



ESTIMATION OF THE LEVEL OF WHOLE SALIVARY CYTOKINES BEFORE AND AFTER REMOVAL OF FAULTY PROSTHESIS - AN IN-VIVO COMPARATIVE STUDY

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Abstract

Purpose: The aim of this study was to estimate and compare the levels of pro-inflammatory cytokines Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF- α) in whole unstimulated saliva of patients with faulty prosthesis before and 45 days after removal of the prosthesis.

Materials and Methods: This in vivo comparative study was conducted in the Department of Prosthodontics, Dr. Ziauddin Ahmad Dental College, AMU in collaboration with the Department of Biochemistry, JNMC, AMU. A total of 62 patients aged 40-60 years with faulty prosthesis for more than one month were randomly selected. Exclusion criteria included smoking, systemic diseases, anti-inflammatory or antibiotic therapy within three months, and pregnancy. Whole unstimulated saliva samples were collected following standardized protocols (2-hour fasting, mouth rinsing, 10-minute waiting period, and 5-minute collection). Samples were centrifuged at 4°C for 10 minutes and stored at -20°C until analysis. IL-6 and TNF- α levels were estimated using analyzing kits. Statistical analysis was performed using paired samples t-test.

Results: A statistically significant reduction in both cytokine levels was observed in the overall study population following prosthesis removal. IL-6 levels decreased from a mean of 51.76 ± 39.21 pg/mL to 14.97 ± 14.82 pg/mL ($t=8.82$, $p<0.001$). TNF- α levels reduced from 98.58 ± 59.77 pg/mL to 43.21 ± 15.14 pg/mL ($t=7.76$, $p<0.001$). However, gender-stratified analysis showed non-significant reductions in both male and female subgroups individually.

Conclusion: Removal of faulty prosthesis results in a significant reduction in salivary IL-6 and TNF- α levels, indicating resolution of oral inflammation. Salivary cytokine analysis serves as a valuable, non-invasive tool for monitoring inflammatory changes following prosthetic rehabilitation. Timely intervention to replace faulty prostheses may contribute to reducing both local and systemic inflammatory burden.

Keywords- Dental Prosthesis Failure; Saliva; Cytokine; Interleukin 6; Tumor Necrosis Factor Alpha; Salivary Biomarker; Oral Inflammation

Introduction

A dental prosthesis is a prosthesis used to restore residual ridge defects, missing teeth, missing structure of teeth and associated structures around it.[1] Because of a lack of willingness and financial restrictions, the number of rural residents who require tooth replacement seek treatment from a local, unqualified dentist.[2] This leads to botched procedures that are not only painful but also destructive. The common malpractice in India includes the use of wires to stabilize the teeth or denture with the support of adjacent teeth, which just damages the remaining healthy teeth. This procedure can be traumatic to patients as they can lead to excess bone loss and adjacent tooth loss. Another common malpractice is replacement of a missing tooth with artificial teeth with auto polymerizing resin directly in the mouth. The self-cure acrylic resin used for this purpose does not completely cure and can cause damage to the underlying gums and lead to bone resorption on adjacent teeth, and it is also a known carcinogenic material with high monomer content.[3] According to a study by Harrita et al., faulty prosthesis leads to periodontally compromised abutments, to decay of abutment tooth, gingival inflammation, denture stomatitis, non-vital abutment tooth. Faulty prosthesis may also lead to oral premalignant lesions and even oral malignancies have occurred on some occasions. Faulty prosthesis damages the abutment tooth and surrounding structure of oral mucosa.[4]

Interleukin-6 (IL-6) is known to be a proinflammatory cytokine, and it may be produced by various activated cells, including monocytes, macrophages, endothelial cells, fibroblasts, keratinocytes, and T and B lymphocytes. Interleukin-6 (L-6) is an important mediator of host response to tissue injury and infection. It plays a major role in differentiation of B cell and also enhances proliferation of T cell and bone resorption. The correlation between tissue levels of IL-6 and the severity of the coincident inflammation is significant. In mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis, spontaneous production of IL-6 has been reported. The levels of IL-6 may correlate with the severity of periodontal disease. [5] Studies have shown that in case of periodontal inflammation there is higher systemic levels of C-reactive protein, interleukin (IL)-6, and neutrophils. These elevated inflammatory factors may increase inflammatory activity in atherosclerotic lesions, potentially increasing the risk for cardiac or cerebrovascular events.[6] Periodontitis has been considered as a separate risk factor for some cardiovascular, respiratory, endocrine, musculoskeletal, and reproductive system related abnormalities.[7]

TNF- α is a major signal for cellular apoptosis, bone resorption, MMP secretion, intercellular adhesion molecule (ICAM) expression, and interleukin-6 (IL-6) production. Any condition producing elevations in serum IL-1 β /TNF- α has potential to cause hyperlipidemia. TNF- α has been implicated as a causative factor in insulin resistance and Type 2 diabetes in animal models and in human studies.[8,9] Elevated levels of TNF- α alter intracellular insulin signalling (inhibiting tyrosine kinase activity of the insulin receptor) and reduce synthesis of the insulin- responsive glucose transporter, creating an insulin resistance syndrome similar to the insulin resistance that characterizes Type 2 diabetes.[9,10] Additionally, TNF- α has been implicated in the development of macrophage-dependent cytotoxicity of pancreatic islets in diabetes.

The subsequent production of cytokines such as interleukins (IL-1, IL-6), Tumor necrosis factor (TNF- α), and prostaglandins (PGE₂), which have been implicated in the mechanism of labour.[11] The recently proposed mechanism of labour suggests that the intra-amniotic levels of these mediators rise steadily throughout pregnancy until a threshold is reached at which labour is induced.[12] Thus,

it raises the possibility that the presence of infection results in an abnormally elevated production of the normal physiological mediators of parturition, which may trigger births, also resulting in low birth weight.

Systemic Saliva is said to be a "mirror of the body". It is an indicator of health not just in the oral cavity but throughout the body.[13] About 99.5% of its total volume is water and the rest of 0.5% includes different ions, biochemical and many other important biomarkers.[14] Salivary analysis has become an important resource for monitoring general health and the state of disease due to its origin, composition equivalent to serum, and interactions with other organs.

Thus the aim of this study was to estimate the level of pro-inflammatory mediator cytokines IL-6 and TNF- α , in whole unstimulated saliva of patients with faulty prosthesis before and 45 days after removal, and perform a comparative analysis.

Materials and Methods

The present in vivo study will be conducted in the Department of Prosthodontics and Crown & Bridge, Dr. Ziauddin Ahmad Dental College, AMU in collaboration with Department of Biochemistry, JNMC, AMU.

Armamentarium

1. Test tube
2. Centrifugal machine
3. Eppendorfs
4. Deep freezer
5. Analysing kit for IL-6 and TNF- α

Methodology

To avoid the possibility of contamination of saliva the following instructions were given to the patients: Do not eat, drink, and use oral hygiene measures for 2 hours before collection of saliva, rinse mouth with water to remove food residue before sample collection, swallow to increase hydration and wait at least (10 minutes) after rinsing before collecting saliva to avoid sample dilution. After rinsing, patients were seated comfortably in the dental chair with eyes open and head tilted slightly forward. Saliva was collected in the mouth while patient kept his lips closed saliva was collected for five minutes (5-10 ml) from each patient into a sterile vessel. Saliva was immediately centrifuged at for 10 minutes and stored at (-20°C) until subsequent analysis.

A total number of 62 patients with faulty prosthesis, aged 40-60 years, visiting the department of Prosthodontics and Crown & Bridge, ZADC (AMU) were randomly selected for the study. All individuals were in good general health and had not received any previous dental treatment or taken antibiotics, immunomodulatory or anti-inflammatory drugs in the 3 months before the study.

Exclusion criteria

1. Smoking, alcohol drinking
2. Systemic antibiotic therapy or anti-inflammatory therapy within the last three months
3. Patients with any systemic disease including diabetes. viral, fungal, or bacterial infections and cardiovascular diseases
4. Pregnant and lactating women

Inclusion criteria

1. Patients with faulty prosthesis for more than one month
2. Patients with the age between 40-60 years
3. Patients who are willing to be a part of this study
4. Mentally and systemically healthy patients

This was a comparative study and results from the data collected were obtained by student t- test. Any necessary changes, modification and requirements appropriate for study were made according to the conditions.

Results

A paired-samples t-test was conducted to examine differences in inflammatory markers before and after treatment. Results indicated a statistically significant reduction in Interleukin-6 levels following treatment. The mean score decreased from 51.76 (SD = 39.21) prior to treatment to 14.97 (SD = 14.82) after treatment, $t(61) = 8.82, p < .001$. Similarly, TNF- α levels demonstrated a significant decline from pre-treatment (M = 98.58, SD = 59.77) to post-treatment (M = 43.21, SD = 15.14), $t(61) = 7.76, p < .001$. These findings indicate that the treatment was associated with a substantial reduction in inflammatory responses among participants.

Table 2 presented the results of paired-samples *t*-tests examining pre- and post-treatment differences in IL-6 and TNF- α levels among female participants ($n = 22$). For IL-6, the mean value decreased from 29.36 (SD = 25.77) before treatment to 19.37 (SD = 18.52) after treatment. However, this reduction was not statistically significant, $t(21) = 1.39, p = .177$. Similarly, TNF- α levels showed a slight increase from a pre-treatment mean of 62.95 (SD = 43.14) to a post-treatment mean of 67.48 (SD = 70.06). This change was also not statistically significant, $t(21) = 0.25, p = .804$. Overall, the findings indicate that the intervention did not produce statistically significant changes in IL-6 or TNF- α concentrations among female participants.

Table 3 summarized the paired-samples *t*-test analyses comparing biomarker levels before and after treatment among male participants ($n = 37$). IL-6 levels increased slightly from a mean of 28.22 (SD = 25.22) at baseline to 31.31 (SD = 36.82) following treatment. This difference was not statistically significant, $t(36) = 0.44, p = .664$. In the case of TNF- α , the mean level declined from 60.84 (SD = 30.70) prior to treatment to 53.08 (SD = 33.83) after treatment. However, this reduction did not reach statistical significance, $t(36) = 1.01, p = .317$. These findings suggest that the treatment did not result in meaningful changes in IL-6 or TNF- α levels among male participants.

Table 1

Mean, Standard Deviation, and Paired Samples t-Test for Before and After Treatment on Measures Interleukin-6 and TNF- α

Variable	Before Treatment		After Treatment		<i>t</i> (61)	<i>p</i>
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
Interleukin-6	51.76	39.21	14.97	14.82	8.82***	<.001
TNF- α	98.58	59.77	43.21	15.14	7.76***	<.001

Note. $N = 62$.

*** $p < .001$.

Table 2

Before and After Comparison of Biomarkers IL-6 and TNF-Alpha in Female Participants

Variable	Before Treatment		After Treatment		<i>t</i> (21)	<i>p</i>
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
IL-6	29.36	25.77	19.37	18.52	1.39	.177
TNF-Alpha	62.95	43.14	67.48	70.06	0.25	.804

Note. $n = 22$.

Table 3

Before and After Comparison of Biomarkers IL-6 and TNF-Alpha in Male Participants

Variable	Before Treatment		After Treatment		<i>t</i> (36)	<i>p</i>
	<i>M</i>	<i>SD</i>	<i>M</i>	<i>SD</i>		
IL-6	28.22	25.22	31.31	36.82	0.44	.664

TNF-Alpha	60.84	30.70	53.08	33.83	1.01	.317
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Note. $n = 37$.

Discussion

The present study aimed to evaluate the levels of pro-inflammatory cytokines IL-6 and TNF- α in the whole unstimulated saliva of patients with faulty prosthesis before and 45 days after removal of the prosthesis. The findings demonstrated a statistically significant reduction in both IL-6 and TNF- α levels following removal of the faulty prosthesis in the overall study population ($p < 0.001$). However, when stratified by gender, the reduction did not reach statistical significance within the male and female subgroups individually, suggesting that the overall significance was driven by the pooled sample size and possibly influenced by gender-related factors.

The significant decline in salivary IL-6 levels from a mean of 51.76 pg/mL to 14.97 pg/mL ($p < 0.001$) following prosthesis removal represents a clinically meaningful reduction in oral inflammation. This finding aligns with the established role of IL-6 as a key pro-inflammatory cytokine produced by various activated cells including monocytes, macrophages, fibroblasts, and lymphocytes in response to tissue injury and infection [5]. The elevated baseline levels of IL-6 in patients with faulty prosthesis can be attributed to the chronic inflammatory stimulus posed by ill-fitting prostheses, which cause mechanical trauma, plaque accumulation, and subsequent gingival inflammation.

The findings of the present study are consistent with existing literature examining inflammatory responses to dental prostheses. A systematic review and meta-analysis by various authors examining cytokine profiles in patients with fixed dental prostheses demonstrated that metal-ceramic prostheses tend to elicit higher levels of inflammatory mediators including IL-6 and TNF- α compared to all-ceramic prostheses, confirming that prosthetic materials and their adaptation significantly influence periodontal inflammatory responses. [15] Similarly, Bradić-Vasić et al. in their 2025 study on patients with denture stomatitis reported significantly increased levels of salivary TNF- α and other inflammatory biomarkers in denture wearers with mucosal inflammation compared to controls, reinforcing the association between faulty prostheses and elevated salivary cytokines. [16]

The reduction in TNF- α levels observed in our study from 98.58 pg/mL to 43.21 pg/mL ($p < 0.001$) following prosthesis removal is particularly significant given the multifaceted role of this cytokine. TNF- α is a major signal for cellular apoptosis, bone resorption, MMP secretion, and interleukin-6 production [8,9]. Elevated TNF- α has been implicated in insulin resistance and Type 2 diabetes pathogenesis by altering intracellular insulin signaling [9,10]. The substantial decrease following removal of the faulty prosthesis suggests that eliminating the chronic inflammatory source may have systemic implications beyond oral health.

Pervov et al. investigated cytokine changes during dental orthopedic fixed prosthetics and observed that mucosal immunity of oral mucous membranes was significantly influenced by the type of prosthetic material used, with metal-ceramic crowns inducing greater inflammatory responses compared to zirconium dioxide crowns. Their finding that changes in salivary cytokine indices reflect the level of reaction of oral mucosal immunity supports our use of saliva as a valid medium for assessing prosthesis-related inflammation. [17]

The gender-based differences observed in our study, where neither males nor females showed statistically significant reductions when analysed separately despite the overall significant finding, warrant consideration. This may be attributed to the smaller subgroup sample sizes reducing statistical power, or potentially to hormonal influences on inflammatory responses. Rai et al. have previously noted the role of cytokines in pregnancy-related gingival conditions, suggesting that gender-specific hormonal variations may modulate inflammatory mediator production [11].

The clinical implications of our findings extend beyond the oral cavity. Periodontitis and chronic oral inflammation have been recognized as risk factors for cardiovascular, respiratory, endocrine, and reproductive system abnormalities [7]. Loos demonstrated that periodontal inflammation is associated with higher systemic levels of C-reactive protein, IL-6, and neutrophils, potentially increasing inflammatory activity in atherosclerotic lesions [6]. The significant reduction in salivary cytokines

following prosthesis removal suggests that eliminating faulty prostheses may contribute to reducing systemic inflammatory burden.

Saliva has emerged as a valuable diagnostic medium, often described as a "mirror of the body" [13]. Its non-invasive collection, composition comparable to serum, and ability to reflect both local and systemic health make it an ideal substrate for monitoring inflammatory conditions. The significant changes detected in salivary IL-6 and TNF- α in our study reinforce the utility of salivary analysis for assessing treatment outcomes in prosthodontic patients.

Limitations

The present study has certain limitations. The relatively small sample size, particularly when stratified by gender, may have limited the statistical power to detect significant differences within subgroups. The 45 days follow-up period, while adequate to detect initial changes, may not capture long-term inflammatory resolution. Additionally, potential confounding factors such as oral hygiene practices, dietary habits, and socioeconomic status were not extensively controlled.

Conclusion

Within the limitations of this study, it can be concluded that removal of faulty prosthesis results in a statistically significant reduction in salivary IL-6 and TNF- α levels, indicating resolution of oral inflammation. These findings underscore the importance of timely intervention to replace faulty prostheses, not only for oral health but potentially for reducing systemic inflammatory burden. Salivary cytokine analysis proves to be a valuable, non-invasive tool for monitoring inflammatory changes following prosthetic rehabilitation. Further studies with larger sample sizes and longer follow-up periods are warranted to validate these findings and explore the systemic implications of prosthesis-related inflammation.

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