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Original Article

Serum Level of Interleukin-17 in Patients with Recurrent Aphthous Stomatitis

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is an inflammatory nature, while interleukin 17 (IL-17) has a pre-inflammatory role. In the present study, serum level of IL-17 was measured in a group of individuals with RAS, compared to healthy individuals.

Methods: This case-control study was done on 36 patients with RAS as cases and 36 healthy subjects as controls. The level of IL-17 was measured, using ELISA method.

Results: The mean serum level of IL-17 in cases was 0.14±0.21 pg/ml, which was insignificantly lower than 0.21±0.17 pg/ml in control group (P=0.12). Meanwhile there was a significant reverse correlation between age and serum level of IL-17 (r=-0.32, P=0.006).

Conclusion: Although the serum level of IL-17 in RAS patients was lower than the controls, the difference was not significant. However, significantly lower serum level of IL-17 in older subjects.

Keywords: Aphthous Stomatitis; Interleukin-17; Inflammation; RAS

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Introduction

Recurrent aphthous stomatitis (RAS) is one of the most common oral diseases (1), characterized by recurrent aphthous lesions in oral mucosa and usually appears in the second and third decades of life (2). Although few pathologic processes such as immunodeficiency, decreased integrity of mucosa, and increased exposure to antigens have been proposed as etiologies of RAS, The exact pathophysiology of disease has not been completely understood yet.

IL-17 is a protein with 32 KDa molecular weight, belonging to a family with the same name, which contains 6 cytokines, including IL-

17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. IL-17 cell receptors (IL-17R) exist in endothelial, peripheral T, B cells, fibroblasts, lung, myelomonocytic, and bone marrow stem cells. IL-17 plays an important role in delayed type reaction, and like gamma interferon, recruits neutrophils and monocytes to the inflammation area. Subsequent to tissue destruction and delayed inflammatory response, IL-23 secretion induces T-helper cells to produce IL-17. These cytokines acting synergistically with TNF-α and IL-1 are secreted in response to extra cellular pathogens and destroy their cellular matrix (3, 4). It is comprised of 155 amino-acids (5), and interestingly, its protein structure does not resemble to any other interleukins or other known proteins (6).

IL-17 receptors comprises five main groups of which, IL-17R is the most important one that IL-17A and IL-17F are attached to it. Due to the reactions of these cytokines with several molecules, many roles have been defined for them. However, the most important role of them is contribution to pre-inflammatory processes. They also play an important role in allergic reaction and induce secretion of many other cytokines such as GM-CSF, IL-1β, TGF-β, TNF-α, IL-6, and G- CSF; such chemokines as MCP-1, GRO-α, and IL-8; and PGE2 prostaglandin. The cells secreting these factors due to induction by this cytokine are epithelial cells, endothelial cells, fibroblasts, macrophages, and keratinocytes. Thus, this cytokine can recruit other inflammatory cells and induce changes in the mucosa of airways. The other important role of this cytokine is its effect on T helper 17 cells, a certain type of CD4+ cells, leading to its key role in such autoimmune conditions as Rheumatoid Arthritis, asthma, lupus, allograft rejection, and anti-tumor immunity (6).

With regard to pre-inflammatory role of IL-17 and inflammatory nature of RAS, the present study investigated the possible relation between serum level of IL-17 and occurrence of RAS.

Materials and Methods

This case-control study was done on 36 RAS patients referred to Oral and Maxillofacial Diseases Department, School of Dentistry, Tehran University of Medical Sciences, as case group, and 36 healthy subjects from the same departments as controls. The exclusion criteria included presence of any other local or systemic diseases with oral manifestations such as Bechet's syndrome, celiac disease, AIDS, etc; presence of any mucocutaneous diseases; and presence of periodontal diseases.

Three ml blood sample was obtained from the subjects. Centrifuge process was done on the clotted blood samples, and the plasma was collected and preserved in -20° C. The level of IL-17 was determined, using Sandwich ELISA method (eBioscience, USA) according to manufacturer's instructions. The concentration of IL-17 (picogram/milliliter) was calculated based on standard curves from OD of standard wells.

T-test and linear regression model served for statistical analysis. P value of less than 0.5 was considered as a level of significance.

Results

The case group comprised 18 men and 18 women, with a mean age of 37.97±8.43 (range 23 to 60) (**Table 1**). The mean age in control group, with the same men/women ratio was 36.19±8.61 (range 23 to 58).

The mean serum level of IL-17 in case and control group was 0.14±0.21 pg/ml, and 0.21±0.17 pg/ml, respectively, with no statistically significant difference (P=0.12) (**Figure 1**).

According to linear regression analysis, no statistically significant correlation existed neither between gender and serum level IL-17 (P=0.41) nor between RAS and serum level IL-17 (P=0.18). However, a significant reverse correlation existed between age and serum level IL-17 (r=-0.32, P=0.006).

Discussion

Recent evidences have shown that RAS is initiated by vast release of cytokines. This process leads to primary cell-mediated immune response against local sites of oral mucosa (7, 8). The first changes during the development of aphthous ulcers are local destruction of epithelial cells and formation of intra-epithelial vesicles (9). Then a dense infiltration of lymphocytes-monocytes occurs around disturbed cells, with T CD8+ lymphocytes comprising the majority of the cells at the beginning, replacing with T CD4+ lymphocytes at ulceration stage. However, the majority of

Table 1. Characteristics of the patients with RAS.

Patient's code	Age (years)	Sex	History of aphthous	Number of aphthous lesions	IL17 (pg/ml)
A1	44	Male	Since 17 years ago	Always	0
A2	44	Female	Since 20 years ago	1 per month	0
A3	42	Female	Since early childhood	1 per month	0
A4	23	Female	Since early childhood	2 per month	0.29
A5	53	Female	Since 5 years ago	Always	0.11
A6	32	Female	Since last year	5-6 per year	0.65
A7	43	Male	Since 4 years ago	Always	0
A8	41	Male	Since 4 years ago	4 per year	0
A9	37	Male	Since 3 years ago	Always	0
A10	38	Female	Since 4 years ago	Always	0
A11	27	Female	Since 5 years ago	4-5 per year	0.55
A12	35	Male	Since 3 years ago	1 per month	0.51
A13	36	Male	Since early childhood	1 per month	0.01
A14	44	Female	Since early childhood	Always	0.1
A15	37	Female	Since early childhood	Always	0
A16	32	Male	Since 10 years ago	7-8 per month	0
A17	39	Female	Since 10 years ago	8-10 per year	0
A18	45	Male	Since 20 years ago	Always	0
A19	50	Male	Since 3 years ago	1 per month	0.01
A20	40	Male	Since last year	Unknown	0.39
A21	34	Female	Since 9 years ago	6 per year	0.015
A22	31	Female	Since 3 years ago	3-4 per month	0.45
A23	35	Male	Since 10 years ago	5-6 per month	0
A24	32	Female	Since 3 years ago	1 per month	0
A25	36	Male	Since 10 years ago	7-10 per year	0
A26	38	Female	Since first pregnancy	2 per month	0.3
A27	40	Female	Since early childhood	1 per month	0
A28	25	Male	Since early childhood	1 per month	0.41
A29	28	Male	Since early childhood	4 per year	0.015
A30	28	Female	Since 5 years ago	3-4 per month	0.29
A31	57	Male	Since 10 years ago	10 per year	0
A32	39	Male	Since early childhood	2 per month	0.59
A33	42	Female	Since 3 years ago	1 per month	0
A34	60	Female	Since first pregnancy	Always	0.1
A35	29	Male	Since early childhood	Always	0.015
A36	31	Male	Since early childhood	3-4 per month	0.42

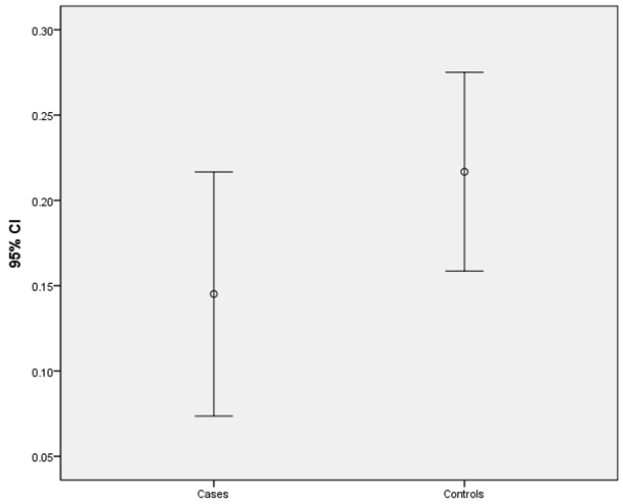

Figure 1. The mean serum level of IL-17 in cases with RAS and controls.

the cells at this stage are memory CD4 SRO+ cells (10).

These changes are associated with cell-mediated immune response and secretion of such pre-inflammatory as TNF, Interleukin-2 (IL-2), and IFN- (11). It is notable that circulating leukocytes in patients with RAS compared to those in healthy subjects release elevated amount of TNF (12). Development of Th1 and Th2 is mediated by IL-12 and IL-10, respectively (13). Recent scans with cDNA microrrary analyses have shown more activity by gene cluster of Th1 compared to that of Th2 in patients with RAS (8). Thus, it can be supposed that the T cells engaged in RAS express increased level of IL-12 and decreased level of IL-10. This hypothesis is supported by such findings as decreased level of IL-10 related mRNA in Keratinocytes in normal mucosa of patients with RAS compared to that in mucosa of healthy subjects, and deficient activity of mRNA in RAS patient (8).

The mucosal barrier is very important against RAS development as the more frequency of RAS occurrence in non-keratinized parts of the mouth shows (14).

To the best of our knowledge, the present study was the first study investigating relation between RAS and serum level of IL-17. According to the results, no statistically significant difference existed between the serum level of IL-17 in RAS patients and that in control group, although the serum level of IL-17 in case group was lower. The insignificancy might come from the sampling, which was done regardless of presence of RAS lesions in the mouth of the patients. Sampling at the time of presence of active lesions, and after healing may results in significant differences between the two groups.

Najafi et al. in 2014 compared repetition of alleles and genotypes of IL-10 in the blood of 60 RAS patients with that among healthy subjects. They found no statistically significant difference, although certain SNPs of IL-10 gene was associated with the susceptibility to RAS (15). Their results resemble to ours, and such findings suggest that backgrounds other than inflammatory background might contribute to occurrence of RAS.

Caproni et al. measured the level of serum IL-17 in patients with psoriasis before and after treatment using ELISA method, and compared it with that among healthy controls (16). Serum level of IL-17 was higher among the cases, and decreases after completion of the treatment (16).

Shaker and Hassan compared the level of serum IL-17 in patients with cutaneous lichen planus with that among healthy controls (17). Contrary to our study, the serum level of IL-17 was higher among patients in that study (17). This difference may result from the different nature of the two diseases, so that the role of IL-17 in lichen planus is more important compared to that in RAS with probable other factors.

Lower IL-17 levels among older subjects in our study can be attributed to the decreased capability of immune system in older ages. On the other hand, the similarity of IL-17 serum levels among the two genders may show that the gender is not an important factor in occurrence of RAS.

In the present study the serum level of IL-17 in RAS patients did not differ significantly with that in healthy subjects. The significantly lower serum level of IL-17 in older subjects may be due to weakness of immune system in older ages.

Conflict of interest

The authors have no conflicts of interest.

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