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ORIGINAL ARTICLE

CLINICO-PATHOLOGICAL COMPARISON OF SALIVARY TNF-α AMONG MENOPAUSE WOMAN WITH AND WITHOUT PERIODONTITIS - A SHORT STUDY

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Abstract

Background: Menopause is associated with significant hormonal changes that can influence systemic and oral health, including an increased risk of inflammatory conditions such as periodontitis. Tumor Necrosis Factor-alpha (TNF-α) is a key pro-inflammatory cytokine involved in periodontal disease progression and has been detected in saliva, serving as a potential biomarker for disease severity.

Objectives: To determine the association between elevated salivary TNF-α levels and periodontitis in menopausal women.

Materials and Methods: An in vivo cross-sectional study was conducted on 40 menopausal women, divided into two groups: Group I (with periodontitis; n=20) and Group II (without periodontitis; n=20). Saliva samples were collected using randomized sampling, and TNF-α levels were quantified using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using SPSS software, with Independent t-tests employed for group comparisons; significance was established at $p < 0.05$.

Results: All participants exhibited detectable salivary TNF-α levels. Group I demonstrated significantly higher TNF-α concentrations with Mean ± SD of 20.48 ± 3.10 compared to Group II with 11.32 ± 1.74, with the difference being statistically significant ($p = 0.001$). These results revealed the association between elevated TNF-α levels in periodontitis in menopausal women, reflecting the combined impact of hormonal changes and local inflammation.

Conclusion: Elevated salivary TNF-α levels in menopausal women with periodontitis suggest that TNF-α could serve as a potential biomarker for periodontal inflammation influenced by estrogen deficiency. The findings contribute to a better understanding of the interplay between systemic hormonal alterations and local inflammatory responses in periodontal disease, warranting further research with larger cohorts and exploration of targeted therapeutic strategies.

Keywords: TNF-α, periodontitis, menopause, salivary biomarkers, inflammation

INTRODUCTION

Menopause is a significant physiological milestone in a woman's life, marked by the cessation of ovarian function and subsequent estrogen depletion. This hormonal change profoundly impacts various body systems, including the oral cavity. One of the lesser-known consequences of menopause is its association

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with oral health alterations, particularly the susceptibility to periodontal diseases.[1] Periodontitis, a chronic inflammatory condition that destroys the supporting structures of teeth, are influenced by both local and systemic factors, including hormonal fluctuations and immune responses. Among the immune-related mediators implicated in periodontitis, tumor necrosis factor-alpha (TNF-α) plays a pivotal role. TNF-α is a pro-inflammatory cytokine that regulates immune responses and contributes to the destruction of periodontal tissues by enhancing the recruitment of inflammatory cells, inducing osteoclastogenesis, and disrupting the balance of bone metabolism. Elevated levels of TNF-α have been detected in various bodily fluids, including saliva, in individuals with periodontitis, highlighting its potential as a biomarker for periodontal inflammation (Figure 1). [2,3]

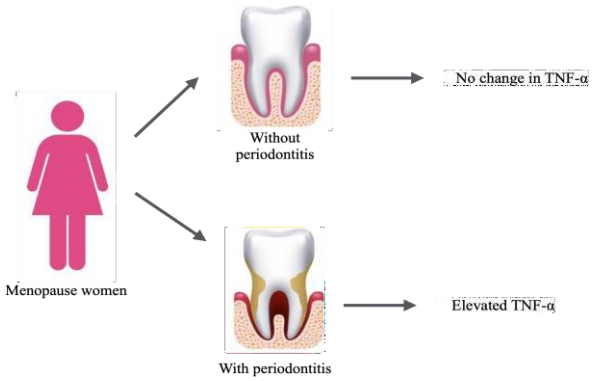

Figure1. Relationship between Menopause, Periodontal health, and Salivary TNF-α levels

Menopausal women are particularly vulnerable to periodontal diseases due to estrogen deficiency, which exacerbates inflammatory responses and disrupts the protective role of estrogen in maintaining bone and soft tissue health.[4] Estrogen receptors are present in periodontal tissues, and their activation modulates inflammatory cytokine expression. A decline in estrogen levels, therefore, creates a pro-inflammatory environment, which may increase the risk and severity of periodontitis in menopausal women. Consequently, investigating the levels of inflammatory mediators such as salivary TNF-α among menopausal women with and without periodontitis can provide insights into the interplay between systemic hormonal changes and oral inflammatory conditions.[5,6]

Several studies have explored the association between menopause, systemic inflammation, and periodontal health. For instance, Chakrabarti et al. (2014) reported elevated levels of TNF-α in postmenopausal women with periodontitis, suggesting that hormonal changes influence the inflammatory milieu of periodontal tissues.[7]

Similarly, Feres et al. (2016) demonstrated that menopausal women exhibited a higher prevalence and severity of periodontitis compared to premenopausal women, which was correlated with increased systemic and local inflammatory markers .[8] Furthermore, salivary biomarkers, including TNF-α, as non-invasive tool for assessing periodontal disease activity in systemic conditions such as menopause.[9]

Despite the growing body of evidence linking menopause to periodontal inflammation, clinicopathological comparisons of inflammatory markers like TNF-α in saliva among menopausal women with and without periodontitis remain underexplored. Saliva offers a convenient and non-invasive medium for assessing systemic and local inflammatory states, making it an attractive option for clinical research.[10] Studies comparing salivary TNF-α levels in menopausal women with varying periodontal statuses can bridge the knowledge gap and aid in understanding the underlying mechanisms linking menopause, inflammation, and periodontal health.[11]

The interaction between menopause-induced hormonal changes, inflammatory mediators such as TNF-α, and periodontal health represents a critical area of research with significant clinical implications. This study aims to investigate the clinicopathological differences in salivary TNF-α levels between menopausal women with and without periodontitis. By examining the relationship between salivary TNF-α levels and periodontal status, this research seeks to elucidate the role of systemic hormonal changes in modulating inflammatory responses in periodontal diseases. Additionally, the findings may contribute to the development of targeted strategies for the prevention and management of periodontal diseases in menopausal women, thereby improving their overall oral and systemic health.

MATERIALS AND METHODS

Study Design

This in vivo cross-sectional study was conducted on saliva samples collected from menopausal women,

both with and without periodontitis. The study was non-invasive and designed to minimize inconvenience to participants. Ethical approval was obtained from the Scientific Review Board (IHEC/SDC/BDS/1977/01). Despite its strengths, the study had a limitation of a small sample size.

Study Setting

A total of 20 samples (n=20) were collected from patients coming to clinics at Saveetha Dental College and Hospitals who were divided into two groups Group I consists of menopause women with periodontitis n=10, Group II consists of menopause women without periodontitis n=10.

Randomized sampling ensured unbiased selection, and validation of samples was performed by an expert pathologist. All participants were from the Dravidian ethnic group of South India. Informed consent was obtained from all participants, ensuring anonymity and compliance with ethical standards.

Selection Criteria for Study Subjects

Participants included menopausal women diagnosed with periodontitis and those without the condition. Patients with systemic comorbidities or terminal illnesses were excluded.

Sample Collection

Unstimulated saliva samples (1 ml) were collected from participants into Eppendorf tubes. Samples were immediately stored at -20°C. Before analysis, the samples were thawed and centrifuged. Collection took place between August 2024 and October 2024.

Principle of the Test

The assay was based on an enzyme-linked immunosorbent assay (ELISA) using a competitive binding technique. Tumor necrosis factor-alpha (TNF-α) in the samples competed with horseradish peroxidase (HRP)-labeled TNF-α for binding to a human monoclonal antibody immobilized in the wells. The bound TNF-α was detected using a biotinylated anti-human TNF-α antibody followed by HRP-conjugated streptavidin. The substrate 3,3′,5,5′-tetramethylbenzidine (TMB) was added to develop color, which changed from blue to yellow upon the addition of Stop Solution. The intensity of the yellow color was measured at 450 nm.

Reagent Preparation

Reagents and samples were brought to room temperature (18-25°C) prior to use. Assay Diluent B was prepared as a 5-fold dilution with deionized water, while Assay Diluent A was used for sample dilution, with a suggested dilution range of 2-20 folds for standard preparation, a 50 pg/ml TNF-α standard was serially diluted using Assay Diluent A for serum/plasma samples, with Assay Diluent B serving as the zero standard. The Wash Buffer was prepared by diluting a 20X concentrate with deionized water to yield 400 ml of 1X Wash Buffer. The detection antibody and HRP-streptavidin were briefly spun, mixed gently, and diluted as required for the assay.

Assay Procedure

Reagents and samples were equilibrated to room temperature before use, and all standards and samples were run in duplicate. A volume of 100 µL of each standard and sample was added to the wells and incubated at room temperature for 2.5 hours with gentle shaking. The wells were then washed four times with 1X Wash Buffer, followed by the addition of 100 µL of biotinylated antibody to each well, which was incubated for 1 hour with gentle shaking. After another washing step, 100 µL of prepared Streptavidin-HRP solution was added and incubated for 45 minutes. Subsequently, 100 µL of TMB substrate was added and incubated in the dark for 30 minutes. The reaction was stopped with 50 µL of Stop Solution, and the absorbance was measured at 450 nm.

Statistical Analysis

Statistical analysis was performed using SPSS Version 23.0. Salivary TNF-α levels were calculated with independent t-test to compare the groups and $p<0.05$ was considered significant.

RESULTS

Prevalence of Salivary TNF-α Levels in Group I menopausal women with periodontitis and Group II menopausal women without periodontitis exhibited detectable levels of salivary TNF-α (pg/ml), with Group I showing a higher mean concentration of 20.48 ± 3.10 compared to 11.32 ± 1.74 in Group II (Table 1). There was a notable and statistically significant difference in TNF-α levels between the two groups. Women with periodontitis exhibited

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significantly elevated TNF-α levels compared to those without periodontitis, suggesting a potential link between elevated TNF-α and the presence of periodontitis. A statistically significant difference ($p < 0.05$) was observed between the groups, with higher salivary TNF-α levels detected in menopausal women with periodontitis compared to those without periodontitis (Figure 2).

Table 1. Table showing Mean ± SD of Salivary TNF-α of Menopause women

Group	Salivary TNF-α levels (pg/ml) (Mean ± SD)	P value
Association between Menopause women with Periodontitis	20.48 ± 3.10	P = 0.001*
Association between Menopause women without Periodontitis	11.32 ± 1.74	

** statistically significant*

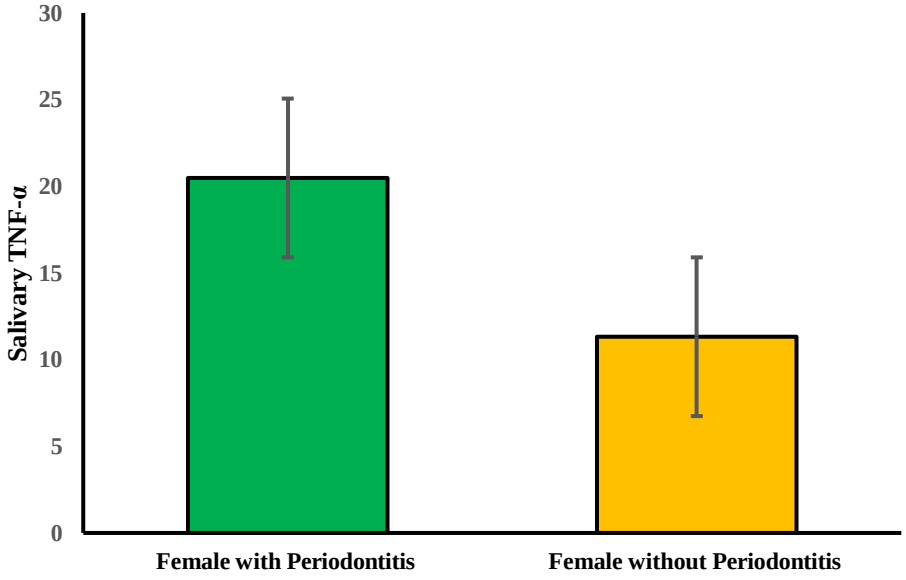

Figure 2. The bar graph illustrates the comparison of salivary TNF-α levels between women with and without periodontitis. The findings reveal that salivary TNF-α levels are higher in females with periodontitis compared to those without periodontitis, with error bars indicating standard deviations.

DISCUSSION

The present study aimed to compare salivary TNF-α levels between menopausal women with and without periodontitis. Results revealed a significantly higher concentration of salivary TNF-α in menopausal women with periodontitis (20.48 ± 3.10 pg/mL) compared to those without periodontitis (11.32 ± 1.74 pg/mL), with a *p*-value < 0.05. These findings are consistent with previous studies that highlight TNF-α as a key pro-inflammatory cytokine involved in periodontal pathogenesis and its exacerbation under systemic conditions such as menopause. Several studies in recent years have corroborated the role of TNF-α in both menopause and periodontal diseases. Sharma et al. (2020) reported elevated TNF-α levels in postmenopausal women with periodontitis, suggesting that estrogen depletion exacerbates periodontal inflammation by increasing pro-inflammatory cytokines.[12] Similarly, Rai et al. (2021) emphasized that reduced estrogen levels during menopause lead to systemic inflammation, manifesting as increased TNF-α in saliva and serum,

which contributes to periodontal tissue destruction.[13]

Further, Gupta et al. (2022) explored salivary biomarkers in menopausal women and observed significantly higher TNF-α levels in those with periodontal disease compared to their healthy counterparts. Their findings align with the current study, suggesting that menopause creates a pro-inflammatory environment that predisposes women to periodontal inflammation.[14]

While López-Marcos et al. (2021) proposed that salivary TNF-α can serve as a non-invasive biomarker for periodontal disease progression, particularly in systemic conditions like menopause, where inflammation have already heightened.[15]

From a pathophysiological perspective, estrogen deficiency in menopausal women disrupts immune homeostasis by promoting osteoclastogenesis and inflammatory cytokine expression, including TNF-α.[16] TNF-α, a potent pro-inflammatory mediator, contributes to periodontal tissue degradation by inducing matrix metalloproteinase (MMP) activity and enhancing the recruitment of inflammatory cells to the periodontium [17]. Taba et al. (2023) emphasized that elevated salivary TNF-α levels reflect ongoing periodontal tissue destruction and can differentiate periodontal disease severity.[18]

The present study also highlights the utility of saliva as a diagnostic fluid for detecting inflammatory markers. Saliva has been widely recognized as a non-invasive alternative to blood in assessing systemic and local inflammation.[19] Recent studies, including those by Al-Rawi et al. (2022), have demonstrated that salivary TNF-α levels correlate well with periodontal status and can be effectively used to monitor inflammatory conditions in menopausal women.[20]

Despite these findings, the small sample size of the present study remains as a limitation. Future studies with larger cohorts are required to confirm the generalizability of these results. Longitudinal studies assessing TNF-α levels before and after periodontal therapy in menopausal women could further clarify its diagnostic and prognostic value. Additionally, exploring other salivary inflammatory markers, such as IL-1β and IL-6, alongside TNF-α, may provide a more comprehensive understanding of the inflammatory burden in menopausal women with periodontitis.

CONCLUSION

The elevated salivary TNF-α levels observed in menopausal women with periodontitis interplay between systemic hormonal changes and local inflammatory responses. These findings align with recent literature and highlight the potential of TNF-α as a biomarker for periodontal disease assessment, particularly in vulnerable populations like menopausal women.

DECLARATIONS

Abbreviations

TNF-α – Tumour Necrosis Factor - Alpha

Conflict of Interest

There is no Conflict of Interest

Acknowledgement

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