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Examining the Relationship between Oral Microbiome Dysbiosis and Systemic Inflammation: A Clinical Laboratory Investigation in a Tertiary Care facility

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Abstract

Background: Dysbiosis of oral microbiome may be associated with chronic inflammation, although clinically integrated studies within multidisciplinary laboratories are scarce in developed countries.
In tercial care settings.

Objectives: Examine the association between oral microbiome dysbiosis and systemic inflammation in patients at a tertiary care hospital.

Methods: The study was a cross sectional comprising 120 participants, 60 with periodontitis and 60 age and sex matched healthy controls. Clinical periodontal examinations were performed, accompanied sample collection of unstimulated salivary and blood. 16S rRNA sequencing was conducted for oral microbiome analysis alongside serum inflammatory markers, including CRP, IL-6, and TNF-alpha which were analyzed using ELISA. Results were presented with descriptive statistics and differences between means were tested using t-test.

Results: Periodontitis subjects were significantly low diverse in microbial diversity with a Shannon diversity index of 3.2 ± 0.6 with an increase in bacterial taxa, including Porphyromonas gingivalis and Fusobacterium nucleatum ($p<0.001$) while control subjects had an index of 4.7 ± 0.5. Moreover, periodontitis patients exhibited significantly high markers of systemic inflammation. Levels were strongly positively correlated with pathogenic taxa and inflammatory markers with CRP levels yielding $r=0.68$ $p<0.001$. Analysis of beta diversity confirmed significant clustering of microbes within groups $p=0.002$.

Conclusion: This work illustrates the link of oral microbiome dysbiosis to heightened systemic inflammation. Results emphasize the role of oral health care in reducing the burden of inflammation and systemically integrated approaches for early diagnosis and intervention in tertiary care models.

Keywords: Oral microbiome, Dysbiosis, Systemic inflammation, Periodontitis, 16S rRNA sequencing, Tertiary hospital, CRP, IL-6, TNF-α

Introduction

Oral cavity refers to the part of the human body that includes the teeth and jaws. This cavity is the home for a range of microorganisms, second only to the gut in complexity and density. The oral microbiome is crucial for both oral cavity health as well as general wellbeing and strengthens both. Chronic inflammatory responses are known to advance local diseases like periodontitis. More obtrusively however, it will lead to inflammation on a whole-body scale (Georges et al., 2022).

The inflammation system is known to be affected by oral microbiomes. They are useful in controlling how inflammation manifests and acts. It can be hypothesized that shifts within this particular microbial community can alter the stability of pathogenic inflammatory networks. Kleinstein and Nelson stated that pathological inflammatory responses are activated in advanced stages of chronic disorders, which include cardiovascular disorders (Kleinstein & Nelson, 2020).

Increased inflammation is suspected when there is disruption in the normal functioning of the oral microbiota, now referred to as dysbiosis. In addition to this microbial community, skeletal dysregulation presents an imbalance in osteoclastic and osteoblastic activities controlling bone resorption and formation respectively, and also pro-inflammatory elements circulate in various parts of the body imbalance weakens immunity. Systemically inflammatory resolution when it can is policed and moderated by certain responsible bacteria like Porphyromonas gingivalis. Bacterial formation that governs changes within the body's circadian pace actively works towards inflammatory responsive structure (Slocum et al., 2016).

Moreover, the reciprocal relations between oral and gut microbiomes, known as the "oral–gut axis," are receiving increased attention for their contribution to the exacerbation of inflammation in the body, especially in case of chronic inflammatory diseases. Emerging evidence highlights the dysbiosis of salivary and fecal microbiomes as inflammatory bowel disease, accentuating the role of oral–gut axis in systemic inflammation (Abdelbary et al., 2022).

In the inflammatory chronic disease context with ever-increasing burden and combined with the recently acknowledged oral-systemic connection, there is an evident need for multidisciplinary studies that incorporate clinical synopses with microbial and immune system analysis conducted in the lab. Thus, we aim to gather clinical data on patients from a tertiary hospital, in combination with systemic inflammation measurements, to analyze dysregulated oral microbiomes for possible causal relations. With this approach, we hope to provide possible mechanistic explanations and evaluate prospects for proactive diagnosis and treatment.

Literature Review

1. The Oral Microbiome in Health and Dysbiosis

The oral microbiome is a dynamic ecological system that is important for sustaining oral and overall health. It defends against homeostatic imbalance and prevents pathogenic species from opportunistically colonizing. However, changes to this balance—referred to as dysbiosis—can increase the abundance of pathogenic bacteria underlying inflammatory oral diseases, especialmente periodontitis (Georges et al., 2022). Dysbiosis has been described as a condition of reduced microbial diversity and increased abundance of pathological species, such as Porphyromonas gingivalis, that cause tissue destruction and immune system disorder (Slocum et al., 2016).

2. Mechanisms Linking Oral Dysbiosis to Systemic Inflammation

The implications of dysbiotic oral microbiota extend beyond local concerns within oral pathology. Components and metabolites of bacteria may translocate into blood circulation, initiating inflammatory response pathways as well as contributing to systemic inflammatory processes such as endothelial dysfunction and metabolic disorders (Kleinstein & Nelson 2020). Furthermore, periodontopathogens also promote immune dysregulation and result in chronic low-grade inflammation, increasing the susceptibility to diabetes mellitus, cardiovascular diseases, and rheumatoid arthritis (Slocum et al., 2016).

3. The Oral–Gut Axis: Amplification of Systemic Inflammation

Recent research has highlighted the importance of the oral–gut axis in relation to inflammation. Oral dysbiosis influinces gut microbiota composition, resulting in gut barrier dysfunction and systemic endotoxemia (Amato, 2022). In particular, Porphyromonas gingivalis and other oral pathogenic microbes are capable of translocating to the gut, altering local microbiota, and worsening more extreme inflammatory diseases manifesting in the body. Abdelbary et al. (2022) showed that patients suffering from inflammatory bowel disease simultaneously display dysbiosis in saliva and feces, which illustrates the progression of a shared microbiome imbalance between the mouth and the intestines (Abdelbary et al., 2022).

4. Clinical Evidence from Recent Studies

These microbiological insights have received considerable clinical research confirmation. Soffritti et al. (2021) reported that dysbiosis of oral microbiomes is associated with heightened immune responses and worsened symptoms in COVID-19 patients, underscoring the impact of dysregulation within the oral flora on systemic health (Soffritti et al., 2021). In a similar manner, Plachokova et al. (2021) associated the severity of periodontitis with higher levels of inflammatory markers, strengthening the hypothesis of inflammation bridging the oral cavity and systemic circulation (Plachokova et al., 2021).

5. Gaps in Research and Need for Integrated Approaches

The nuances of the oral-systemic connection remains understudied, particularly concerning the underlying causes and possible bidirectional relationships involving oral dysbiosis and systemic diseases. Previous studies have focused on capturing a moment in time which leaves out the potential to determine a cause-and-effect relationship and further analysis focusing on complex co-morbidities and polymicrobial patient profiles is uncommon in tertiary care settings. Bridging these gaps is likely to

require advanced lab techniques like microbiome sequencing, cytokine profiling, metabolomics, and integrative clinical-database approaches alongside interdisciplinary collaboration.

My objective was to advance this line of research focusing on the dualistic nature of oral microbiome dysbiosis and systemic inflammation, utilizing a clinical framework in combination with thorough lab analysis within the context of a tertiary hospital.

Methodology

Design and Setting of the Study

The Department of Dentistry and Clinical Laboratory Services in a tertiary care hospital served as the setting for this observational cross-sectional study. This study was ethically reviewed and approved, and all participants provided written informed consent prior to enrolment.

Participants

One hundred twenty adult patients aged 18 to 65 years and attending the outpatient dental clinic were enrolled. Patients were divided into two groups according to the periodontal health status:

- Group A: Healthy individuals with no clinical symptoms of periodontal disease (n=60).
- Group B: Patients diagnosed with moderate to severe chronic periodontitis according to 2017 World Workshop classification (n=60).

The exclusion criteria included:

- Recent antibiotic therapy (in the last three months),
- Pregnancy or lactation,
- Systemic inflammatory conditions as autoimmune diseases (unless part of research focus),
- Ongoing immunosuppressive therapy,
- History of recent oral surgery.

Clinical Evaluation

Every study participant underwent a complete periodontal examination by a specialist periodontist. The following clinical parameters were recorded for all participants:

- Probing depth (PD),

- Clinical attachment loss (CAL),
- Bleeding on probing (BOP),
- Plaque index (PI).

Demographic information including age, sex, medical history and smoking history were included as well.

Sample Collection

Salivary Samples

Participants were asked to provide unstimulated whole saliva samples in the morning to reduce the influence of circadian rhythm in sterile tubes. All samples were placed on ice and then transferred to a lab where they were stored at -80 degrees Celsius until analysis.

Blood Samples

Blood was drawn from each participant to determine the levels of systemic inflammatory markers. Blood serum was obtained after centrifugation at 3000 rpm for 15 minutes and placed in a freezer at -80 degrees Celsius.

Laboratory analysis

Microbiome Profiling

Salivary samples were processed with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) which enables extraction of microbial DNA, following the procedure described by the manufacturer.

The V3–V4 region of the 16S rRNA gene was amplified and sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). For the bioinformatics part, the QIIME 2 pipeline was utilized and for the taxonomic classification, the SILVA 138 reference database was used.

The indices of alpha diversity metrics (Shannon and Simpson diversity) together with beta diversity (Bray-Curtis dissimilarity) were computed. In periodontitis and systemic inflammation, taxa that were found to be nonrandomly associated were analyzed for differential abundance.

Inflammation Marker Assay

The serum concentration of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) were measured with selected enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

The analysis of the data was performed using SPSS version 26.0 (IBM Corp, Armonk, NY, USA).

Quantitative variables were reported as mean ± standard deviation (SD) and qualitative datasets were reported as counts and percentages.

The Shapiro-Wilk test was employed to validate the normality assumption of the data. Group comparisons were performed with independent t-test or Mann–Whitney U test for continuous variables and chi-square test for categorical variables.

The relationship between indices of microbiome diversity and systemic inflammatory markers was analyzed using Spearman's correlation coefficient.

Statistical significance was set at $p < 0.05$.

Ethical consideration

The study was carried out in line with the Declaration of Helsinki. All data related to the patients were stripped of identifier labels, ensuring anonymity and confidentiality was maintained during the course of the research.

Results

1. Study Population

A total of 120 participants were enrolled, comprising 60 periodontitis patients (Group B) and 60 healthy controls (Group A). Demographic and clinical characteristics are summarized in **Table 1**.

Table 1. *Demographic and Clinical Characteristics of Study Participants*

Variable	**Healthy Controls (n=60)**	**Periodontitis Patients (n=60)**	**p-value**
Age (years), mean ± SD	42.3 ± 9.1	45.7 ± 8.6	0.045*
Male, n (%)	28 (46.7%)	31 (51.7%)	0.58
Smoking status (smokers), n (%)	12 (20.0%)	21 (35.0%)	0.048*
Probing depth (mm), mean ± SD	2.1 ± 0.5	4.8 ± 0.9	<0.001*
Clinical attachment loss (mm), mean ± SD	0.3 ± 0.1	3.6 ± 1.2	<0.001*

$p < 0.05$ statistically significant

2. Microbiome Diversity Analysis

Alpha diversity, assessed by the Shannon index, was significantly lower in periodontitis patients compared to healthy controls (3.2 ± 0.6 vs. 4.7 ± 0.5, $p < 0.001$), indicating reduced microbial diversity in disease states (**Table 2**).

Beta diversity analysis using Bray-Curtis dissimilarity revealed clear clustering between groups (PERMANOVA, $p = 0.002$), suggesting distinct microbial community structures.

Table 2.*Microbial Diversity Indices*

Diversity Metric	Healthy Controls (n=60)	Periodontitis Patients (n=60)	p-value
Shannon Index	4.7 ± 0.5	3.2 ± 0.6	<0.001*
Simpson Index	0.91 ± 0.03	0.78 ± 0.05	<0.001*
Bray-Curtis Dissimilarity	Reference	Significant clustering	0.002*

3. Differential Abundance of Key Microbial Taxa

Significant differences in microbial composition were observed between groups:

- *Porphyromonas gingivalis* was highly enriched in the periodontitis group (log2 fold change: +4.3, $p < 0.001$).
- *Fusobacterium nucleatum* was also elevated (+3.8 log2 fold change, $p < 0.001$).
- Conversely, *Streptococcus sanguinis*, a health-associated species, was reduced in periodontitis patients (log2 fold change: –2.7, $p = 0.004$).

Table 3.*Differentially Abundant Microbial Taxa*

Taxa	Fold Change (log2)	p-value	Direction
Porphyromonas gingivalis	+4.3	<0.001*	↑ Increased
Fusobacterium nucleatum	+3.8	<0.001*	↑ Increased
Streptococcus sanguinis	–2.7	0.004*	↓ Decreased

4. Inflammatory Marker Levels

Serum inflammatory markers were markedly elevated in the periodontitis group:

- CRP: 6.2 ± 2.1 mg/L vs. 2.8 ± 1.3 mg/L in controls ($p < 0.001$)
- IL-6: 18.4 ± 5.7 pg/mL vs. 9.3 ± 3.1 pg/mL ($p < 0.001$)
- TNF-α: 32.7 ± 8.9 pg/mL vs. 14.8 ± 4.5 pg/mL ($p < 0.001$)

Table 4. *Systemic Inflammatory Markers*

Marker	Healthy Controls (n=60)	Periodontitis Patients (n=60)	p-value
C-Reactive Protein (mg/L)	2.8 ± 1.3	6.2 ± 2.1	<0.001*
Interleukin-6 (pg/mL)	9.3 ± 3.1	18.4 ± 5.7	<0.001*
TNF-alpha (pg/mL)	14.8 ± 4.5	32.7 ± 8.9	<0.001*

5. Correlation Between Microbiome and Inflammatory Markers

Significant positive correlations were observed between *P. gingivalis* abundance and serum inflammatory markers:

- CRP ($r = 0.68$, $p < 0.001$),
- IL-6 ($r = 0.61$, $p < 0.001$),
- TNF-α ($r = 0.59$, $p < 0.001$).

Microbial diversity (Shannon index) was inversely correlated with CRP ($r = –0.55$, $p < 0.001$), indicating that reduced microbial diversity was associated with heightened systemic inflammation.

Discussion

The objective of this study is to determine the association of oral microbiome dysbiosis with systemic inflammation in patients from a tertiary hospital. Our results are equally compelling that people suffering from periodontitis have marked changes in their oral microbial profile with lower diversity and higher counts of known pathogenic organisms like Porphyromonas gingivalis and Fusobacterium nucleatum. These changes were significantly associated with elevated systemic inflammation measured as CRP, IL-6, and TNF-α.

The lower microbial diversity among periodontitis patients is consistent with previous research that documented dysbiotic microbial communities as a defining feature of oral inflammatory diseases (Georges et al., 2022). The increase in pathogenic bacteria, especially P. gingivalis, is known to disrupt host-microbiota equilibrium and results in chronic inflammation and the systemic release of inflammatory mediators (Slocum et al., 2016). These findings strongly affect our understanding of the issue because we documented strong positive correlations of P. gingivalis abundance with the systemic inflammatory biomarkers.

In addition, our findings support the increasing understanding of the oral–gut axis as an important mediator of systemic inflammation. Inflammatory bowel disease (IBD) patients demonstrate dysbiosis of both their salivary and fecal microbiomes, which underscores the passage of oral microbiota to distal mucosal surfaces (Abdelbary et al., 2022). Alongside this, our research noted clear microbial clustering differentiating between health and disease, demonstrating the further reach of oral microbiota disruption.

The increase in systemic inflammatory markers as an underlying condition among periodontitis patients is of profound clinical relevance. The chronic burden of CRP, IL-6, and TNF-α increases the risk for cardiovascular disease, insulin resistance, and worsened outcomes in infectious diseases (Kleinstein &

Nelson, 2020). The study also reinforces the findings of Soffritti et al. (2021), who stated that oral dysbiosis worsens inflammation and the immune response in COVID-19 patients (Soffritti et al., 2021).

In the clinic, our research highlights the active management of oral health as part of the preventive measures in controlling systemic diseases. Periodontal maintenance and microbiome-driven therapies may lessen the burden of the systemic inflammatory load and prove to be important components of preventative medicine.

Despite its intriguing outcomes, the study is limited in several aspects. It is impossible to determine causation with this type of study, and it would be necessary to conduct longitudinal research to establish whether oral dysbiosis occurs prior to or follows systemic inflammation. Moreover, our focus on salivary samples provides a non-invasive method; however, it restricts access to deeper periodontal pockets and other possible microbial niches, which could provide valuable information.

Moving forward, researchers should investigate the longitudinal relationship between oral microbiome and systemic inflammation using multi-omics techniques like metatranscriptomics and metabolomics. Furthermore, interventional studies adopting periodontal therapy or microbiome modulation to evaluate changes in systemic inflammatory responses would be instrumental in elucidating the underlying mechanisms.

Conclusion of Discussion:

As noted previously, this study provides strong evidence for the association of inflammation and the oral microbiome dysbiosis in patients admitted to a tertiary hospital. This integrated approach of clinical and laboratory work sheds light on the potential avenues of The oral-systemic connection which require definition alongside the frameworks of targeted recognition-focused prevention and treatment.

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