales into a paper bag and then the judge examines the odor of the bag [176].

· Dental floss test

In the dental floss test, the passage is made on the mesial and distal faces of teeth 16, 26, 36, 46, subsequently evaluating the odor.

For all these organoleptic evaluation techniques described, Rosenberg established a scoring system from 0 to 5 classifying the intensity of bad odor:

Grade 0: No perceptible odor;

Grade 1: Slightly perceptible odor;

Grade 2: Perceptible odor;

Grade 3: Moderate odor;

Grade 4: Strong odor;

Grade 5: Very strong odor [183].

Individuals with values ≥ 2 are considered positive to halitosis.

The reason why the organoleptic test has been considered the *Gold Standard* method for diagnosing halitosis is its ease of execution and the fact that the human nose is capable of detecting more than 10,000 odors, much more than any device available on the market [177]. It is also able to define odors as

pleasant and unpleasant, evaluating not only the VSC but also other organic compounds from the expiration and identified as unpleasant [184]. However, the examiner does not smell a pure sample of mouth air, but a mixture of mouth air and air from airways. The organoleptic examination does not distinguish between the two, it only subjectively assesses the general level of odors [174]. To avoid any bias, it is preferable that this assessment precedes all other assessments [177]. This method is cheap, does not need an electronic device and allows the clinican to detect VSC and unpleasant organic compounds, present in bad breath. Several studies have shown that the assessment of organoleptic scores is effective in the assessment of halitosis, as significant positive correlations were found between this assessment and VSC levels measured by gas chromatography devices and sulphide monitoring [185].

Before its application, the evaluator must undergo a qualitative assessment, testing its ability to feel and recognize different odors, as well as a quantitative assessment, verifying its ability to detect odors with low concentrations [177].

The usefulness of organoleptic examination has recently been contested, due to its subjectivity. It also adds the fact that it does not have calibration or international standardization and is not clinically reproducible [174].

The perception of odorous substances depends on several factors, including constant fluctuations regarding the individual clinician's threshold level for that specific odor, the concentration and volatility of the molecules themselves, ambient temperature (gases are less volatile at lower temperatures), humidity of the exhaled breath, how strongly the breath is blown into the examiner's nose (the less forcefully exhaled breath will consist of less air volume and fewer odorant molecules will be transported to the examiner's olfactory epithelium), and lastly, the concentration of the examiner at the time of the exhaled breath. All of these parameters vary with time and individual, making it a subjective measure [174].

Despite all the disadvantages, organoleptic measurements are the *Gold Standard* for assessing halitosis when combined with VSC measurement [186].

- Gas detectors

For the diagnosis to be complete and correct, it is necessary to detect VSC [187]. In fact, direct and objective clinical evaluation can be performed using

gas detectors such as gas chromatography or a portable sulphide monitor, which are considered the most sensitive and objective methods to evaluate these compounds [188].

Both devices measure VSC. The Halimeter® (portable sulphide monitor) provides an overall VSC concentration value, while the OralChroma™ (gas chromatography) distinguishes between the three main components of bad breath: hydrogen sulphide, dimethyl sulphide and methylmercaptan [189].

Gas chromatography

Quantitative analysis of odor-causing VSC (dimethylsulfide, methylmercaptan and hydrogen sulfide gases) is performed by gas chromatography [88]. Gas chromatography is a highly sensitive technique, considered ideal for quantifying and differentiating VSC present in the oral cavity.

- **OralChroma®**

The OralChroma® is a portable device, created in 2003, capable of distinguishing the three sulphur compounds most responsible for halitosis, hydrogen sulphide, methylmercaptan and dimethyl sulphide. It is equipped with an indium oxide semiconductor gas sensor and, unlike standard gas chromatographs, does not have a carrier gas, using air itself as a vehicle for the compounds for the chromatographic columns. It is a test that performs highly objective, reproducible, and reliable measurements, even when extremely low gas concentrations are present. However, it is a relatively expensive test, requiring sensor and column replacement every two years and requiring equipment calibration. Although it distinguishes the different sulphur gases, it cannot detect other compounds [190].

In this method, a photometric detector is used to measure the concentration of VSC [191]. It is possible to separately measure low concentrations of gases and determine their quantities. Samples collected from saliva, tongue coating and exhalation are analyzed by a detector and the mass spectra of existing compounds are compared and determined by a computer database. An automatic aspiration system was developed in gas chromatography to remove differences caused by sample or expiration techniques [88].

For the collection of samples, the patient closes his mouth and retains the air for 30 seconds, then the air in the mouth (10 mL) is collected with a hermetic

syringe. After taking the samples, air is injected into the gas chromatograph column at 70°C [192].

Threshold levels for bad breath are 112 parts per billion (ppb) for hydrogen sulfide, 26 ppb for methyl mercaptan and 8 ppb for dimethyl sulfide [188].

This OralChroma™ device has several limitations such as specificity, confounding factors, and stability [188]. It is not very easy to implement as it requires trained examiners, is time-consuming, expensive and has an unportable size. However, the ability of gas detection equipment to analyze specific target analytes is a clear advantage over the organoleptic method alone, so this equipment is a valuable aid for periodic analysis and prognosis [178].

It is suggested that gas chromatography, due to its ability to separately identify and measure gases qualitatively and quantitatively, is considered a more objective and sensitive method for VSC analysis [188]. It is also used in association with organoleptic measurement in order to overcome the subjectivity of the latter technique [81], with high correlations being found between organoleptic measurement and gas chromatography [88]. VSC monitors detect 18% to 67% of odors that can be effectively detected by organoleptic measurement. While most of these compounds can be measured, certain compounds such as volatile fatty acids (butyrate, propionate), diamines (cadaverine, putrescine) and other malodor products can only be measured by laboratory testing [178].

Portable sulfide monitor

The sulfide monitor is a portable device that allows easy measurement of VSC found in expired air outside the laboratory environment. The device was developed over time and presented on the market under the names of Halimeter® and Breathtron® [88]. It measures the amount of VSC emitted by the patient in ppb, not sensitive to dimethylsulfide [193].

- **Halimeter®**

The Halimeter® is a portable sulphide monitor capable of assessing oral malodour. It features a voltammetric sensor that emits an electrical signal when exposed to compounds such as hydrogen sulfide and methylmercaptan,

for which it has high and low sensitivity, respectively. It can analyze the total VSC content of the breath, in ppb. It is easy to use and does not require any calibration by the operator. It is relatively inexpensive and is considered less embarrassing for patients compared to the organoleptic method. The disadvantage of this technique is the existence of false positive results since the monitor does not differentiate between the different types of sulphides. Examples of these are acetone, ethanol and methanol, which are measured by the Halimeter®, and which do not contribute to halitosis [190].

To enable the VSCs to be read, the patient must keep the mouth closed for 1 to 2 minutes before the first sample. A cannula is then placed on the back of the tongue up to the reference mark, approximately four centimeters. This procedure must be performed carefully so that the opening of the cannula does not come into contact with any surface of the oral mucosa. The patient should breathe through the nose to allow the reader to measure the amount of total volatile sulphur compounds in the oral cavity and convert the value to ppb. Three readings are taken, and the result is obtained by taking the arithmetic mean of the three measured readings. The VSC results are interpreted according to the manufacturer's instructions: less than 80 ppb denoted no perceptible odor, 80 to 100 ppb denoted perceivable odor, 100 to 120 ppb denoted moderate halitosis, 120 to 150 ppb denoted more pronounced halitosis, and > 150 ppb denoted severe halitosis [190,194,195].

The electrochemical reaction that occurs in sulphur-containing compounds in breathing produces an electrical current, directly proportional to the levels of the compounds [177].

Halimeters are gas detectors that, although they measure specific gases, do not measure halitosis. When present, halitosis is composed of almost 700 organic gases, nitrogen or sulphur. The concentration of a captured gas at the time does not reflect the patient's average level of halitosis, because these levels fluctuate throughout the day, even every 2 minutes [179].

The portable sulfide monitor is easily operable and reproducible, also featuring a sensitivity and specificity of 92.2% and 91.7%, respectively [87]. However, it does not differentiate between the three VSC, being only sensitive to sulphur-containing compounds, more sensitive to hydrogen sulphide than to methylmercaptan, and almost insensitive to dimethyl sulphide. As oral malodor can comprise agents other than VSC, it may provide an imprecise assessment

of the source and intensity of oral malodor [176]. In addition, the presence of compounds such as alcohols, phenyl compounds and polyamines can interfere with readings [87].

Due to its portability, simplicity and speed, it is suitable for clinical studies [196].

Gas chromatography may be preferred if the clinical situation requires VSC differentiation. The sulfide monitor may be sufficient for an initial objective assessment of halitosis [187].

- ***Breathtron®***

It is a sulphide monitor, created in 1996, consisting of a semiconductor sensor, based on a thick zinc oxide membrane, sensitive to VSC. It features a silica gel filter, capable of retaining other undesirable volatile organic compounds, such as ketones and alcohol, which are mainly responsible for the incorrect results of the Halimeter®. It is an easy to perform, reliable and relatively inexpensive test. Another advantage is the fact that patients do not feel embarrassed by this test, in relation to the organoleptic evaluation [190].

It is performed by inserting a mouthpiece into the oral cavity, so that the patient closes his mouth and breathes through his nose. Air aspiration should be performed at 40 to 60 ml per minute. Values above 250 ppb, according to the manufacturer, indicate the existence of halitosis [190].

Indirect measurement tests

Indirect measurement methods include the BANA test, chemical sensors, salivary incubation test, quantification of β-galactosidase activity, ammonia monitoring, ninhydrin method and polymerase chain reaction [178].

These chemical and enzymatic tests only detect oral bacteria or bacterial metabolites instead of halitosis. The presence of bacteria, however, does not truly reflect the existence of halitosis [179]. They are rarely used in routine clinical practice because they are expensive and time consuming [176].

- **Benzoyl-DL-arginine naphthylamide Test (BANA)**

The BANA test is a test that evaluates the proteolytic activity of strictly anaerobic proteolytic Gram-negative bacteria, mainly those that form the red complex, such as *Treponema denticola*, *Porphyromonas gingivalis* and *Tannerella forsythia* and can be used as an adjunct in the measurement of VSC

and thus, in the detection of halitosis [176]. These bacteria are detected by an enzyme that degrades benzoyl-DLarginine-naphthylamide (BANA), a synthetic substrate of trypsin, which forms a compound, thus providing additional information on compounds other than VSC [178].

The sample is collected by swabbing the main areas of accumulation of bacteria, being subsequently placed on a BANA test strip and taken to the incubator for 5 minutes at 55°C. Changing the color of the test strip to blue represents the presence of these bacteria in the sample. The intensity acquired by the test strip is directly proportional to the bacterial concentration of the sample [190,197].

It has been shown that the BANA test, in the oral cavity, tongue and saliva, significantly correlates with the organoleptic tests [178].

- **Chemical sensors**

The chemical sensors, also called "electronic nose", have an integrated probe to measure the amount of VSC in the periodontal pockets and on the surface of the tongue. The principle is similar to sulfide monitors. In the presence of sulfide ions, an electrochemical voltage proportional to ion concentrations is created. The measurement is indicated by the digital score on the device's screen [198]. It has six sensors with different selectivity and sensitivity, and the information received is transferred to software. This method has a positive correlation with other measurement methods and is still under development [88].

With these devices it is possible to measure ammonia and methylmercaptan compounds from exhaled air and there are even some new types of sensors that measure each sulphur-containing compound separately. The sensitivity is similar to that of gas chromatography and the measurement results are very close to organoleptic measurements [192].

Using this method, it is possible to identify complex gaseous samples at low cost, obtaining immediate fast results (in real time). It is non-invasive and easy to use compared to other chemical analysis instruments and methods currently available for sample analysis, which makes them useful in clinical situations [198].

- **β-Galactosidase Test**

β-Galactosidase is one of the main enzymes responsible for eliminating the carbohydrate side chains of glycoproteins, making them more susceptible to proteolytic degradation by bacteria, which originates VSC. Levels of the enzyme beta-galactosidase have been found to correlate with oral malodor.The β-Galactosidase test uses a chromogenic substrate that allows quantifying the activity of β-Galactosidase in saliva [190,197]. When saliva comes into contact with the paper disk, a color change in the chromatography paper is detected, according to the amount of enzymatic activity [88].

- **Salivary incubation test**

In the salivary incubation test, saliva is collected in a glass tube and incubated at 37°C for 3 to 6 hours in an anaerobic medium containing 80% nitrogen, 10% carbon dioxide and 10% hydrogen. Then, the odor is evaluated by the examiner. The saliva incubation test is less affected by external factors, such as scented food, use of scented cosmetics, and tobacco consumption, when compared to the organoleptic assessment [88].

- **Ammonia monitor**

In addition to VSC, ammonia is another important component in halitosis. VSC can be detected by a portable sulfide monitor, however ammonia cannot be measured using this method. To measure ammonia produced by oral bacteria [88], a new portable monitor has been developed.

At least 2 hours before measurements, patients should refrain from eating and drinking. Then, patients rinse with mouthwash for 30 seconds and close their mouth for 5 minutes. This mouthwash includes a urea solution, as bacteria produce ammonia from urea. To measure the ammonia concentration, a disposable tube that is part of the device is placed inside the patient's mouth. The disposable tube is connected to an ammonia gas detector that contains a pump that extracts 50 mL of air and the ammonia concentration is recorded directly in the detector tube [192].

- **Amine Detection Test**

The gases responsible for halitosis result from the degradation of proteins into amino acids by bacteria, which are subsequently metabolized into amines and polyamines. This is a fast and inexpensive method capable of detecting low

molecular weight amino acids and amines present in saliva [190,197]. The detection of low molecular weight amines and polyamines, which cannot be done using the sulphur monitor, is then achieved using the ninhydrin method. With this method, isopropanol is mixed with the sample taken from the patient and centrifuged. Subsequently, through a spectrometer, the reading is carried out, according to its permeability to light. Ninhydrin calorimetric reaction is fast, easy to apply and inexpensive [88].

- **PCR**

The Polymerase Chain Reaction (PCR) refers to a widely used technique in basic and biomedical sciences. It is a laboratory technique used to amplify specific segments of DNA with a wide range of laboratory and/or clinical applications [199,200].

The perception that bacteria can be rapidly and accurately identified by obtaining the sequence of their rRNA genes introduced a new era in bacterial identification. The first success of the revolution in environmental microbiology was the detailed identification algorithms in clinical microbiology, where there was previously nothing equivalent. The procedure was simple: use primers that recognize conserved regions of the 16S rRNA genes to amplify the gene, obtain a partial sequence, and then compare it to increasing sequence databases. It is now possible to identify an unknown bacterial isolate in 24 hours using this approach; once the process is automated, the time will be even shorter [201].

Polymerase chain reaction (PCR) has been shown to be a sensitive, rapid, and specific method for detecting and quantifying individual oral, VSC-producing microbial species from saliva, tongue coating, and subgingival biofilm [202].

Final conclusions about the diagnosis

Diagnosing and solving this condition is important for patients' self-esteem and quality of life. Thus, it is important to take patient self-assessment into account, which may include feedback from others, rather than relying solely on objective examination methods [178]. The measurement of VSC levels detected by portable devices can be used as an adjunct tool to organoleptic tests in individuals with halitosis complaints [186].

In conclusion, several methods for measuring halitosis are available on the market, but more than an objective assessment, halitosis is a subjective perception of the individual [184]. Despite the great improvement of these devices in recent years, organoleptic tests, together with VSC measurement, remain the *Gold Standard* method for diagnosing malodor [186].

1.2.5 Halitosis and periodontal disease

Patients with periodontal disease often complain of bad breath. Several clinical studies have investigated a possible relationship between halitosis and periodontal disease [88].

Periodontitis is a chronic destructive inflammatory condition that affects the supporting tissues of the teeth and can lead to tooth loss [203]. It is known to be the second most common cause of intraoral halitosis, after tongue coating [204].

Tissue damage, characteristic of periodontal disease, results from unregulated and prolonged inflammatory responses due to persistent subgingival biofilm [203]. Bacteria associated with gingivitis and/or periodontitis, such as *Porphyromonas gingivalis* or *Prevotella intermedia*, are mainly Gram-negative anaerobes, capable of producing VSC, like the bacteria associated with halitosis [189].

A microbiological correlation between halitosis and periodontal disease was proposed, in which hydrogen sulphide and methyl mercaptan (VSCs produced by microorganisms) facilitate the penetration of lipopolysaccharides into the gingival epithelium, inducing inflammation. VSC also aids in bacterial invasion of connective tissue due to their toxic effects on epithelial cells, while methyl mercaptan hinders the growth and proliferation of epithelial cells. This phenomenon is accentuated by the decrease in oxygen tension, resulting from an increase in the depth of the periodontal pocket, with a concomitant decrease in pH, necessary for the putrefaction of amino acids that create VSC [87].

It has also been suggested that the saliva of patients suffering from periodontitis may produce increased amounts of VSC, including hydrogen sulfide (H_2S) and methylmercaptan (CH_3SH), which are the main causes of intraoral malodor [205].

One of the first to describe a correlation between the production of H_2S and the occurrence of inflamed periodontal pockets was Rizzo, in 1967. In this study it was demonstrated that the highest concentrations of H_2S were present in the deepest pockets [81].

Ten years later, Tonzetich showed that the increase in VSC correlated with the number and depth of periodontal pockets greater than 3 mm. Through root planing and periodontal surgery, these concentrations can be reduced [81]. Studies have shown that the occurrence of oral malodour is considerably higher in periodontal patients when compared to periodontally healthy individuals [205].

Several hypotheses have been proposed that VSC may be responsible for an accelerated destruction of periodontal tissues. In addition, the morphology of periodontal pockets creates an ideal environment for sulphur-producing bacteria [88]. Methylmercaptan has a pronounced effect on the permeability of the oral mucosa. Can be dimerized to dimethylsulfide, as sulfides are considered to be highly cytotoxic, methylmercaptan may accelerate the progression of periodontitis [81].

There is also a contribution of gingival inflammation to the intensity of oral malodor [205]. The increased blood supply to inflamed areas or bleeding conditions that accompany periodontal disease provides the bacteria with supplemental substrates (e.g. iron), creating favorable conditions for the growth of periodontopathogenic Gram-negative anaerobic bacteria, leading to excessive production of VSC in the oral cavity [206].

In an *in vitro* experiment, porcine epithelial tissues were treated with CH_3SH, resulting in severe cell damage or apoptosis. Even extremely low concentrations of VSC have proven to be toxic to periodontal tissues, and it is presumed that these compounds may facilitate bacterial invasion into deeper tissues [189].

VSC penetrate oral tissues, increase the permeability of the oral mucosa, and have an impact on fibroblast growth, affecting collagen synthesis and degradation, inhibiting the healing process. This fact facilitates the invasion of the gingival epithelium and allows periodontopathogenic microorganisms to penetrate the tissues. Stimulate production of the proinflammatory cytokine IL-

1, inducing a reaction that promotes prostaglandin E2-mediated bone resorption [207].

Regarding H_2S, it has been shown to induce apoptosis of human gingival cells as well as apoptosis of human gingival fibroblasts by increasing the level of reactive oxidative species [207]. Thus, VSC-induced mucosal permeability may play a role in the transition from gingivitis to periodontitis [204].

A study by Washio et al. showed that there is a significant correlation between VSC concentrations and the percentage of *P. intermedia* producing H_2S. This result suggested that specific periodontopathogenic bacteria present in the tongue coating are closely associated with VSC concentrations in the oral cavity air. Among cultivable oral bacteria, the three most active producers of H_2S in vitro are *P. gingivalis*, *T. denticola* and *T. forsythia*. These Gram-negative anaerobic microorganisms are associated with periodontal disease [81].

As already mentioned, toxic VSC are capable of damaging periodontal tissues, and thus lead to greater attachment loss. There is a mutual relationship between periodontal attachment loss and VSC production. To interrupt this cycle, adequate oral hygiene is necessary [81]. Periodontal treatment combined with halitosis treatment begins with patient instruction on the most appropriate oral hygiene techniques, continuing with conventional periodontal treatment, scaling and root planing, and use of chlorhexidine mouthwashes [88]. This approach proves to be an effective method to improve periodontal clinical status as well as reduce halitosis [205].

On the other hand, the role of periodontal disease in halitosis is still not fully understood. Epidemiological data have shown that periodontal disease may be an additional cause for the development of oral malodor, as not all patients suffering from periodontal disease suffer from this condition and, in turn, periodontally healthy patients may have malodor [81].

In a small number of patients, gingivitis or periodontitis may be the sole cause of halitosis. Several studies suggest that inflamed periodontal tissue (as measured by the gingival index) is more likely to be related to VSC formation rather than pocket depth. As the interdental spaces increase, in periodontally affected patients, there is an increase in food impaction and thus a predominance of putrefaction. Thus, specific periodontopathogenic

microorganisms, associated with malodor, can colonize other surfaces, which leads to their growth and multiplication [81].

In conclusion, patients with periodontal disease have a higher risk of developing halitosis than healthy individuals. Malodor is mainly associated with gingival inflammation and tongue coating [202].

Consequently, not only do VSC cause halitosis in terms of a lifestyle problem, but they are also considered a periodontal pathogenic factor [189].

1.3 Oral health-related quality of life

Oral disease, such as dental caries, periodontal disease or oral cancer, is the fourth most expensively treated disease worldwide, although it is one of the easiest to prevent [208]. The Ministry of Health's oral health budget policies in Portugal lead to one of the lowest dental treatment rates in Europe, hence the importance of conducting studies in this field in order to gather information that characterizes oral health and improves not only the results of dental treatment but also prevention [209]. The health-related quality of life evaluation aims to understand how different domains are influenced by the characteristics of changes (diseases) affecting the individual. The use of clinical resources in the evaluation of oral conditions has been shown to be very reducing, making it essential to demonstrate the importance that oral diseases have on the physical and psychological well-being of individuals and, therefore, in the Oral Health-Related Quality of Life [210–212]. By evaluating the subjective experiences of individuals to determine the impact of oral health conditions on well-being and self-esteem, it is possible to improve clinical interventions and thus the quality of life. One of the most used indicators for evaluating the OHRQoL is the Oral Health Impact Profile (OHIP), because it has good psychometric qualities and allows measuring the self-perception of the consequences inherent to oral conditions [210,211,213]. The OHIP-14 is based on the "International Classification of Impairments, Disabilities and Handicaps" (ICIDH) developed by the WHO (1980) and adapted for oral health by Locker, allowing for the collection of information on the severity, extent and prevalence of negative impacts on oral health-related quality of life (OHRQoL) in a single administration [212]. It is based on Locker's oral health model, which considers that diseases cause impairments (Impairment) and functional

limitations at the organ level, and as a result, the individual may become disabled (Disability) or disadvantaged in society (Handicap) [212]. The OHIP-14, which is a reduced version of the OHIP-49 containing only 14 items from the 7 evaluated dimensions (Functional limitation, Physical pain, Psychological discomfort, Physical incapacity, Psychological incapacity, Social incapacity, and Disadvantage), was described by Slade in 1997 [212] and has been validated in the Portuguese population [214]. The internal reliability of the original OHIP-14 version was high, with a Cronbach's alpha coefficient of 0.88 [213]. In the multivariate analysis, eight oral condition variables and sociodemographic variables were found to be associated, both in the OHIP-49 and the OHIP-14 ($p<0.05$), suggesting good construct validity [213].

Currently evidence shows a correlation between clinically diagnosed periodontitis and deterioration of self-perceived OHRQoL in adult individuals [215,216]. In addition, severe and extensive stages of periodontitis have a more pronounced impact in the OHRQoL [215,217]. Hence, periodontitis negatively impacts OHRQoL perceptions due to the deterioration and loss of periodontal tissues [218], tooth mobility and in ultimately tooth loss [215,219]. 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 [220,221]. 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 [218]. To the best of our knowledge, research in this area is scarce and has never been conducted in Portuguese patients suffering from periodontal disease.

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2

Aims

2. Aims

Halitosis and periodontitis are two conditions with proven psychosocial impact which lead many patients to seek treatment. However, is still poorly studied and valued in the medical community. It is important to understand these diseases from diagnosis to treatment, as well as the need to broadcast to the scientific community.

Thus, the aim of this thesis was to evaluate the impact of periodontal treatment on halitosis and quality of life as well as to characterize the microbial biodiversity of periodontal pockets before and after non-surgical periodontal treatment.

In this way our research goals were divided into two stages:

STAGE 1: Studies to understand intra-oral halitosis in patients with periodontitis.

1.1 Review of the state of the art on halitosis, causes and treatment (Publication 1).

1.2 To evaluate the prevalence of intra-oral halitosis in patients with periodontitis (Publication 2).

1.3 Check how clinical periodontal parameters correlate with intra-oral halitosis and the patient's quality of life. (Publication 2).

1.4 Compare the patient's self-perception of halitosis with its diagnosis (Publication 2).

STAGE 2: Studies to understand the impact of periodontal treatment on intra-oral halitosis.

2.1 Analise the subgingival microbiome and intra-oral halitosis before and after periodontal treatment (Publication 3).

2.2 Evaluate and compare intra-oral halitosis and quality of life before and after periodontal treatment (Publication 3).

2.3 Establish new hypotheses for future research.

3

Revisiting Standard and Novel Therapeutic Approaches in Halitosis: A Concise Review

3. Revisiting standard and novel therapeutic approaches in halitosis: a concise review

This chapter was based from the published work:

Publication I: Catarina Izidoro, João Botelho, Vanessa Machado, Ana Mafalda Reis, Luis Proença, Ricardo Castro Alves, José João Mendes. Revisiting Standard and Novel Therapeutic Approaches in Halitosis: A Review. *International Journal of Environmental Research and Public Health*. **2022**;19(18):11303.

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Revisiting standard and novel therapeutic approaches in halitosis: A concise review

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Abstract:

Halitosis or chronic bad breath is an oral health problem characterized by the unpleasant malodor emanated from the oral cavity. This condition has multiple origins and causes a negative burden in social interactions, communication and quality of life, and may be indicative of underlying non-oral non-communicable diseases. Most cases of halitosis are due to inadequate oral hygiene, periodontitis and tongue coating, yet the remaining proportion of cases are due to ear-nose-throat-associated (10%) or gastrointestinal/endocrine (5%) disorders. For this reason, the diagnosis, treatment and clinical management of halitosis often require a multidisciplinary team approach. This comprehensive review revisits the etiology of halitosis as well as standard and novel treatment that may contribute to higher clinical success.

Keywords: Halitosis; Periodontal disease; Periodontitis; Tongue coating; Mouthwashes; Probiotics.

3.1 Introduction

Halitosis is characterized by an unpleasant odor emanating from the mouth, from oral or non-oral sources [1,2]. This malodor results from the presence of odoriferous substances – named volatile sulphur compounds (VSCs) – present in the exhaled air as a result of the action of anaerobic oral Gram-negative bacteria (*Bacteroides loescheii*, *Centipedaperiodontii*, *Eikenella corrodens*, *Treponema denticola*, *Prevotella intermedia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Selenomonas*, *Eubacterium*, *Bacteroides forsythus* and *Tannerella forsythyia*) on substrates containing sulphur [3–5]. These VSCs result from bacterial metabolism and its pathways are well described (Figure 3.7). The understanding and the development of VSCs measurement procedures allowed to be used as clinical levels for the diagnosis of halitosis [6,7].

Epidemiological studies report the prevalence of halitosis is estimated to range between 2.4-78% [3,4], and the American Dental Association outlined near 50% of American adults suffer from oral malodor [8]. The reason for such large variability in the prevalence rates are the large variability in methods used for halitosis assessment, the geographic location, and the year when the study was developed. With such an elevated prevalence, its etiology and patient-reported outcomes become a topic of interest. Halitosis negative impacts patients' quality of life, particularly the interpersonal relationships [9], and when patients experience social and personal embarrassment, they tend to seek treatment by a professional [6].

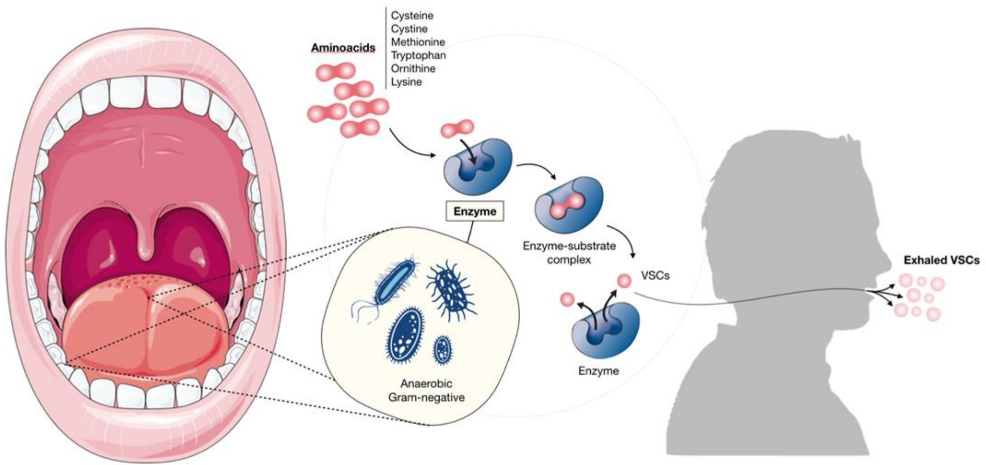

Figure 3.7: Pathophysiology of halitosis. Bacteria present in the gingival pockets and the dorsum of the tongue metabolize amino acids through enzymes into volatile sulphur compounds that are exhaled.

This review aims to provide a snapshot on halitosis etiology and classification, as well as discussing standard and innovative alternative treatments and clinical management strategies.

3.1.1 Classification of Halitosis

The current classification system for halitosis is based on the origin of the VSCs and was established by the International Society for Breath Odor Research (ISBOR) [7,10]. As such, halitosis can be categorized as genuine halitosis or delusional halitosis (Figure 3.8):

1. Genuine halitosis is an oral malodour that is noticeable and exceeds the socially acceptable level. Genuine halitosis can be classified into physiological halitosis or pathological halitosis [11]. Pathological halitosis can originate from oral diseases - intra-oral halitosis, (e.g., tongue coating, periodontal infections, odontogenic infections, xerostomia, mucosal lesions) or systemic diseases - extra-oral pathologic halitosis (e.g.,respiratory tract infections, gastrointestinal disease, metabolic disorders, endocrine system disorders, medication).

Physiological halitosis, mainly originating from the dorso posterior region of the tongue, consists of a bad odor originating from putrefaction processes in the oral cavity, without any association with a pathological condition.

2. Delusional halitosis includes pseudohalitosis and halitophobia.

Pseudohalitosis is a condition in which patients are convinced they have oral malodour, but it is not noticed by others. Complaints usually improve after counseling and simple oral hygiene measures. Halitophobia is the condition in which patients maintain the conviction that they have bad breath even after diagnosis and treatment, without any physical or social evidence suggestive of the presence of halitosis. Also called non-real halitosis, halitophobia is understood by the compulsive idea of suffering from bad breath and irritating others with it [12].

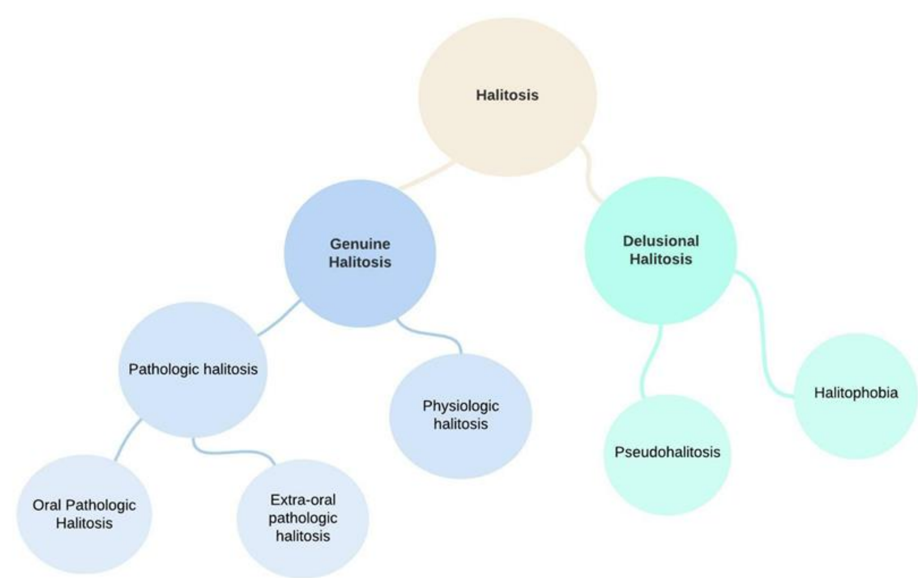

Figure 3.8: Current classification of halitosis.

In the following subsections, we revisit halitosis risk factors (subsection 2.1) and causes (subsection 2.2).

3.1.2. Risk Factors for Halitosis

Behavioral Factors

Among the behavioral factors that increase the risk for developing halitosis are smoking, dietary habits and alcohol consumption [13]. Smoking is linked with increased incidence and severity of halitosis [14,15]. This largely stems from the existence of cigarette’s high content involatile sulphur compounds (VSCs)

[16], particles responsible for the malodor. Also, cigarette smoke active alters the subgingival microbial ecosystem balance causing an increase in the absolute numbers of VSC-producing bacteria [17,18]. Also, smoking causes secondary hyposalivation and dry mouth (explained on section 2.1.2). In what diet concerns, VSCs-enriched foods (such as, garlic, onions, durians, and spices), can cause transient unpleasant oral malodor, yet this is often not comprehended as a malodor, and is influenced by people's acceptable breath smell threshold [13]. Chronic alcohol consumption is another potential risk factor for halitosis as a result of oral and hepatic alcohol oxidation, producing acetaldehyde and other odorous byproducts, or as secondary association hyposalivation and dry mouth [14].

Dry Mouth / Xerostomia

Saliva's functions include the destruction of microorganisms, digestion, lubrication, and cleaning of the hard and soft tissues of the mouth. Secreted saliva contains intrinsic salivary components and desquamated epithelial cells, lysed leukocytes, hemopoietic cells, food debris, and microorganisms [22]. Dry mouth is characterized by reduced salivary flow favoring anaerobic bacterial putrefaction of food debris that remain in the oral cavity after meals [15,23]. The lack of salivary flow causes the disappearance of saliva's antimicrobial activity and the transition from Gram-positive to Gram-negative bacteria species [24]. Consequently, VSC production increases, the main contributor to halitosis [25].

Medication is the most common cause of xerostomia, namely anticholinergics, anti-histaminic, antipsychotics, anxiolytics, antidepressant, antihypertensives, diuretics and narcotics. Chronic mouth breathing, radiotherapy, dehydration, vitamin deficiencies, menopause, emotional disturbances, and autoimmune conditions (systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis and scleroderma) can also reduce salivation, as can systemic illness such as diabetes *Mellitus*, nephritis and thyroid dysfunction. About 25% of the elderly suffer from dry mouth [19]. Furthermore, in geriatric age, oral changes occur that can influence the development of malodour: an increase in salivary pH due to amino acid ingestion and a change in oxygen depletion. These changes stimulate the metabolism of Gram-negative bacteria, responsible for the increased production of VSC [20,21]. Although xerostomia is associated with aging, several studies have shown that salivary gland function is

maintained in healthy elderly people. Therefore, dry mouth is probably a condition of systemic or extrinsic origin.

3.1.3. Causes of Halitosis

Extra-Oral Causes

It is estimated that 10-20% of halitosis has non-oral causes. The origins of extraoral halitosis include sources of respiratory tract infections, gastrointestinal diseases, endocrine and hematologic system disorders [26]. The main VSC associated with extra-oral halitosis is dimethyl sulphoxide whereas the main VSCs contributing to intra-oral halitosis are methyl mercaptan and hydrogen sulphide [26,27]. The most common sources are discussed below according to the system involved.

Respiratory System

To distinguish the origin of malodor between oral or nasal origin, the expired air must be separated. The technique described consists of asking the patient to close the lips and exhale through the nose. The examiner will notice if the bad odor is coming from the nose. Then, the patient is asked to pinch the nose and exhale through the mouth. The patient is asked [26]. In this way, we can distinguish whether the cause of halitosis is of nasal, nasopharynx or paranasal sinuses origin. Bacterial sinusitis mainly develops from acute viral sinusitis, with *Streptococcus pneumonia* and *Haemophilus influenza* being the main bacteria involved. When purulent mucus is produced, a typical odor appears. The diagnosis is confirmed using radiological or computed tomography (CT) images, where fading is revealed.

In 10% of sinusitis cases, one tooth or several teeth are involved. *Peptostretococcus spp.*, *Fusobacterium spp.*, *Prevotella spp.* and *Porphyromonas spp.* are the bacteria most frequently involved. Since these bacteria are capable of producing VSCs, a clear association with halitosis is available. In the treatment of acute sinusitis, antibiotics are often used, reducing the prevalence of anaerobic pathogens as well as malodour problem [28].

Chronic sinusitis may be the main cause of bad breath coming from the nose [20,21]. The most common symptoms include post-nasal drip, cough, covered

tongue and constant urge to clear the throat [31,32]. It is thought that the purulent material falls on the base of the tongue, which already contains microbiota, predisposing to the production of VSC [33]. Causes of chronic sinusitis can include an upper respiratory tract infection, chronic mucosal disease, or malformed intranasal structures. Patients with halitosis and chronic nasal / paranasal sinus diseases should be referred to an ear-nose-and-throat specialist for causal treatment [33,34].

Repetitive infections of the tonsils and adenoids cause chronic follicular tonsillitis [35]. There are deep crypts that form in the tonsillar crypts which, due to their anatomy, favor the accumulation of food, saliva, and necrotic matter. If these materials are not eliminated naturally, tonsilloliths develop, which pose a 10-fold increased risk of abnormal VSC levels [36]. On examination, the tonsils may or may not be hypertrophied and are usually not hyperemic. The microorganisms most often involved are streptococci, but viral infections are also a possible etiology (eg, infectious mononucleosis). Anaerobic bacteria detected in tonsilloliths belonged to the species of *Eubacterium*, *Fusobacterium*, *Porphyromonas*, *Prevotella*, *Selenomonas* and *Tanerella*, all of which appear to be associated with the production of VSCs [37].

Bronchitis and pneumonia from aerobes, mycoplasma, or viruses, even if productive of a substantial amount of sputum, do not usually cause halitosis. Several clinical situations of the respiratory tract that present halitosis as a relevant symptom are described. Among them, anaerobic lung abscess, foreign bodies lodging along the respiratory tract, necrotizing pneumonia, emphysema, lung cancer, bronchiectasis, and tuberculosis [29,38-40].

The lungs function as a source of odors that arise from metabolism. Aromatic foods such as garlic; alcohol; high-fat diets; and ketosis (diabetic ketoacidosis), as well as nitrates, chloral hydrate, and iodine-containing drugs, are examples of products that reach the lungs through the circulation and are released through expired air [34].

Gastrointestinal

Although the medical community and many patients believe that halitosis mostly originates in the stomach, it is known that only 0.5% of halitosis cases originate from gastrointestinal tract. There are, however, gastrointestinal

pathologies that can be the cause of bad breath, including esophageal reflux, achalasia, pyloric stenosis or hiatal hernia. In these pathologies, there is a weakening or inhibition of esophageal closure, incomplete emptying into the stomach, resulting in retention of food, liquid, and saliva, causing oral malodour [7,41,44].

On the other hand, gastroesophageal reflux, an extremely common disorder, rarely causes halitosis. The most frequent symptom is heartburn, and the associated odor is like a simple belch, similar to the odor of the most recent meal [42].

Malabsorption syndromes, gastric carcinomas, and some enteric infections, bezoars have been noted to contribute to halitosis [42,43].

Metabolic Disorders

There are several metabolic diseases that manifest as a cause of bad oral odors, namely, Diabetes Mellitus, renal disease and liver cirrhosis [45,46]. Patients with type 2 diabetes manifest a typical sweet and fruity odor [47].

Using gas chromatography-mass spectrometry, it is possible to diagnose different extra-oral causes of halitosis, such as diabetes [48]. Diabetic ketoacidosis leads to a typical breath odor [49]. Patients with chronic renal failure have high blood urea nitrogen levels and reduced salivary flow. The odor is typically uremic associated with dry mouth. Treatment with peritoneal dialysis reduces the problem [45]. Feller and Blignaut in 2005 described pancreatic insufficiency as a cause of extraoral halitosis as well [50].

Whittle et al., describe metabolic disorders in the intestines as a cause of halitosis. Trimethylaminuria, also known as "fish odour syndrome", is a genetic disorder characterized by the body's inability to metabolize trimethylamine. The accumulation of this volatile compound leads to its excretion in the urine, but it is also found in sweat and breath, giving it a fishy smell. According to the authors, this genetic disorder is the biggest cause of undiagnosed body odor, with social consequences, leading to isolation and even depression [51].

The symptoms of trimethylaminuria can be improved by changes in the diet to avoid precursors, in particular trimethylamineN-oxide which is found in high concentrations in marine fish. Treatment with antibiotics to control bacteria in

the gut, or activated charcoal to sequester trimethylamine, may also be beneficial [52].

Hepatology and Endocrinology

When liver function is reduced, waste products are eliminated through the lungs, causing 'fetor hepticus': a sweet, excremental odor (the breath of death) [53]. Liver failure inhibits detoxification throughout the body, causing unpleasant odors, called Fetor hepticus [54]. In addition, some hereditary disorders can influence breathing: tyrosinemia is the most important example (cabbage odor). Not only the hormonal cycle seems to influence the mouth odor, but also a lot of other intestinal diseases [55,56]. Recently, van Steenberge mentioned a completed list of metabolic and endocrinological aspects in correlation to halitosis: Type-2-diabetes in adults, Type-1-diabetes in children, Intestinal obstruction, Alcoholic ketoacidosis, Kidney-insufficiency, Trimethylaminuria, Phenylketonuria, Methionine adenosyl transferase deficiency, Isovaleriaan acidity, Deficiency on chromosome 15, Maple syrup urine disease, Homocystinuria, Disease of Lignac [47].

Medication

In addition to medication resulting in a dry mouth, such as anticholinergics, anti-histaminic, antipsychotics, anxiolytics, antidepressant, antihypertensives, diuretics and narcotics; recently the use of bisphosphonates can contribute to oral malodor. Bisphosphonate is used in treatment of malignant bone tumors and their metastases and can induce osteonecrosis of jaws [57]. The necrotic sequesters should be removed and it is tried to cover up the necrotic area with a steeled flap [58].

Intra-Oral Causes

Several review articles have shown that the oral cavity is the main contributor to bad breath in 85%-90% of patients with halitosis [59–61]. The humid environment and temperature up to 37°C inside the oral cavity favors the growth of bacteria and their ability to metabolize sulphur-containing amino acids (L-cysteine + L-methionine) to generate hydrogen sulfide and methyl mercaptan [18,26,62].

Oral malodor is mainly caused by microbial degradation of both sulphur-containing and non-sulphur-containing amino acids derived from proteins in exfoliated human epithelial cells and white blood cell debris, or present in

plaque, saliva, blood, and tongue coating [59,63]. The subgingival periodontal biofilm is mainly composed of Gram-negative anaerobic bacterial species, which are proteolytic in nature [46].

These species are able to degrade sulphur-containing substrates on different surfaces of the oral cavity, including periodontal pockets, releasing volatile sulphur compounds (VSCs). Clinical studies have shown that CSVs are major contributors to halitosis [60,64]. Hydrogen sulfide, methyl mercaptan and, to a lesser extent, dimethyl sulfide, account for 90% of the VSC in bad breath [60,64,65].

Odontogenic Halitosis

Insufficient or inadequate oral hygiene, plaque, tooth decay, food impaction and poorly sanitized acrylic dentures (used at night or not regularly cleaned or with rough surfaces) are the main causes of odontogenic halitosis [66]. The dentist should be the first to diagnose these causes and follow up with the appropriate treatment for the cause.

Tongue Coating

Several studies have implicated the dorsum of the tongue as the main site of putrefaction of the microflora and the production of VSC [29], which is why it is considered the major contributor to intra-oral halitosis [29,67,68].

The dorsum of the tongue has a complex papillary structure that favors the accumulation of bacteria [69,70]. These microorganisms (e.g. *Veillonella* and *Actinomyces*), especially the Gram-negative and proteolytic nitrate-producing anaerobes, have the ability to produce odorous substances from food remnants and epithelial cell debris [4,5].

It is thought that instead of a few dominant species involved, interactions between various bacterial species occur on the dorsum of the tongue that result in oral malodour [4].

The score-based classification of tongue coating was developed by Kojima [71,72]. Clinically, there is a correlation between halitosis and tongue coating, and this association is particularly strong in the region posterior to the circumvallate papillae, an area with the highest load of Gram-negative bacteria that contribute to oral malodour [61].

This area is the most inaccessible to tongue hygiene procedures, hence the greatest accumulation of bacteria in this area.

The coating on the tongue is difficult to remove. Daily tongue scraping or brushing helps to reduce the substrate for putrefaction, bacterial load, and improve taste sensation [73].

There are several local factors, including salivary pH, reduced ambient oxygen concentration, bacterial production, and the substrate available for bacterial metabolism, that favor increases in salivary concentrations of VSC precursors, such as cystine and methionine. The production and release of these volatile compounds gives rise to the subsequent detection of these malodorous oral substances [55].

Decreased salivary flow; saliva stagnation; reduction of the carbohydrate content available as a bacterial substrate; and increased oral pH (the malodour occurs mainly in an alkaline microenvironment), create a favorable environment for the change from Gram-positive to Gram-negative bacteria [74].

Periodontal Disease

Periodontal disease (gingivitis and periodontitis) are the oral inflammatory conditions that most often contribute to oral malodor, with the production of a very distinct, fetid or putrid smell [38]. Necrotizing gingivitis or periodontitis cause extreme soiled odours.

In patients with active periodontitis there is a significantly higher prevalence of damaged epithelial cells, leukocytes and bacteria in saliva compared to normal subjects [75].

Many studies support a direct correlation between periodontal disease and halitosis [67,76]. Other studies also verify a positive correlation between the depth of the pockets and the concentration of sulphur compounds [76,77].

The proposed microbiological link between halitosis and periodontal disease is based on three assumptions:

1) Periodontal patients have a higher prevalence of intraoral bacteria (bacterial plaque and tongue coating), decreased pH, which is necessary for amino acid putrefaction and formation of VSCs [80].

2) The microbially generated VSCs (hydrogen sulfide and methylmercaptan) facilitate the penetration of lipopolysaccharide into the gingival epithelium, inducing inflammation [78,79].

3) VSCs also aid in bacterial invasion of connective tissue by their toxic effects on epithelial cells, while methylmercaptan prevents the growth and proliferation of epithelial cells [25].

This mechanism is enhanced by the decrease in oxygen tension due to the increase in the depth of the periodontal pocket, with a concomitant decrease in pH, which is necessary for the putrefaction of the amino acids that create VSCs [25].

However, some studies have not found a correlation between periodontal disease and halitosis, considering that the periodontal pocket is a closed environment, not representing a sufficient cause for the release of smelly gases that are able to escape into the mouth [18,78,81,82].

Oral Candidiasis

Long-term treatment with antibiotics or corticosteroids, immunosuppressed patients with HIV, Diabetes *Mellitus*, undergoing chemotherapy/radioteraphy, may develop fungal infections in the oral cavity. Oral candidiasis is a frequent infection, which presents several clinical manifestations in the oral mucosa.

In most cases of oral candidiasis, the diagnosis is based on clinical signs and symptoms through physical examination and medical history. Confirmation of the diagnosis can be made with complementary tests, especially when the clinical examination is uncertain or the patient does not respond to antifungal therapy. Confirmation of the diagnosis can be made by obtaining a smear (exfoliative cytology). Candida infections produce a distinct sweet, fruity odor. Antifungal agents, such as nystatin, topical ketoconazole, clotrimazole and miconazole, can resolve the condition and treat oral malodor [29].

Oral Cancer

Since malignant or benign primary tumors of the oral cavity are very often associated with the presence of necrotic tissue, blood exudation, opportunistic infections, accumulation of food debris, an environment is created that favors increased putrefaction and VSC production.

Patients undergoing cancer treatment are more susceptible to tissue destruction, multiple infections and bleeding. All these factors contribute to the accumulation of anaerobic bacteria and the release of foul-smelling gases [83,84].

Other Oral Sources

There are other factors that contribute to intraoral halitosis which include: non-vital teeth, exposed tooth pulps, healing wounds, stomatitis, intra-oral neoplasia, extraction wounds (with blood cloth or purulent discharges), or crowding of teeth (favouring food entrapment) canal sobinvolved and fixed orthodontic appliances. Moreover, peri-implantitis, pericoronitis, recurrent oral ulcerations and herpetic gingivitis, are described as origin for bad breath [85,86].

All these factors create a food retention site or plaque that allows for bacterial putrefaction of amino acids, causing halitosis [66]. Acute clinical situations such as pericoronal infections, oral ulcerations and necrotizing ulcerative gingivitis can also cause oral malodour with the production of a characteristic oral stench. There is also temporary halitosis that lasts only a few hours, caused for example by eating foods that contain VSCs, such as garlic or fast food [87]. On the other hand, a high fiber diet such as vegetables, fruit and green tea accelerate gastric emptying, leading to reduced VSC levels over a period of time [88,89].

3.1.4. Treatment

A patient who suffers from halitosis and seeks help is a person who is always worried about it, who has often tried treatments and has not found an answer [90]. An accurate objective and diagnosis must be made to manage the appropriate treatment for the causal factors. All patients diagnosed with halitosis, regardless of the cause of halitosis, should be evaluated by an oral health professional.

The authors describe a treatment approach based on halitosis types in Figure 3.9.

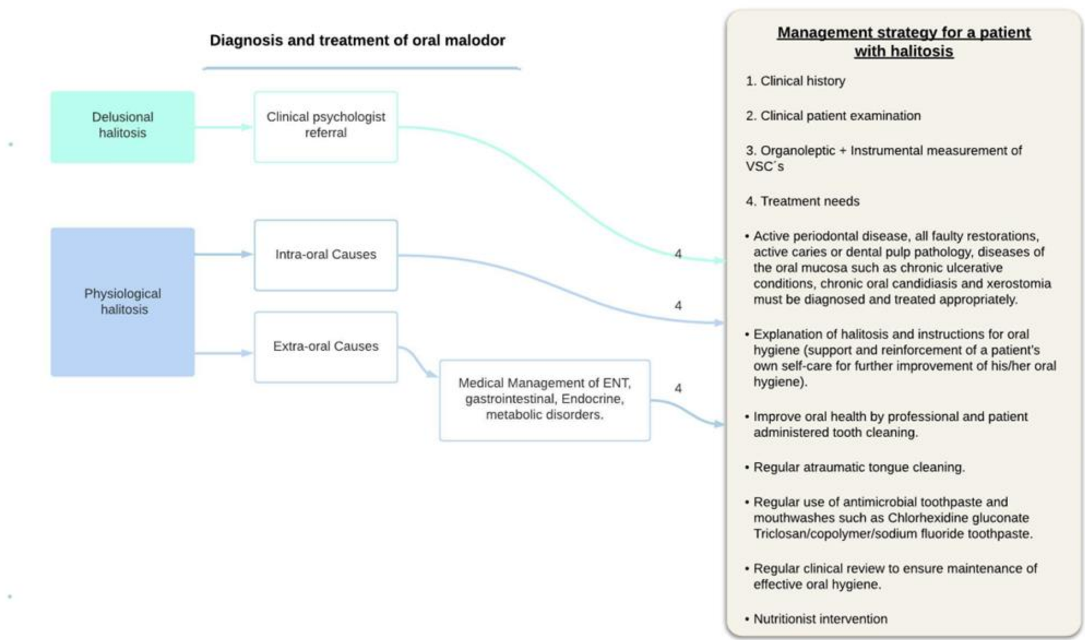

Figure 3.9: Treatment approach based on halitosis types and etiology.

Treatments for Intra-Oral Causes

The course of treatment for halitosis is determined after a thorough oral clinical examination, including the dentition, soft tissue, and periodontal health status. All active caries, secondary caries, pulp pathology, oral pathologies such as chronic ulcerative conditions, oral candidiasis, and xerostomia, must be identified, diagnosed, and treated appropriately. Also, the diagnosis of periodontal disease such as gingivitis, periodontitis or necrotizing periodontal disease, should be performed and adequately treated, since these pathologies are a major contributor to the levels of oral VSC [78]. The treatment of intra-oral halitosis include 4 phases: 1) mechanical reduction of nutrients and intraoral microorganisms; 2) chemical reduction of microorganisms; 3) inversion of volatile gases into non-volatile components (chemical neutralization of VSC) or 4) masking the malodour [91].

Mechanical Reduction

Bearing in mind that microorganisms and their metabolites are involved in halitosis etiopathogenesis, mechanical removal of biofilm and microorganisms is the first step in halitosis control [92]. Tongue coating is the main causal

factor of intraoral halitosis, hence the importance of extensive cleaning of the tongue. Scraping the dorsum of the tongue reduces both available nutrients and available microorganisms, leading to improved odour [93]. All patients should receive clear instructions on the most appropriate oral hygiene care for their case, as well as an explanation of tongue cleaning. The patient should gently brush the dorsum of the tongue with a soft bristle brush and a toothpaste in 5 to 15 movements. The area that tends to accumulate bacterial deposits and keratin and food debris, contributing to physiological halitosis is terminal sulcus, the division between the posterior and middle thirds of the tongue. Removal of these materials decreases the release of VSCs.

There are two ways to do daily tongue cleaning: using a regular toothbrush or using a tongue scraper [94]. It was described in a systematic review published by Van der Sleen et al. that tongue brushing or tongue scraping allows the reduction of tongue coating and improvement of halitosis. According to these authors, tongue scrapers are suitable for the anatomy of the tongue and reduce 75% of VSCs compared to 45% when a toothbrush is used [95].

On the other hand, in a Cochrane review in 2006, which compared randomized controlled trials for different tongue cleaning methods, concluded that there was a weak, but statistically significant, difference in the reduction of VSC levels when scrapers or cleaners were used instead of toothbrushes [96]. More studies are needed for clearer conclusions.

Since periodontitis is a major cause of oral malodour, treating periodontitis will also improve halitosis.

One-stage full-mouth disinfection can be performed, as described by Bollenet al. [97], combining scaling and root planing with the use of chlorhexidine. There is a significant microbiological reduction up to 2 months, with reduced organoleptic scores [98].

The accumulation of bacterial plaque due to lack of interproximal cleaning leads to a high incidence of malodour, so it is essential to use dental floss/interproximal brush to control bacterial plaque and oral microorganisms [103].

The oral health professional should dedicate time from their consultation to the motivation and instruction of oral hygiene care to patients. This is the only way to achieve good treatment adherence results [99].

Chemical Reduction

Antibacterial agents for mouthwashes include chlorhexidine (CHX), cetylpyridinium chloride (CPC), and triclosan. Its mechanism acts on bacteria capable of producing volatile sulphur compounds [26].

Mouthwashes containing CHX and CPC can inhibit the production of VSCs, while mouthwashes containing chlorine and zinc dioxide have a neutralizing action on sulphur compounds that produce halitosis, according to a systematic review published by Cochrane [101].

Rinsing is a common practice in the management of oral malodor. The most used rinse components are:

· Chlorhexidine (CHX): considered the gold standard mouth rinse for halitosis treatment [102]. Its use at a concentration of 0.2% causes a 43% reduction in VSCs and a 50% reduction in organoleptic scores throughout the day [103]. CHX in combination with CPC produce greater fall in VSCs level, and both aerobic and anaerobic bacterial counts showed the lowest percentage of survival in a randomized, double-blind, cross-over study design [102]. Combined effects of zinc and CHX were studied in a study conducted in 10 participants, Zinc (0.3%) and CHX (0.025%) in low concentration led to 0.16% drop in H_2S levels after 1h, 0.4% drop after 2h and 0.75% drop after 3h showing a synergistic effect of the two [104]. However, patients may be reluctant to use CHX long-term as it has an unpleasant taste and can cause (reversible) staining of the teeth [105].

· Essential oils: these products give only a short-term and restricted effect (25% reduction) for 3h. Also, the reduction in odor-producing bacteria is limited [106]. Usage of Listerine containing essential oils resulted in significant reduction in halitosis producing bacteria in healthy subjects [107].

· Chlordioxide: It is a strong oxidant that can reduce halitosis by oxidizing H_2S, CH_3SH, cysteine and methionine. A 29% reduction in odor was reported after 4h [108].

· Triclosan, is a widely used antimicrobial agent with good results in reducing dental plaque, gingivitis and halitosis [109]. Its use in toothpastes in

combination with a tongue scraper and toothbrush revealed a significant reduction in organoleptic scores and sulphur levels in the mouth air [109].

A formulation of triclosan/copolymer/ sodium fluoride in 3 weeks randomized double blind trial by Hu et al. seemed to be particularly effective in reducing VSC, oral bacteria, and halitosis [110].

Toothpastes containing stannous fluoride, zinc or triclosan have a beneficial effect on reducing oral malodor for a limited period of time [112-114].

In a recent Cochrane review by Fedorowicz, with five randomized controlled trials involving 293 participants, 0.05% chlorhexidine + 0.05% cetylpyridinium chloride + 0.14% zinc lactate was compared to placebo. With the use of this mouthwash, there was a significant reduction in organoleptic scores, but also a more significant presence of stains on the tongue and teeth. A meta-analysis of the data was not possible due to clinical heterogeneity between trials [101].

It is concluded that this mouthrinse (0.05% chlorhexidine + 0.05% cetylpyridinium chloride + 0.14% zinc lactate) plays an important role in reducing the levels of halitosis producing bacteria on the tongue and can be effective in neutralization of odoriferous sulphur compounds. But well-designed, randomized controlled trials with larger sample size, a longer intervention and follow-up period are still needed to confirm these results.

Probiotics

Several studies have shown that probiotic bacterial strains, originating from the indigenous oral microbiota of healthy humans, may have potential application as adjuvants for the prevention and treatment of halitosis [115]. The aim is to prevent the re-establishment of unwanted bacteria and thus limit the recurrence of oral malodour for an extended period. Recently, several studies have been carried out to replace the bacteria responsible for halitosis with probiotics such as *Streptococcus salivarius* (K12), *Lactobacillus salivarius* or *Weissellacibaria.*

Oral administration of probiotic lactobacilli has shown good results in the treatment of physiological halitosis, as well as improved bleeding on probing of periodontal pockets [116].

Furthermore, *in vivo,* and *in vitro* studies revealed that *Weisellacibaria* isolates have the ability to inhibit the production of VSC, demonstrating that they have

potential for the development of new probiotics for use in the oral cavity [117]. The use of a suspension of living non-pathogenic Escherichia coli bacteria also seems to have good results in the treatment of gut-caused halitosis [118].

Transformation of Volatile Sulphur Components

Products containing chlorite anion and chlorine dioxide have been shown to be effective in oxidizing and inactivating the oral VSC demonstrating long-lasting effects [119,120]. Positively charged metal ions such as zinc, mercury and copper bind to sulphur radicals inhibiting the expression of VSCs [121,122]. For this reason, the combination of zinc and CHX appears to have a synergistic effect on the elimination of VSCs. According to the authors Young et al, a commercial rinse (containing 0.005% CHX, 0.05% cetylpyridinium chloride (CPC) and 0.14% zinc lactate) appears to be much more efficient than CHX alone, due to the zinc effect [121].

Masking Effect

Rinsing products, sprays, mint tablet, chewing gum increase the saliva production, thereby retaining more soluble sulphur components for a short period of time, having only a short-term masking effect [123].

Treatments for Extra-Oral Causes

Bearing in mind that halitosis presents a multifactorial complexity, treatment should be individualized and directed to each patient, rather than generalized [1].

Diagnosis and treatment involve a multidisciplinary team: primary healthcare clinician, dentist, otolaryngologist, nutritionist, gastroenterologist and clinical psychologist [124].

After a detailed clinical oral examination and anamnesis, that excludes intra-oral causes for halitosis, and patients with signs or symptoms of systemic diseases that may be the cause of oral malodour, should be referred to the appropriate medical specialty (ENT, pulmonogist, endocrinologist or gastroenterologist). Patients with pseudohalitosis or halitophobia should be counseled appropriately and referred for psychologic evaluation and treatment.

In the clinical approach to halitosis, a relationship of trust and empathy between the patient and a general practitioner is extremely important. In this reliable medical approach, the patient will feel comfortable to communicate their complaints and the doctor will be able to encourage the patient to undergo treatment, improving the quality of life of the patient as a whole, and improving their interactions and social relationships [105].

Halitophobia

Imagined halitosis is poorly documented in the psychiatric literature [126]. Many of the cases with imagined halitosis described in the literature resemble the psychiatric syndrome of social phobia [127]. Generally, these patients believe that their oral malodour is related to social rejection or avoidance behaviors of the people with whom they interact [125]. Patients with halitophobia require referral for clinical psychology investigation and treatment for mental assessment and appropriate treatment [105,124].

The ‘treatment’ of these patients is impossible, as they are not within the arguments presented by a physician. Mostly, these patients hop from clinic/specialist to clinic/specialist to find an argument for their self-esteemed problem.

Xerostomia/ Dry Mouth

The treatment of xerostomia will also contribute to the treatment of halitosis. The dry mouth symptom can be treated with hydration and sialogogues or with artificial saliva substitutes [128].

When the cause of xerostomia is medication, it becomes important to find other pharmacological alternatives without compromising the patient's health.

The patient should be encouraged to increase water intake and avoid drinking caffeinated beverages.

For salivary stimulation, we can resort to the use of sugar-free candies or gums and the use of an artificial salivary substitute, which is usually composed of carboxymethylcellulose.

In severe cases of dry mouth, e.g. patients with Sjögren’s syndrome or patients undergoing radiotherapy, therapy with a cholinergic agonist is prescribed. The most frequently used has been pilocarpine at a dosage of 5 to 10 mg/day [1],

and more recently cevimeline hydrochloride (Evoxac), 30 mg three times a day, also with good results in the treatment of dry mouth [128,129].

3.2. Conclusion

Halitosis is highly prevalent with multifactorial origins, and high burden for social and auto-esteem. This review emphasizes the importance for a multidisciplinary approach. Despite the current decision trees for the clinical management of halitosis, there is still some inconsistencies that require robust randomized clinical trials comparing standard and innovative therapies.

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4

Periodontitis, halitosis and oral health-related quality of life: Findings from a cross-sectional study

4. Periodontitis, halitosis and oral health-related quality of life: findings from a cross-sectional study

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4. Periodontitis, halitosis and oral health-related quality of life – a cross-sectional study

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Abstract:

We aimed to explore the association between volatile sulphurous compounds (VSCs) with periodontal epithelial surface area (PESA) and periodontal inflamed surface area (PISA) on a cohort of periodontitis patients. Consecutive patients were assessed for periodontitis and halitosis. Full-mouth periodontal status assessed probing depth (PD), clinical attachment loss (CAL), gingival recession (REC), bleeding on probing (BoP), PISA and PESA. Halitosis assessment was made using a VSC detector device. Periodontal measures were regressed across VSC values using adjusted multivariate linear analysis. From a total of seventy-two patients (37 females/35 males), the PESA from posterior-lower regions was found to be significantly higher in halitosis cases than their no-halitosis counterparts (p=0.031). Considering all patients, PESA of the posterior-lower region (B=1.3, 95% CI:0.2-2.3, p=0.026) and age (B=-1.6, 95% CI:-3.1-0.2, p=0.026) showed significant association with VSCs. In halitosis patients, PESA of the posterior-lower region (B=0.1, 95% CI:0.0-0.1, p=0.001), PISA Total (B=-0.1, 95% CI:-0.1-0.0, p=0.008) and the OHIP-14 domain of physical disability (B=-2.1, 95% CI:-4.1-0.1, p=0.040) were the most significant

variables to this model. PESA from the posterior-lower region may be associated with VSCs, when other causes of extra-oral halitosis are excluded. Further intervention studies are mandatory to confirm this association.

4.1 Introduction

Periodontitis is a plaque induced chronic inflammatory disease affecting the periodontium. The destruction of the periodontium is the most common cause of tooth loss worldwide [1]. In a representative Portuguese study, the prevalence of periodontitis was estimated at 59.9%, with 24.0% and 22.2% of the participants exhibiting severe and moderate periodontitis, respectively [2]. The clinical manifestation of periodontitis may include tooth mobility, gingival bleeding, halitosis, masticatory impairment, pain and ultimately, tooth loss [3], with a negative impact on oral health-related quality of life (OHRQoL) [4], particularly associated with halitosis [5].

Halitosis results from unpleasant odor arising from the oral cavity when breathing or speaking. Several lines of evidence estimate 80–90% of the causes of bad breath arose from the oral cavity [6–8], and the remaining occur in conditions involving the gastrointestinal tract, the upper and lower respiratory system, the use of medications, diabetes *mellitus*, liver cirrhosis, uremia, and idiopathic conditions [9, 10]. Oral malodor is primarily caused by the microbial degradation of both sulphur-containing and non-sulphur-containing amino acids derived from proteins in exfoliated human epithelial cells and white blood cell debris, or present in plaque, saliva, blood, and tongue coatings [7, 11]. Subgingival periodontal biofilm is mainly composed by Gram-negative anaerobic bacterial species, which present a proteolytic nature [12]. Those species are able to degrade sulphur-containing substrates on different surfaces of oral cavity, including the periodontal pockets, releasing volatile sulphur compounds (VSCs).

Clinical studies have shown that VSCs are the major contributors to halitosis [13, 14]. Hydrogen sulfide, methyl mercaptan and, to a lesser extent, dimethyl sulfide, represent 90% of the VSC in bad breath [9, 13, 14]. From a patient's point of view, halitosis negatively impacts oral health-related quality of life (OHRQoL), specifically on interpersonal relationships, which is the main reason for seeking professional care [15].

Individuals with periodontitis present higher odds of having oral malodor [16]. Alas, the association between periodontitis and halitosis is still far from being fully understood because evidence comes from studies with different periodontitis criteria and halitosis measurement techniques [17]. Additionally, two periodontal research measures have been recently presented, periodontal epithelial surface area (PESA) and periodontal inflamed surface area (PISA) [18], that represent more accurately the periodontal status of the patient and have never been explored with the quantification of VSCs.

Therefore, the main aim of this cross-sectional was to explore the association between VSCs with PISA and PESA on a cohort of periodontitis patients. We further assessed whether measures of halitosis (VSCs and self-reported) were associated with OHRQoL.

4.2 Materials and Methods

4.2.1 Study Design and Setting

This cross-sectional study was conducted at the Egas Moniz Dental Clinic (EMDC). Participants were consecutively recruited from the Periodontology Department of EMDC for periodontal assessment, between October 2019 and March 2021. This study was approved by the Egas Moniz Ethics Committee (nº 781) in accordance with the Helsinki Declaration of 1975, as revised in 2013. After a full explanation, patients agreed to participate in the study and signed a written consent form.

This study was carried out following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [19].

4.2.2. Participants and Eligibility criteria

We enrolled participants with the following inclusion criteria: diagnosis of periodontitis; aged between 18 and 65, who fulfilled the recommendations given for halitosis assessment, and who gave their informed consent. Patients were excluded if: had previously been treated for periodontitis; consumed antibiotics within 4 weeks; past history of radiotherapy of the head and neck; chemotherapy (previous 6 months); extra-oral halitosis (such as, sinusitis, bronchitis, rhinitis, pharyngitis, laryngitis, cancer, diabetes *mellitus*, kidney

diseases and antidepressants drugs) [9, 10]; pregnancy; systemic medication resulting in hyposalivation; or, with diabetes *mellitus*.

Participants were unaware that a halitosis assessment would be done (to reduce any bias), and were instructed to, prior to the periodontal assessment, avoid spicy foods and foods such as onions and garlic (for 24 to 48 hours before examination); avoid smoking 4 to 12 hours before the exam; perform oral hygiene 12 hours before, if the exam is performed in the morning, or 4 hours before if it is performed in the afternoon; consume water until 1 hour before treatment; avoid the use of perfumes and deodorants within 24 hours before the test [20].

4.2.3 Variables

Sociodemographic and Medical Questionnaires

The sociodemographic data collected included gender, age, educational level (no education, elementary, middle or higher), occupation status (student, employed, unemployed or retired), marital status (single, married, divorced or widowed), smoking habits (quantity and duration), alcoholic habits (quantity and frequency), and average family monthly income (in euros). In the medical questionnaire, patients reported the presence of systemic diseases and medications, oral hygiene habits (frequency and devices used). Dental general examination evaluated the presence of: caries, retained roots, fixed prosthesis, removable prosthesis, poorly adapted restorations, supragingival calculus, presence of implants, peri-implantitis, recent extractions and presence of dental abscesses).

Periodontal Assessment

Full-mouth periodontal examination was conducted to assess the following clinical parameters at six sites per tooth using a manual periodontal CP-12 probe (Hu-Friedy®, Chicago, USA). Probing pocket depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP). Plaque index (PI)[21], 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 [22]. Tooth mobility was further appraised [23]. Periodontitis cases were defined according to the AAP/EFP 2018 consensus, with a patient being a periodontitis case if interdental CAL is detectable at ≥2 mm non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth [24].

Halitosis Assessment

The diagnosis of halitosis was carried out in two steps: 1) self-reported questionnaire, to exclude possible causes for extra-oral halitosis (referred in section 2.2) [9, 10]; 2) halitosis assessment through a monitoring VSCs device (Halimeter®, Interscan Corp, Chatsworth, CA, USA).

Prior to the assessment, the mouth remained closed for 1 minute. The end of the cannula was then inserted into the patient's mouth, and the VSCs score recorded at the maximum peak displayed by the device. The result was interpreted according to the manufacturer’s instructions and as previously reported: less than 80 ppb denoted no perceptible odor, 80 to 100 ppb denoted perceivable odor, 100 to 120 ppb denoted moderate halitosis, 120 to 150 ppb denoted more pronounced halitosis, and > 150 ppb denoted severe halitosis [25, 26].

OHRQoL questionnaire

In order to assess Oral health-related Quality of Life (OHRQoL), we applied Oral Health Impact Profile Questionnaire (OHIP-14) [27] validated for Portuguese language [28]. Answers were given on a Likert scale ranging from 0 to 4 (0 = never, 1 = almost never, 2 = occasionally 3 = quite often, 4 = very often), and the survey was completed by the patient alone, without interference. In the case of five or more items were missing or more than two missing items in a subscale, the questionnaire was considered invalid, yet all responses were answered in full and considered for statistical analysis [29].

4.2.4 Measurement Reliability and Reproducibility

The clinical data collection was performed by a single examiner previously subjected to a calibration process (intra and inter-examiner) for the periodontal

assessment in five patients not included in the study. Measurement reliability and reproducibility were assessed by the intra-class correlation coefficient (ICC). Inter-examiner agreement was 0.97 for both PD and CAL, and intra-examiner agreement was 0.94 for both PD and CAL.

4.2.5 Statistical analysis

Data analysis was performed using SPSS for Windows (IBM SPSS Statistics version 26.0 for Windows, IBM Corporation, Armonk, NY, USA). Descriptive and inferential statistics methodologies were applied. Mean values and standard deviation (SD) were calculated for continuous values. For analysis purpose OHRQoL scores were considered as continuous variables. Explicit comparison of mean values was not performed by parametric tests, since data assumptions for the application of the tests were not met (normality and homoscedasticity). Group data comparison was alternatively performed by Mann-Whitney and Kruskal-Wallis tests. Chi-square test was used for comparisons of categorical variables across the groups. The level of significance was set at 5% in all inferential analyses. All patients completed the questionnaires and missing data management was not required.

4.3 Results

4.3.1 Participants

From an initial sample of 132 patients referred to periodontal diagnosis, 60 participants were excluded when inclusion and exclusion criteria were applied (Figure 4.10).

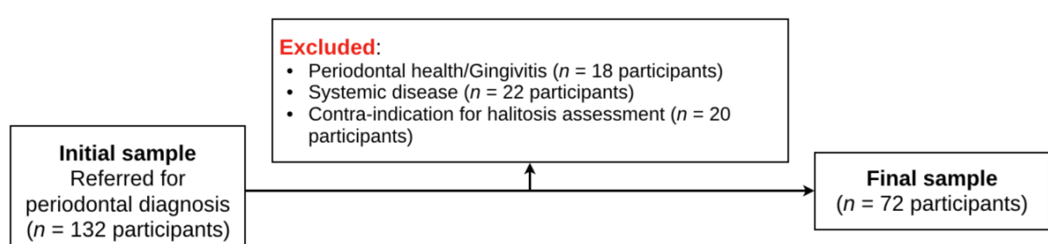

Figure 4.10: Flowchart of participants.

Thus, a final sample of 72 participants was obtained. Overall, this sample had 48.6% males (n=35), with a mean age of 54.5 (±17.6) years (Table 4.4). A total

of 27.8% were smokers (n=20), 16.7% and 5.6% had reported cardiovascular disease and asthma, respectively.

	No halitosis (n=40)	Halitosis (n=32)	p-value	Total (n=72)
Age (years), mean (± SD)	59.5 (± 3.6)	49.9 (±3.2)	0.066	54.6 (±17.6)
Gender, n (%)				
Male	20 (50.0)	15 (46.9)	0.817	35 (48.6)
Female	20 (50.0)	17 (53.1)		37 (51.4)
Smoking habits, n (%)				
Current smoker	13 (32.5)	7 (21.9)	0.429	20 (27.8)
Non-smoker	27 (67.5)	25 (78.1)		52 (72.2)
Comorbidities, n (%)				
Cardiovascular disease	10 (25.0)	7 (21.9)	0.788	12 (16.7)
Asthma	0 (0.0)	4 (12.5)	**0.035**	4 (5.6)
Self-perceived halitosis, mean (±SD)	4.0 (2.6)	4.4 (2.6)	0.567	4.2 (2.6)
Oral health conditions, n (%)				
Poorly adapted restorations	6 (15.0)	7 (21.9)	0.543	13 (18.1)
Fixed prosthesis	6 (15.0)	7 (21.9)	1.000	13 (18.1)
Removable prosthetic	17 (42.5)	5 (15.6)	**0.012**	22 (30.6)
Caries	21 (52.5)	13 (40.6)	0.350	34 (47.9)
Retained roots	6 (15.0)	5 (15.6)	1.000	11 (15.3)
Supragingival calculus	30 (75.0)	25 (78.1)	0.788	55 (76.4)
Peri-implantitis	0 (0.0)	3 (9.4)	0.083	3 (4.2)
Presence of implants	0 (0.0)	4 (12.5)	**0.035**	4 (5.6)
Recent extraction	3 (7.5)	2 (6.3)	1.000	5 (6.9)
Presence of dental abscesses	0 (0.0)	2 (6.3)	0.194	2 (2.8)
VSCs, mean (SD)	38.5 (18.0)	168.1 (121.3)	**<0.001**	96.1 (103.9)

Table 4.4: Participant's characteristics, stratified according to halitosis status.

*1Chi-square test for categorical variables and Mann-Whitney test for continuous variables, p<0.05 denoted in bold.

Of the 72 participants, 44.4% (n=32) had halitosis, of which 53.1% (n=17) were female. With regard to smoking habits, we observed that 78.1% (n=25) of participants with halitosis were non-smokers and 21.9% (n=7) smokers. Halitosis was not found to be significantly associated with gender (p=0.817) and smoking habits (p=0.429). Regarding oral health conditions, the use of removable prosthesis (p=0.012) and presence of implants (p=0.035) were significantly more prevalent in halitosis cases.

When analyzing periodontal clinical characteristics according to the halitosis status (Table 4.5), we found no differences for tooth brushing frequency, interdental cleaning, periodontal staging/extent and most periodontal clinical measures (p>0.05). However, the PESA from posterior-lower regions was found to be significantly higher in halitosis cases than their no-halitosis counterparts (p=0.031).

	No halitosis (n=40)	Halitosis (n=32)	p-value	Total (n=72)
Tooth brushing frequency, n (%)				
Once a day or less	5 (12.5)	7 (21.9)	0.076	12 (16.7)
Twice a day	19 (47.5)	7 (21.9)		26 (36.1)
More than twice a day	16 (40.0)	18 (56.2)		34 (47.2)
Interdental cleaning, n (%)				
No	22 (55.0)	15 (46.7)	0.636	26 (51.0)
Yes	18 (45.0)	17 (53.3)		25 (49.0)
Periodontal staging, n (%)				
I/II	2 (5.0)	4 (12.5)	0.191	6 (8.3)
III	15 (37.5)	16 (50.0)		31 (43.1)
IV	23 (57.5)	12 (37.5)		35 (48.6)
Periodontal extent, n (%)				
Localized	13 (32.5)	11 (34.4)	1.000	24 (33.3)
Generalized	27 (67.5)	21 (65.6)		48 (66.7)
Periodontal clinical parameters, mean (SD)				
BOP (%)	20.9 (18.4)	19.9 (18.3)	0.865	20.5 (18.2)
PI (%)	35.5 (21.5)	30.6 (18.0)	0.371	33.3 (20.0)

CAL (mm)	6.0 (±0.08)	6.0 (±0.05)	0.562	6.0 (0.07)
PPD (mm)	3.1 (±0.8)	3.2 (±0.7)	0.493	3.2 (0.7)
Recession (mm)	1.0 (0.9)	1.0 (1.1)	0.504	1.0 (0.9)
Missing teeth	10.1 (5.7)	7.8 (5.1)	0.066	9.1 (5.5)
PISA Total	89.9 (120.0)	95.3 (107.5)	0.227	92.3 (113.9)
PESA Total	311.8 (179.7)	344.3 (151.5)	0.230	326.3 (167.4)
PISA Posterior	69.8 (102.9)	76.7 (94.7)	0.200	72.9 (98.7)
PISA Anterior	20.1 (22.7)	18.6 (19.9)	0.977	19.4 (21.4)
PISA Posterior-Upper	60.2 (93.4)	65.8 (86.9)	0.268	62.7 (89.9)
PISA Anterior-Upper	7.8 (11.0)	8.5 (14.0)	0.964	8.1 (12.4)
PISA Posterior-Lower	9.6 (13.0)	11.0 (11.7)	0.283	10.2 (12.4)
PISA Anterior-Lower	12.3 (14.1)	10.1 (11.4)	0.623	11.3 (12.9)
PESA Posterior	224.8 (158.2)	261.2 (141.2)	0.239	241.0 (150.9)
PESA Anterior	88.6 (32.0)	94.2 (20.3)	0.272	91.1 (27.4)
PESA Posterior-Upper	184.0 (143.0)	209.7 (134.8)	0.344	195.4 (139.0)
PESA Anterior-Upper	36.9 (21.0)	42.0 (17.2)	0.106	39.1 (19.5)
PESA Posterior-Lower	40.8 (23.9)	51.6 (17.0)	0.031	45.6 (21.7)
PESA Anterior-Lower	51.8 (17.4)	52.2 (16.7)	0.721	52.0 (16.9)

Table 4.5: Periodontal clinical characteristics, stratified according to halitosis status.

* Chi-square test for categorical variables and Mann-Whitney test for continuous variables, p<0.05 denoted in bold.

As with OHIP total score and all seven OHIP-14 domains (Table 4.6), no significant differences were found regarding the halitosis assessment. When we compared different periodontitis stages, a difference was depicted regarding physical pain (p=0.048).

Then we assessed which variables were associated with the measurement of VSCs both in the overall sample as in a sample of patients clinically diagnosed with halitosis (Table 4.7). Considering all patients, our stepwise linear regression pointed PESA of the posterior lower region (B=1.3, 95% CI: 0.2-2.3, p=0.026) and age (B=-1.6, 95% CI: -3.1-0.2, p=0.026) as the most significant

variables. As with halitosis patients, PESA of the posterior lower region (B=0.1, 95% CI: 0.0-0.1, p=0.001), PISA Total (B=-0.1, 95% CI: -0.1—0.0, p=0.008) and the OHIP-14 domain of physical disability (B=-2.1, 95% CI: -4.1—0.1, p=0.040) were the most significant variables to this model. A graphical representation of PESA posterior lower teeth and age towards the measurement of VSCs was made (Figure 4.11).

OHIP-14 Domains	Halitosis assessment			Periodontitis stage				Total (n=72)
	No halitosis (n=40)	Halitosis (n=32)	p-value *	I/II (n=6)	III (n=31)	IV (n=35)	p-value *	
Functional Limitation	1.58 (1.85)	1.66 (2.42)	0.502	2.67 (2.16)	1.06 (1.57)	1.91 (2.42)	0.214	1.61 (2.11)
Physical Pain	2.88 (2.26)	3.16 (2.45)	0.709	4.67 (2.07)	2.39 (2.11)	3.26 (2.43)	**0.048**	3.00 (2.33)
Psychological Discomfort	3.75 (2.63)	3.94 (2.49)	0.744	3.83 (1.60)	3.39 (2.40)	4.23 (2.79)	0.458	3.83 (2.55)
Physical Disability	2.50 (2.55)	1.97 (2.46)	0.321	3.00 (2.19)	1.55 (1.86)	2.77 (2.92)	0.184	2.26 (2.51)
Psychological Disability	2.30 (2.33)	2.25 (2.16)	0.995	2.83 (2.04)	1.55 (1.46)	2.83 (2.67)	0.129	2.28 (2.24)
Social Disability	1.33 (2.02)	1.28 (1.84)	0.889	1.33 (1.63)	0.74 (1.18)	1.80 (2.36)	0.269	1.31 (1.93)
Handicap	1.85 (2.21)	1.84 (2.05)	0.822	2.00 (2.10)	1.26 (1.61)	2.34 (2.44)	0.202	1.85 (2.13)
Total	16.18 (13.48)	16.19 (12.26)	0.843	20.33 (10.58)	12.00 (7.88)	19.17 (15.69)	0.139	16.18 (12.87)

Table 4.6: Oral health related quality of life (OHRQoL) according to the halitosis status and to the periodontal status, presented as mean (standard deviation) of OHIP-14 domain scores.

* Mann-Whitney test.

	Overall (n=72)			Halitosis patients (n=32)		
Variable	B (SE)	95% CI	p-value	B (SE)	95% CI	p-value
Constant	131.2 (49.4)	32.8;229.7	**0.010**	36.8 (5.1)	26.4;47.1	**<0.001**
PESA posterior-lower	1.3	0.2;2.3	**0.021**	0.1	0.0;0.1	**0.001**
Age	-1.6	-3.1;-0.2	**0.026**	-	-	-
PISA Total	-	-	-	-0.1	-0.1;-0.0	**0.008**
Physical Disability	-	-	-	-2.1	-4.1;-0.1	**0.040**

Table 4.7: Stepwise multivariate linear regression analysis of age and PESA posterior-lower teeth for the outcome variable of VSCs (N = 405).

p<0.05 denoted in bold.

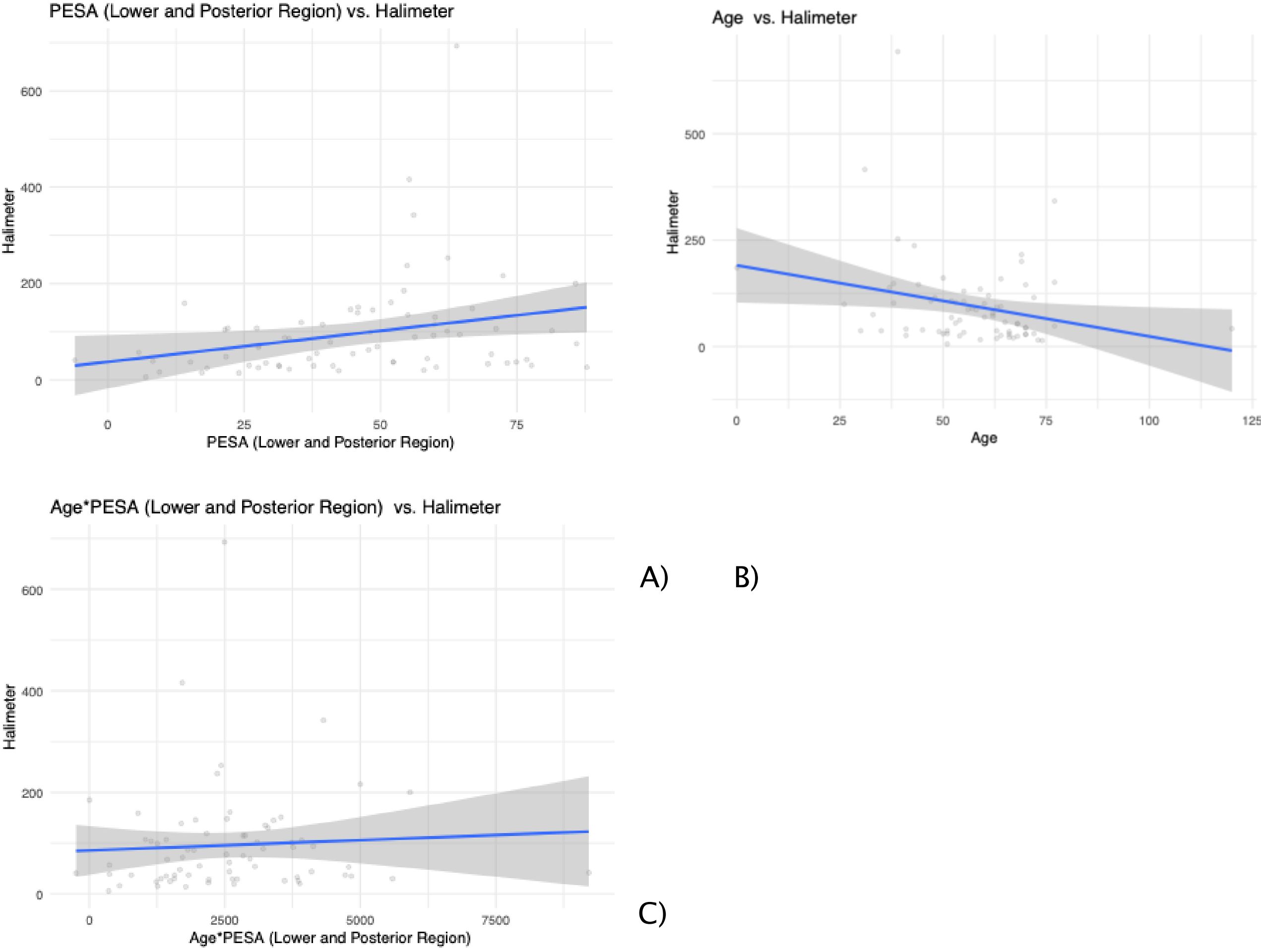

Figure 4.11: Graphical representation of PESA posterior lower teeth and age towards the measurement of VSCs.

4.4 Discussion

In this cross-sectional study, we sought to explore the relationship of PISA and PESA with VSCs. We hypothesize exploring such far-reaching measures would provide a more comprehensive understanding on how periodontal destruction relates with VSCs. Our results confirmed VSC counts may be associated with the amount of PESA of the posterior-lower region. To the best of our knowledge, this study may be the first to demonstrate such association.

Halitosis occurs from the complex interaction between bacterial biofilm and protein substrates [12]. Subgingival biofilm is composed mainly of anaerobic Gram-negative bacteria, of proteolytic nature, capable of degrading the substrates present in the oral cavity, originating VSCs [16]. The levels of VSC have been shown to increase with the periodontitis severity [30]. Biologically, deeper periodontal crevices emerge with the progression of periodontitis, increasing the area of bacterial colonization, and intensifying the release of VSCs [31]. Additionally, the hypoxic milieu of deepest pockets, acidification and the consequent activation of the decarboxylation of amino acids, give rise to VSCs [31]. In turn, VSCs shift the permeability of the oral mucosa and the solubility of collagen, and also for decreasing the synthesis of proteins and collagen, resulting in the destruction of periodontal tissues [31–33].

The significance of the posterior-lower region may be seen as innovative and shall be clarified in future and prospective studies. According to these results, it may be possible, in the future, to establish a protocol for the treatment of periodontitis and halitosis. Starting periodontal treatment at the posterior-lower sites/regions, in order to motivate patients and respond to the patient's complaints, may come to make sense. Conventional periodontal therapy (scaling and root planing) contributes to mitigate Gram-negative anaerobic bacteria, consequently decreasing the concentration of VSCs in the oral cavity and, with this, the prevalence of halitosis. This decrease in VSCs produces a significant improvement in halitosis. The treatment of halitosis can be used by clinicians, as a motivating instrument for the patient, for periodontal treatment [15, 34, 35]. Reasoning from the fact halitosis may affect quality of life, our results revealed no significant differences among the various domains of OHIP-14 according to the halitosis status of patients. This may relate with the self-perception of bad breath, as these participants manifested clear inability to this as measured through self-perception of bad breath between patients with

and without halitosis. Several studies have addressed self-perceived halitosis (SPH), most of them in dental students [33–37]. Inadequate oral hygiene and infrequent tooth brushing [38, 39] and unawareness towards halitosis prevention [38] were among the most important related factors. Also, awareness and concern regarding halitosis have resulted in better extraoral self-care practices [34, 40].

Thus, greater public awareness and education should be encouraged. Informing and educating patients for oral malodor should be accentuated and new generations should be qualified to address this matter effectively [41].

Strengths and Limitations

This study presents a convenience sample from a Periodontology department hindering the potential for generalization. Selection bias related to such narrow inclusion and exclusion criteria, as well as difficulties in measuring and diagnosing halitosis, may also have occurred. Thus, new studies in different and larger populations can contribute to validate our findings. As well, future prospective randomized clinical trials are mandatory to measure the biological and biochemical differences in periodontal and halitosis assessment before and after periodontal treatment and its impact on oral health-related quality of life.

Halitosis assessment was based on the measurement of VSCs and should be complemented with organoleptic assessment [16], as VSCs have low sensitivity [36]. Nevertheless, these results demonstrated significance even though the association between periodontitis and halitosis is greater in studies where organoleptic examination was carried out [16]. Alas, we shall highlight the subjectivity upon the organoleptic test [26], despite remaining the “gold standard” diagnostic method [37]. To our view, measuring VSC levels stands for an objective method [8], and therefore has higher potential clinical applicability.

The main advantages of the present study can be considered its design, which differs from the others by the definition of cases of periodontitis, where the most up-to-date case definition was considered [42], ensuring comparability with future studies. The exclusion of causes of extra-oral halitosis is also a strength. The assessment of tridimensional periodontal measures (PISA and PESA) provides new results that deserve further attention.

4.5 Conclusions

Within the limitations of this observational study, the PESA from the posterior-lower region seems to be associated with VSC levels, when other causes of extra-oral halitosis are excluded. Further intervention studies are mandatory to verify a possible causal association.

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5

Non-surgical periodontal treatment impact on subgingival microbiome and intra-oral halitosis

5. Non-surgical periodontal treatment impact on subgingival microbiome and intra-oral halitosis

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5. Non-surgical periodontal treatment impact on subgingival microbiome and intra-oral halitosis

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Abstract:

The purpose of this study was to characterize and compare subgingival microbiome before and after periodontal treatment to learn if any changes of the subgingival microbiome were reflected in intra-oral halitosis. We tested the hypothesis that intra-oral halitosis (Volatile sulphur compounds levels) correlates with corresponding subgingival bacterial levels before and after periodontal treatment. Twenty patients with generalized periodontitis completed the study. Subgingival plaque samples were collected at baseline and 6–8 weeks after nonsurgical periodontal therapy. Full-mouth periodontal status assessed probing depth (PD), clinical attachment loss (CAL), gingival recession (REC), bleeding on probing (BoP), PISA and PESA. Halitosis assessment was made using a volatile sulphur compounds (VSC) detector device. Periodontal measures were regressed across VSC values using adjusted

multivariate linear analysis. The subgingival microbiome was characterized by sequencing on an Illumina platform. From a sample of 20 patients referred to periodontal treatment, 70% were females (n = 14), with a mean age of 56.6 (±10.3) years; full-mouth records of PD, CAL, BOP (%) allowed to classify the stage and grade of periodontitis, with 45% (n = 9) of the sample having Periodontitis Stage IV grade C and 95% (n=19) had generalized periodontitis. The correlation of bacterial variation with VSCs measured in the periodontal diagnosis and in the reassessment after treatment were evaluated. Fusobacterium nucleatum, *Capnocytophaga gingivalis* and *Campylobacter showaei* showed correlation with the reduction of VSC after periodontal treatment (p-value = 0.044; 0.047 and 0.004, respectively). *Capnocytophaga sputigena* had a significant reverse correlation between VSCs variation from diagnosis (baseline) and after treatment. Microbial diversity was high in the subgingival plaque on periodontitis and intra-oral halitosis participants of the study. Furthermore, there were correlations between subgingival plaque composition and VSC counting after periodontal treatment. The subgingival microbiome can offer important clues in the investigation of the pathogenesis and treatment of halitosis.

Keywords: halitosis; subgingival microbiome; periodontitis; periodontal disease; periodontal medicine; volatile sulphurous compounds

5.1 Introduction

Periodontitis is a plaque-induced chronic inflammatory disease affecting the periodontium [1]. It is a biofilm-mediated and multifactorial disease, in which the subgingival microbiome plays a critical role in its onset and progression of the disease [2]. The subgingival niche offers optimal ecological conditions for microbial growth [3,4]. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are considered keystone periodontal pathogens [5], yet other relevant microorganisms are present suggesting its subgingival screening is highly relevant [6,7]. In addition, periodontitis consistently links with sytemic health through its pathogenic bacteria, bioproducts and associated low-grade general inflammatory state [8]. Among clinical signs and symptoms, halitosis is a negative consequence of periodontitis [9,10] and

contributes to the deteriorating effect on oral health-related quality of life (OHRQoL) [11,12]. Halitosis is characterized by an unpleasant odor emanating from the mouth, either from oral or non-oral sources [13]. About 80–90% of the causes are estimated to derive from the oral cavity [14,15]. Depth pockets act as reservoirs of microorganisms responsible for secreting sulphur components that contribute to this unpleasant smell [10,16]. Volatile sulphur compounds (VSC) can be toxic for human cells even at low concentrations [17]. Several studies demonstrate the importance of studying volatile compounds (VSCs and VOCs) in detecting oral diseases [18]. While observational studies could associate periodontal pathogens to VSC levels production, subgingival microbiome characterization after periodontal treatment and changes to the VSC level is scarce. This may contribute to unveiling whether expected subgingival plaque shift after treatment impacts the production of VSCs. Nonsurgical periodontal treatment (that is, scaling and root planing), induces subgingival ecological and composition changes [19]. To the best of our knowledge, the effect of scaling and root planing on the subgingival microbiome and its outcome on intraoral VSCs is poorly understood. Thus, the purpose of this non-randomized controlled trial was to characterize the subgingival microbiome and quantify the VSC levels before and after periodontal treatment.

5.2 Materials and Methods

5.2.1 Study Design

This non randomized intervention study recruited participants consecutively from the Periodontology Department of Egas Moniz Dental Clinic (EMDC), between December 2021 and June 2022. This study was approved by the respective Institutional Review Board (Egas Moniz Ethics Committee ID no. 781) and within the Helsinki Declaration of 1975 (as per the 2013 revision). Patients were informed of the aims of the study and following the agreement to participate in the study, signed the written consent form. All clinical examinations and treatments were performed by the same clinician (C.I.).

5.2.2 Participants and Eligibility Criteria

Participants were enrolled if they fulfilled the following inclusion criteria: diagnosis of periodontitis; age between 18 and 65 [20], and ability to provide

informed consent. Patients were excluded if they had received treatment for periodontitis in the last 12 months; if they had taken antibiotics in the last month; previous record of head and neck radiotherapy; chemotherapy in the previous 6 months; extra-oral causes of halitosis (for instance, respiratory tract inflammatory conditions, cancer, kidney diseases, diabetes *mellitus* or antidepressants) [21,22]; pregnancy; systemic medication resulting in hyposalivation; or, a diagnosis of diabetes mellitus. Without mentioning that the halitosis measurement was planned (to minimize any bias source) at the baseline, participants were informed two days prior to the periodontal assessment [20] to: avoid particular foods (namely, spicy aliments, onions and garlic) 24 to 48h prior examination; avoid smoking 4 to 12h before the exam; perform oral hygiene 12h before, if the exam was performed in the morning, or 4h before if it was performed in the afternoon; consume water until 1h before treatment; avoid the use of perfumes and deodorants within 24h before the test.

5.2.3 Variables

Sociodemographic and Medical Questionnaires

Among the sociodemographic information, we collected sex, age, schooling (no education, elementary, middle or higher), job status (student, employed, unemployed or retired), marital status (single, married, divorced or widowed), smoking habits (amount and length), alcoholic habits (amount and frequency), and average family monthly income (in euros). Among the medical information, participants reported existing systemic diseases, prescriptions, and oral hygiene habits (frequency and devices used). The overall dental observation included presence of: caries, retained roots, fixed prosthesis, removable prosthesis, poorly adapted restorations, supragingival calculus, presence of implants, peri-implantitis, recent extractions and presence of dental abscesses.

Periodontal Assessment

The periodontal diagnosis was based on a circumferentially full-mouth protocol using a manual periodontal CP-12 probe (Hu-Friedy®, Chicago, IL, USA). Measurements were made at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) [23]. Periodontal pocket depth (PPD) measured the distance from the free gingival margin to the bottom

of the pocket and recession from the cemento enamel junction (CEJ) to the free gingival margin. Clinical attachment loss was the result of the sum PPD and recession for each site. Measurements were rounded to the lowest whole millimeter. Furcation involvement (FI) was assessed using a Naber's probe [24]. Tooth mobility was further appraised [25]. We defined periodontitis according to the AAP/EFP 2018 consensus, that is, if interdental CAL was detectable at≥2 mm non-adjacent teeth, or buccal or oral CAL≥3mm with pocketing >3mm was detectable at ≥ 2 teeth [26]. We further completed the diagnosis with the staging and grading (see Supplementary Materials).

Halitosis Assessment

The diagnosis of halitosis was carried out in two steps: (1) self-reported questionnaire, to exclude possible causes for extra-oral halitosis (referred in Section 2.2) [21,22]; (2) halitosis assessment through a monitoring VSCs device (Halimeter®, Interscan Corp, Chatsworth, CA, USA). Before measurement, patients kept their mouth closed for 1 min. The end of the cannula was then inserted into the patient's mouth, and the VSC score recorded at the maximum peak displayed by the device. The result was interpreted according to the manufacturer's instructions and as previously reported: less than 80 ppb denoted no perceptible odor, 80 to 100 ppb denoted perceivable odor, 100 to 120 ppb denoted moderate halitosis, 120 to 150 ppb denoted more pronounced halitosis, and >150 ppb denoted severe halitosis [27,28].

Treatment Protocol

After completion of baseline monitoring, individuals received scaling and root planing (SRP) in two sessions (one side per session) under local anesthetic and instruction in proper home care procedures. Approximately 2 months after SRP, individuals were re-examined (periodontal clinical parameters; VSC levels; subgingival sample collection) as part of their periodontal maintenance.

Subgingival Microbiome

Samples were collected using sterile curettes, suspended directly in Tris-EDTA Buffer, pH 8.0 in a sterile Eppendorf, and stored at−20◦C during a week (short-term) and then at −80◦C (long term) until analysis.

Genomic DNA was extracted according to the manufacturer's instructions (ExtractMe DNA Tissue Kit, Blirt, Gdansk, Poland). The extracted DNA was stored eluted in Elution Buffer at−20◦C until sequencing.

Sequencing was performed by Novogene (Cambridge,UK). 16S rRNA genes (16SV3-V4) were amplified. All PCR reactions were carried out with the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). PCR products were mixed with the same volume of 1X loading buffer (contained SYB green) and were separated by electrophoresis on 2% agarose gel for detection. Samples with a bright main strip between 400–450bp were chosen for purification using a Qiagen Gel Extraction Kit (Qiagen,Germany). Sequencing libraries were generated using the NEB Next Ultra DNA Library Pre® Kit (New England Biolabs) for Illumina sequencing, following manufacturer's recommendations and index codes were added during library preparation. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated. Paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequence, being merged using Flash. Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags [29] according to the QIIME (v1.7.0) [30] quality controlled process. Tags were compared with the reference database (Gold database) using the UCHIME algorithm (UCHIME Algorithm) [31] to detect chimera sequences, and these were removed [32]. Then, the Effective Tags were finally obtained. Sequence analysis was performed using the Uparse software (v7.0.1001) [33]. Sequences with ≥97% similarity were assigned to the same Operational Taxonomic Unit (OTUs). A representative sequence for each OTU was screened for further annotation. For each representative sequence, the GreenGenes Database [34] was used based on the RDP classifier algorithm [35] to annotate taxonomic information. In order to study phylogenetic relationship of different OTUs, and the difference of the dominant species in different samples (groups), multiple sequence alignment were conducted using the MUSCLE software (v 3.8.31) [36]. The abundance of OTUs was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of

alpha diversity and beta diversity were all performed basing on this output normalized data, using QIIME. Cluster analysis was preceded by a principal component analysis (PCA), which was applied to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 2.15.3). Principal Coordinate Analysis (PCoA) was performed to obtain principal coordinates and visualize the complex, multidimensional data. A distance matrix of weighted or un-weighted unifrac among samples obtained before was transformed to a new set of orthogonalaxes, by which the maximum variation factor was demonstrated by the principal coordinate, and the second maximum one by the second principal coordinate, and so on. The unweighted Pair-group Method with Arithmetic Means (UPGMA) clustering was performed as a type of hierarchical clustering method to interpret the distance matrix using average linkage and was conducted by QIIME software (v 1.7.0).

5.2.4 Measurement Reliability and Reproducibility

One examiner (C.I.) was previously calibrated for the periodontal examination in five patients not included in the study. Intra-class correlation coefficient (ICC) was computed to ascertain reliability and reproducibility. For both PPD and CAL, the inter-examiner agreement was 0.97 and 0.94, respectively.

5.2.5 Statistical Analysis

All statistical analyses were conducted in R (v4.1.0). After testing for data normality, clinical data and questionnaire results were compared between baseline and follow-up. The Mann–Whitney test was used in continuous variables. Then, we the explored correlation of baseline (B), follow-up (FW) and the difference between B and FW (ΔB-FW) with the relative abundance levels of known periodontopathogenic bacteria (*P.gingivalis*,*T.forsythia*, *T. denticola*, *F. nucleatum*, *C. showae*, *C. gingivalis*, *C. sputigena*, *P. intermedia*, *P. nigrescens*, *F. periodonticum*, *P. micra*, *C.ochracea*, *A. actinomycetemcomitans*, *A. israelii*, *Actinomyces gerencseriae*). For this purpose, Spearman correlation test was used. A p-value < 0.05 was considered statistically significant.

5.3 Results

From a sample of 20 patients referred to periodontal treatment, 70% were females (n = 14), with a mean age of 56.6 (±10.3) years; a total of 65% were

non-smokers (n = 13), 20% (n = 4) had reported controlled hypertension (Table 1). With regard to oral hygiene habits, 85% (n = 17) of participants were referred to brush their teeth twice a day, with 90% (n = 18) using manual toothbrush, 65% (n = 13) using mouthwash, and 20% (n = 4) using interdental flossing (Table 5.8)

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Variable	
Age, mean (SD) (min-max]	56.6 (10.3) (41-80)
Females, % (n)	70.0 (14)
Systemic conditions, % (n)	
Hypertension	20.0 (4)
Arthritis	5.0 (1)
Toothbrushing per day, % (n)	
1	15.0 (3)
2	85.0 (17)
Oral hygiene habits, % (n)	
Manual brush	90.0 (18)
Tongue scraper	0.0 (0)
Mouthwash	65.0 (13)
Interdental flossing	20.0 (4)
Interdental brush	25.0 (5)
Denture	15.0 (3)
Smoking habits, % (n)	
Never	65.0 (13)
Active	35.0 (7)
Alcohol consumption, % (n)	60.0 (12)
Periodontitis staging and grading, % (n)	
II-C	5.0 (1)
III-B	35.0 (7)
III-C	5.0 (1)
IV-B	10.0 (2)
IV-C	45.0 (9)
Generalized periodontitis	95.0 (19)

Table 5.8: Participants baseline sociodemographic and clinical characteristics.

Full-mouth records of PD, CAL, BOP (%) and PI (%) allowed to classify the stage and grade of periodontitis, with 45% (n = 9) of the sample having Periodontitis Stage IV grade C and 95% (n = 19) had generalized periodontitis. VSCs, Winkler Index, OHIP-14 (functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability and handicap); mean of CAL and PPD at Baseline and follow-up (6–8 weeks before periodontal treatment) are presented in Table 5.9. Conventional periodontal treatment resulted in a decrease in the mean of the VSCs, statistically significant (p=0,007). The mean values of Winkler index and OHIP-14 scores decrease after periodontal treatment, but no significant differences was found in analyzed sample.

Variable	Baseline	Follow-Up	p-Value *
VSCs, mean (SD) (min-max]	115.2 (113.7)	58.0 (52.3)	0.007
Winkler Index, median (min-max)	3.0 (0–8)	2.0 (0.8)	0.231
OHIP-14, mean (SD)			
Total score	18.7 (11.4)	17.8 (11.4)	0.588
Functional limitation	1.9 (1.7)	2.2 (1.7)	0.277
Physical pain	3.5 (2.0)	3.4 (2.0)	0.937
Psychological discomfort	4.4 (2.5)	4.1 (2.2)	0.546
Physical disability	3.6 (2.4)	3.2 (2.1)	0.382
Psychological disability	2.7 (2.3)	2.2 (2.2)	0.253
Social disability	1.9 (2.3)	1.8 (2.4)	0.774
Handicap	1.9 (1.9)	1.9 (2.4)	0.863
Mean CAL, mean (SD)	3.3 (0.8)	2.6 (0.6)	0.457
Mean PPD, mean (SD)	4.0 (1.7)	3.8 (1.6)	0.406
Distribution of PPD >5 mm, % (n)	18.8 (15.9)	4.8 (10.2)	0.368
Distribution of CAL >7 mm, % (n)	14.5 (20.3)	10.8 (18.9)	0.194

Table 5.9: Participants baseline and follow-up periodontal and halitosis measurements.

* Mann–Whitney test for continuous variables.

When analyzing the number of species of samples of the baseline and after treatment, we observed a relative maintenance without statistical differences (p = 0.9033).

5.3.1 Impact of Periodontal Treatment on Subgingival Microbiome

Relative abundance of the 10 predominant subgingival bacterial taxa phylum before and after nonsurgical periodontal treatment is presented in Figure 5.12 (per patient) and Figure 5.13 (overall). The predominant bacterial taxa phylum in subgingival plaque were *Bacteroidota*, *Firmicutes*, *Proteobacteria*, *Fusobacteriota*, *Actinobacteriota* and *Sprirochaetota*, which constituted approx. ninety percent of all DNA reads before and after nonsurgical periodontal therapy (Figure 5.13). In addition, no significant differences in relative abundance of predominant bacterial taxa phylum were recorded the follow-up period (Figure 5.13).

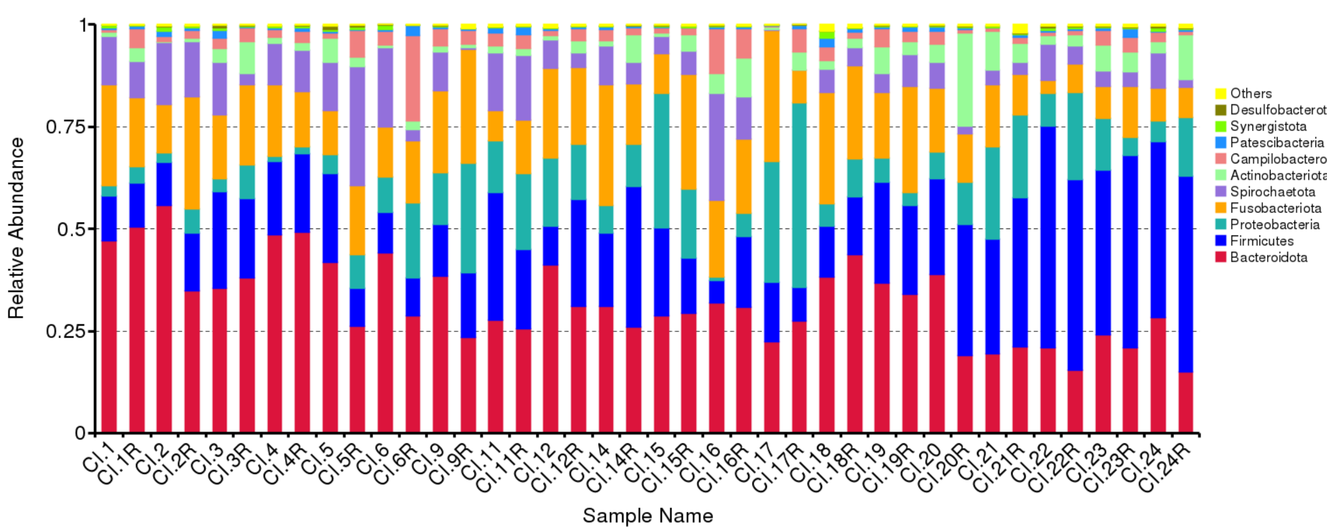

Figure 5.12: Predominant bacterial taxa phylum in subgingival plaque. Each variable of the Sample Name is named without R (baseline) and with R (follow-up).

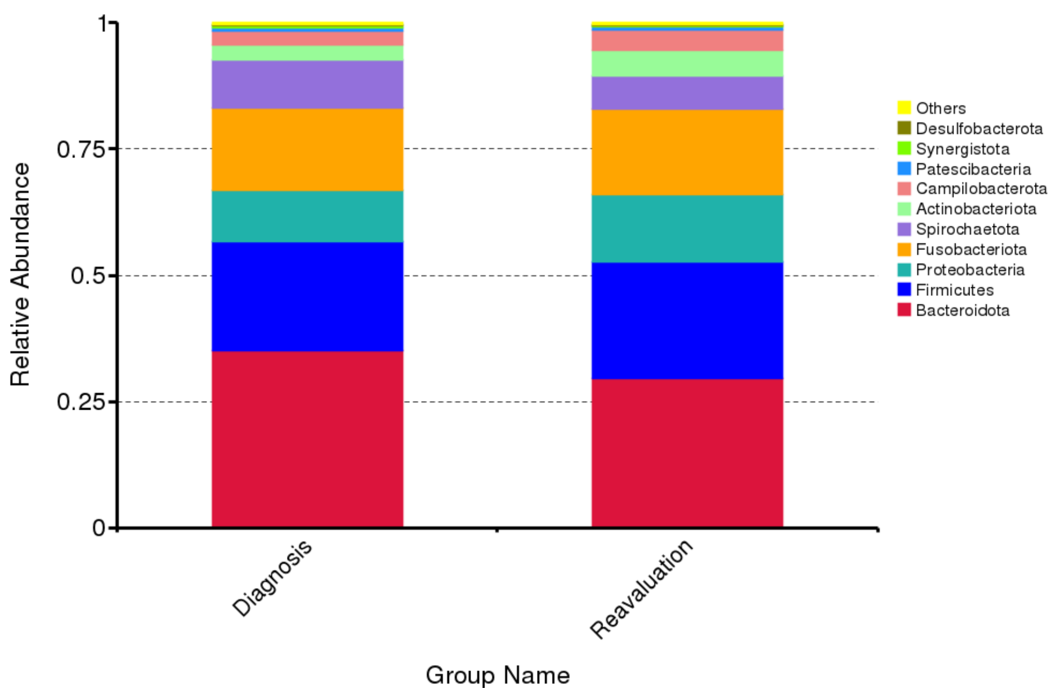

Figure 5.13: Mean levels of relative abundance of the 10 predominant phyla in subgingival samples at baseline and 6-8 weeks after treatment.

The predominant bacterial species in subgingival plaque were *Prevotella*, *Porphiromonas*, *Fusobacterium*, *Treponema*, *Neisseria* and *Leptotrichia* (Figure 5.14).

Figure 5.14: Predominant bacterial species in subgingival plaque. Mean levels of relative abundance of the 100 predominant species in subgingival samples at baseline and 6-8 weeks after treatment.

5.3.2 Correlation between Subgingival Microbiome Abundance and VSC before and after Periodontal Treatment

The correlation of bacterial variation with VSCs levels before and after periodontal treatment was evaluated (Table 5.10). *Fusobacterium nucleatum*, *Capnocytophaga gingivalis* and *Campylobacter showaei* showed correlation with the reduction in VSC after periodontal treatment (p-value = 0.044; 0.047 and 0.004, respectively). *Capnocytophaga sputigena* had a significant reverse

correlation between VSCs variation from diagnosis (baseline) and after treatment (Table 5.10).

Complex	Bacteria	VSC Levels					
		ΔB-FW	*p*-Value	B	*p*-Value	FW	*p*-Value
Red	***P. gingivalis***	0.044	0.855	−0.160	0.500	−0.261	0.267
	T. forsythia	−0.254	0.281	0.207	0.382	−0.056	0.816
	T. denticola	0.013	0.957	−0.006	0.980	0.013	0.960
Orange	***F. nucleatum***	0.047	0.844	0.166	0.483	0.456	**0.044**
	C. showae	−0.744	0.149	0.824	0.086+	0.976	**0.004**
Green	***C. gingivalis***	0.062	0.795	0.149	0.530	0.449	**0.047**
	C.sputigena	−0.451	**0.046**	0.281	0.229	−0.287	0.219
	P. intermedia	0.130	0.584	−0.120	0.613	−0.002	0.994
	P. nigrescens	0.342	0.141	−0.247	0.293	0.143	0.548
	F. periodonticum	0.014	0.955	0.112	0.638	0.271	0.248
-	***P. micra***	0.094	0.694	−0.138	0.563	−0.112	0.637
	C. ochracea	0.099	0.678	−0.042	0.860	0.106	0.657
	A. actinomycetemcomitans	−0.232	0.520	−0.023	0.949	−0.265	0.460
	A. israelii	−0.200	0.427	0.109	0.666	−0.163	0.517
	Actinomyces gerencseriae	0.363	0.139	−0.184	0.465	0.326	0.187

Table 5.10: Correlation of percentage microbiome with VSCs variation.

Abbreviations: B: baseline; FW: Follow-up.

5.4 Discussion

This interventional study explored the variation of the subgingival microbiome and of the intraoral VSCs among adults with periodontitis, before and after treatment. Overall, an expected disruption of the subgingival microbiome was noticed as a result of mechanical root planing. When correlating these levels with intra-oral VSCs, our results showed that two orange complex bacteria (*F.*

nucleatum and *C. showae*) and one green complex bacterium (*C. gingivalis*) had strong correlation with VSCs after therapy. On the contrary, one green complex bacterium (*C. sputigena*) was correlated to lower VSC differences of baseline to follow-up. These correlations may be relevant because they identify four specific bacteria from the dental plaque of pathological periodontal pockets that correlate to clinical levels of halitosis. Additionally, these results may guide future research on the mechanisms involved in the production of VSCs by these bacteria. On the other hand, novel therapies may be designed to target these specific microorganisms and their VSC production; therefore, resulting in novel approaches for mitigating halitosis of periodontal reason. The subgingival biofilm of the periodontium is mostly a Gram-negative anaerobic population niche with proteolytic capacity [37]. Biologically, these bacteria are capable of degrading sulphur-containing substrates, including the periodontal pockets, releasing volatile sulphur compounds (VSCs). There are several mechanisms that can explain the link between halitosis and periodontal disease, usually based on properties of the main microbially generated VSCs, where hydrogen sulfide and methylmercaptan facilitate the penetration of lipopolysaccharide into the gingival epithelium, inducing inflammation [38]. The VSCs also aid bacterial invasion of the connective tissue by their toxic effects on epithelial cells, while methylmercaptan hinders epithelial cell growth and proliferation [39]. This is accentuated by decreasing oxygen tension arising from an increase in periodontal pocket depth, with a concomitant decrease in pH, which is necessary for the putrefaction of amino acids that create VSCs. *Fusobacterium nucleatum* is an anaerobic oral commensal and a periodontal pathogen (orange complex) associated with a wide spectrum of human diseases [40]. *F. nucleatum* is one of the most abundant species in the oral cavity, in both diseased and healthy individuals [41–44]. It is implicated in various forms of periodontal disease including the mild reversible form of gingivitis and the advanced irreversible forms of periodontitis [42–46]. The prevalence of *F. nucleatum* increases with the severity of disease, progression of inflammation and pocket depth [42,45,46]. In this study, we observed that the reduction in this bacterial species after periodontal treatment showed a strong correlation with the reduction in VSCs, which is confirmed by other authors, who associate this bacterium with the production of hydrogen sulfide [47]. *Campylobacter showaei*, a bacterium historically linked to gingivitis and periodontitis (orange complex), has recently been associated with inflammatory

bowel disease and colorectal cancer [48]. This bacterial species correlated significantly with VSC reduction after treatment. Additionally, the microbiome composition of the tongue microbiome was reported to present *Aggregatibacter*, *Campylobacter*, *Capnocytophaga*, *Clostridiales*, *Leptotrichia*, *Parvimonas*, *Peptostreptococcus*, *Peptococcus*, *Prevotella*, *Selenomonas*, *Dialister*, *Tannerella*, and *Treponema* bacteria in the group of patients with IOH [49]. Capnocytophaga gingivalis (green complex) is a facultative anaerobic, capnophilic, fusiform, Gram-negative bacilli exhibiting gliding motility [50]. This species is saccharolytic and manifests an increased biomass and proteolytic potential when grown in elevated glucose conditions [51,52]. Low VSC levels present cellular toxicity potential in human cells. They contain thiols (-SH groups) that interact with other proteins and support the negative interaction of bacterial antigens and enzymes. The result of this effect is chronic inflammation, periodontal gingivitis, and periodontitis [17]. In human gingival fibroblasts, H_2S induces mitochondrial apoptosis [53] and is a known genotoxic agent, with impact on genomic instability and cumulative mutations [54]. In preclinical animal studies, hydrogen sulfide led to ultrastructural changes in epithelial cells and periodontal destruction [55]. Other consequences may result from high levels of H_2S such as activation of proliferation, migration, and invasion that may lead to carcinogenesis [56,57]. Several species have been strongly correlated with oral dysbiosis and oral carcinoma, such as *Capnocytophaga gingivalis*, *Fusobacterium sp*., among others, due to the fact that these bacteria may promote inflammation, cell proliferation and the production of some oncogenic substances, [58] and VSCs may be involved in this process.

Strengths and Limitations

Some limitations apply to this investigation, including the withdrawal of three patients who did not attend the reassessment visit 6 to 8 weeks after periodontal treatment. As mentioned, oral hygiene and feeding instructions were given before the VSC measurement. However, we cannot guarantee that participants strictly adhered to the given recommendations. We consider that this may be a limitation of the study, but that it seems to be common to most clinical studies involving the evaluation of halitosis. For practical and economical reasons, analysis of a pooled subgingival sample from the four

deepest periodontal lesions was used. However, as all participants had more than the four diseased sites from where samples were taken, information on the complete subgingival microbiome was not obtained. This limitation highlights the major dilemma of using local microbial sampling in clinical periodontology, namely that ideally single-site sampling analysis should be performed. However, this may not always be practically feasible.

5.5 Conclusions

Microbial diversity was high in the subgingival plaque on periodontitis and intraoral halitosis participants of the study. Furthermore, there were correlations between subgingival plaque composition and VSC quantification after periodontal treatment. The subgingival microbiome can offer important clues in the investigation of the pathogenesis and treatment of halitosis.

5.6 References

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6

General Discussion, Concluding remarks and Future Directions

6. General Discussion, Concluding Remarks and Future Directions

6.1. General Discussion

The aim of this thesis was to evaluate the impact of periodontal therapy on halitosis and quality of life, and to compare the initial subgingival microbial composition of periodontal pockets with that following treatment.

We started by overviewing the literature on halitosis etiology, diagnosis and multidisciplinary approach to treatment (**Publication I**). This first article allowed us to develop a clinical questionnaire for the diagnosis of intra-oral halitosis and to exclude all extra-oral causes of halitosis. Through this literature review, we have updated all available information on the prevalence, risk factors, etiology, classification, and treatment of halitosis.

This oral health problem is characterized by an unpleasant odor emanating from the mouth. This condition can have many causes and negatively affect social interactions, communication, and overall quality of life. It can also be an indicator of underlying non-oral and non-communicable diseases. Most halitosis cases are caused by poor oral hygiene, periodontal disease, and tongue coating, while the remaining cases are due to ear-nose-throat (10%) or gastrointestinal/endocrine disorders (5%). As such, diagnosis, treatment, and management of halitosis often requires a multidisciplinary approach. Although there are current methods for managing halitosis, there are still some inconsistencies that need to be addressed through robust clinical trials comparing standard and novel therapies.

Halitosis is caused by the complex interaction between bacterial biofilm and protein substrates [1]. The subgingival biofilm is mainly composed of anaerobic Gram-negative bacteria with proteolytic properties that can degrade substrates in the oral cavity, producing volatile sulphur compounds (VSCs) [2]. VSC levels have been shown to increase with the severity of periodontitis [3]. As periodontitis progresses, deeper periodontal pockets provide more space for bacterial colonization, resulting in increased VSC release [4]. The hypoxic environment of deep pockets, acidification, and the subsequent activation of amino acid decarboxylation also contribute to the production of VSCs [4]. In turn, VSCs alter the permeability of the oral mucosa, decrease the solubility of

collagen, and hinder the synthesis of proteins and collagen, leading to destruction of periodontal tissues [4-6].

On the other hand, the relationship between periodontal disease and halitosis is not yet fully understood. Epidemiological data suggest that periodontal disease may contribute to oral malodor, but not all patients with periodontal disease experience this condition, and some periodontally healthy patients may have oral malodor [7].

Therefore, to better understand the link between periodontal destruction and VSCs prodution, in our cross-sectional study in an Egas Moniz dental clinic population (**Publication II**), we examined the relationship between periodontal inflamed surface area (PISA) and periodontal epithelial surface area (PESA) with VSC measurements. We also evaluated the oral health-related quality of life and self-perception of halitosis in patients with periodontitis.

Our results confirmed that VSC levels may be associated with the amount of PESA in the posterior-lower region. To the best of our knowledge, this study may be the first to demonstrate such an association.

The importance of the posterior-lower region is novel and will be further investigated in future studies. Based on these results, it may be possible to develop a protocol for the treatment of periodontitis and halitosis. Starting periodontal treatment in the posterior-lower regions may be a useful way to address patient discomfort and increase patient motivation. Conventional periodontal therapy, such as scaling and root planing, reduces Gram-negative anaerobic bacteria and therefore lowers VSC concentrations in the oral cavity, resulting in a decrease in halitosis. Improvement in halitosis can be used to motivate patients to seek periodontal treatment [8-10].

As halitosis can affect quality of life, we also evaluated the quality of life in people with periodontitis in this observational study. The overall quality of life related to oral health and its domains did not show significant association to the halitosis status. Despite the observational nature of this study, one might argue whether this may be related to the self-perception of bad breath, as participants demonstrated a clear inability to accurately assess their own halitosis.

Oral health education was found to be neglected in the population studied (people with periodontitis from the EMDC) which explains the lack of self-

perception of bad breath, lack of knowledge about the need for treatment, and inadequate oral hygiene habits obtained, which are in line with national data [11]. This may help to clarify the long-term consequences for oral health, as patients without self-perception of the disease may only seek treatment when they are in an irreversible clinical state.

Considering that halitosis is a clinical manifestation of other diseases, either oral or extra-oral, we believe that it is important to take measures to raise awareness about the topic among the population, and health professionals should be more attentive to this problem to advise patients to seek evaluation and treatment if necessary.

Several studies have investigated self-perceived halitosis (SPH), mostly in dental students [12–16]. Inadequate oral hygiene, infrequent tooth brushing [17,18], and a lack of awareness about halitosis prevention [17] were found to be significant risk factors. Greater awareness and concern about oral malodor have been associated with better self-care practices [13,19]. Therefore, increased public education and awareness about oral malodor should be encouraged. Patients should be informed and educated about halitosis and future generations of dental professionals should be trained to deal with this issue effectively [20].

This study was based on a convenience sample from the Periodontology department of the EMDC, which limits the ability to generalize the results. The narrow inclusion and exclusion criteria and difficulties in measuring and diagnosing halitosis may also have introduced selection bias. Further studies in larger and more diverse populations are needed to validate these findings. In addition, future prospective randomized clinical trials are necessary to assess the biological and biochemical differences in periodontal and halitosis assessment before and after periodontal treatment and to evaluate its impact on oral health-related quality of life.

The "gold standard" for the assessment of halitosis is based on measuring VSC levels and supplemented with organoleptic examination [2]. However, on March 2020, the World Health Organization declared Coronavirus 2019 (COVID-19) a pandemic, one that has since spread across the globe. Since clinical studies were conducted during the Covid-19 pandemic, we had to stop using the organoleptic method for diagnosing halitosis due to the risk of transmission [21]. Despite this, our results have shown significance, even

though the relationship between periodontitis and halitosis is stronger in studies where organoleptic examination has been performed [2]. However, it is important to note the subjectivity of the organoleptic test [16,22]. To our knowledge, measuring VSC levels is an objective method [8] and therefore has a higher potential clinical applicability.

The present study has several advantages, including its design which differs from previous studies and uses the most recent definition of periodontitis [23] to ensure comparability with future studies. Additionally, the exclusion of extra-oral causes of halitosis is another strength. Furthermore, the assessment of three-dimensional periodontal measures (PISA and PESA) provides novel results that merit further research.

Finally, we aimed to explor the variation of the subgingival microbiome in relation to the quantification of intraoral volatile sulphur compounds (VSCs) among adults with periodontitis (**Publication III**), before and after treatment. Overall, a noticeable disruption of the subgingival microbiome was observed as a result of mechanical root planing. When these levels were compared with intra oral VSCs, our results showed strong correlations between two orange complex bacteria (*F. nucleatum* and *C. showae*) and one green complex bacterium (*C. gingivalis*) with VSCs after therapy. Conversely, one green complex bacterium (*C. sputigena*) was correlated with lower differences in VSCs from baseline to follow-up.

These correlations are important because they pinpoint four specific bacteria from the dental plaque of pathological periodontal pockets that are related to clinical levels of bad breath. These results could guide future studies on the mechanisms behind the production of VSCs by these bacteria. Additionally, new treatments could be developed to target these specific microorganisms and their VSC production, leading to innovative ways of addressing bad breath caused by periodontal disease.

The subgingival periodontal biofilm is mainly composed of Gram-negative anaerobic bacterial species that have a proteolytic nature [1]. These species are capable of breaking down sulphur-containing substances on various surfaces of the oral cavity, including periodontal pockets, leading to the release of VSCs.

There are multiple mechanisms that can explain the connection between bad breath and periodontal disease, which are typically based on the properties of the main microbially generated VSCs. Hydrogen sulfide and methyl mercaptan can facilitate the penetration of lipopolysaccharide into the gingival epithelium, causing inflammation [24]. The VSCs also enable bacterial invasion of connective tissue by their toxic effects on epithelial cells, while methyl mercaptan interferes with epithelial cell growth and proliferation [25]. This is exacerbated by decreased oxygen tension caused by an increase in periodontal pocket depth, which leads to a decrease in pH, necessary for the breakdown of amino acids that produce VSCs.

Fusobacterium nucleatum is an anaerobic bacterium oral commensal and periodontal pathogen, from orange complex that has been linked to a range of human health problems [26]. F. nucleatum is abundant in the oral cavity of both healthy and unhealthy individuals [27–30] and is involved in different types of periodontitis, including mild reversible form of gingivitis and severe, irreversible forms of periodontitis [28–32]. The presence of this bacteria tends to increase as the severity of the disease and inflammation grows, and as the depth of periodontal pockets increases [28,31,32].

In our study, we found that a decrease in this particular bacterial specie after periodontal treatment was strongly linked to a decrease in VSCs. This is supported by previous research that links this bacterium with the production of hydrogen sulfide [33].

We also observed that *Campylobacter showae*, a bacterium previously linked to gingivitis and periodontitis and now also associated with inflammatory bowel disease and colorectal cancer [34], was correlated with a reduction in VSCs after periodontal treatment. A review by Seerangaiyan et al. in 2017 also showed the presence of this bacterium, along with other species of the genus *Aggregatibacter*, *Capnocytophaga*, *Clostridiales*, *Leptotrichia*, *Parvimonas*, *Peptostreptococcus*, *Peptococcus*, *Prevotella*, *Selenomonas*, *Dialister*, *Tannerella*, and *Treponema*, in the tongue microbiome of patients with IOH [35]

Capnocytophaga gingivalis, a bacterium belonging to the green complex, is a facultative anaerobic, capnophilic, fusiform, Gram-negative bacilli exhibiting gliding motility [36]. These species are saccharolytic organisms, some of which

manifest an increased biomass and proteolytic potential when grown in elevated glucose conditions [37,38].

VSCs can be toxic for human cells even at low levels, due to their thiols (-SH groups) that react with proteins and support the negative effect of bacterial antigens and enzymes. This leads to chronic inflammation, gengivitis and periodontitis [39]. In human gingival fibroblasts, hydrogen sulfide (H_2S) activates the mitochondrial pathway of apoptosis [40]. H_2S is known to cause genetic damage and accumulate mutations [41]. Studies in rats have shown that H_2S causes structural changes in epithelial cells and periodontal destruction [42]. Increased amounts of H_2S can also drive cell growth, migration and invasion, leading to the development of cancer [43,44].

Several species of bacteria, including *Capnocytophaga gingivalis* and *Fusobacterium sp.*, have been strongly linked to oral health problems and oral cancer. These bacteria can cause inflammation, cell growth, and the production of carcinogenic substances [45]. The role of VSCs in this process is not yet fully understood.

There are some limitations to this research: the participants were instructed about oral hygiene and dietary recommendations before the VSC measurement, but compliance with these instructions could not be guaranteed. This may be a limitation of the study but is a common challenge in clinical studies involving evaluating oral malodor.

For practical and economic reasons, a pooled subgingival sample from the four deepest periodontal lesions was analyzed. However, since all participants had more than four diseased sites, information on the complete subgingival microbiome was not obtained. This limitation highlights the dilemma of using local microbial sampling in clinical periodontology, where ideally single-site sampling analysis should be performed but may not always be feasible.

In the conducted study, we evaluated the subgingival microbiome and its changes after periodontal treatment. It is known that the microbiota present on the tongue coating also influences intra-oral halitosis [46]. Despite strict instructions on tongue hygiene given to all participants, we cannot guarantee that the results of halitosis are solely due to the subgingival microbiome.

Despite some limitations of this study and the need for a larger sample size, this research allowed for the collection of a significant amount of dental data

from participants that reflects their lifestyle. It allows us to reflect on the potential of dentistry in analyzing records in the context of forensic medicine and the history of medicine. For this reason, we believe that a more detailed knowledge of dental records, both nationally and internationally, would be useful for characterizing the population and its oral health patterns [47,48].

6.2. Concluding Remarks

- **Publication I:** Halitosis is highly prevalent and of multifactorial origin, with a high social and self-esteem burden. This scientific endpoint summarized the available evidence on the etiology of halitosis, treatment approaches and emphasized the importance of a multidisciplinary approach. It allowed the implementation of a complete clinical questionnaire for the diagnosis of halitosis, increased the robustness of the eligibility criteria, which was later used in the following studies.
- **Publication II:** Through an observational study we showed that oral VSC counts may be associated with area of epithelial periodontal surface of the posterior-lower region. The existence of a hypothethical region linked to oral VSC counting may unveil a potential therapeutical area of intervention. Oral health-related quality of life and its domains was not affected by their halitosis status, evidencing signs of the lack of accuracy in evaluating their own bad breath. Therefore, the promotion of more public education and awareness about halitosis should be emphasized. Patients should be informed and educated about halitosis and future generations of dental professionals should be trained to deal effectively with this problem.
- **Publication III:** This interventional non-randomized trial reported a high subgingival microbial diversity in people with periodontitis and intra-oral halitosis. Specific subgingival bacteria correlated with VSC counts after periodontal treatment, and these species may offer important clues for future investigations into the pathogenesis and treatment of halitosis.

Overall, this thesis concludes that:

- The intra-oral and extra-oral causes of halitosis are well documented in the literature. The approach to halitosis is multidisciplinary, but regarding treatment, there are still some gaps in the literature, with a need for more randomized clinical trials comparing standard and innovative therapies.
- VSC counts may be associated with the amount of PESA in the posterior-lower region when other causes of extra-oral halitosis are excluded. Further intervention studies are needed to verify a possible causal link.
- The promotion of increased public education and awareness of halitosis should be a priority. Patients should be informed and educated about the condition, and future generations of dental professionals should be trained to effectively manage it.
- The subgingival microbiome may provide important clues to the pathogenesis and treatment of halitosis.

6.3. Future Directions

Concerning the correlation between periodontitis and halitosis, the evaluation of three-dimensional periodontal measures (PISA and PESA) provides new results that deserve to be further explored. Future studies should standardize terms and definitions in the diagnosis of halitosis and periodontitis and increase the number of participants. Future prospective randomized clinical trials are needed to assess the biological and biochemical differences in halitosis after periodontal treatment.

Assessment of the subgingival microbiome yielded important correlations because it pinpoints four specific plaque bacteria in pathological periodontal pockets that are related to clinical levels of bad breath. These results may guide future studies on the mechanisms behind the production of VSCs by these bacteria. On the other hand, new treatments can be developed to target these specific microorganisms and their VSC production, leading to innovative ways of dealing with bad breath caused by periodontal disease. It would be interesting to conduct randomized controlled studies that evaluate the subgingival and tongue coating microbiome before and after periodontal treatment, in a larger sample with longer follow-up periods.

There should be a focus on promoting greater public education and awareness about halitosis. Patients should be given information and education about the condition, and future generations of dental professionals should receive training to deal with it effectively.

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ANNEXES

Anexxe 1. Ethical approvment.

Comissão de Ética EGAS MONIZ

COOPERATIVA DE ENSINO SUPERIOR, CRL • EGAS MONIZ

Proc. Interno nº 781

Ex.ma Senhora
Catarina Pequito Pinto

Monte de Caparica, 26 de junho de 2019.

Ex.ma Senhora,

Em resposta ao Pedido de Parecer que submeteu à apreciação da Comissão de Ética da Egas Moniz, com o tema denominado **"Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal"**, foi aprovado por unanimidade.

Com os melhores cumprimentos,

A Presidente da Comissão de Ética da Egas Moniz

Profª. Doutora Maria Fernanda de Mesquita

EGAS MONIZ – COOPERATIVA DE ENSINO SUPERIOR, CRL
Campus Universitário – Quinta da Granja – Monte de Caparica
2829-511 Caparica

Annexe 2. Informed consent.

Monte de Caparica, ___(dia) de____________ (mês) de _____ (ano)

Exmo.(a)
Sr.(a),____

No âmbito do Projecto de Doutoramento intitulado " Impacto do tratamento periodontal" sob a responsabilidade da Mestre Catarina Pequito Izidoro de Sousa Pinto, e Orientação do Professor Doutor José João Mendes e Professor Doutor Ricardo Castro Alves, solicita-se a sua autorização para a participação no mesmo.
O objetivo do estudo é avaliar a qualidade de vida, halitose e microbioma subgengival de pacientes com periodontite antes e após tratamento periodontal
A participação neste estudo é voluntária. A sua não participação não lhe trará qualquer prejuízo.
Para além dos dados clínicos recolhidos habitualmente na consulta de periodontologia, será convidado a preencher um questionário sobre a qualidade de vida que terá uma duração apróximada de 10 minutos no inicío e no fim do tratamento. Será também convidado a realizar um teste para avaliação de halitose (mau hálito), e será realizada uma recolha de placa bacteriana subgengival de forma não invasiva, antes e após tratamento periodontal. Este estudo pode trazer benefícios na medida em que visa compreender qual o impacto da doença periodontal na qualidade de vida, analisar o grau de severidade da doença periodontal e a qualidade de vida e a forma como a sua qualidade de vida interfere com o estado de saúde periodontal.
Permitirá ainda avaliar as consequências da doença periodontal como a halitose, desconforto mastigatório e alterações estéticas antes e após tratamento periodontal e ainda avaliar a sua percepção sobre o sucesso do tratamento.
Todos estes pressupostos visam dar contributo para o progresso do conhecimento.
A informação recolhida destina-se unicamente a tratamento estatístico e/ou publicação e será tratada pelo(s) investigadores. A sua recolha é anónima e confidencial.
(Riscar o que não interessa)
ACEITO/NÃO ACEITO participar neste estudo, confirmando que fui esclarecido sobre as condições do mesmo e que não tenho dúvidas.
____

(Assinatura do participante)

Anexxe 3: Clinical questionnaire used in periodontal diagnosis, halitosis diagnosis and assessment of oral health-related quality of life**.**

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

PARTE I - HISTÓRIA MÉDICA:

Consentimento informado: Assinado? Sim ☐ Não ☐

Sexo: M__ F__ Data de Nascimento (DD/MM/AAAA): ___/___/_____

Antecedentes pessoais

Esteve internado no Hospital no último ano? Sim☐ Não☐

Está em Tratamento médico? Sim☐ Não☐ Qual a doença? ________________________

Tomou regularmente algum medicamento no último ano? Sim☐ Não☐

Se SIM, qual?____

- ☐ Anti-hipertensor
- ☐ Broncodilatador
- ☐ Anti-depressor
- ☐ Anti-ácidos
- ☐ Estatinas
- ☐ Anti-diabéticos
- ☐ Análgésicos
- ☐ Aspirina
- ☐ Esteróides

Tem alergia a algum medicamento? Sim ☐ Não☐ Se sim, qual?______________________

Toma a pílula? Sim☐ Não☐

Coloque uma cruz nas doenças que tem ou teve:

- ☐ Artrite
- ☐ Candidíase oral
- ☐ Doença cardíaca congénita
- ☐ Doença da tiróide
- ☐ Doença venérea
- ☐ Enfarte miocárdio
- ☐ Febre reumática
- ☐ Gânglios aumentados de volume
- ☐ Glaucoma
- ☐ Osteoporose
- ☐ Úlcera Gástrica/duodenal
- ☐ Leucemia
- ☐ Diabetes
- ☐ Endocardite infecciosa
- ☐ Infecção pelo vírus da Sida (HIV)
- ☐ Anemia
- ☐ Asma
- ☐ Cancro
- ☐ Hipertensão arterial
- ☐ Hepatite viral (tipo____)
- ☐ Doença válvula cardíaca
- ☐ Prótese válvula cardíaca
- ☐ Tratamento psiquiátrico
- ☐ Tuberculose
- ☐ Alergias
- ☐ Tosse persistente
- ☐ Portador de Pacemaker
- ☐ Epilepsia

1

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

Alguma vez efectuou tratamento com radiação (radioterapia)? ? Sim☐ Não☐

Alguma vez realizou tratamento com substâncias químicas (quimioterapia)? Sim☐ Não ☐

Tem alguma doença importante não mencionada em cima? Sim ☐ Não☐

Hábitos de Higiene oral:

- Quantas vezes escova os dentes por dia?Nunca

☐ 1 vez por mês ☐ 2-6 vezes por semana

☐ 2-3 vezes por mês ☐ 1 vez por dia

☐ 1 vez por semana ☐ 2 vezes por dia

- Usa algum destes utensílios para limpar os dentes?

☐ Escova de dentes manual ☐ Fio/fita dentário(a)

☐ Escova de dentes elétrica ☐ Raspador lingual ☐

☐ Escovilhão

- Costuma usar colutório? Sim ☐ Não ☐ Se sim, qual?___________________________

- É portador de prótese dentária? Sim ☐ Não☐

☐ Fixa sobre dentes ☐ Prótese removível esquelética

☐ Fixa sobre implantes ☐ Prótese removível acrílic

- Hábitos tabágicos: Sim☐ Não☐ Quantidade: ____cig/ dia____ Há quantos anos ____

- Hábitos alcoólicos: Sim ☐ Não ☐

	1 dose*	2 doses	3-4 doses	>5 doses
Uma vez por dia				
Uma vez por semana				
Duas ou mais vezes por semana				
Uma vez por mês				

*1 dose: 1copo de cerveja (330ml) ou 1 cálice vinho (100ml) ou 1 copo de bebida destilada (30ml).

<table>
<tr><td rowspan="7">Periodontite</td><td rowspan="2" colspan="2">Estádio</td><td>I</td><td>II</td><td>III</td><td>IV</td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="2" colspan="3">Grau</td><td>A</td><td>B</td><td>C</td></tr>
<tr><td></td><td></td><td></td></tr>
<tr><td colspan="3">Localizada</td><td colspan="3"></td></tr>
<tr><td colspan="3">Generalizada</td><td colspan="3"></td></tr>
<tr><td colspan="3">Padrão Molar/incisivos</td><td colspan="3"></td></tr>
</table>

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

PARTE II - DIAGNÓSTICO PERIODONTAL

IP% ____ IG%____

Nº de dentes perdidos por periodontite: __________

Quais os dentes perdidos?___________

Mobilidade																	
Margem gengival																	
Sondagem																	
Dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
Lesão de Furca																	
Identificar os Implantes com espiras a vermelho																	V P
Dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	
Sondagem																	
Margem gengival																	
Mobilidade.																	
Margem gengival																	
Sondagem																	
Dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	
Lesão de Furca																	
Identificar os Implantes com espiras a vermelho																	L V
Dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	
Sondagem																	
Margem gengival																	

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº ______

PARTE III - AVALIAÇÃO DA QUALIDADE DE VIDA (OHIP-14) - Consulta de Diagnóstico

	NÃO	QUASE NUNCA	OCASIONALMENTE	BASTANTE FREQUENTE	MUITO FREQUENTEMENTE
1. Já teve dificuldade em pronunciar palavras devido a problemas com os seus dentes, boca ou a sua prótese?					
2. Já sentiu que seu paladar piorou devido a problemas com os seus dentes, boca ou prótese dentária?					
3. Você já teve dor na boca?					
4. Já achou desconfortável comer algum alimento devido a problemas com os seus dentes, boca ou próteses?					
5.Sente-se consciente de seus dentes, boca ou prótese?					
6.Já se sentiu tenso devido a problemas com os seus dentes, boca ou próteses?					
7. Já sentiu que a sua alimentação foi insatisfatória devido a problemas com os seus dentes, boca ou prótese dentária?					
8. Já teve de interromper refeições devido a problemas com os seus dentes, boca ou próteses?					
9. Já teve dificuldade em relaxar devido a problemas com os seus dentes, boca ou prótese dentária?					
10. Já ficou um pouco envergonhado/ atrapalhado devido a problemas com os seus dentes, boca ou prótese?					
11. Já ficou um pouco irritado/a com outras pessoas devido a problemas com os seus dentes, boca ou prótese?					
12. Já sentiu dificuldade em realizar as suas tarefas diárias devido a problemas com os dentes, a boca ou prótese?					
13. Já sentiu que a vida no geral é menos boa devido a problemas com os seus dentes, boca ou prótese?					
14. Já se sentiu totalmente incapaz devido a problemas com dentes, boca ou prótese?					

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

PARTE IV - DIAGNÓSTICO HALITOSE

1. INGERIU ALGUM DESTES ALIMENTOS NAS ÚLTIMAS 48HORAS? - ALHO, CARIL, PIMENTA, CEBOLA, PRESUNTO, COMIDA PICANTE, BEBIDAS ALCOÓLICAS, AIPO, ESPECIARIAS

SIM ☐ NÃO ☐

2. FUMOU NAS ÚLTIMAS 4 HORAS? SIM ☐ NÃO ☐

3. ESCOVOU OS DENTES OU USOU ELIXIR NAS ÚLTIMAS 2 HORAS? SIM ☐ NÃO ☐

4. SOFRE DE ALGUMA DAS SEGUINTES PATOLOGIAS?

- ☐ Sinusite
- ☐ Bronquite
- ☐ Rinite
- ☐ Faringite (viral ou bacteriana)
- ☐ Laringite
- ☐ Abscessos retrofaríngeos
- ☐ Presença de criptas tonsilares profundas
- ☐ Presença de corpo estranho na cavidade nasal ou sinusal
- ☐ Noeplasia
- ☐ Diabetes
- ☐ Problemas renais
- ☐ Medicação com anti-depressivos

5. QUAL A SUA AUTO-PERCEPÇÃO DE MAU HÁLITO DE 0 A 10?

1____2 ____3____4____5____6____7____8____9____10____

6. JÁ FOI INFORMADO POR ALGUÉM QUE TINHA MÁU HÁLITO? SIM ☐ NÃO ☐

6.1 POR QUEM? ______

7. ACHA QUE PRECISA DE TRATAMENTO PARA O MAU HÁLITO? SIM ☐ NÃO ☐

8. OBSERVAÇÃO CLÍNICA:

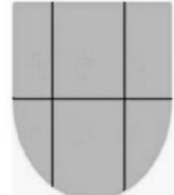
- ☐ Restaurações mal adaptadas
- ☐ Pontes/ Prótese fixa
- ☐ Prótese removível
- ☐ Aparelho ortodôntico
- ☐ Cáries
- ☐ Raízes retidas
- ☐ Pericoronarite
- ☐ Abcessos
- ☐ Extracções recentes (1 mês)
- ☐ Implantes
- ☐ Peri-implantite
- ☐ Presença de Cálculo supra-gengival
- ☐ Xerostomia
- ☐ Lesões orais

5

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

9. ÍNDICE DE WINKEL (atribuir pontuação nas 6 divisões da língua consoante a acumulação de placa bacteriana)



0 – Nada (sem biofilme)

1 - Biofilme leve

2 – Biofilme severo

Escala de avaliação organoléptica:

Grau 0 = sem odor

Grau 1 = odor quase impercetível

Grau 2 = odor ligeiro mas apreciável

Grau 3 = odor moderado

Grau 4 = odor forte

Grau 5 = odor muito desagradável

10. AVALIAÇÃO HALITOSE

		GRAU 0	GRAU 1	GRAU 2	GRAU 3	GRAU 4	GRAU 5
Diagnóstico	AVALIAÇÃO ORGANOLÉPTICA						
	HALIMETER®						

11. AVALIAÇÃO XEROSTOMIA

Para Cada questão indique com uma cruz a alternativa que melhor se ajusta á sua situação.

	Nunca	Ocasionalmente	Com Frequência	
1. Sinto a boca seca durante as refeições				
2. Sinto a boca seca				
3. Tenho dificuldade em comer alimentos secos				
4. Sinto dificuldade em engolir cerros alimentos				
5. Tenho os lábios secos				
	Nunca	Ocasionalmente	Com Frequência	Sempre
6. Com que frequência sente a boca seca?				

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

PARTE V - CONSULTA DE REAVALIAÇÃO

IP% ____ IG%____ Nº de dentes perdidos por periodontite: __________ Quais os dentes perdidos? ___________

Diagnóstico Periodontal: ____

Mobilidade																
Margem gengival																
Sondagem																
Dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Lesão de Furca																
Identificar os Implantes com espiras a vermelho																
Dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
Sondagem																
Margem gengival																

V

P

Mobilidade.																
Margem gengival																
Sondagem																
Dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Lesão de Furca																
Identificar os Implantes com espiras a vermelho																
Dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Sondagem																
Margem gengival																

L

V

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal

Questionário Nº _______

PARTE VI - AVALIAÇÃO DA QUALIDADE DE VIDA (OHIP-14) - Consulta de Reavaliação

	NÃO	QUASE NUNCA	OCASIONALMENTE	BASTANTE FREQUENTE	MUITO FREQUENTEMENTE
1. Já teve dificuldade em pronunciar palavras devido a problemas com os seus dentes, boca ou a sua prótese?					
2. Já sentiu que seu paladar piorou devido a problemas com os seus dentes, boca ou prótese dentária?					
3. Você já teve dor na boca?					
4. Já achou desconfortável comer algum alimento devido a problemas com os seus dentes, boca ou próteses?					
5.Sente-se consciente de seus dentes, boca ou prótese?					
6.Já se sentiu tenso devido a problemas com os seus dentes, boca ou próteses?					
7. Já sentiu que a sua alimentação foi insatisfatória devido a problemas com os seus dentes, boca ou prótese dentária?					
8. Já teve de interromper refeições devido a problemas com os seus dentes, boca ou próteses?					
9. Já teve dificuldade em relaxar devido a problemas com os seus dentes, boca ou prótese dentária?					
10. Já ficou um pouco envergonhado/ atrapalhado devido a problemas com os seus dentes, boca ou prótese?					
11. Já ficou um pouco irritado/a com outras pessoas devido a problemas com os seus dentes, boca ou prótese?					
12. Já sentiu dificuldade em realizar as suas tarefas diárias devido a problemas com os dentes, a boca ou prótese?					
13. Já sentiu que a vida no geral é menos boa devido a problemas com os seus dentes, boca ou prótese?					
14. Já se sentiu totalmente incapaz devido a problemas com dentes, boca ou prótese?					

Questionário: Avaliação da qualidade de vida de pacientes com periodontite antes e após tratamento periodontal | Questionário Nº _______

PARTE VII - DIAGNÓSTICO HALITOSE - CONSULTA DE REAVALIAÇÃO

1. INGERIU ALGUM DESTES ALIMENTOS NAS ÚLTIMAS 48HORAS? - ALHO, CARIL, PIMENTA, CEBOLA, PRESUNTO, COMIDA PICANTE, BEBIDAS ALCOÓLICAS, AIPO, ESPECIARIAS

SIM ☐ NÃO ☐

2. FUMOU NAS ÚLTIMAS 4 HORAS? SIM ☐ NÃO ☐

3. ESCOVOU OS DENTES OU USOU ELIXIR NAS ÚLTIMAS 2 HORAS? SIM ☐ NÃO ☐

4. SOFRE DE ALGUMA DAS SEGUINTES PATOLOGIAS?

- ☐ Sinusite
- ☐ Bronquite
- ☐ Rinite
- ☐ Faringite (viral ou bacteriana)
- ☐ Laringite
- ☐ Abscessos retrofaríngeos
- ☐ Presença de criptas tonsilares profundas
- ☐ Presença de corpo estranho na cavidade nasal ou sinusal
- ☐ Neoplasia
- ☐ Diabetes
- ☐ Problemas renais
- ☐ Medicação com anti-depressivos

5. QUAL A SUA AUTO-PERCEPÇÃO DE MAU HÁLITO DE 0 A 10?

1____2 ____3____4____5____6____7____8____9____10____

6. JÁ FOI INFORMADO POR ALGUÉM QUE TINHA MÁU HÁLITO? SIM ☐ NÃO ☐

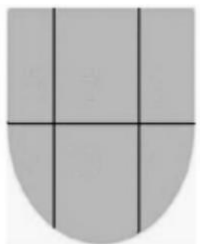
6.1 POR QUEM? ______

7. ACHA QUE PRECISA DE TRATAMENTO PARA O MAU HÁLITO? SIM ☐ NÃO ☐

8. OBSERVAÇÃO CLÍNICA:

- ☐ Restaurações mal adaptadas
- ☐ Pontes/ Prótese fixa
- ☐ Prótese removível
- ☐ Aparelho ortodôntico
- ☐ Cáries
- ☐ Raízes retidas
- ☐ Pericoronarite
- ☐ Abcessos
- ☐ Extracções recentes (1 mês)
- ☐ Implantes
- ☐ Peri-implantite
- ☐ Presença de Cálculo supra-gengival
- ☐ Xerostomia
- ☐ Lesões orais

9. ÍNDICE DE WINKEL (atribuir pontuação nas 6 divisões da língua consoante a acumulação de placa bacteriana)



0 – Nada (sem biofilme)

1 - Biofilme leve

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Escala de avaliação organoléptica:

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Grau 3 = odor moderado

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Grau 5 = odor muito desagradável

10. AVALIAÇÃO HALITOSE

		GRAU 0	GRAU 1	GRAU 2	GRAU 3	GRAU 4	GRAU 5
Reavaliação	AVALIAÇÃO ORGANOLÉPTICA						
	HALIMETER®						

11. AVALIAÇÃO XEROSTOMIA

Para Cada questão indique com uma cruz a alternativa que melhor se ajusta á sua situação.

	Nunca	Ocasionalmente	Com Frequência	
1. Sinto a boca seca durante as refeições				
2. Sinto a boca seca				
3. Tenho dificuldade em comer alimentos secos				
4. Sinto dificuldade em engolir cerros alimentos				
5. Tenho os lábios secos				
	Nunca	Ocasionalmente	Com Frequência	Sempre
6. Com que frequência sente a boca seca?				

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