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# Examining the role of salivary IL-2 and IL-22 in recurrent aphthous stomatitis: a diagnostic approach

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## Abstract

**Background:** Recurrent aphthous stomatitis (RAS) is a prevalent inflammatory condition, that manifests as mouth ulcers. This study aims to evaluate IL-2 and IL-22 levels in saliva samples of individuals with recurrent aphthous stomatitis (RAS) compared to healthy volunteers.

**Methods:** A case-control study was conducted between April 1st and September 30th, 2023, at the Dental Center of New Baquba, Diyala, Iraq. Saliva samples were collected from 40 patients diagnosed with recurrent aphthous stomatitis and 40 healthy controls. IL-2 and IL-22 levels were assessed in both groups using enzyme-linked immunosorbent assay (ELISA). Statistical analysis, employing paired sample 't' test, was conducted with a significance threshold below.

**Results:** In the case group, the mean age was 53.4 years (SD=1.58), ranging from 26 to 81 years, while in the control group, it was 47.18 years (SD=1.55), ranging from 24 to 78 years. Females predominated in both groups, comprising 62.5% in cases and 52.2% in controls. Most aphthous ulcers (77.5%) were found in the buccal mucosa, with minor aphthous ulcers being the most common (67.5%). Results from paired-samples t-tests revealed significantly higher levels of IL-2 in RAS cases (M=62.090, SD=20.242) compared to controls (M=36.366, SD=14.596), (t(39)=7.101, p < 0.001). Similarly, IL-22 levels were significantly elevated in RAS cases (M=106.537, SD=44.112) compared to controls (M=73.399, SD=32.852), (t(39)=3.553, p=0.001). These results suggest a potential association between RAS and increased IL-2 and IL-22 levels, indicating their involvement in RAS pathophysiology.

**Conclusion:** In summary, females showed a significant predominance in RAS cases. Significant differences were observed in IL-2 and IL-22 levels between case and control groups.

**Keywords:** Gender, Saliva, Interleukin-2 (IL-2), Interleukin-22 (IL-22), Recurrent Aphthous Stomatitis, Oral, Dental, Iraq

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## Background

Aphthous stomatitis, a prevalent inflammatory condition affecting the oral mucosa, occurs across diverse racial, cultural, and geographic demographics [1]. While colloquially termed "canker sores," scientifically, these lesions are referred to as "recurrent aphthous stomatitis" (RAS [2]. RAS manifests as painful recurring ulcers in the oral mucosa, often resolving within 1-2 weeks [3]. However, the severity, duration, and frequency of these ulcers significantly impact morbidity [2]. Furthermore, beyond being symptomatic of autoimmune or inflammatory conditions, RAS or similar lesions may indicate an underlying disease or deficiency [3]. Recurrent aphthous stomatitis (RAS) manifests in three subtypes: minor, major, and herpetiform aphthous ulcers. Minor ulcers, the most prevalent type, recur every 1 to 4 months, typically appearing as small, oval, or round lesions surrounded by an erythematous halo and a grey-white pseudomembrane. They commonly affect non-keratinized mucosa like the buccal and labial mucosa, healing within two weeks without scarring [4]. Major aphthous ulcers, affecting 10% of RAS patients, are larger (>10 mm), last 5 to 10 weeks, and may scar. Any oral area, including the oropharynx, can be affected [4,5]. Herpetiform ulcers, the rarest form, occur in 1% to 10% of RAS cases, resembling primary herpetic stomatitis but unrelated to herpes viruses. Typically affecting older females, these painful ulcers may number up to 100, healing within one to two weeks, occasionally leaving scars [6]. The cytokines or interleukins are biologically active glycoproteins that play an

active role in facilitating communication among leukocytes [[7,8]. These interleukins are chiefly derived from activated lymphocytes and macrophages [9,10]. Interleukin-2 (IL-2), also recognized as a T cell growth factor, is primarily produced by activated T helper cells. IL-2 serves a crucial function in modulating chronic inflammatory responses, both cellular and humoral. Its interaction with the IL-2 receptor on T lymphocytes prompts cell proliferation and heightened secretion of lymphokines [11]. RAS pathogenesis involves cell-mediated responses, including T cells and TNF-α produced by various leukocytes. TNF-α induces inflammation by affecting endothelial cell adhesion and neutrophil chemotaxis [12,13]. Type-1 cytokines like IL-2, IL-12, IFN-ᵞ, and TNF-α drive pro-inflammatory responses, promoting cell-mediated immunity [14]. IL-2, in inflammation, stimulates the secretion of pro-inflammatory cytokines like IL-1, TNF-α, and TNF-β [15]. This study aims to assess IL-2, and IL-22 levels in RAS patients compared to a sample of healthy volunteers.

## Methods

### Study design and participants

A case-control study was conducted between April 1st and September 30th, 2023 at the Dental Center of New Baquba, Diyala, Iraq. Based on previously published similar studies in Iraq [16], and some other countries [17], a convenient sampling technique was employed to choose forty confirmed cases of RAS and forty healthy Individuals as control.

### Inclusion and exclusion criteria

The case group comprised individuals over 18 years, of both genders, visiting dental clinics, with a positive history or current presentation of RAS. Exclusion criteria encompassed those with other oral inflammations or systemic ailments like rheumatoid arthritis, diabetes, cardiovascular, renal, or hepatic diseases, and unwilling participants. The control group included healthy individuals willing to participate. Informed consent form was obtained from patients and healthy individuals. Comprehensive medical, dental, and social histories were gathered, with all participants assured of their right to withdraw from the study at any point.

### Procedure

Saliva collection occurred during clinic visits when patients were available. All participants who exhibited active ulcerative lesions of RAS were asked to rinse their mouths and provided saliva samples while seated. Each individual expectorated 10 ml of unstimulated saliva into sterile tubes. Saliva was stored at -80°C and subsequently analyzed. Salivary IL-2 and IL-22 levels were determined using enzyme-linked immunosorbent assay (ELISA), with results expressed in pg/mL.

### Statistical analysis

Data analysis was conducted using SPSS software version 16. Biochemical parameter values were presented as mean ± standard deviation (SD). Significance levels were assessed using independent sample t-tests, with statistical significance set at $p < 0.05$.

## Results

### Sociodemographic factors

The case group had a mean age of 53.4 years (SD ± 1.58), ranging from 26 to 81 years, while the control group had a mean age of 47.18 years (SD ± 1.55), ranging from 24 to 78 years. In both groups, females were predominant, comprising 62.5% in the case group and 52.2% in the control group. The majority of aphthous ulcers were located in the buccal mucosa (77.5%), with minor aphthous ulcers being the most common type (67.5%).

**Table 1:** Sociodemographic and clinical features of case and control groups (n=80).

| Variables | Categories | Case group (n=40) | Control group (n=40) |
|---|---|---|---|
| Age | Mean (SD);<br>Range in years | 53.4 (1.58)<br>(26-81) | 47.18 (1.55)<br>(24-78) |
| Age groups | 19-38 years | 10 (25.0) | 15 (37.5) |
| | 39-58 years | 15 (37.5) | 16 (40.0) |
| | 59 years and more | 15 (37.5) | 9 (22.5) |
| Gender | Female | 25 (62.5) | 21(52.5) |
| | male | 15 (37.5) | 19 (47.5) |
| Site of aphthous ulcer | Buccal mucosa | 31 (77.5) | - |
| | Tongue | 6 (15.0) | - |
| | Lips | 1 (2.5) | - |
| | The floor of the mouth | 1 (2.5) | - |
| | Palat | 1 (2.5) | - |
| Type of aphthous ulcer | Minor aphthous ulcer | 27 (67.5) | - |
| | Major aphthous ulcer | 5 (12.5) | - |
| | Herpetiform aphthous ulcer | 8 (20.0) | - |

### Bivariate analysis

A paired-sample t-test was utilized to compare IL-2 and IL-22 levels between RAS and non-RAS (control) groups. For IL-2, significant differences were found between the case (M=62.090, SD=20.242) and control (M=36.366, SD=14.596) groups ($t(39)=7.101$, $p < 0.001$), indicating higher IL-2 levels in RAS cases. Similarly, for IL-22, significant differences were observed between the case (M=106.537, SD=44.112) and control (M=73.399, SD=32.852) groups ($t(39)=3.553$, $p=0.001$), suggesting elevated IL-22 levels in RAS cases. These findings imply a potential association between RAS and increased IL-2 and IL-22 levels, highlighting their role in the pathophysiology of RAS. The cross-tabulation analysis revealed no significant differences in RAS types based on age groups ($\chi2 = 3.243$, $p = 0.518$), and gender ($\chi2 = 1.742$, $p = 0.418$). These results are summarized in Table 3.

**Table 2:** Comparison of IL-2 and IL-22 in saliva pg/mL in recurrent aphthous stomatitis and healthy controls (n=80)

| Group in Interleukin | n | Mean | Standard deviation | Standard error | t-value | p-value | 95% C.I. (Upper-Lower) |
|---|---|---|---|---|---|---|---|
| Case (IL-2) | 40 | 62.090 | 20.242 | 3.200 | 7.101 | <0.001 | 18.396-33.051 |
| Control (IL-2) | 40 | 36.366 | 14.596 | 2.307 | | | |
| Case (IL-22) | 40 | 106.537 | 44.112 | 6.975 | 3.553 | 0.001 | 14.274-52.001 |
| Control (IL-22) | 40 | 73.399 | 32.852 | 5.194 | | | |

**Table 3:** Bivariate analysis of type of aphthous ulcer in age groups and gender (n=40).

| Variables | Categories | Minor aphthous ulcer N (%) | Major aphthous ulcer N (%) | herpetiform aphthous ulcer N (%) | Total N (%) | $\chi 2$ | p-value |
|---|---|---|---|---|---|---|---|
| Gender | Female | 15(60.0) | 4(16.0) | 6(24.0) | 25 (62.5) | 1.742 | 0.418 |
| | Male | 12 (80.0) | 1(6.7) | 2(13.3) | 15 (37.5) | | |
| Age groups | 19-38 years | 7 (70.0) | 2(20.0) | 1(10.0) | 10 (25.0) | 3.243 | 0.518 |
| | 39-58 years | 9(60.0) | 1(10.0) | 5(33.3) | 15 (37.5) | | |
| | 59 and more years | 11(73.3) | 2(20.0) | 2(20.0) | 15 (37.5) | | |

## Discussion

The majority of patients in the case group (67.5%) exhibited minor aphthous ulcers, consistent with findings by Scully and Porter, where 80% of RAS patients had minor ulcers recurring every 1 to 4 months. Predominantly, 92.5% of cases involved the buccal mucosa and tongue. Similarly, Preeti et al. [18] noted that RAS commonly occurs on nonkeratinized mucosal surfaces like the labial and buccal mucosa, as well as the floor of the mouth, typically healing within 10-14 days without scarring. This study utilized saliva to measure IL-2 and IL-22 levels due to its accessibility, ease of collection, and non-invasiveness. Our findings align with research by Kalpana et al. [17] and Porter et al. [19], who noted that recurrent aphthous ulceration typically begins in the second decade of life. Interestingly, contrary to previous findings, we observed a predominance of females in both patient and control groups. Findings of other studies suggest hormonal changes in women may be associated with RAS, indicating a link between oral ulceration and menstruation onset or the luteal phase of the menstrual cycle, potentially explaining the observed female predominance [18]. Furthermore, the concentration of salivary IL-2 in the RAS group was aligned with those reported in a study by Simcic et al. [20]. The authors suggested that elevated IL-2 levels contributed to the pathogenesis of burning mouth syndrome through immunological reactions during inflammation, indicating a potential mechanism for RAS development. Therefore, RAS might be aroused from changes induced by inflammatory mediators in the oral mucosa [1]. Additionally, Individuals with RAS may experience uncontrolled or excessive release of locally active inflammatory mediators, including IL-2 and IFN-γ, which are most likely related to various etiological factors. IL-22 is vital for mucosal barrier defense, epithelial cell survival, and tissue repair. However, its role extends beyond protection, as it also exhibits pathogenic traits in autoimmune diseases, infections, and malignancies [21]. In the current study, significant disparities in IL-22 levels between RAS and control groups indicate heightened IL-22 levels in RAS cases. Despite the common occurrence of aphthous ulcers in the oral mucosa, their etiology remains unclear, and the pathogenesis of RAS is still elusive. Our results suggest a potential link between RAS and elevated IL-22 levels, underscoring their involvement in RAS pathophysiology. One hypothesis proposes that RAS stems from cell-mediated immunity dysfunction, leading to the accumulation of pro-inflammatory cytokines and T cells. Factors such as hormonal fluctuations, emotional stress [22], familial predisposition, and trauma may increase RAS susceptibility. Dysregulation in cell-mediated hormonal fluctuations, emotional stress [22], familial predisposition, and trauma may increase RAS susceptibility. Dysregulation in cell-mediated immunity and the buildup of proinflammatory cytokines and T cells might also have implications for cancer development [23]. Exposure to TNF-α and IL-6 can prompt the differentiation of naïve CD4 T cells into Th22 cells, which produce IL-22 and TNF-α [28,29] [24,25]. The presence of proinflammatory cytokines and Th1-associated chemokine receptors, observed at protein levels, shows comparable trends in patients with RAS and cancer [26].

## Conclusion

In summary, females exhibited a higher prevalence of RAS compared to males. Significant disparities in IL-2 and IL-22 levels were observed between RAS and control groups, suggesting their involvement in RAS pathogenesis and potential diagnostic utility. IL-2's synergistic effect on cytokine activation proposes a novel approach for RAS treatment by targeting IL-2 inhibition to prevent recurrences. Additionally, IL-22 antagonists could offer therapeutic benefits, though further research is warranted. Gender and age did not show significant differences in RAS occurrence between the control and case groups.

### Abbreviation

RAS: Recurrent aphthous stomatitis; ELISA: Enzyme-Linked Immunosorbent Assay; IL-2: Interleukin-2, IL-22: Interleukin-22; SD: Standard Deviation; TNF-α: Tumor Necrosis Factor Alpha; TNF-β: Tumor Necrosis Factor Beta; IFN-ᵞ: Interferon-Gamma

### Declaration

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### Availability of data and materials

Data will be available by emailing mustafa.gheni.taher@gmail.com

### Authors' contributions

Mustafa Gheni Taher (MGT) conceived and designed the study, analyzed and interpreted the data; drafted the manuscript; and revised the manuscript. The author read and approved the final manuscript.

### Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The ethical approval was obtained from the Ethics Review Committee of the College of Medicine, University of Diyala [Ref No. R.2/9/275 of February 7, 2023] Diyala, Iraq.

## Consent for publication
Not applicable

## Competing interest
The authors declare that they have no competing interests.

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