e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(6); 188-193

Original Research Article

Assessment of the Salivary Interleukin-2 Levels Among the Patients Diagnosed with Recurrent Aphthous Stomatitis: An Original Study.

Praveena Adusumilli[1], Mary Oshin[2], Syed Ali Abedi[3], Siya K [4], Suhas R Nayak[5], Fousiya Ali[6]

[1]MDS, Department of Oral Pathology and Microbiology, Senior Lecturer, Drs. Sudha & Nageswararao Siddhartha Institute of Dental Sciences, Chinoutpalli.
[2]Senior Lecturer, Tirumala Institute of Dental Sciences and Research Centre., Department of Oral Pathology and Microbiology
[3]MDS, Oral and Maxillofacial Pathologist Private Practitioner
[4] Senior Lecturer, Educare Institute of Dental Sciences, Dept of Oral Pathology & Microbiology
[5]Head of the Department, Dept of Oral Pathology & Microbiology, Educare institute of Dental Sciences
[6]Senior Lecturer, Malabar Dental College & Research Centre, Dept of Oral Pathology & Microbiology

Received: 15-04-2022 / Revised: 23-05-2022 / Accepted: 05-06-2022
Corresponding author: Dr. Praveena Adusumilli
Conflict of interest: Nil

Abstract

Aim: The goal of this study was to calculate and compare salivary interleukin-2 (IL-2) levels in patients with recurrent aphthous stomatitis and healthy controls, as well as their fluctuation with age and gender.

Design of the Study: Saliva was collected from 100 individuals between the ages of 15 and 50, including 50 patients with recurrent aphthous stomatitis (27 females and 23 men) and 50 healthy control subjects (28 Females and 22 Males). The enzyme linked immunosorbent test was used to estimate IL-2 in both groups (ELISA). The data was statistically analyzed using the Independent 't' test.

Results: showed that patients with recurrent aphthous stomatitis had higher salivary IL-2 levels than healthy controls. In comparison to other age groups, IL-2 levels were also higher in patients aged 15 to 30 years. In female patients, a rise in IL-2 was also seen.

Conclusion: Changes in IL-2 levels in recurrent aphthous stomatitis patients were shown to be age and sex related.

Keywords: Interleukin-2, Enzyme linked immunosorbent assay (ELISA), Recurrent Aphthous Stomatitis.

Introduction

The development of painful, recurring solitary or multiple ulcerations of the oral mucosa is defined by recurrent aphthous stomatitis (RAS), an unhappy common

condition. [1] Hippocrates coined the term "aphthous" to describe mouth problems around 460-370 BC. [2] Cooke divided RAS lesions into three categories, which Lehner named minor aphthous ulcers, big aphthous ulcers, and herpetiform ulcers. [3] Interleukins are cytokines that have been molecularly characterized and are thought to mediate communication between leukocytes. Interleukins are physiologically active glycoproteins produced by activated lymphocytes and macrophages. [5] Interleukin-2 (IL-2) is a 15-kilodalton glycoprotein that was previously known as T-cell growth factor (TCGF). Activated T helper cells are the principal producers of this substance. It's important for controlling both cellular and humoral chronic inflammatory responses. When IL-2 binds to the IL-2 receptor on T cells, cell proliferation and lymphokine release increase. [6] T lymphocytes and the generation of tumor necrosis factor (TNF) by these and other leucocytes are involved in the pathophysiology of RAS. The action of TNF- on endothelial cell adhesion and neutrophil chemotaxis causes inflammation. [7] Type-1 cytokines include interleukins like IL-2 and IL-12, as well as interferons like interferon (IFN) and TNF, which are pro-inflammatory cytokines that generate cell-mediated immunity. [8] IL-2 promotes the release of pro-inflammatory cytokines such IL-1, TNF-, and TNF- during inflammation. In RAS patients, there was an increase in local expression of Th1 genes as well as systemic production of cytokines such IL-2, TNF-a, and IL-6. [9] The goal of this investigation was to detect the presence of IL-2 in an inflammatory condition like RAS.

Materials and Methods

A total of fifty RAS patients were enrolled in the trial. A control group of fifty age-matched healthy volunteers was chosen. Cases were chosen based on the individuals' medical histories and a comprehensive clinical assessment. Patients between the ages of 15 and 50 who had a history of RAS and/or clinical symptoms were eligible (active lesion in ulcerative phase). Subjects with additional inflammatory oral lesions or systemic disorders were excluded from the study. The patient's informed consent was acquired, and medical, dental, and social histories were gathered.

There were 27 males and 23 females in the RAS group. The patients ranged in age from 15 to 50 years old. They were split into three age groups: 15-30 years old (Group-1), 31-40 years old (Group-2), and 41-50 years old (Group-3) (Group-3). There were 22 males and 28 females in the control group.

All of the patients had active RAS lesions that were in the ulcerative stage. The participants were instructed to rinse their mouths. In a sitting position, each participant's saliva was collected. In a sterile tube, each participant was asked to expectorate 10 mL of whole, unstimulated saliva. The saliva was kept at 80°C until it was analyzed. An enzyme linked immunosorbent assay (ELISA) was used to measure salivary IL-2, and the results were expressed in pg/mL. The data was statistically analyzed using an independent t test.

ELISA stands for enzyme-linked immunosorbent assay

This ELISA kit included a microtiter plate that had been pre-coated with an anti-IL-2 antibody. After that, a biotin-conjugated polyclonal antibody preparation specific for IL-2 was applied to the relevant microtiter plate wells. After that, each microplate well was incubated with Avidin coupled to Horseradish Peroxidase (HRP). Then, in each well, a 3,3,5,5-Tetramethyl benzidine (TMB) substrate solution was added. The hue of wells containing IL-2, biotin conjugated antibody, and enzyme-conjugated Avidin changed. The enzyme-substrate reaction was stopped with the addition of a stop solution, and the color

change was detected at 450 nm using an ELISA microplate reader. Our study's material to be determined was IL-2, hence known quantities of IL-2 solutions were prepared for testing. 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml were the different concentrations of IL-2 [10]

Analytical statistics

The statistical package for social sciences (SPSS) software version 14.00 was used to analyze the data. The mean + standard deviation of biochemical parameters was calculated (SD). The Independent't' test was used to establish the levels of significance. P 0.001 was used to determine statistical significance.

Results

There was a significant increase in IL-2 in RAS patients compared to controls [Table 1]. Average values of IL-2 concentration were signifi cantly higher in the group of patients with RAS: Mean 30.23 + 3.46 pg/mL compared to the controls: Mean 11.91 + 1.7 pg/mL ($P < 0.001$). Age distribution in RAS patients Group A (16-30 years) were affected more of about 63.3% compared to Group B (31-45 years) and Group C (46-60 years) which is of 20% and 16.7% respectively [Table 2]. Gender distribution in RAS group shows females were more affected compared to the males [Table 2].

Table 1: Comparison of the levels of IL-2 among the subjects in the study.

Group	Mean ±SD	P
Healthy	29± 10.11	0.000
RAS	29±31.22	

Table 2: Comparison of the demographics in the RAS groups

Group	Number	Percentage
Age group		
Group 1	20	64
Group 2	8	20
Group 3	6	18
Sex		
Male	13	44
Female	17	56

Discussion

Multiple recurring tiny, round or ovoid ulcers with constricted edges, erythematous haloes, and yellow or gray floors arise first in childhood or adolescence in RAS (aphthae; canker sore). [10] It is a prevalent illness that affects 5% to 66 percent of adult patient groups studied. [11] RAS is primarily a cell-mediated immune response that leads in the creation of T-cells and TNF- by other leucocytes (macrophages and mast cells). The TNF-cytokine, a key inflammatory mediator, causes the inflammatory process to start by affecting endothelial cell adhesion and causing neutrophils to chemotact. Treatment with drugs that block the manufacture of endogenous TNF-, such as thalidomide and pentoxifylline, has been found to prevent RAS. [12]

Because TNF- is thought to be involved in the etiology of RAS, anti-TNF-agents like pentoxifylline and thalidomide may be useful, especially in patients who require systemic immunosuppressive medication due to RAS. After 1 month of treatment, pentoxifylline (400 mg three times day) dramatically reduced the number of RAS for up to 9 months (Pizarro et al., 1995,

1996; Wahba-Yahav 1995). Thalidomide works through affecting the immune system's Th1 and Th2 responses, as well as having angiogenic capabilities. (Porter et al., 2002). However, because of its teratogenicity, it is not recommended for use during pregnancy. [12] In the active stage of RAS, the plasma level of IL-2 was shown to be significantly higher. IL-2-activated natural killer (NK) cells may have a role in the disease's progression. These cells' activity was found to be higher in active lesions and to be lower during periods of remission. [12] Diverse oral micro-environments or domains, such as the oral mucosa, salivary glands, saliva, and the gingival crevice, have different host defenses against infection. This has shifted the attention to IL-2 detection in saliva and its significance in determining the disease's origin and course. Saliva was employed in this investigation to estimate IL-2 levels because it is widely available, easy to collect, and the process is non-invasive. Our findings are consistent with those of Stephen R. Porter et al.,[11], who found that recurrent aphthous ulceration typically begins in the second decade, with a female predominance in some adult and child patient groups. There have been reports of a link between RAS and hormonal changes in women. Oral ulceration has been linked to the commencement of menstruation or the luteal phase of the menstrual cycle in studies. [13] This could be one of the reasons for the current study's female predominance. In a study conducted by Daria Simcic et al., salivary IL-2 levels in the RAS group coincided with those in the study group (burning mouth syndrome). [14] The author demonstrated that a rise in IL-2 concentration provides a possible explanation for the causal mechanism of burning mouth syndrome via immunological reactions during inflammation in which the cytokine IL-2 concentrations were elevated. This suggests that RAS could be induced by changes in the oral mucosa produced by inflammatory mediators. In response to the etiological variables, patients with RAS may experience uncontrolled or excessive production of locally active inflammatory mediators such as IL-2 and IFN-[12]. RAS is a prevalent oral mucosal illness characterized by alterations in humoral and cellular immunity. [15] When given by macrophages showing the right major histocompatibility antigens, certain antigens can activate a clonal population of resting T lymphocytes. Antigen binds to particular receptors on the surface of resting T lymphocytes, which triggers activation. Antigen binding causes de novo production and secretion of IL-2 or T-cell growth factor, as well as transitory expression of high and low affinity IL-2 receptors, in the presence of macrophage-derived interleukin-1 (IL-1). The following interaction of IL-2 with its high affinity membrane receptors boosts cellular proliferation, resulting in T-cell population expansion,[16,17] potentiates B-cell development, and enhances NK-cell and monocyte activation. [18] TNF- is a pro-inflammatory cytokine released by activated monocytes that induces the activation of cytotoxic T-cells and neutrophils, as well as epithelial necrosis and aphthous lesion formation. [20]

This explains why IL-2 levels in patients with RAS are higher than in the healthy control group in the current study. [21] Increased IL-2 expression in RAS could explain the causal mechanism and its consequences during infection. However, it is still unclear whether RAS is primarily caused by the production of IL-2 or is the result of a combination of other cytokines. Given that RAS is a complex condition, the most feasible strategy is to investigate other immunologically active chemicals that could serve as a good target for analyzing the causes and treatment quality. Because some medications, such as pentoxifylline, have been linked to the treatment of RAS and the prevention of endogenous TNF- (a cytokine), it is

important to note that other cytokines must be identified in order to prevent the disease process.

Conclusion

In comparison to the control group, RAS patients have a higher level of IL-2 in this study. Patients in Group A (15-30 years) were impacted more than other groups with female inclination, according to age and gender distribution in RAS. As a result, IL-2 changes in RAS patients were found to be age and sex related.

This study's main goal was to inform patients on the nature of their ailment, particularly the fact that RAS can be controlled with treatment. RAS recurrences can be prevented by early diagnosis and identification of the triggering events that produced the lesion. Because IL-2 has a synergistic influence on other cytokines' activation, treating RAS with medicines that block IL-2 could be a novel way to prevent recurrences.

References

1. Rajendran R, Sivapathasundharam B. Shafer‘s textbook of Oral Pathology-6th ed. New Delhi (India): Elsevier (Reed) publications; 2009
2. Femiano F, Lanza A, Buonaiuto C, Gombos F, Nunziata M, Piccolo S *et al*. Guidelines for diagnosis and management of aphthous stomatitis. Pediatric Infect Dis J 2007; 26:728-32.
3. Rogers RS. Recurrent aphthous stomatitis in the diagnosis of Behcet’s disease. Yonsei Med J 1997; 38:370-79.
4. Vinay kumar V, Abbas Ak, Fausto N. Robbins and Cotran. Pathologic basis of disease. 7th ed: Elsevier (Saunders);2008.
5. Elmslie RE, Dow SW, Ogilvie GK. Interleukins: Biological properties and therapeutical potential. Vet Intern Med 1991; 5:283-93.
6. June CH, Jackson KM, Ledbetter JA, Leiden JM, Lindsten TL, Thompson CB. Two distinct mechanisms of interleukin-2 gene expression in human T lymphocytes. J Autoimmun 1989; 2:55-65.
7. Scully C, Gorsky M, Lozada Nur F. The diagnosis and management of recurrent aphthous stomatitis: A consensus approach. J Am Dent Assoc 2003; 134:200-07.
8. Albanidou-Farmaki E, Markopoulos AK, Kalogerakou F, Antoniades DZ. Detection, enumeration and characterization of T helper cells secreting type 1 and type 2 cytokines in patients with recurrent aphthous stomatitis. Tohoku J Exp Med 2007; 212:101-05.
9. Guimarães AL, Correia-Silva Jde F, Sá AR, Victória JM, Diniz MG, Costa Fde *et al*. Investigation of functional gene polymorphisms IL-1□, IL-6, IL-10 and TNF-□ in individuals with recurrent aphthous stomatitis. Arch Oral Biol 2007; 52:268-72.
10. Kalpana R, Thubashini M, Sundharam BS. Detection of salivary interleukin-2 in recurrent aphthous stomatitis. J Oral Maxillofac Pathol 2014; 18:361-4.
11. Porter SR, Hegarty A, Kaliakatsou F, Hodgson TA, Scully C. Recurrent aphthous stomatitis. Clin Dermatol 2000; 18:569-78.
12. Jurge S, Kuffer R, Scully C, Porter SR. Mucosal disease series. Number VI. Recurrent aphthous stomatitis. Oral Dis 2006; 12:1-21.
13. Preethi L, Magesh K, Rajkumar K, Karthik R. Recurrent aphthous stomatitis. J Oral MaxillofacPathol 2011; 15:252-56.
14. Simcic D, Pezelj-Ribarić S, Grzić R, Horvat J, Brumini G, Muhvić-Urek. Detection of salivary interleukin-2 and interleukin-6 in patients with burning mouth syndrome. Mediators Inflamm 2006;1-4.
15. Sun A, Chia JS, Wang WB, Chiang CP. Immunomodulating effects of “Tien-Hsien liquid” on peripheral blood mononuclear cells and T-Lymphocytes from patients with

recurrent aphthous ulcerations. Am J Chin Med 2004; 32:221-34.
16. Greene WC, Depper JM, Krönke M, Leonard WJ. The human interleukin-2 receptor: Analysis of structure and function. Immunol Revi 1986; 92:29-48.
17. Glaser R, Kennedy S, Lafuse WP, Bonneau RH, Speicher C, Hillhouse J, *et al*. Psychological stress-induced modulation of interleukin 2 receptor gene expression and interleukin 2 production in peripheral blood leucocytes. Arch Gen Psychiatry 1990; 47:707-12.
18. Khan, A., & Darmal, D. I. Frequency of Type II Diabetes Mellitus in ST Segment Elevated MI patients presented to French Medical Institute for Mothers and Children: A Cross Section Study. Journal of Medical Research and Health Sciences, 2022:5(6), 2023–2038.
19. Sun A, Chia JS, Wang WB, Chiang CP. "Tien-Hsien liquid" can modulate antigen-stimulated cytokine production by T-cells isolated from patients with recurrent aphthous ulcerations. Am J Chin Med 2005; 33:559-71.
20. Fröde TS, Tenconi P, Debiasi MR, Medeiros YS. Tumour necrosis factor-□, interleukin-2 soluble receptor and different inflammatory parameters in patients with rheumatoid arthritis. Mediators Inflamm 2002; 11:345-49.
21. Eguia-del Valle A, Martinez-Conde-Llamosas R, López-Vicente J, Uribarri-Etxebarria A, Aguirre-Urizar JM. Salivary levels of Tumour Necrosis Factor-alpha in patients with recurrent aphthous stomatitis. Med Oral Patol Oral Cir Bucal 2011;16: e33-6.