

INFLUENCE OF OBESITY ON SUBGINGIVAL MICROBIOTA COMPOSITION IN SUBJECTS WITH DIFFERENT PERIODONTAL STATUS: A SYSTEMATIC REVIEW

INFLUENCIA DE LA OBESIDAD SOBRE LA COMPOSICIÓN DE LA MICROBIOTA SUBGINGIVAL EN SUJETOS CON DIFERENTE CONDICIÓN PERIODONTAL: UNA REVISIÓN SISTEMÁTICA

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ABSTRACT

Objective: This systematic review aimed to investigate the changes in the composition of the subgingival microbiota among subjects with normo-weight, overweight and obesity, in conditions of periodontal health and disease. **Materials and Methods:** The protocol for this study was designed following PRISMA guidelines. Records were identified using different search engines (PubMed/MedLine, Scopus and Web of Science). Observational studies, in human subjects diagnosed with obesity (BMI >30kg/m²) and periodontal disease (gingivitis and periodontitis), on the analysis of subgingival microbiota were selected. Eight articles were included. **Results:** The subgingival microbiota of 1,229 subjects (n=894 exposure group and n=335 control group) was analyzed. Periodontal pathogens were the most common bacteria detected in subjects with obesity and periodontitis (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Campylobacter gracilis*, *Eubacterium nodatum*, *Fusobacterium nucleatum* spp. *vincentii*, *Parvimonas micra*, *Prevotella intermedia*, *Campylobacter rectus*, and *Aggregatibacter actinomycetemcomitans*), as along with some accessory pathogens such as: *Streptococcus gordonii*, and *Veillonella parvula* that favor the virulence of late colonizers. **Conclusions:** Although there are evident alterations in the composition of the subgingival microbiota in subjects with obesity and periodontitis, it is still a challenge to identify a specific pattern of microbiota in these subjects. If associations between subgingival plaque microorganisms and obesity are confirmed, microbiome analysis could be a useful tool to improve preventive measures and the management of people with obesity.

Keywords: subgingival microbiota, obesity, periodontitis, checkerboard for DNA-DNA hybridization, next generation sequencing, systematic review

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RESUMEN

Objetivo: Esta revisión sistemática tuvo como objetivo investigar los cambios en la composición de la microbiota subgingival entre sujetos con normopeso, sobrepeso y obesidad, en condiciones de salud y enfermedad periodontal. **Materiales y métodos:** El protocolo de este estudio se diseñó siguiendo las directrices PRISMA. Los registros se identificaron utilizando diferentes motores de búsqueda (PubMed/MedLine, Scopus y Web of Science). Se seleccionaron estudios observacionales en sujetos humanos diagnosticados con obesidad (IMC >30kg/m²) y enfermedad periodontal (gingivitis y periodontitis), sobre el análisis de la microbiota subgingival. Se incluyeron ocho artículos. **Resultados:** Se analizó la microbiota subgingival de 1229 sujetos (n = 894 grupo de exposición y n = 335 grupo de control). Los patógenos periodontales fueron las bacterias más comunes detectadas en los sujetos con obesidad y periodontitis (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Campylobacter gracilis*, *Eubacterium nodatum*, *Fusobacterium nucleatum* spp. *vincentii*, *Parvimonas micra*, *Prevotella intermedia*, *Campylobacter rectus* y *Aggregatibacter actinomycetemcomitans*), junto con algunos patógenos accesorios, como *Streptococcus gordonii* y *Veillonella parvula*, que favorecen la virulencia de los colonizadores tardíos. **Conclusiones:** Aunque

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existen alteraciones evidentes en la composición de la microbiota subgingival en sujetos con obesidad y periodontitis, sigue siendo un reto identificar un patrón específico de microbiota en ellos. Si se confirman las asociaciones entre los microorganismos de la placa subgingival y la obesidad, el análisis del microbioma podría ser una herramienta útil para mejorar las medidas preventivas y el manejo de las personas con obesidad.

Palabras clave: microbiota subgingival, obesidad, periodontitis, *checkerboard* para hibridación ADN-ADN, secuenciación de nueva generación, revisión sistemática

INTRODUCTION

Obesity is a clinical condition defined as an excessive accumulation of adipose tissue, with a body mass index $>30\text{kg}/\text{m}^2$ that compromises the health of the individual [1]. Currently, 39% of the world's population is overweight or obese, if this trend continues, it is expected that by 2025, the prevalence of obesity worldwide will be 18% in men and 21% in women, becoming the epidemic of the 21st century [2] with exaggerated health risks and serious systemic complications such as the occurrence of type 2 diabetes mellitus, cardiovascular disease, cancer, coronavirus disease and periodontal disease [3]. A recent meta-analysis found a positive association between obesity and periodontitis regardless of country or age; however, evidence is still lacking to determine the cause-effect relationship [4].

Periodontal disease (PD) comprehends all diseases and conditions of the supporting tissues of the teeth. The two forms mainly induced by the constant challenge of a polymicrobial dysbiotic film with predominance of Gram-negative anaerobes are gingivitis and periodontitis. Gingivitis corresponds to an inflammatory process confined to the gingiva, which in susceptible individuals, can progress to a more severe and destructive form, periodontitis [5]. PD has a prevalence of 62% globally, is the sixth most common osteolytic disease affecting humans and in its most severe form can affect up to 23.6% of the world's population [6]. PD is multifactorial and is influenced by modifiable risk factors such as smoking, diet, use of multiple prosthetic restorations with poor marginal fit [7], low levels of physical activity, low vitamin D status, type 2 diabetes mellitus, and obesity [8]. Undoubtedly, this disease can impair the quality of life of individuals, especially in very advanced stages where tooth loss is imminent, resulting in a strong impact on the patient's oral health and economy [9].

The management of subjects with obesity and periodontitis requires a thorough knowledge of multiple disciplines (microbiology, pharmacology, immunology and nutrition) on the part of dentists. Therefore, dentists' awareness of the risks associated with the treatment of these subjects and the importance of personalized approaches should be increased. In addition, the improvement and/or refinement of routine periodontal therapy for the successful management of subjects affected with this condition has not yet been fully elucidated [10].

The onset and progression of PD is due to the dental plaque biofilm, which corresponds to a well-organized aggregation of microorganisms (including bacteria, viruses, archaea, fungi, and protozoa) residing within a polymeric extracellular matrix [11]. The dominant phyla in the subgingival region are *Obsidian pool*, *TM7*, *Deferribacteres*, *Spirochaeta*, *Fusobacteria*, *Actinobacteria*, *Firmicutes*, *Clostridia*, *Proteobacteria*, and *Bacteroidetes* [12]. Although there are more than 500 bacterial species colonizing the gingival sulcus, in reality only a small portion of these microorganisms can trigger the destruction of periodontal tissues by activating an aberrant immune response in a genetically susceptible host [13].

A large number of hypotheses have been proposed over time on the relationship between biofilm and its role in PD pathogenesis [14-16]. In fact, Socransky and Haffe [12], provided a fairly broad understanding of the bacterial communities in health and disease conditions associated with the biofilm of the subgingival microbiota using the Checkerboard technique for DNA-DNA hybridizations. The bacterial species were divided into several complexes that received a unique color code according to the damage produced. Thus, the complexes; yellow (composed of *Streptococcus*

species), green (composed of *Capnocytophaga* species), blue (composed of *Actinomyces* species) and purple (composed of *Veillonella* species) are primary colonizers and are associated with periodontal health, while the orange complex (composed of *Fusobacterium*, *Prevotella* and *Campylobacter* species) are bridging colonizers and the red complex (composed of *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) are late colonizers and are found in subjects with periodontitis [17,18]. The composition and diversity of the subgingival microbiota may be altered by obesity, in fact, several studies have reported a high prevalence of orange and red complex bacteria, producing increased damage to the periodontium [19-25]. However, to date there are very few studies characterizing the composition of the subgingival microbiota in overweight and obese subjects with periodontal health and disease. Therefore, this paper would be the first systematic review that seeks to determine a specific phenotype. This study investigated the changes in the composition of the subgingival microbiota among subjects with normo-weight, overweight and obesity, in conditions of periodontal health and disease.

MATERIALS AND METHODS

Protocol and registration

The present study followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) [26]. The protocol was recorded with the OSF enrollment (Registration DOI: 10.17605/OSF.IO/JXWF4).

Pico question

The research question of this systematic review was designed based on PICO items: Are there specific differences in the composition of the subgingival microbiota among subjects with normo-weight, overweight and obesity, in conditions of periodontal health and disease? P (Population): Periodontally healthy subjects with normo-weight, overweight and obesity. I (Intervention): Alterations in the composition of the

subgingival microbiota. C (Comparison): Subjects with periodontal disease (gingivitis or periodontitis) with normo-weight, overweight and obesity. O (Outcome): There is a difference in bacterial counts/proportions/abundance/prevalence between (1) NWP versus NW, (2) OWP versus OW and (3) OP versus O.

Eligibility criteria

The inclusion criteria were as follows: Case-control and cross-sectional studies, articles published in English, adult patients with at least 10 teeth in the mouth, obese, overweight and periodontally healthy non-obese patients, obese, overweight and non-obese patients with gingivitis and/or periodontitis. Exclusion criteria were as follows: Case reports, cohort studies, review articles and animal studies were excluded, edentulous patients, patients under antibiotic treatment, patients who had received periodontal treatment during the study or in the last three months, pregnant and lactating women, current smokers, patients with any systemic disease other than obesity and patients with orthodontic appliances.

Literature search and study selection

A search strategy was used to identify articles on the composition of the subgingival microbiota among subjects with normo-weight, overweight and obesity, in conditions of periodontal health and disease. Four databases were selected for the search of relevant articles: PubMed/MedLine, Google Scholar, Scopus and Web of Science. The digital search was performed from December 15th, 2007, to October 15th 2023. Medical subject headings (MeSH) and terms related to obesity and periodontitis: "Obesity, Overweight, Normal weight, Subjects" [Mesh] AND "Periodontitis, Patients" [Mesh] "Microbiome", [Mesh], "Subgingival Microbiota" [Mesh] AND "gingival sulcus" [Supplementary Concept], "Patients with Obesity and Periodontitis" [Mesh] "and/or Periodontally Healthy Patients" [Mesh], "Patients without Obesity and Periodontitis" [Mesh] "and/or Periodontally healthy patients" [Mesh], "Bacterial Complex" [Mesh] "Socransky Complex" [Mesh] "Non-grouped bacteria" [Mesh] AND "New

Bacterial Species” [Mesh]. Initially, articles related to the composition of the subgingival microbiota in periodontally healthy normo-healthy, overweight and obese patients with gingivitis and/or periodontitis were identified, eliminating duplicates. In the filtering phase, articles were excluded by title and abstract, in the eligibility phase, the full text was reviewed and articles that did not correspond to the scope of this review were excluded, and in the inclusion phase, articles were selected for the final analysis. The main researcher was in charge of searching and screening the articles. After eliminating duplicates, the articles went through three stages; reading the title, reading the abstract, and reading the full text.

Data extraction

Microsoft Excel was used for data extraction. The variables included and analyzed were the following:

- Characteristics of the studies: first author's last name, name of the country where the study was performed, study title, study design, journal of publication.
- Sociodemographic characteristics of the study groups: gender, mean age, case population and control population.
- Methodology: Periodontal clinical parameters such as plaque index (PI), bleeding on probing (BP), probing depth (PD) and clinical attachment level (CAL), periodontal status (gingivitis and/or periodontitis), body mass index (BMI), sample collection methods, identification methods and bacterial species found.
- Results: Bacterial counts/proportions/abundance/prevalence.

Summary of measurements.

The measures included were the mean \pm standard deviation of each continuous quantitative variable, while the qualitative variables were represented by absolute and relative frequency, considering a value of $p < 0.05$ as statistically significant.

Synthesis of the results

A database of the selected articles was created using Microsoft Excel software and the variables of interest were organized in order to simplify the interpretation and comparison of the results.

Assessment of individual risk of bias

The methodological quality and risk of bias of the studies selected by the NIH for observational cohort and cross-sectional studies were evaluated.

RESULTS

Selection of studies

The initial search strategy identified 42 articles, of which 14 studies were excluded because they were duplicate records. The screening process of 28 articles was performed on the basis of the title and abstract and 18 articles were excluded. Subsequently, a full-text evaluation of 10 studies was performed, and 3 articles were excluded because of the clarity of the results and the characteristics of the study population. Finally, all 7 remaining articles were included in the current review. Figure 1 provides the detailed flow chart of the article selection process.

Characteristics of the selected studies

Seven articles were included for this systematic review. The main characteristics of the studies evaluated are described in Table 1. In detail, Table 2 describes the clinical parameters of the normo-weight and obese subjects with periodontal disease, Table 3 describes the methodology used for the analysis of the composition of the subgingival microbiota, as well as the statistically significant abundance of the bacterial species found. Table 4 corresponds to the assessment of the risk of bias of the studies selected in this systematic review.

Demographic characteristics

All the included articles were observational studies published between 2006 and 2023. Five were cross-sectional studies and two were case-control studies. In total, 43% of the studies were conducted in Brazil and

14.3% were conducted in the United Arab Emirates, France, Sweden and the United States. The journals of publication are also shown.

According to the available data a total of 1,229 subjects participated in the seven included articles, of which 894 were patients affected with obesity (exposure group) and 335 were patients with normo-weight (control group). There were 477 (39%) females and 752 (61%) males, with an age range from 14 to 58 years (Table 1).

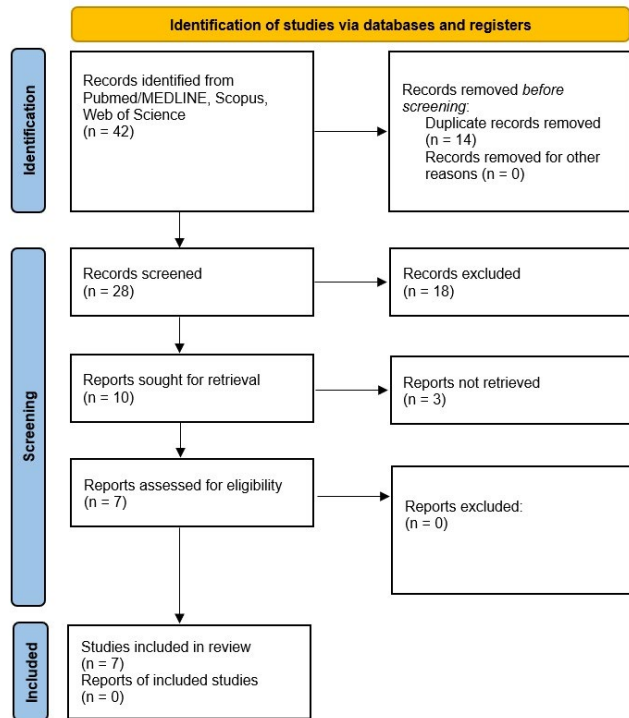


Figure 1. PRISMA flow diagram. PRISMA: Preferred Reporting Items for Systematic and Meta-Analyses.

Clinical characteristics

In relation to clinical parameters, only two articles evaluated the % PI, being higher in subjects with normal weight and periodontitis (68.55 ± 27.5) compared to subjects with obesity and periodontitis (64.91 ± 25.1) [23], however a more recent study reported inverse values, being higher in subjects with obesity and periodontitis (16.80 ± 14.41) compared to the control group (14.67 ± 14.56) [20]. The % BP was reported in five of the seven studies, being higher in subjects with obesity and periodontitis compared to the control groups [20-24]. Only one study reported higher % BP in normo-weight subjects with periodontitis (61.2 ± 13.3) compared to the exposure group (59.2 ± 22.3) [23]. The mean PD and CAL was higher in subjects with obesity and periodontitis compared to the control groups (Table 2) [19,23-25] According to periodontal condition, only

Table 1: Main characteristics of the selected articles

Author/Year	Country	Type of Study	Journal of Publication	Gender F*/M**	Average Age	Cases	Controls	Total Population
Rahman et al., 2023	United Arab Emirates	Cross-Sectional	Nutrients-MDPI	16(21.3)*/ 59(78.7)**	31±10.4	75	0	75
Thomas et al., 2021	France	Cross-Sectional	Diagnostics-MDPI	19(100)*	58.25±11.07	10	9	19
Rodrigues de Andrade et al., 2021	Brazil	Cross-Sectional	Journal of Periodontology	77(100)**	23.16±3.9	48	29	77
Silva-Boghossian et al., 2017	Brazil	Cross-Sectional	Journal of Periodontology	110(100)*	42.6±11.2	76	34	110
Da Silva Maciel et al., 2016	Brazil	Cases and Controls	Journal of Clinical Periodontology	166(100)*	46.90±7.65	82	84	166
Zeigler et al., 2012	Sweden	Cases and Controls	Pediatric Obesity	33(38)*/ 54(62)**	14.55±1.6	29	58	87
Haffe and Socransky, 2006	USA	Cross-Sectional	Journal of Clinical Periodontology	133(19)*/ 562(81)**	41.64±11	574	121	695

Abbreviations: F= Female, M= Male

29 patients (2%) were obese without periodontitis and 58 patients (5%) were normo-weight and periodontally healthy [24], the rest (93%) had periodontal disease (periodontitis and/or gingivitis) [19,23-25]. The BMI for the exposure group was $\geq 30\text{kg/m}^2$, whereas, for the control group it was $\leq 25\text{kg/m}^2$ respectively (Table 3).

Collection methods, techniques and microbial abundance

Subgingival plaque samples were collected and for their analysis, two studies [19,20] used the 16S rRNA sequencing technique, while five studies [21-25] used DNA-DNA hybridization technique. All microorganisms found in the form of counts/proportions/abundance/prevalence for the comparison of the exposure group versus the control group were statistically significant.

From the previously evaluated studies, we found that, in normo-weight subjects with periodontitis, an increase of proportions bacterial of two species of the yellow complex was found: *Streptococcus oralis* and *Streptococcus*

sanguinis. While, in periodontally healthy patients an increase of count bacterial of two species of the blue complex: *Actinomyces naeslundii* and *Actinomyces oris* [23].

In overweight subjects with gingivitis, an increase of count bacterial of two species of the red complex was found: *Porphyromonas gingivalis* and *Treponema denticola*, as well as *Lactobacillus* [21].

In subjects with obesity and periodontitis an increase of count bacterial the following bacterial species was found: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Campylobacter gracilis*, *Eubacterium nodatum*, *Fusobacterium nucleatum spp. vincentii*, *Parvimonas micra*, *Prevotella intermedia*, *Campylobacter rectus*, *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, *Capnocytophaga ochracea*, *Streptococcus gordonii*, *Streptococcus intermedius*, *Streptococcus mitis*, *Veillonella parvula*, *Actinomyces gerencseriae*, *Actinomyces israelii*, *Prevotella melaninogenica*, *Treponema socransky* and *Leptotrichia buccalis*. Only two studies [19,20,22,23,25,26] did not evaluate the composition of the subgingival microbiota in patients with obesity and periodontitis [21,24] (Table 3).

Table 2: Clinical parameters of study subjects

Author/Year	Plaque Index (%)	Bleeding on probing (%)	Mean PD (mm)	Mean CAL (mm)
Rahman et al., 2023	N/E	N/E	O+P/Stage I= 2.29 ± 0.57 O+P/Stage II,III,IV= 2.96 ± 0.64	O+P/Stage I= 0.7 ± 0.42 O+P/Stage II,III,IV= 1.76 ± 1.40
Thomas et al., 2021	O+P= 16.80 ± 14.41 NW+P= 14.67 ± 14.56	O+P= 34.20 ± 22.16 NW+P= 23.00 ± 17.73	O+P= 3.75 ± 1.25 NW+P= 3.72 ± 1.02	O+P= 5.39 ± 2.05 NW+P= 4.68 ± 1.29
Rodrigues de Andrade et al., 2021	N/E	O+G= 26.1 ± 5.1 NW+G= 13.3 ± 2.7	O+G= 2.2 ± 0.08 NW+G= 2.1 ± 0.1	O+G= 2.1 ± 0.12 NW+G= 1.98 ± 0.1
Silva-Boghossian et al., 2017	N/E	O+P= 36.5 ± 2.3 NW+P= 27.1 ± 3.4	O+P= 2.6 ± 0.1 NW+P= 2.3 ± 0.1	O+P= 2.9 ± 0.1 NW+P= 2.4 ± 0.1
Da Silva Maciel et al., 2016	O+P= 64.91 ± 25.1 NW+P= 68.55 ± 27.5	O+P= 59.2 ± 22.3 NW+P= 61.2 ± 13.3	O+P= 3.5 ± 0.5 NW+P= 3.4 ± 0.7	O+P= 4.3 ± 1.0 NW+P= 4.2 ± 1.0
Zeigler et al., 2012	N/E	0-25%= O: 20 0.25%= NW: 54 >25%= O: 6 >25% NW: 4	N/E	N/E
Haffe and Socransky, 2006	N/E	N/E	O+P= 3.13 ± 0.64 NW+P= 1.97 ± 0.29	O+P= 3.36 ± 1.02 NW+P= 1.35 ± 0.42

Abbreviations: O+P= Obesity + Periodontitis; NW+P= Normal Weight + Periodontitis; O+G= Obesity + Gingivitis; NW+G= Normal Weight + Gingivitis; PD= Probing Depth; CAL= Clinical Attachment Loss; N/E= Not Evaluated

Table 3: Microorganisms found in statistically significant higher counts/proportions/abundance/prevalence in obese and periodontally healthy subjects and in obese and periodontitis subjects

Author/Year	Periodontal Status	BMI(kg/m ²)	Type of Sample	Bacterial Identification Method	Bacterial Species Detected	
					Periodontal health*/gingivitis** and Obesity	Periodontitis and Obesity
Rahman et al., 2023	Moderate-Severe Periodontitis (MS): Stages II to III/IV	EG: 30.08±5.95	Subgingival plaque	16S rRNA sequencing	N/E	Patients with NM: <i>F. nucleatum</i> and <i>T. forsythia</i>
	No-Mild Periodontitis (NM): Stage I	CG: 26.97±7.05				Patients with MS: <i>A. actinomycetemcomitans</i>
Thomas et al., 2021	Periodontitis	EG: 30.02±1.48 CG: 23.11±1.29	Subgingival plaque	16S rRNA sequencing	N/E	A higher abundance of the genus <i>Capnocytophaga</i> and <i>Streptococcus</i>
Rodrigues de Andrade et al., 2021	Gingivitis	EG: 35.1±4.3 CG: 21.9±1.7	Subgingival plaque	DNA-DNA hybridization technique	<i>P. gingivalis</i> **	N/E
Silva-Boghossian et al., 2017	Periodontitis	N/E	Subgingival plaque	DNA-DNA hybridization technique	<i>P. gingivalis</i> , <i>L. buccalis</i> and <i>S. oralis</i> *	<i>C. ochracea</i> , <i>P. gingivalis</i> , <i>T. forsythia</i> , <i>F. nucleatum</i> spp. <i>vincentii</i> , and <i>E. corrodens</i>
Da Silva Maciel et al., 2016	Chronic Periodontitis	EG: 32.9±3.6	Subgingival plaque	DNA-DNA hybridization technique	<i>A. naeslundii</i> , <i>A. oris</i> *	<i>A. israelii</i> , <i>V. parvula</i> , <i>S. gordonii</i> , <i>S. mitis</i> , <i>A. actinomycetemcomitans</i> , <i>E. corrodens</i> , <i>C. rectus</i> , <i>E. nodatum</i> , <i>F. nucleatum</i> spp. <i>vincentii</i> , <i>T. forsythia</i> , <i>L. buccalis</i> , <i>P. micra</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>S. anginosus</i> , <i>T. socranskii</i>
		CG: 23.2±1.9				<i>A. israelii</i> , <i>V. parvula</i> , <i>S. gordonii</i> , <i>S. mitis</i> , <i>A. actinomycetemcomitans</i> , <i>E. corrodens</i> , <i>C. rectus</i> , <i>E. nodatum</i> , <i>F. nucleatum</i> spp. <i>vincentii</i> , <i>T. forsythia</i> , <i>L. buccalis</i> , <i>P. micra</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>S. anginosus</i> , <i>T. socranskii</i>
Zeigler et al., 2012	Without Periodontitis	EG: 37.4±6.8	Subgingival plaque	DNA-DNA hybridization technique	<i>A. israelii</i> , <i>A. naeslundii</i> , <i>A. odontolyticus</i> , <i>S. aureus</i> spp. <i>anaerobius</i> , <i>S. haemolyticus</i> , <i>S. anginosus</i> , <i>S. constellatus</i> , <i>S. gordonii</i> , <i>S. mitis</i> , <i>S. mutans</i> , <i>S. oralis</i> , <i>S. pneumoniae</i> , <i>S. sanguinis</i> , <i>P. micra</i> , <i>L. acidophilus</i> ,	N/E
		CG: 19.8±2.3				<i>V. parvula</i> , <i>C. gingivalis</i> , <i>C. ochracea</i> , <i>C. sputigena</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>F. nucleatum</i> spp. <i>nucleatum</i> , <i>vincentii</i> , <i>polymorphum</i> , <i>C. gracilis</i> , <i>C. rectus</i> , <i>C. showae</i> , <i>N. mucosa</i> , <i>E. corrodens</i> , <i>P. gingivalis</i> , <i>T. forsythia</i> *
Zeigler et al., 2012	Without Periodontitis	EG: 37.4±6.8	Subgingival plaque	DNA-DNA hybridization technique	<i>V. parvula</i> , <i>C. gingivalis</i> , <i>C. ochracea</i> , <i>C. sputigena</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>F. nucleatum</i> spp. <i>nucleatum</i> , <i>vincentii</i> , <i>polymorphum</i> , <i>C. gracilis</i> , <i>C. rectus</i> , <i>C. showae</i> , <i>N. mucosa</i> , <i>E. corrodens</i> , <i>P. gingivalis</i> , <i>T. forsythia</i> *	N/E
		CG: 19.8±2.3				<i>V. parvula</i> , <i>C. gingivalis</i> , <i>C. ochracea</i> , <i>C. sputigena</i> , <i>P. intermedia</i> , <i>P. melaninogenica</i> , <i>F. nucleatum</i> spp. <i>nucleatum</i> , <i>vincentii</i> , <i>polymorphum</i> , <i>C. gracilis</i> , <i>C. rectus</i> , <i>C. showae</i> , <i>N. mucosa</i> , <i>E. corrodens</i> , <i>P. gingivalis</i> , <i>T. forsythia</i> *
Haffe and Socransky, 2006	Periodontitis	EG: 27.9±5.74 CG: 24.6±5.63	Subgingival plaque	DNA-DNA hybridization technique	↑ <i>T. forsythia</i> *	↓ <i>T. forsythia</i>

EG, Experimental Group; CG, Control Group; N/E, Not Evaluated; ↑, Increase; ↓, Decrease

Whereas, in patients with obesity and periodontally healthy, an increase of count bacterial the following bacterial species was found: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum spp. polymorphum*, *nucleatum* and *vincentii*, *Prevotella intermedia*, *Parvimonas micra*, *Capnocytophaga gingivalis*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Campylobacter rectus*, *Campylobacter showae*, *Eikenella corrodens*, *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Streptococcus sanguinis*, *Streptococcus gordonii*, *Streptococcus oralis*, *Veillonella parvula*, *Actinomyces odontolyticus*, *Actinomyces naeslundii*, *Actinomyces oris*, *Actinomyces israelii*, *Prevotella melaninogenica*, *Leptotrichia buccalis*, *Staphylococcus aureus ss anaerobius*, *Staphylococcus haemolyticus*, *Neisseria mucosa* and *Lactobacillus acidophilus* [22-24].

Risk of bias

Table 4 shows the risk of bias analysis of the studies included in this systematic review according to NIH for observational cohort and cross-sectional studies. No article complied with items 8, 10 and 11. Thus, all the articles included complied with 90% of the items evaluated.

DISCUSSION

The present systematic review analyzed only human studies, particularly observational cross-sectional and case-control studies. This review sought to analyze the current knowledge regarding changes in the composition of the subgingival microbiota among normal-weight, overweight, and obese subjects with healthy periodontium and/or periodontal disease.

Scientific evidence has shown that overweight and obese individuals with a high BMI ($\geq 25\text{kg/m}^2$) have worse periodontal health parameters and poor oral hygiene status, which favors an increased risk of developing dental caries, periodontitis and tooth loss, however, the underlying mechanism remains unclear. It has been suggested that insulin resistance as a consequence of a chronic inflammatory state and oxidative stress could be involved in this association [27]. In the present review, it was found that, in general, all periodontal clinical parameters: IP, BP, PD and CAL were increased in subjects affected with obesity and periodontitis compared to subjects with normal weight and the same periodontal condition. However, it would be interesting to plan a study where overweight patients are also included and the clinical parameters are analyzed to know if this metabolic condition also greatly modifies these

Table 4: Quality assessment of the included studies according to the NIH Quality Assessment Tool for Observational Cohort and Cross-Selectional studies

Questions i?	Author/Year						
	Rahman et al., 2023	Thomas et al., 2021	Rodrigues de Andrade et al., 2021	Silva-Boghossian et al., 2017	Da Silva Maciel et al., 2016	Zeigler et al., 2012	Haffe and Socransky, 2006
Research question	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Study population	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Participant rate of eligible persons	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Eligibility criteria	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sample size	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Exposure assessment	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Exposure levels	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Repeated exposure assessment	No	No	No	No	No	No	No
Outcomes measures	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Assessors blinding	No	No	No	No	No	No	No
Follow-up rate	No	No	No	No	No	No	No
Statistical analyses	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Total	9	9	9	9	9	9	9

measurements with respect to normal weight and obese patients, this could be through a follow-up study (cohort study) and analyze their periodontal characteristics and microbiota over time. In a study published by Pataro *et al.*, [28] 2016 whose main objective was to compare the frequency of periodontopathogenic bacteria and *H. pylori* in mouth and stomach of obese individuals with or without periodontitis undergoing bariatric surgery. They found that, indeed in patients who had undergone this surgical treatment for weight reduction, clinical parameters decreased significantly, showing an inverse microbial effect in the oral and stomach environments, i.e., a higher frequency of oral bacteria and a lower frequency in gastric tissue.

As the global burden of obesity and periodontitis is continuously increasing, the management of these inflammatory diseases has become increasingly important. Obese subjects with periodontitis may benefit from different therapeutic options, ranging from surgical procedures such as bariatric surgery, to more conservative procedures such as antimicrobial photodynamic therapy and nonsurgical periodontal treatment [29]. The aim of these therapeutic procedures has the same common goal which is reflected in the decrease of periodontal clinical parameters and inflammatory and destruction biomarkers in different oral fluids such as saliva, blood serum and gingival crevicular fluid, however, the health care costs are higher and the improvement is inferior compared to non-obese subjects [30].

Despite the controversies in the clinical findings after the therapy of choice in obese subjects, dentists should be aware that obesity is a chronic metabolic disease and that periodontal treatment should be part of a comprehensive treatment of obesity, not only for the improvement of the systemic inflammatory state but also in the quality of life of the affected individuals [31].

Seven studies from different parts of the world were included, most of them from Brazil. Although all the studies analyzed the composition of the subgingival microbiota in normo-weight and obese subjects, in healthy and periodontal disease conditions, different methods of detection of the microorganisms were employed. Among them, next-generation sequencing,

specifically 16S rRNA amplicon sequencing. This technique reads the DNA sequences of microbes present in a sample and compares them to a sequence database to quantitatively establish the different organisms present in that sample. It is useful for characterizing the structure of the microbiome at the phylum, genus, and species level [32]. Interestingly, only two studies employed this technique [19,20]. An interesting finding, is that, despite the great advances in new molecular biology techniques for the identification of pathogenic microorganisms, five investigators preferred to use the Checkerboard technique for DNA-DNA hybridizations, which was introduced more than 25 years ago by Socransky *et al.* [18], has remained one of the most frequently used methods for studying of the subgingival microbiota in patients with different systemic condition that is closely related to periodontitis such as; obesity, rheumatoid arthritis, cancer, cardiovascular diseases, among others. This technique is based on the simultaneous hybridization of 18 to 40 digoxigenin-labeled whole genomic DNA probes with the whole genomic DNA present in dental plaque samples (supragingival or subgingival as the case may be). Therefore, it has become one of the techniques of choice by researchers for the study of periodontal microbiota, which indicates that despite being a technique with many years of having been implemented, it continues to be a useful and effective technique, in addition to being a quantitative method.

To the authors' knowledge, this is the first study that has compared the results of different studies in an attempt to identify a possible association between a specific subgingival microbiota and obesity-periodontitis status.

Thus, despite the heterogeneity of the studies, the most common genera detected in patients with obesity and periodontitis were *Capnocytophaga* and *Streptococcus*. While, at the species level, we found a higher abundance of three species of the orange complex: *C. gracilis*, *E. nodatum* and *F. nucleatum spp. vincentii*, one species of the yellow complex: *S. Intermedius*, one species of the blue complex: *A. gerencseriae* and two species of the ungrouped complexes: *A. actinomycetemcomitans* and *T. socransky* in comparison with periodontally healthy obese subjects, who presented a higher bacterial load and a higher frequency of blue and yellow complex species

possibly because the authors analyzed a larger bacterial population. Therefore, future studies should include a larger and more controlled microbiological analysis where different comparisons can be made with the study population (figure 2).

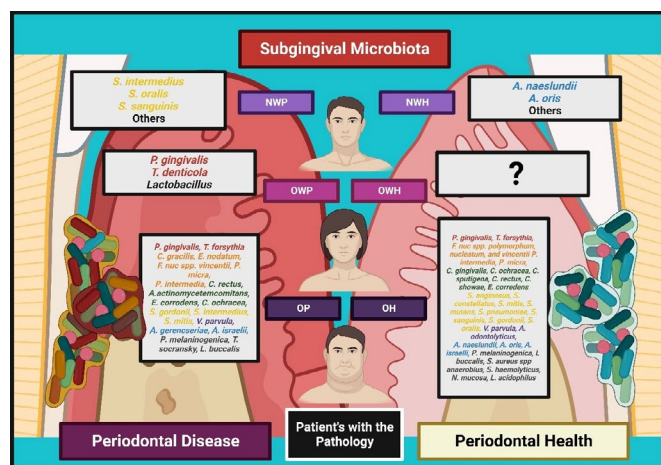


Figure 2. Main bacterial species colonizing the gingival sulcus of subjects with normo-weight, overweight and obesity, in conditions of periodontal health and disease. NWP= Normal Weight with Periodontitis; OWP= Over Weight with Periodontitis; OP= Obesity with Periodontitis; NWH= Normal Weight with Periodontal Health; OWH= Over Weight with Periodontal Health; OH= Obesity with Periodontal Health. www. biorender.com (Accessed on 24 August 2023)

Orange complex bacteria are bridging colonizing species, which through coaggregation processes mediated by cell surface adhesion and cooperative metabolism interact with recognized periodontopathogenic species favoring their growth and the expansion of their habitat in the subgingival biofilm microenvironment, which tips the balance towards periodontitis [12,19].

The persistence of *C. gracilis* after non-surgical therapy has been demonstrated in patients with peri-implantitis and even a higher prevalence of this bacterial species in the extraradicular biofilm than in periapical lesions caused by an infectious process or dental trauma [33]. *C. gracilis* is a Gram-negative, facultative anaerobic bacillus that has also been detected in a higher proportion in patients with type 2 diabetes mellitus and generalized periodontitis, which highlights its role in other metabolic diseases [17]. *C. gracilis*, could be a key pathogen that in obstructed patients may be playing a specific role in the destruction of the periodontium by

activating the immune response. It has been shown that *C. gracilis*, as well as *Leptotrichia wadei* and *F. nucleatum* strongly up-regulate the expression of chemokines (CXCL10, CXCL11 and CCL20) with antimicrobial activity and proinflammatory mediators (IL-6 and IL-8) in gingival fibroblasts (GF) compared to recognized periodontopathogenic species such as *P. gingivalis* that stimulated low levels or often suppressed the expression of these factors. This suggest that the invasion of red complex bacteria into gingival connective tissue may suppress the immune response of GF and may be a source of persistent infection in gingival tissue [34]. On the other hand, it has also been shown that the invasiveness of orange complex species such as *C. gracilis* and *F. nucleatum* in GF was markedly higher than that of *P. gingivalis*, suggesting that bacterial invasion by these periodontal pathogens in GF might be the first survival strategy to escape the host immune system [35]. Another important pathogen is *E. nodatum*, a Gram-positive, anaerobic, Gram-positive bacillus associated with the progression of periodontal disease and cardiovascular disease [12,17,18]. It has symbiotic activities with *P. gingivalis*, *T. forsythia* and *T. denticola*. Thus, as the concentration of orange complex species increases, the amount of red complex species also increases in the oral biofilm, favoring greater damage to the periodontium [36].

On the other hand, *A. actinomycetemcomitans* is a periodontopathogenic bacterium associated with aggressive periodontitis. It is a Gram-negative coccobacillus, facultative anaerobe, capnophilic, non-motile and late colonizer of the biofilm [18]. In relation to different metabolic conditions, a higher prevalence of this bacterial species has been detected in subjects with arterial hypertension compared to normotensive subjects. In addition, two studies have reported its presence in obese patients and different systemic and treatment conditions. Al-Rawi *et al.* [37], 2017 reported higher amounts of this pathogen in saliva samples from obese subjects, while, nevertheless, other authors have observed a high frequency (90%) of periodontal pathogens present in gastric tissue biopsies, even *A. actinomycetemcomitans* which in periodontal health conditions is only found in low numbers in the mouth, was commonly detected in the stomach (58%) [38]. This periodontopathogenic

species has antagonistic activities with *S. sanguinis*, as it reduces the levels of hydrogen peroxide produced by *S. sanguinis*, through the production of the cytoplasmic catalase enzyme by expression of the *katA* gene, favoring the growth and survival of *P. gingivalis* [36]. However, an interesting finding in patients with obesity and periodontitis is the high prevalence of *S. intermedius*, a cocci, Gram-positive, anaerobic and strongly associated with chronic periodontitis [18]. In fact, in relation to cooperative metabolism and as part of the beneficial effects of this bacterium, it has been shown that *S. intermedius* and *S. cristatus* inhibit the expression of FimA of *P. gingivalis* by the arginine deaminase (ArcA) system that catalyzes the conversion of Arginine to Citrulline leading to reduced biofilm formation [39].

Both in obese patients with healthy periodontium and periodontitis, a high prevalence of *S. gordonii* and *V. parvula* was found, which are early colonizing species that act as accessory pathogens since they facilitate the colonization of periodontopathogens that cause greater tissue damage. In fact, it has been shown that the food web in oral biofilm ecosystems affects their maturation process by cross-feeding between *S. gordonii* that produces ornithine. This ornithine is exported by the arginine-ornithine antiporter ArcD and is taken up by *F. nucleatum* that presents the enzyme ornithine decarboxylase, responsible for the conversion of ornithine/arginine to putrescine. Whereas, *V. parvula* produces lysine in the microenvironment and is taken up by *F. nucleatum* favoring cadaverine production. The sustained delivery of both polyamines favors the growth and habitat expansion of species such as *P. gingivalis* and produce increased pathogenicity in oral biofilms, i.e., this cooperative metabolism underlies the conversion of periodontal microbiota from a symbiotic to a dysbiotic state [39]. On the other hand, also *S. gordonii*, in addition to its symbiotic interactions with red complex species, antagonizes the effects of other species such as *A. naeslundii*, *S. mutans*, *S. sanguinis* which are beneficial bacteria in the periodontium.

A. gerencseriae has also been implicated in PD, mainly in patients with necrotizing ulcerative gingivitis (NUG). This species belongs to the autochthonous microbiota of hard surfaces (supragingival plaque), in the mucosa of the oral cavity and is one of the main microorganisms causing cervicofacial actinomycoses, which like NUG, actinomycoses have a complex etiology. They are usually polymicrobial infections with a flora usually comprising many suspected periodontopathogens [40].

This review has some limitations due to the great heterogeneity of the few studies present in the literature to date. There is currently no study that evaluates the composition of the subgingival microbiota in overweight patients with periodontitis, which it would be interesting to know the microbial profile of these patients and compare it with the other groups. In addition, creating a form of follow-up, by planning a longitudinal study, where changes in the composition of the subgingival microbiota and periodontal condition in obese subjects subjected to surgical procedures such as bariatric surgery, strict diets and in general a different lifestyle could be relevant to analyze the gradual changes that occur in the recovery of periodontal tissues.

CONCLUSION

There is a growing interest in analyzing the composition of the subgingival microbiota in patients with obesity and periodontal disease to identify the possible association between the two diseases. However, although there are evident alterations in microbial diversity, i.e.; an increase in the bacterial count and proportion of *C. gracilis*, *E. nodatum*, *F. nucleatum spp. vincentii*, *S. intermedius*, *A. gerencseriae*, *A. actinomycetemcomitans* and *T. socransky* species in patients with obesity and periodontitis compared to obese and periodontally healthy patients, it remains a challenge to identify a specific microbiota pattern. Another interesting fact, is the lack of information on the composition of the subgingival microbiota in overweight patients in healthy and diseased condition, therefore, we encourage future standardized studies to increase the knowledge on the subgingival microbiota and its possible implications.

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