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Evaluation of Salivary Interleukin 2 (IL-2) and Interleukin 12(IL-12) in Recurrent Aphthous Stomatitis

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Abstract

Recurrent aphthous stomatitis (RAS) is a common kind of excruciating mouth mucosal disease. Despite their high prevalence, etiopathogenesis remains unclear. This study was to investigate the concentration of Interleukin 2 (IL-2) and Interleukin 12 (IL-12) in the saliva of RAS. Eighty people were involved In this study (forty RAS patients and forty healthy controls), their ages ranged (from 16 - 60) years. The sandwich enzyme-linked immune-sorbent assay was used to assess Interleukin 2 (IL-2) and Interleukin 12 (IL-12) concentrations in saliva in each group. The salivary concentrations of Interleukin 2 (IL-2) and Interleukin 12 (IL-12) was significantly higher in the patient group (159.06±20.37), (331.32±94.42) respectively in comparison to the control group at ($p \leq 0.05$). These results indicate that saliva provides the perfect medium for the detection of pro-inflammatory markers of the oral cavity, Additionally, salivary Interleukin 2 (IL-2) and Interleukin 12 (IL-12) may play a significant role in the pathophysiology of this illness.

Keywords: Recurrent aphthous stomatitis, salivary evaluation, interleukin, pro-inflammatory.

1. Introduction

Until now, the etiology of recurrent aphthous stomatitis (RAS) has remained unknown, although genetic susceptibility, infectious agents, and alterations in immune mechanics have been implicated [1, 2]. Recurrent aphthous stomatitis (RAS) is a prevalent oral mucosal disease characterized by a yellowish-gray base with elevated margins and an erythematous halo. RAS typically manifests in the lining or mucosa that is not keratinized [3, 4], it has a prevalence of 50–66% and it is one of the most prevalent conditions affecting the oral mucosa [5, 6]. Recurrent aphthous stomatitis is frequently observed and is immune-mediated [7, 8] . RAS can be managed through a wide variety of preventative measures and therapies, intending to reduce ulcer pain, stimulate ulcer healing, and/or prevent ulcer recurrence [9]. First-line treatment options include topical medications in the form of corticosteroids (triamcinolone acetonide), anti-inflammatory drugs (amlexanox), antibiotics (doxycycline), and antiseptics (lidocaine) [10]. In more severe cases of RAS where local treatment is insufficient, systemic drugs in the form of corticosteroids (prednisone), immunomodulatory drugs (thalidomide), and antibiotics/antimicrobials (clofazimine) can prove effective [11]. Because they are essential mediators of the immune

response, cytokines have received a lot of attention in the immunological pathophysiology of RAS [12]. Recent studies suggest that RAS may be brought on by a cellular defect in the oral mucosa membrane, which results in a cellular immune response focused on the portion of the oral mucosa membrane at its center [13-16]. Two different types of cytokines pro-inflammatory cytokines that induce cell-mediated immunity and are produced by Th1 (IL-2, IL-12, IFN-g and TNF-a) and anti-inflammatory cytokines (IL-4, IL-5, IL-10 and IL-13) produced by Th2 and promote humoral immunity and suppression of cell-mediated immunity [17, 18]. The imbalance of Th1/ Th2 immune response is strongly influenced by their cytokine environment [19]. To fight infections, cancers and self-antigens, the primary immune response mediators are cytokines. They have a significant impact in examining the pathophysiology of immune responses [20]. Among the cell types that make cytokines are type I and type II helper T cells. T helper cells release two types of cytokines: type 2 (IL-4, IL-5, IL-6, IL-10, and IL-13), which improve humoral immunity and tolerance, and type 1(interleukin (IL-2), IL-12, interferon (IFN), and tumor necrosis factor (TNF), which enhance cellular immunity [21]. The cytokine profile controls immune activation and tolerance [22]. IL-2 is a 15-kDa glycoprotein that was originally referred to as T-cell growth factor (TCGF). Helper T cells that have been activated are the main sources of IL-2 secretion. For controlling cellular and cellular-chronic inflammatory responses, it is essential. T cells have an IL-2 receptor that IL-2 binds to, which causes the production of lymphokines and the promotion of cell proliferation [23, 24]. Natural killer cells, as well as antigen-presenting cells like dendritic cells (DCs) and macrophages, produce the interleukin-12 [25]. The aim of this study was to investigate the concentration of Interleukin 2 (IL-2) and Interleukin 12(IL-12) in saliva of patients with RAS.

2. Materials and methods

The study included 40 patients with RAS ranged between (16-60) years., compared with 40 apparently healthy individuals ranged between (16-60years) considered as a controls. Saliva was collected from patients and control group between 9-12am to evaluate the concentrations of Interleukin 2 (IL-2) and IL-12 in saliva Were measured by enzyme-linked immunosorbent assay (ELISA) kit that is commercially available. This was completed in accordance with the booklet of the kit's instructions. Interleukin 12 (IL-12) and IL-2 ELISA kit for humans (My BioSource, USA).

2.1. Statistical analysis

Data description, analysis and presentation have been performed using computerized software statistical package for social science (SPSS version-22). Shapiro Wilk test was used to test the normality distribution of the quantitative variable. Both descriptive and inferential statistics were used [26], the descriptive statistics included: frequency, percentage, minimum, maximum, mean, standard deviation (SD) and graphical presentation by bar charts. Inferential statistics were used to accept or reject the statistical hypotheses which included: Analysis of variance student t-test and Chi-square test. The statistical significance of difference of mean between 2 groups was calculated by T-test and Chi-square test. Correlation among different parameters was calculated by the Pearson correlation coefficient test. In the statistical evaluation, the following levels of significance are used: Not significant $p>0.05$, Significant $p\leq0.05$, Highly significant $p\leq0.01$.

3. Results

3.1. The concentration of IL-2 in saliva

The results of this study showed, there is elevation in the concentration of IL-2 in saliva of patients with (RAS)(159.06) Comparing with it is concentration in saliva of control group (**Table 1**), (**Figure 1**).

Table 1. Descriptive and Analytic Statistics of Mean ±SD IL-2 concentration level for patients and Control.

IL-2 pg/ml	Group of the Patients N=40	Group of the Control N=40	T-test (P.value)
'Minimum'	125.84	65.66	
'Maximum'	236.21	98.95	
Mean	159.06	82.09	0.000**
SD	20.37	8.35	
SE	3.22	1.32	

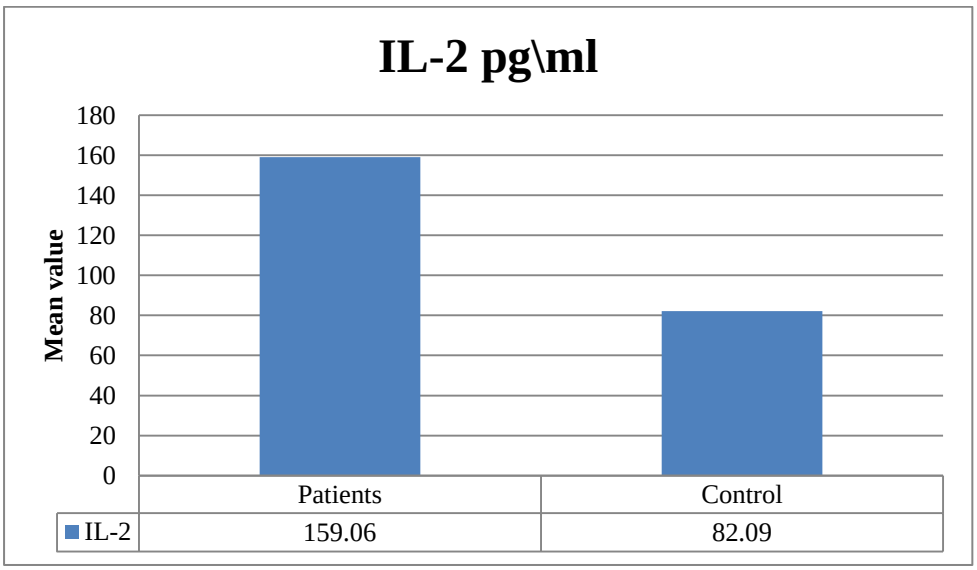

Figure 1. Descriptive and Analytic Statistics of Mean Value of IL-2 concentration for patients and Control.

3.2. The concentration of IL-12 in saliva

The results of this study showed ,there is elevation in the concentration of IL-12 in saliva of patients with (RAS)(331.32) Comparing with it is concentration in saliva of control group (**Table 2**, **Figure 2**).

Table 2. Descriptive and Analytic Statistics of Mean ±SD IL-12 concentration for patients and Control.

IL-12 pg/ml	Group of the Patients N=40	Group of the Control N=40	T-test (P.value)
Minimum	228.93	79.29	
Maximum	811.55	199.98	
Mean	331.32	130.31	0.000**
SD	94.42	34.38	
SE	14.93	5.43	

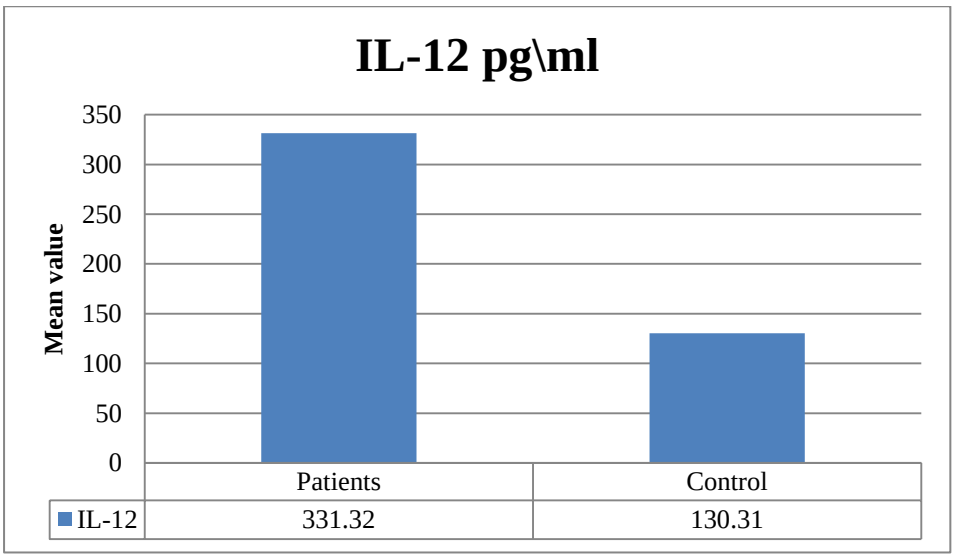

Figure 2. Effect of Age groups in parameters of sample studied.

4. Discussion

4.1. The concentration of IL-2 and IL-12 in saliva

In this study, the patient group had significantly greater concentrations of IL-2 and IL-12 in saliva than the control group. This is essential because RAS frequently affects the oral mucosa and is caused by alterations in cellular and humoral immunity. These results are in accordance with other results The level of IL-2 and IL-12 is highly increased while IL-10 is decreased in patients. The age group 20 to 40 years showed a higher prevalence [27]. These observations may be explained by the fact that The pathogenesis of RAS involves cell-mediated responses, involving T cells and tumor necrosis factor (TNF)-α production by these and other leucocytes. TNF is a pro-inflammatory cytokine .Secreted by activated monocytes, causing activation of cytotoxic T lymphocytes and neutrophils; epithelial necrosis and eventually the development of an aphthous lesion [28]. This study may also be explained by the fact that type-1 Th1 cytokines include pro-inflammatory cytokines like IL-2, IL-12, IFN, and TNF, wihich activate cell-mediated immunity [19]. Significant elevation in secretion of Th1 cytokine that produces pro-inflammatory cytokines (IL-2, IL-12 and IFN-y) increased in patients with RAS in comparison to healthy patients was demonstrated in earlier studies [29, 30].

5. Conclusions

According to these results, saliva is the optimal medium for detecting oral pro-inflammatory markers. The concentration of the inflammatory interleukin 2 (IL-2) and interleukin 12 (IL-12) in the saliva may also have a substantial effect on the pathogenesis of this disease.

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Conflict of Interest

There is no conflict of interest.

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