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RESEARCH ARTICLE

Elevated Salivary IL-8 Levels in Patients with Aphthous Ulceration and Rheumatoid Arthritis: A Cross-Sectional Study

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ABSTRACT

Background: **Aphthous ulcers (AU) and Rheumatoid Arthritis (RA) are conditions marked by inflammation, with Interleukin 8 (IL-8) playing a significant role in their pathogenesis. This study aims to evaluate the levels of IL-8 in saliva among individuals with AU, RA, both conditions and healthy controls, providing insights into its potential as a diagnostic biomarker and therapeutic target.**

Methods: **The study, endorsed by the Ethics Committee of the University of Kufa, College of Dentistry, Department of Oral Pathology, employed a descriptive, analytical, and cross-sectional approach. Participants included patients with AU, RA, both conditions and a control group, totalling 94 individuals. Exclusion criteria encompassed medication use, other systemic conditions, and lifestyle factors like smoking. Saliva and blood samples were collected for IL-8 concentration measurement using ELISA and for Rheumatoid Factor (RF) and anti-CCP analysis.**

Results: **Analysis showed the highest average salivary IL-8 levels in patients with both AU and RA (483.33 ± 141.20 pg/mL), followed by the RA group (338.57 ± 79.11 pg/mL), the AU group (381 ± 108.48 pg/mL), and the control group (213.69 ± 84.65 pg/mL). Significant differences in IL-8 levels were observed between these groups, with the combined AU and RA groups showing the highest concentrations. However, the variation in IL-8 levels between those who have both AU and RA and those with only RA was not statistically significant.**

Conclusion: **The study highlights a distinct elevation in salivary IL-8 levels in patients with AU and RA, particularly in those suffering from both conditions.**

Keywords: Aphthous Ulceration (AU), Inflammatory Cytokines, Interleukin 8 (IL-8), Rheumatoid Arthritis (RA).

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1. Introduction

Aphthous ulcers, also known as canker sores are sores that often occur in the mouth. The exact cause of these ulcers is not fully. It is believed to involve a combination of factors, immune system problems and environmental triggers. Research suggests that cellular immunity T cell responses play a role, in the development of these ulcers. Other factors such as deficiencies (such as levels of vitamin B12, iron and folate) hormonal changes, stress and certain food sensitivities may also contribute to their occurrence. Although the involvement of microbes in ulcers is not yet clearly established, they could potentially play a part. Due to the nature of this condition and its various causes managing ulcers requires a comprehensive approach involving multiple treatment strategies [1].

In this context, Interleukin 8 (IL 8) plays a part as a mediator in responding to tissue damage and inflammation by activating immune cells like lymphocytes. It is involved in processes. Can be produced by different cell types such as monocytes/macrophages, lymphocytes and endothelial cells under pathological conditions [2].

Under circumstances IL 8 levels are typically low in tissues; however, they can significantly increase when there are inflammatory cytokines like tumor necrosis factor-alpha (TNF α) and IL 1 present or due to microbial

infections and cellular stress. In patients with ulceration, an elevation in IL 8 production has been observed. Additionally, TNF α can be produced by mononuclear cells around the lesions [3].

Different cells that are stimulated by IL 1 and TNF α, in these lesions can release amounts of IL 8 further promoting the infiltration of cells and playing a role in the development of aphthous ulcers [4].

According to some studies, patients have considerably greater blood and saliva levels of IL 8 than do healthy people. This suggests that when testing for inflammatory cytokines in these individuals, saliva may be a better option than blood. The increased levels of IL 8 imply that it may be used as a marker to detect ulcers in Rheumatoid Arthritis [5].

Rheumatoid arthritis (RA) is a term disorder that mainly affects the joints but can also impact other parts of the body. One of the characteristics of RA is the presence of inflammation, which is caused by a complex interplay of cytokines and immune cells. Among these cytokines, Interleukin 8 (IL 8) plays a role [6].

IL 8 belonging to a group of chemokines called CXC chemokines is primarily known for its role, in attracting and activating neutrophils. In the context of RA elevated levels of IL 8 can be found in both the fluid and blood serum of individuals affected by the condition. This increase, in IL 8 has been associated with the severity of the disease, especially regarding inflammation and damage [7].

Rheumatoid arthritis (RA) is a condition where the joints are primarily affected by inflammation due, to a response. However, it's important to note that RA can also have an impact beyond the joints. The role of IL 8 in RA is quite complex. Firstly, it plays a role in attracting neutrophils to the areas of inflammation. Once these neutrophils are activated, they release enzymes and reactive oxygen species, which contribute to the characteristic tissue damage seen in RA. Moreover, IL 8 also promotes the growth and survival of fibroblasts, which play a role in RAs development. These fibroblasts contribute to the formation of pannus. A layer of tissue that invades and damages both cartilage and bone, within the joint [8].

The evidence that is currently available suggests that IL 8 may have a function in both rheumatoid arthritis and ulceration. This study aims to quantify the amounts of IL 8 in saliva from four groups: healthy persons, rheumatoid arthritis patients, ulcer patients, and patients with both illnesses together. Given that IL 8 is involved in both rheumatoid and ulceration, it's plausible that those who have both diseases have greater amounts of IL 8. If this theory is validated, it may create opportunities for treating patients by emphasizing immunomodulation techniques.

2. Materials and Methods

2.1. Study Design

This investigation received the endorsement of the Ethics Committee at the University of Kufa, College of Dentistry, Department of Oral Pathology. Employing a descriptive, analytical, and cross-sectional approach, this study engaged patients with aphthous ulceration (AU), Rheumatoid Arthritis (RA), both conditions, and a control group of healthy individuals. Participants were recruited from attendees at the dental clinic of the college.

2.2. Sampling

The control group comprised individuals undergoing routine health checkups at laboratories, with no history of medication use or systemic illnesses. Rheumatic subjects were identified among those visiting the Rheumatology Center, characterized by Rheumatoid Factor levels of >14 IU/ml and anti-CCP (cyclic citrullinated peptide) level of >20 u/ml. These individuals also presented with typical RA symptoms such as joint pain and tenderness, joint swelling and inflammation, morning stiffness, fatigue, fever and loss of appetite, joint deformities, reduced range of motion and rheumatoid nodules. Patients manifesting symptomatic AU were identified from the same dental clinics based on clinical assessments and history of present illness, those with AU were distinguished from others with other types of ulcerations such as traumatic ulceration, ulcerative lichen planus and herpetic ulceration according to the clinical findings.

Exclusion criteria for all groups included the use of medications, the presence of systemic conditions other than AR and AU, inflammation in other body parts, and periodontal diseases. Lifestyle factors like smoking, substance abuse, and alcohol consumption were also considered for exclusion.

2.3. Serological and Salivary Tests

Rheumatoid Factor and Anti-CCP tests were conducted for AU patients. Based on the results, they were categorized into either the AU group or the combined AU and AR group. This recruitment continued until the desired sample size of 42 was reached.

The use of drugs, the existence of systemic disorders other than AR and AU, inflammation in other parts of the body, and periodontal diseases were the exclusion criteria for all the groups. Lifestyle characteristics such as alcohol usage, drug addiction, and smoking were also taken into account for exclusion.

Participants provided a 3 mL blood sample between 7 and 9 a.m., minimizing circadian influences. 3 mL of unstimulated saliva was collected from each participant using the spitting technique. The blood samples were centrifuged at 3000 rpm for 15 minutes post-clotting for anti-CCP and RF analysis using the separated plasma. Saliva samples were immediately frozen at −20 °C for subsequent IL-8 concentration measurement using the ELISA kit (Innovative ELISA, R and D Systems Inc., USA).

2.4. Statistical Analysis

In this study, only data with a P value below 0.05 were deemed to have statistical significance. For the analysis of this data, we employed the Statistical Package for Social Sciences (SPSS) version 20, along with Microsoft Excel 2010. A one-way ANOVA test was utilized to compare data across different groups.

TABLE I: The Concentration of Salivary IL-8 in the Study Groups

Groups	Mean ± SD	IL-8 range	F-test*	P-value
Control	213.69 ± 84.65	195–459	187.66	0.0001
AU	381 ± 108.48	254–700		
AU & RA	483.33 ± 141.20	253–572		
RA	338.57 ± 79.11	89–389		

Note: *One-Way ANOVA test.

TABLE II: Multiple Comparison Between the 4 Groups Usin Post hoc Analysis

Multiple comparison	Mean difference	P-value
Control group AU group	−167.31	0.016
Control group RA group	−124.61	<0.001
Control group AU + AR group	−269.64	<0.0001
AU group AU + AR group	−102.33	0.06

TABLE III: Mean and Standard Deviation of RF and Anti-CCP for the 4 Groups

Groups	RF mean ± SD	Anti-CCP mean ± SD
Control	3.39 ± 0.40	12.81 ± 1.49
AU	4.03 ± 0.46	15.78 ± 2.27
AU & AR	23.91 ± 3.18	33.10 ± 5.82
AR	19.19 ± 4.93	29.24 ± 7.73

3. Results

26 Rheumatoid Arthritis (RA) patients, 26 healthy controls, and 42 patients with aphthous ulcer (AU) were evaluated. Among the AU patients, 28.42% also had Rheumatoid Arthritis; As indicated in Table I, the average salivary level of IL-8 was highest in patients with both AU and Rheumatoid Arthritis (483.33 ± 141.20 pg/mL), followed by the AR group (338.57 ± 79.11 pg/mL), the AU group (381 ± 108.48 pg/mL), and the control group (213.69 ± 84.65 pg/mL). A one-way ANOVA revealed significant differences between these groups in terms of their salivary IL-8 levels ($P = 0.0001$).

Salivary IL-8 levels were found to differ significantly between the AU and control groups ($P = 0.016$), between the Rheumatoid Arthritis and control groups ($P < 0.001$), between the control group and the combined AU + AR group ($P < 0.0001$), and between the AU and AU + AR groups ($P = 0.013$) after further analysis using the post hoc least significant difference test. Even though individuals with both AU and AR had saliva with an average IL-8 content greater than those with Rheumatoid Arthritis, this difference was not statistically significant ($P = 0.06$) [Table II].

The mean values for RF and Anti-CCP was highest in AU patients with AR, followed by the AR, AU, and control groups, respectively [Table III].

4. Discussion

The underlying causes of aphthous ulceration are yet to be fully determined. Emerging theories suggest that the disease's development is closely linked to the immune system, specifically T-cell-mediated responses involving macrophages and T cells in the lesion areas. The production and release of a variety of cytokines by cells in the blood and oral mucosa are thought to be key factors in the initiation and advancement of the condition [9].

The scientific field has long been engaged in the search for a dependable and nonintrusive method to monitor the progression and treatment of aphthous ulceration. A significant amount of research has been dedicated to finding a noninvasive, economical diagnostic method to accurately measure disease-related cytokines in blood and other bodily fluids [10], [11].

The findings of our recent study reveal a significant elevation in the mean levels of IL-8 in the saliva of individuals with aphthous ulceration compared to those in a control group, with a statistical significance of $P = 0.016$.

Rhodus and colleagues have identified that the levels of IL-8, both in saliva and serum, are markedly increased in patients with aphthous ulceration compared to a control group [12]. This points to the potential of IL-8 as a valuable biomarker for diagnosis, ongoing monitoring, and the planning of treatment strategies for this condition.

Research by Chiang and their team has shown that IL-8 levels in the oral fluid of patients suffering from aphthous ulceration are significantly higher than those in healthy individuals [13]. Their findings advocate for the effectiveness of using saliva samples for detecting increased cytokine levels in aphthous ulceration, suggesting that salivary IL-8 is a dependable indicator for evaluating the disease's severity. Furthermore, Tavangar *et al.* have also observed a notable increase in serum IL-8 levels in patients with this condition compared to healthy subjects ($P = 0.002$).

The heightened levels of cytokines found in the saliva of individuals with aphthous ulceration may be due to increased production by inflammatory cells or keratinocytes [14]. Additionally, in severe cases of aphthous ulceration, the damaged oral mucosa may lose its effectiveness as a barrier, which could further contribute to elevated cytokine levels in saliva [15], [16].

In cases of aphthous ulceration, keratinocytes are known to produce IL-1 and TNF-α. Similarly, mononuclear cells around the ulcerated mucosal areas are capable of releasing TNF-α [17]. When stimulated by IL-1 and TNF-α, various cells within the ulceration sites, including keratinocytes, macrophages, T cells, endothelial cells, and fibroblasts, can produce significant amounts of IL-8. This cytokine plays a critical role in attracting T cells, especially

cytotoxic T cells, to the site of the ulceration, contributing to the disease's pathogenesis [18].

The second aim of our study is to compare the salivary levels of interleukin-8 between individuals with type Rheumatoid Arthritis and non-Rheumatoid individuals in the context of aphthous ulceration, to understand any potential differences and correlations.

In this study, we observed that the mean salivary level of IL-8 in Rheumatoid Arthritis patients with aphthous ulceration was significantly higher compared to healthy controls ($P < 0.001$). This aligns with numerous reports indicating elevated serum levels of IL-8 in Rheumatoid Arthritis, which are also reflected in higher salivary concentrations. The presence of IL-8 in saliva likely mirrors its serum levels, suggesting that the systemic inflammatory response in these patients contributes to oral manifestations like aphthous ulceration [19].

Research, including a pivotal study by Choi *et al*. [19], has consistently shown that serum levels of IL-8 are significantly higher in Rheumatoid Arthritis patients than in the control group20. The resultant increase in IL-8 exacerbates inflammatory responses, which is particularly relevant in the development and persistence of aphthous ulceration in Rheumatoid Arthritis.

In our investigation, the AU and RA patients' salivary IL-8 concentrations were much greater ($P < 0.05$) than those of the control and AU groups. The pathophysiology of AU and RA and the synergistic effect in individuals with AU and RA are indicated by the increased salivary and serum levels of IL-8 in the control, AU, and AU + RA groups, respectively.

In the present investigation, AU patients, Rheumatoid Arthritis patients, and AU + RA patients had considerably greater salivary levels of IL-8 than did the healthy population. Serum sampling appears to be more intrusive and less convenient than salivary sampling. The salivary sampling technique can be used in addition to or instead of the serum sample.

A constraint of this research was the dearth of participants' cooperation and the challenge of recruiting a sufficient number of eligible AU patients. Furthermore, even though patients without inflammatory or systemic disorders were included, it was not able to completely eradicate moderate internal inflammation, which could have a negligible impact on the level of IL-8.

Future research with a bigger sample size is necessary to evaluate and compare salivary levels of IL-8 in various AU forms and look into its impact on the disease's clinical progression.

5. Conclusion

The study shows that individuals with AU and RA, especially those who have both disorders, have a significant increase in salivary IL-8 levels. These results imply that IL-8 may be a useful biomarker for inflammation in these illnesses. Additional investigation might examine IL-8's potential as a target for therapeutic treatments, which could open up new treatment options for various inflammatory diseases.

Ethical Consideration

The ethical committee at the Oral Pathology Department/College of Dentistry/University of Kufa approved the study.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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