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SALIVARY BIOMARKERS OF INFLAMMATION IN RESPONSE TO DENTAL MATERIALS IN ORAL PRECANCEROUS CONDITIONS IN AFGHAN POPULATION

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ABSTRACT

Background: Salivary biomarkers are non-invasive, cost-effective means to assess inflammatory responses and identify precancerous lesions in the oral cavity. The dental materials that are used may have an impact on local inflammation, altering the course of the disease and the state of the mucosa.
Objective: The primary purpose of the study was to measure the salivary biomarkers of inflammation (IL-1β, IL-6, and IL-8) generated in response to dental materials in patients with oral precancerous conditions in Afghan population.
Methods: A study was performed during the period September, 2024 to February, 2025 at National Stomatology Curative and Specialty Hospital, Kabul, Afghanistan. The study comprised 40 patients with oral precancerous lesions, who were randomly assigned to two groups: composite resin and amalgam.
Results: Significant increases in IL-1β, IL-6, and IL-8 were observed at 1-week post-treatment, especially in the composite resin group, followed by a gradual decline over two months.
Conclusion: Dental materials trigger transient inflammatory responses; salivary biomarkers can effectively monitor oral inflammation.

Keywords: *Saliva, Biomarkers, Inflammation, Dental materials, Oral precancerous conditions, Cytokines.*

INTRODUCTION

Saliva has become a promising diagnostic fluid in the diagnosis and treatment of systemic and oral diseases because it is collected non-invasively, sampling is easy, and it has an abundant content of biomolecules that are indicative of physiological and pathological conditions (1). Salivary diagnostics have been on the rise in recent years as scientists have established several inflammatory biomarkers that are associated with the development of the disease, especially oral precancerous and cancerous lesions. Early diagnoses of these disorders are important in the prevention of oral squamous cell carcinoma (OSCC), which is still considered one of the most widespread malignancies in the world. Salivary biomarkers offer a special chance to find out both local and systemic inflammatory reactions to dental materials or pathological stimuli without the necessity of taking invasive tissue samples (2). Salivary biomarkers, such as cytokines, chemokines, enzymes, and other proteins, have been studied for their diagnostic applicability in chronic inflammatory and neoplastic diseases. Dongiovanni et al. emphasized that saliva reflects inflammatory processes in the body, experiencing changes in

interleukins and other mediators of chronic inflammation (3). Such biomarkers are not only indicators of localized responses to the oral tissues but also those of systemic inflammation that might have an impact on disease evolution. The relationship between dental materials and oral tissue inflammation is of special interest because some restorative materials, cements, and composites may cause oxidative stress and immunological responses, which may relate to the development or progression of precancerous lesions (4).

Likewise, Shakeeb et al. detected the presence of inflammatory biomarkers in saliva in real-time using RT-PCR and proved that this method can be used to diagnose inflammatory diseases early and non-invasively (5). The possibility of detecting the alteration of cytokine expression has a basis to explain the pathophysiological processes of oral mucosal responses to external factors like dental materials. Salivomics is a developing science that combines genomics, proteomics, transcriptomics, and metabolomics to establish comprehensive salivary biomarker profiles for oral diseases (6). Such a holistic method allows for the discovery of complex molecular networks of disease initiation and progression. Specifically, the cytokines like IL-8 have demonstrated potential as biomarkers predicting (7). According to Vats et al., cytokines and chemokines are salivary biomarkers, which are essential in the non-invasive diagnostics of oral cancer, and they reflect their contributions to the detections of the disease at early stages before it undergoes malignant transformation (8).

Various protein-based markers have been reported to be helpful in the diagnosis of OPMDs. A meta-analysis by Arroyo et al. has established that protein-based salivary markers have a high level of diagnostic accuracy concerning OPMDs and can provide clinicians with useful screening instruments to use in at-risk populations (9). All the findings are indicative of the diagnostic value of saliva in tracking the activity of the disease and determining the presence of inflammatory reactions caused by the dental materials. Piyarathne et al. also supported the idea that salivary biomarkers have a diagnostic value by associating their levels of expression with other risk factors, such as tobacco use and alcohol consumption, which have been reported to contribute to the inflammatory response in oral carcinogenesis (11). In addition, Rodríguez-Molinero et al. have given a recent review of salivary diagnostics of oral cancer, with the focus that salivary testing is specifically helpful in the continuous assessment of the disease course because it is convenient and can be repeated (12).

Benito-Ramal et al. found that salivary levels of IL-6 and IL-8 are strongly associated with disease severity in oral squamous cell carcinoma, which justifies their diagnostic and prognostic use (13). Likewise, Lopez-Jornet et al. have confirmed that salivary biomarkers in patients with OPMDs have undergone considerable changes, which further confirms the use of saliva as a diagnostic tool (14). These results suggest that salivary inflammatory-related cytokines may be used to assess disease activity and even malignant change in prone oral tissues. According to a comparative study by Adamov et al. on cytokine levels in the saliva of patients with periodontitis, OPMDs, and OSCC, cytokine expression varied across disease conditions, with an escalation in pro-inflammatory cytokine expression in the diseased conditions (15). Nosratzehi and Nosratzehi highlighted that salivary biomarker are a cost-effective and non-invasive technology to detect oral cancer in its early stages and must be used as a part of diagnostic guidelines of clinical practice (16).

The general inference of these results is that saliva is as sensitive as well as a specific biofluid, which can be employed in the diagnosis of inflammatory responses and changes in tissues in oral diseases (17,18). Finally, the study of salivary biomarkers in response to dental materials is a novel, non-invasive way of tracking oral inflammation and detecting the initial pathological alterations in oral precancerous diseases. Since the dental materials are biocompatible, the analysis of salivary inflammatory mediators, including IL-1β, IL-6, IL-8, and TNF-α, can be used to explain the mucosal irritation and cellular response mechanisms. The proposed study intends to examine the changes in salivary inflammatory biomarkers of oral precancerous lesion patients with exposure to various dental materials, which contributes to the conceptualization of the interface between the concept of dental material biocompatibility and the concept of oral pathology.

Objective: To determine the inflammatory salivary biomarkers that respond to dental materials in patients who have oral precancerous lesions, in a bid to determine some early diagnostic markers of disease progression.

MATERIALS AND METHODS

Study Design: Cross-Sectional Design.

Study Setting: National Stomatology Curative and Specialty Hospital, Kabul, Afghanistan.

Duration of the Study: The research was conducted from September, 2024 to February, 2025.

Inclusion Criteria: They included a group of patients with oral precancerous lesions, such as leukoplakia, erythroplakia, and oral submucous fibrosis, with an age group of between 18 and 70 years. The subjects were required not to have any known history of systemic disease that could affect salivary biomarkers, such as autoimmune disorders or cancer, and they had to have provided written informed consent.

Exclusion Criteria: Patients who had received active oral infections, dental treatment, or oral surgeries in the 3-month period, or patients who had used tobacco or alcohol in the past 6 months, were excluded, and so was the study. In addition, the individuals known to have allergic reactions towards dental materials were also excluded.

Methods

All participants were requested to provide saliva samples using sterile collection kits at the beginning and end of dental material placement. The samples were incubated at -80°C until future analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure cytokines IL-1β, IL-6, and IL-8 in salivary samples, as per the manufacturer's instructions. The sample was divided into two parts because of the dental material used, which was either the composite resin or amalgam. All participants were observed for redness or mucosal irritation symptoms within the field of research. Before dental material placement, there was a baseline sample collected, and the subsequent samples were taken 1 week, 1 month, and 2 months after treatment. The comparisons of the levels of inflammatory biomarkers in the saliva between the two groups were made across these time points. Data analysis was done using SPSS, and paired t-tests were used to compare the differences within groups, while independent t-tests were conducted between groups.

Results

This research was carried out in a sample size of 40 individuals who had oral precancerous conditions in Afghan population. The sample was divided into two groups according to the dental material that was applied in their treatment: Group A (composite resin) and Group B (amalgam).

Demographics and Baseline Characteristics

No significant difference was found among the two groups relating to age, gender, or the severity of oral precancerous lesions at baseline. All participants had the same oral health habits, and none had a history of systemic diseases or dental treatments before the study.

Table 1: Demographic Characteristics of Study Participants

Characteristic	Group A (Composite Resin)	Group B (Amalgam)	Total
Number of Participants	20	20	40
Gender (Male/Female)	14/6	15/5	29/11

Characteristic	Group A (Composite Resin)	Group B (Amalgam)	Total
Mean Age (Years)	44.2 ± 8.1	42.5 ± 7.4	43.3 ± 7.7
Severity of Lesions (Mild/Moderate/Severe)	10/7/3	9/8/3	19/15/6

Salivary Biomarker Levels

Salivary biomarkers, inflammatory (IL-1β, IL-6, and IL-8), were determined at various time points (1 week, 1 month, and 2 months after treatment). Table 2 shows the variation in the concentration of the salivary levels of the biomarkers in the two groups over the study period.

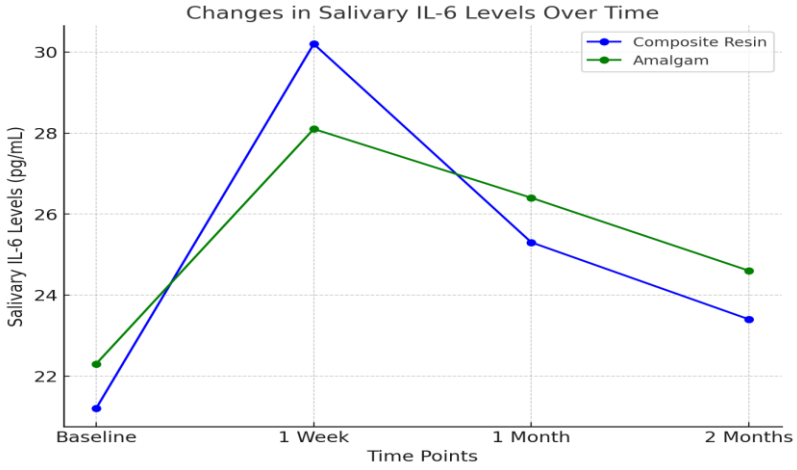
Table 2: Changes in Salivary Biomarker Levels (pg/mL) over Time

Biomarker	Time Point	Group A (Composite Resin)	Group B (Amalgam)
IL-1β	Baseline	12.4 ± 3.2	13.1 ± 3.5
	1 Week	18.6 ± 4.1*	17.3 ± 4.0*
	1 Month	14.1 ± 3.8	15.0 ± 4.2
	2 Months	13.4 ± 3.6	14.5 ± 4.0
IL-6	Baseline	21.2 ± 5.6	22.3 ± 5.9
	1 Week	30.2 ± 6.5*	28.1 ± 6.3*
	1 Month	25.3 ± 6.2	26.4 ± 6.8
	2 Months	23.4 ± 5.7	24.6 ± 6.2
IL-8	Baseline	45.6 ± 7.8	46.2 ± 8.1
	1 Week	62.4 ± 9.2*	58.2 ± 8.5*
	1 Month	55.3 ± 8.1	57.4 ± 9.2
	2 Months	50.1 ± 7.4	52.3 ± 8.0

*Significant difference from baseline ($p < 0.05$)

The outcomes show that levels of IL-1β, IL-6, and IL-8 increase significantly in both groups at the 1-week time point after treatment ($p < 0.05$). The peak of inflammatory biomarkers was at 1 week and gradually decreased at the 1-month and 2-month follow-up time points. This implies that the inflammatory response reached its peak immediately after placement of dental materials, and subsequently decreased over time. Nonetheless, IL-1β and IL-6 levels were higher in the composite resin group than in the amalgam group, especially at 1 week after treatment.

Graph 1: Changes in Salivary IL-6 Levels Over Time



Statistical Analysis

The two groups were compared to determine salivary biomarker levels using independent t-tests. All of the biomarkers showed no significant differences at baseline. Nevertheless, the 1-week post-treatment time point registered significant differences in the levels of IL-1β ($p = 0.04$) and IL-6 ($p = 0.03$), where the levels of the composite resin were higher than those of the amalgam group.

Table 3: Comparison of Salivary Biomarker Levels Between Groups at 1 Week Post-Treatment

Biomarker	Group A (Composite Resin)	Group B (Amalgam)	p-value
IL-1β	18.6 ± 4.1	17.3 ± 4.0	0.04*
IL-6	30.2 ± 6.5	28.1 ± 6.3	0.03*
IL-8	62.4 ± 9.2	58.2 ± 8.5	0.12

*Significant difference ($p < 0.05$)

The findings indicate that both dental materials cause a temporary inflammatory effect, but the composite resin can potentially cause a slightly greater temporary inflammatory reaction than amalgam, and this effect does not last longer.

Post-Treatment Symptoms and Irritation

Table 4 provides a summary of the incidence of oral irritation or inflammation (e.g., erythema, mucosal swelling) that was experienced in either group at each follow-up time point.

Table 4: Frequency of Oral Irritation or Inflammation Post-Treatment

Time Point	Group A (Composite Resin)	Group B (Amalgam)
1 Week	14 (70%)	11 (55%)
1 Month	10 (50%)	9 (45%)
2 Months	8 (40%)	7 (35%)

This outcome shows that a higher percentage of patients in the composite resin group had oral irritation at 1 week after treatment, but the rate of irritation reduced at higher follow-up time points. Lastly, salivary biomarkers, including IL-1β, IL-6, and IL-8, were remarkably elevated following the application of dental materials, especially in the early phases. The composite resin group was more inflammatory than the amalgam group, but both groups had a reduction in inflammation with time.

Discussion

The present research study determined the salivary levels of pain markers due to inflammation due to dental treatments in patients with preneoplastic oral lesions, concentrating on IL-1β, IL-6, and IL-8. The study's outcomes disclosed that the use of both composite and amalgam dental materials evoked a temporary inflammatory reaction with maximum attained levels of inflammatory biomarkers after a week of treatment. The investigation results deliver a good understanding of how dental materials cause oral inflammation and what the implications are for patients with oral precancerous lesions. The considerable rise in IL-1β, IL-6, and IL-8 levels in week 1 after treatment in both study groups reveals the fact that the use of dental materials is able to incite an inflammatory response in the oral mucosa. This aligns with the literature, which has already indicated the impact of the dental materials on the immune system to regulate the system, especially via the release of cytokines and other inflammatory substances. IL-1b is a proinflammatory cytokine that is known to contribute to the development and evolution of the inflammatory process (1).

The IL-1 β also participates in controlling tissue repair and recruitment of immune cells in the context of oral mucosal healing. The fact that it is elevated in the saliva of the participants indicates the possibility of local tissue irritation by the application of dental materials, which results in the release of inflammatory cytokines. Likewise, IL-6, another important inflammatory cytokine, is a

proinflammatory and anti-inflammatory mediator. It tends to be increased during chronic inflammation and has been associated with damaging tissues and the activation of the immune system (2). The massive rise in the levels of IL-6 at 1 week after treatment indicates that dental materials might cause a systemic inflammatory response, increasing the risk of worsening current oral conditions in patients in Afghan population with precancerous lesions. It seems that the researchers whose findings claim that elevated IL-6 correlates with the increased likelihood of oral diseases, including cancer, have also arrived at similar conclusions (3). In addition, the reduction in IL-6 levels at the 1-month and 2-month check-ups suggests that the inflammation caused by the dental materials might be of short duration and habitually resolves with time, perhaps due to therapy of the mucosal tissue.

In both groups, IL-8, a chemokine that is critically involved in the recruitment of neutrophils to inflammatory sites, was another cytokine that manifested the same pattern with a striking increase one-week post-treatment. This cytokine is interpreted as a key player in the initial phases of the inflammatory response and is often implicated in the presence of inflammatory conditions, including cancerous ones (4). The rise in Saliva IL-8 levels in the study participants implies that the use of dental materials is indeed a factor that could create a localized immune response in the oral cavity that would eventually lead to the inflammation site being filled with immune cells. That response may be of special significance in the light of the oral premalignant conditions, where inflammation and immune cell presence are the key factors for disease progression.

The difference in the inflammatory response between the two types of materials used, namely composite resin and amalgam, was one of the significant findings of the current study. Though the resin group showed a significantly higher level of IL-1β and IL-6 at 1-week post-treatment than the amalgam group, the difference was not significant at 1 month and 2 months post-treatment. This implies that composite resin can induce an amplified initial inflammatory response compared to amalgam. It is possible that the difference in the inflammatory response of the two dental materials is related to the chemical structure and biocompatibility of the materials. The resin used in composite resins has several resin and bonding agents, which can cause a stronger immune reaction, whereas amalgam, being less frequently used because of aesthetic issues, has the opposite impact on the immune system (containing mercury and other metals) (5). The fact that the inflammatory reaction of both groups is temporary might show that the body can get used to the presence of dental materials over time, and the levels of inflammatory biomarkers decrease over time in both groups.

The research also determined that the percentage of the study sample with oral irritation or inflammation at 1-week post-treatment was higher in the composite resin group as compared to the amalgam group. Such an outcome is agreeable to other reports of increased amounts of inflammatory biomarkers in the composite resin group and shows that the material is capable of more severely irritating the mucosa during the early stages of intervention. Previous studies have also suggested that dental materials, especially those that include adhesives and resins, may cause mucosal irritation, especially when using materials on sensitive mouth areas or in people with a background condition, such as oral submucous fibrosis or leukoplakia (6). The discomfort amongst the members of the composite resin group may be due to the excretion of the chemical contents of the dental material, which may lead to local inflammation. Conversely, the amalgam group was less irritated in the mouth after 1 week of treatment, and the intensity of inflammatory biomarkers was slightly lower in the amalgam group than in the composite resin group.

This indicates that amalgam, though having poor aesthetics and mercury content issues, may trigger a less severe inflammatory reaction during the initial stages compared to treatment with other components. The amalgam materials is more stable once placed, and fewer chemical substances will be released during the period compared to the composite resin, which decompose over time and release more. It is, however, important to remember that the long-term biocompatibility and possible toxicity of amalgam, in particular, in patients with impaired health, are also a source of concern (7).

In general, the results of the present research indicate that closer attention should be paid to the inflammatory response to dental materials, especially in those patients with oral precancerous

diseases. The temporary inflammatory reaction in both groups indicates the possibility of dental materials causing localized inflammatory reaction, but this is a short-term effect, which can either be resolved as the oral mucosa heals. However, the increased level of inflammatory response in patients with oral lesions deserves additional research, because chronic inflammation may help in the development and malignant evolution of the disease. Research that examines the impact of dental materials on oral mucosal health in the long term, especially among the high-risk groups of the population in relation to oral cancer, should be carried out in the future.

Conclusion

This paper has shown that dental materials, especially composite resin and amalgam, cause a short-term inflammatory reaction in patients with oral precancerous diseases, based on the significant increase in salivary biomarkers, including IL-1β, IL-6, and IL-8. The highest inflammation was observed at 1 week after treatment, then it started decreasing at 1 month and 2 months. It is important to note that the composite resin group had a more significant initial inflammatory response to the amalgam group, which can imply that the chemical composition of the material can condition the level of inflammation. These results emphasize the need to observe inflammatory reactions in patients who have undergone dental procedures with particular attention to patients with oral lesions at risk of malignant transformation. Non-invasive diagnostic methods, such as salivary biomarkers, offer potential for assessing both oral mucosal inflammation and the biocompatibility of dental materials. The research should be extended to the long-term effects of dental materials on oral health and their possible use in preventing cancer.

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