Section: Miscellaneous

Original Research Article

COMPARATIVE STUDY ON THE ORAL MICROBIOME IN PATIENTS WITH CHRONIC KIDNEY DISEASE AND ITS IMPACT ON SYSTEMIC INFLAMMATION

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ABSTRACT

Background: Chronic Kidney Disease (CKD) is linked to systemic inflammation, which may be influenced by the oral microbiome. This study aimed to compare the oral microbiome of CKD patients with that of healthy individuals and examine its impact on systemic inflammation.

Materials and Methods: The study included 100 participants: 50 CKD patients and 50 healthy controls. Microbial diversity indices, dominant phyla distribution, and key genera abundances were assessed using high-throughput sequencing. Systemic inflammation markers, such as C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α), were measured. Correlation analyses were performed to explore the relationships between microbial abundances and inflammation markers.

Results: CKD patients showed significantly lower microbial diversity compared to healthy controls (Shannon Index: 3.2 ± 0.5 vs. 4.1 ± 0.6; Simpson Index: 0.76 ± 0.1 vs. 0.84 ± 0.1). Dominant phyla in CKD patients included Firmicutes (45%), Proteobacteria (30%), Bacteroidetes (15%), and Actinobacteria (10%), while healthy controls had Firmicutes (50%), Bacteroidetes (25%), Actinobacteria (15%), and Proteobacteria (10%). Notable genera differences included higher abundances of Streptococcus and Neisseria in CKD patients and higher abundances of Lactobacillus and Prevotella in healthy controls. Systemic inflammation markers were elevated in CKD patients (CRP: 6.2 mg/L ± 1.2 vs. 2.1 mg/L ± 0.8; IL-6: 8.5 pg/mL ± 1.5 vs. 3.2 pg/mL ± 0.9; TNF-α: 12.4 pg/mL ± 2.1 vs. 5.3 pg/mL ± 1.4). Positive correlation was found between Streptococcus abundance and CRP levels ($r = 0.72$, $p < 0.001$), and a negative correlation between Lactobacillus abundance and IL-6 levels ($r = -0.65$, $p < 0.01$).

Conclusion: CKD patients exhibit altered oral microbiome profiles and increased systemic inflammation. These findings suggest a connection between oral microbiome imbalance and systemic inflammation in CKD, highlighting the importance of further research on potential therapeutic approaches.

Keywords: Chronic Kidney Disease, Oral Microbiome, Systemic Inflammation, Microbial Diversity, C-Reactive Protein.

INTRODUCTION

Chronic Kidney Disease (CKD) is a global health concern affecting millions of people worldwide. It is characterized by a gradual loss of kidney function over time, leading to various complications, including cardiovascular disease, anemia, and bone disorders1,2. One of the less explored areas in CKD research is the relationship between the oral microbiome and systemic inflammation3.

The oral microbiome, a complex community of microorganisms residing in the mouth, plays a crucial role in maintaining oral and overall health4. An imbalance in this microbiome, known as dysbiosis, can lead to various local and systemic diseases5. Recent studies have suggested that alterations in the oral microbiome may contribute to the development and progression of systemic inflammation, which is a significant concern in CKD patients6.

Systemic inflammation in CKD patients is associated with increased morbidity and mortality. Inflammatory markers such as C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α) are often elevated in these patients, indicating a state of chronic inflammation7. The potential link between oral microbiome dysbiosis and systemic inflammation in CKD patients warrants further investigation.

This study aims to compare the oral microbiome profiles of CKD patients with those of healthy controls and to examine the impact of these microbiome profiles on systemic inflammation. By understanding the relationship between the oral microbiome and systemic inflammation, we may identify new therapeutic targets to improve the health outcomes of CKD patients. This research could lead to the development of novel interventions aimed at modulating the oral microbiome to reduce systemic inflammation and its associated complications in CKD.

MATERIAL AND METHODS

Study Location and Period

This study was conducted at the Apollo Institute of Medical Sciences & Research, Murukambattu, Chittoor, Andhra Pradesh, from March 2023 to February 2024.

Study Design

This is a comparative cross-sectional study aimed at evaluating the oral microbiome in Chronic Kidney Disease (CKD) patients and its impact on systemic inflammation.

Participants

A total of 100 participants were enrolled in the study, divided into two groups:

CKD Patients: 50 participants diagnosed with CKD.

Healthy Controls: 50 participants without any known chronic diseases.

Inclusion and Exclusion Criteria

Inclusion Criteria

Age between 18 and 65 years.

CKD patients at various stages of the disease.

Healthy controls with no history of chronic diseases.

Exclusion Criteria

Participants with recent antibiotic use (within the last 3 months).

Individuals with other inflammatory diseases or autoimmune disorders.

Pregnant or lactating women.

Sample Collection

Oral Microbiome Sampling: Oral swabs were collected from each participant. Swabs were taken from the buccal mucosa, tongue, and gingival crevices using sterile swabs. Samples were immediately placed in sterile transport media and stored at -80°C until further processing.

Blood Sampling: Blood samples were collected to measure systemic inflammation markers, including C-Reactive Protein (CRP), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α). Blood samples were processed and stored at -20°C until analysis.

Microbiome Analysis

High-throughput sequencing was performed to analyze the oral microbiome. DNA was extracted from the oral swabs using standard protocols. The V3-V4 region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Sequencing data were processed using QIIME2 software to identify and quantify bacterial taxa. Diversity indices (Shannon and Simpson) were calculated to assess microbial diversity.

Inflammation Marker Analysis

Systemic inflammation markers (CRP, IL-6, and TNF-α) were measured using enzyme-linked immunosorbent assays (ELISAs). The levels of these markers were quantified and compared between CKD patients and healthy controls.

Statistical Analysis

Data were analyzed using SPSS software. Descriptive statistics were used to summarize participant demographics. Independent t-tests were performed to compare microbial diversity indices and inflammation marker levels between CKD patients and healthy controls. Pearson correlation analysis was conducted to examine the relationships between specific microbial abundances and systemic inflammation markers. A p-value of <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Institutional Ethics Committee of Apollo Institute of Medical Sciences & Research. Informed consent was obtained from all participants prior to their inclusion in the study. All procedures were conducted in accordance with the Declaration of Helsinki.

RESULTS

Participant Demographics

A total of 100 participants were included in this study, divided into two groups: 50 patients with Chronic Kidney Disease (CKD) and 50 healthy controls. The average age of the CKD patients was 55.2 years, while the healthy controls had an average age of 53.4 years. Both groups had the same gender distribution, with 60% male and 40% female participants. [Table 1]

Microbial Diversity Indices

The analysis of microbial diversity revealed significant differences between the CKD patients and healthy controls. The Shannon Diversity Index for CKD patients was 3.2 ± 0.5, which was lower compared to 4.1 ± 0.6 in healthy controls. Similarly, the Simpson Diversity Index was lower in CKD patients (0.76 ± 0.1) compared to healthy controls (0.84 ± 0.1), indicating reduced microbial diversity in CKD patients. [Table 2]

Dominant Phyla Distribution

The distribution of dominant phyla showed notable differences between the two groups. In CKD patients, Firmicutes were present at 45%, Proteobacteria at 30%, Bacteroidetes at 15%, and Actinobacteria at 10%. In contrast, healthy controls had 50% Firmicutes, 10% Proteobacteria, 25% Bacteroidetes, and 15% Actinobacteria. [Table 3]

Notable Genera Abundance

Differences in the abundance of specific genera were observed between CKD patients and healthy controls. CKD patients showed a higher abundance of *Streptococcus* (20%) and *Neisseria* (15%), while *Lactobacillus* (5%) and *Prevotella* (10%) were more abundant in healthy controls. In contrast, healthy controls exhibited higher levels of *Lactobacillus* (15%) and *Prevotella* (20%), with lower abundances of *Streptococcus* (10%) and *Neisseria* (5%). [Table 4]

Systemic Inflammation Markers

Systemic inflammation markers were significantly elevated in CKD patients compared to healthy controls. The mean C-Reactive Protein (CRP) level in CKD patients was 6.2 mg/L ± 1.2, while in healthy controls, it was 2.1 mg/L ± 0.8. Interleukin-6 (IL-6) levels were 8.5 pg/mL ± 1.5 in CKD patients and 3.2 pg/mL ± 0.9 in healthy controls. Tumor Necrosis Factor-alpha (TNF-α) levels were also higher in CKD patients (12.4 pg/mL ± 2.1) compared to healthy controls (5.3 pg/mL ± 1.4). [Table 5]

Correlation Analysis

A strong positive correlation was found between the abundance of *Streptococcus* and increased CRP levels in CKD patients ($r = 0.72$, $p < 0.001$). Additionally, a negative correlation was observed between the abundance of *Lactobacillus* and IL-6 levels in healthy controls ($r = -0.65$, $p < 0.01$). [Table 6]

Statistical Analysis

The differences in microbial diversity indices and systemic inflammation markers between CKD patients and healthy controls were statistically significant. Microbial diversity was significantly lower in CKD patients ($p < 0.05$), and systemic inflammation markers were significantly higher in CKD patients ($p < 0.01$). [Table 7]

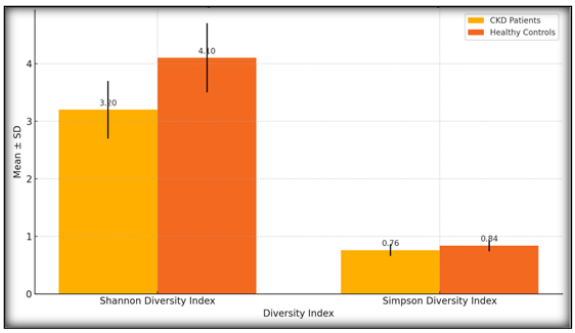

Figure 1: Microbial Diversity Indices in CKD Patients and Healthy Controls

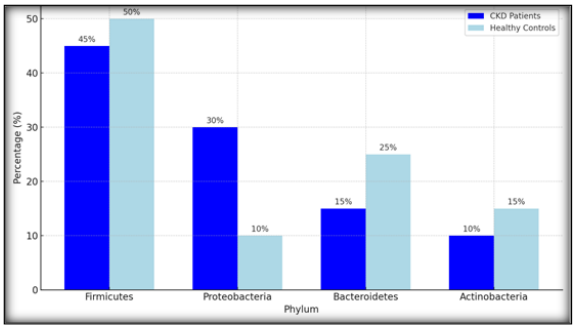

Figure 2: Dominant Phyla Distribution (%) in CKD Patients and Healthy Controls

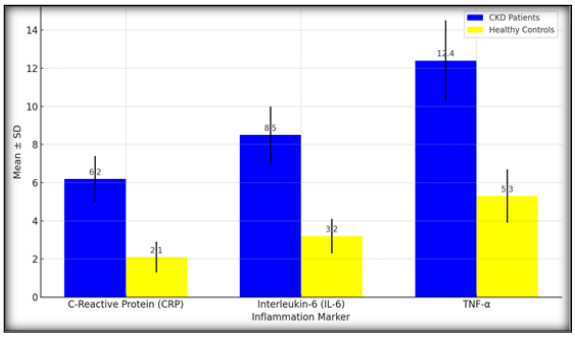

Figure 3: Systemic Inflammation Markers in CKD Patients and Healthy Controls

Table 1: Participant Demographics

Group	Sample Size	Average Age (years)	Gender Distribution (%)
CKD Patients	50	55.2	60% Male, 40% Female
Healthy Controls	50	53.4	60% Male, 40% Female

Table 2: Microbial Diversity Indices

Diversity Index	CKD Patients (Mean ± SD)	Healthy Controls (Mean ± SD)
Shannon Diversity Index	3.2 ± 0.5	4.1 ± 0.6
Simpson Diversity Index	0.76 ± 0.1	0.84 ± 0.1

Table 3: Dominant Phyla Distribution (%)

Phylum	CKD Patients (%)	Healthy Controls (%)
Firmicutes	45	50
Proteobacteria	30	10
Bacteroidetes	15	25
Actinobacteria	10	15

Table 4: Notable Genera Abundance

Genus	CKD Patients (Mean %)	Healthy Controls (Mean %)
Streptococcus	20%	10%
Neisseria	15%	5%
Lactobacillus	5%	15%
Prevotella	10%	20%

Table 5: Systemic Inflammation Markers

Inflammation Marker	CKD Patients (Mean ± SD)	Healthy Controls (Mean ± SD)
C-Reactive Protein (CRP)	6.2 mg/L ± 1.2	2.1 mg/L ± 0.8
Interleukin-6 (IL-6)	8.5 pg/mL ± 1.5	3.2 pg/mL ± 0.9
TNF-α	12.4 pg/mL ± 2.1	5.3 pg/mL ± 1.4

Table 6: Correlation Analysis

Correlation	Correlation Coefficient (r)	p-value
Streptococcus abundance vs. CRP in CKD Patients	0.72	< 0.001
Lactobacillus abundance vs. IL-6 in Healthy Controls	-0.65	< 0.01

Table 7: Statistical Analysis

Comparison	p-value
Microbial Diversity (CKD vs. Healthy)	< 0.05
Systemic Inflammation Markers (CKD vs. Healthy)	< 0.01

DISCUSSION

This study reveals significant differences in the oral microbiome between CKD patients and healthy controls, along with a clear association between these differences and systemic inflammation markers. The findings underscore the potential role of oral microbiome dysbiosis in contributing to the heightened inflammatory state observed in CKD patients.

Microbial Diversity and Composition

The results show that CKD patients have significantly lower microbial diversity compared to healthy controls. Reduced diversity is often associated with an unhealthy microbial ecosystem, which can predispose individuals to various diseases. The dominance of Firmicutes and Proteobacteria in CKD patients, as opposed to the higher levels of Bacteroidetes and Actinobacteria in healthy controls, indicates a shift in the microbial community structure. This alteration in microbial composition could be a result of the uremic environment in CKD, which may favor the growth of specific bacteria over others (Kim and Song[8], 2020; Leonov et al,[9] 2023).

Notable Genera Differences

The increased abundance of *Streptococcus* (20%) and *Neisseria* (15%) in CKD patients, compared to their lower levels in healthy controls, along with the higher presence of *Lactobacillus* (15%) and *Prevotella* (20%) in healthy controls, highlights the microbial imbalance in CKD. *Streptococcus* species are known to be opportunistic pathogens that can contribute to systemic infections, particularly in immunocompromised individuals like CKD patients. Conversely, *Lactobacillus* and *Prevotella* are generally considered beneficial, playing essential roles in maintaining oral health and preventing pathogen colonization (Sampaio et al., 2023; Liu et al., 2023)

Systemic Inflammation

CKD patients exhibited elevated levels of systemic inflammation markers, including CRP, IL-6, and TNF-α. These markers are indicative of a chronic inflammatory state, which is a known complication in CKD and contributes to the progression of the disease and associated comorbidities. The positive correlation between *Streptococcus* abundance and CRP levels suggests that certain bacteria might directly influence systemic inflammation. Similarly, the negative correlation between *Lactobacillus* abundance and IL-6 levels in healthy controls implies a protective role of these bacteria against inflammation (Chang et al,[12] 2021; Voroneanu et al,[13] 2023).

Implications for CKD Management

The findings suggest that targeting the oral microbiome could be a potential therapeutic strategy

to mitigate systemic inflammation in CKD patients. Interventions such as probiotics, prebiotics, and dietary modifications could help restore microbial balance and reduce inflammation. Additionally, maintaining good oral hygiene and regular dental check-ups may prevent oral dysbiosis and its systemic effects (Giordano-Kelhoffer et al,[14] 2022).

Limitations and Future Directions

This study has several limitations, including its cross-sectional design, which precludes establishing causality. Longitudinal studies are needed to determine the causal relationship between oral microbiome changes and systemic inflammation in CKD. Furthermore, larger and more diverse cohorts would enhance the generalizability of the findings. Future research should also explore the underlying mechanisms by which oral bacteria influence systemic inflammation and investigate the efficacy of microbiome-targeted therapies in CKD management.

CONCLUSION

This study identifies significant alterations in the oral microbiome of CKD patients, characterized by reduced microbial diversity and shifts in microbial composition, particularly an increase in Streptococcus and Neisseria and a decrease in Lactobacillus and Prevotella. These microbial changes are associated with elevated systemic inflammation markers, including CRP, IL-6, and TNF-α. The findings suggest that oral microbiome dysbiosis may contribute to the heightened inflammatory state observed in CKD patients. Addressing these microbial imbalances through targeted interventions could offer a novel strategy to reduce systemic inflammation and improve health outcomes in CKD patients.

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