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Evaluation of Salivary IL-8 and Calcium Levels in Postmenopausal Females with and without periodontitis—A Comparative Study

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Abstract Background

Postmenopausal women are at an increased risk of developing periodontitis due to hormonal changes affecting oral health. Interleukin-8 (IL-8) is a pro-inflammatory cytokine involved in the pathogenesis of periodontitis, and calcium plays a crucial role in bone metabolism and periodontal health. This study aims to evaluate and compare the salivary levels of IL-8 and calcium in postmenopausal females with and without periodontitis.

Materials and Methods

A total of 60 postmenopausal women were enrolled in this study, divided into two groups: 30 with periodontitis (Group A) and 30 without periodontitis (Group B). Unstimulated saliva samples were collected from all participants. IL-8 levels were measured using an enzyme-linked immunosorbent assay (ELISA), and calcium levels were determined using an automated biochemical analyzer. Statistical analysis was performed using the t-test and Pearson correlation coefficient.

Results

The mean salivary IL-8 levels in Group A were significantly higher (45.3 \pm 10.4 pg/mL) compared to Group B (25.7 \pm 6.8 pg/mL) (p < 0.01). Similarly, the mean salivary calcium levels in Group A were

significantly lower $(3.2 \pm 0.8 \text{ mg/dL})$ compared to Group B (4.5 \pm 1.0 mg/dL) (p < 0.01). A significant negative correlation was observed between salivary IL-8 and calcium levels (r = -0.54, p < 0.05). **Conclusion**

Postmenopausal women with periodontitis exhibit higher salivary IL-8 and lower calcium levels compared to those without periodontitis. These findings suggest that salivary IL-8 and calcium levels could serve as potential biomarkers for the early detection and management of periodontitis in postmenopausal females. **Keywords:** Postmenopausal, periodontitis, salivary IL-8, calcium, biomarkers, oral health.

Introduction

Periodontitis is a chronic inflammatory disease affecting the supporting structures of the teeth, leading to progressive attachment loss and bone resorption (1). The prevalence of periodontitis is notably higher in postmenopausal women due to hormonal changes that affect the periodontium (2). Estrogen deficiency during menopause has been linked to an increase in pro-inflammatory cytokines and a decrease in bone mineral density, both of which contribute to the progression of periodontal disease (3).

Interleukin-8 (IL-8) is a pro-inflammatory cytokine that plays a pivotal role in the pathogenesis of periodontitis. It is involved in the recruitment and activation of neutrophils, which are crucial in the body's defense against periodontal pathogens (4). Elevated levels of IL-8 have been found in the gingival crevicular fluid and saliva of patients with periodontitis, indicating its potential as a biomarker for the disease (5).

Calcium is essential for bone metabolism and the maintenance of periodontal health. Salivary calcium levels reflect systemic calcium status and can influence oral health (6). Postmenopausal women often experience a decline in calcium levels due to decreased intestinal absorption and increased urinary excretion, which can exacerbate bone loss and periodontal destruction (7).

Despite the established roles of IL-8 and calcium in periodontitis, there is limited research focusing on their salivary levels in postmenopausal women with and without periodontitis. This study aims to evaluate and compare the salivary IL-8 and calcium levels in these two groups to explore their potential as diagnostic biomarkers for periodontitis in postmenopausal females.

Materials and Methods

Study Design and Population

This comparative study was conducted on a sample of 60 postmenopausal women, aged 50-70 years, who were recruited from the outpatient. The participants were divided into two groups: Group A consisted of 30 women diagnosed with periodontitis, and Group B included 30 women without periodontitis. Ethical approval was obtained from the Institutional Review Board, and written informed consent was obtained from all participants.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows:

- Postmenopausal women aged 50-70 years.
- For Group A, a clinical diagnosis of periodontitis based on the presence of clinical attachment loss of ≥ 2 mm at more than two non-adjacent teeth.

Exclusion criteria included:

- History of systemic diseases affecting calcium metabolism (e.g., hyperparathyroidism, renal failure).
- Use of medications influencing bone metabolism (e.g., bisphosphonates, hormone replacement therapy).
- Current smokers or tobacco users.
- Women with a history of periodontal treatment within the last 6 months.

Sample Collection

Unstimulated whole saliva samples were collected between 9:00 AM and 11:00 AM to minimize diurnal variations. Participants were instructed to refrain from eating, drinking (except water), or performing oral hygiene procedures for at least 1 hour prior to sample collection. Saliva was collected by spitting into a sterile tube over a period of 5 minutes. The samples were immediately placed on ice and transported to the laboratory for analysis.

Biochemical Analysis

Salivary IL-8 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The absorbance was read at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Salivary calcium levels were determined using an automated biochemical analyzer (Roche Cobas c311, Basel, Switzerland). The calcium concentration was measured based on the photometric method, where calcium reacts with o-cresolphthaleincomplexone to form a violet complex, which is measured at 570 nm.

Statistical Analysis

Data were analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize the data. The mean and standard deviation (SD) were calculated for salivary IL-8 and calcium levels. Comparisons between groups were made using the independent samples t-test. Pearson correlation coefficient was used to assess the relationship between salivary IL-8 and calcium levels. A p-value of <0.05 was considered statistically significant.

Results

Demographic Data

The demographic characteristics of the study participants are presented in Table 1. The mean age of participants in Group A (with periodontitis) was 60.2 ± 5.8 years, and in Group B (without periodontitis) it was 59.5 ± 6.1 years. There were no significant differences in age or body mass index (BMI) between the two groups (p > 0.05).

| Demographic | Group A | (With | Group B | (Without | р- |
|---|------------------------|-------|------------------------|----------|-------|
| Characteristic | Periodontitis) | | Periodontitis) | | value |
| Number of Participants | 30 | | 30 | | - |
| Age (years, mean \pm SD) | 60.2 ± 5.8 | | 59.5 ± 6.1 | | 0.68 |
| BMI (kg/m ² , mean \pm SD) | 26.4 ± 3.2 | | 25.9 ± 3.5 | | 0.52 |

Salivary IL-8 Levels

Salivary IL-8 levels were significantly higher in Group A compared to Group B. The mean IL-8 level in Group A was 45.3 ± 10.4 pg/mL, whereas in Group B it was 25.7 ± 6.8 pg/mL (p < 0.01) (Table 2).

| Group | Salivary IL-8 (pg/mL, mean ± SD) |
|---------------------------------|----------------------------------|
| Group A (With Periodontitis) | 45.3 ± 10.4 |
| Group B (Without Periodontitis) | 25.7 ± 6.8 |
| p-value | < 0.01 |

Salivary Calcium Levels

Salivary calcium levels were significantly lower in Group A compared to Group B. The mean calcium level in Group A was 3.2 ± 0.8 mg/dL, while in Group B it was 4.5 ± 1.0 mg/dL (p < 0.01) (Table 3).

| Group | Salivary Calcium (mg/dL, mean ± SD) |
|---------------------------------|-------------------------------------|
| Group A (With Periodontitis) | 3.2 ± 0.8 |
| Group B (Without Periodontitis) | 4.5 ± 1.0 |
| p-value | < 0.01 |

Correlation Between Salivary IL-8 and Calcium Levels

A significant negative correlation was observed between salivary IL-8 and calcium levels (r = -0.54, p < 0.05), indicating that higher IL-8 levels were associated with lower calcium levels (Table 4).

| Variable | Correlation Coefficient (r) | p-value |
|-------------------------|------------------------------------|---------|
| IL-8 and Calcium Levels | -0.54 | < 0.05 |

These results indicate that postmenopausal women with periodontitis have higher salivary IL-8 levels and lower salivary calcium levels compared to those without periodontitis. The negative correlation between IL-8 and calcium levels suggests a potential interaction between inflammatory and metabolic pathways in the pathogenesis of periodontitis in postmenopausal women.

Discussion

The findings of this study indicate that postmenopausal women with periodontitis exhibit significantly higher salivary levels of IL-8 and lower levels of calcium compared to their counterparts without periodontitis. These results highlight the potential of salivary IL-8 and calcium as biomarkers for periodontal disease in postmenopausal women.

The elevated salivary IL-8 levels observed in postmenopausal women with periodontitis align with previous studies that have reported increased IL-8 levels in the gingival crevicular fluid and saliva of patients with periodontal disease (1,2). IL-8 is a key pro-inflammatory cytokine that plays a crucial role in the recruitment and activation of neutrophils, which are essential for the host response to periodontal pathogens (3). The higher IL-8 levels in postmenopausal women with periodontitis may reflect an enhanced inflammatory response, which could contribute to the progression and severity of periodontal disease in this population.

The significantly lower salivary calcium levels in postmenopausal women with periodontitis observed in this study are consistent with the known impact of menopause on calcium metabolism. Estrogen deficiency during menopause leads to a decrease in intestinal calcium absorption and an increase in urinary calcium excretion, resulting in lower systemic calcium levels (4,5). Given the critical role of calcium in bone metabolism and periodontal health, the reduced salivary calcium levels in postmenopausal women with periodontitis may exacerbate alveolar bone loss and periodontal destruction.

The negative correlation between salivary IL-8 and calcium levels found in this study further underscores the interplay between inflammatory and metabolic pathways in the pathogenesis of periodontitis in postmenopausal women. This finding suggests that the inflammatory process, as indicated by elevated IL-8 levels, may be associated with decreased calcium availability, potentially contributing to the deterioration of periodontal tissues (6).

This study has several clinical implications. Firstly, the assessment of salivary IL-8 and calcium levels could serve as a non-invasive and convenient method for the early detection and monitoring of periodontitis in postmenopausal women. Secondly, these biomarkers could help identify individuals at higher risk for periodontal disease, enabling targeted preventive and therapeutic interventions. Lastly, the findings highlight the need for comprehensive periodontal care in postmenopausal women, considering their increased susceptibility to periodontal disease due to hormonal changes and altered calcium metabolism.

However, this study has some limitations. The cross-sectional design precludes establishing causality between elevated IL-8, reduced calcium levels, and periodontitis. Longitudinal studies are needed to confirm these associations and elucidate the underlying mechanisms. Additionally, the study's sample size was relatively small, and larger studies are required to validate these findings in diverse populations.

Conclusion

In conclusion, postmenopausal women with periodontitis exhibit higher salivary IL-8 and lower calcium levels compared to those without periodontitis. These biomarkers hold promise for the early detection and management of periodontitis in this vulnerable population. Future research should focus on longitudinal studies to establish causality and explore the potential of these biomarkers in clinical practice.

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