The Role of Serum Levels of Interleukin 17 in Patients with Pemphigus

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Abstract

Introduction:
Pemphigus is a relatively rare autoimmune bullous disease involving skin and mucous epithelia. The disease occurs as a result of production of autoantibodies against intercellular epithelia especially cadherin and desmoglein. Interleukin 17 (IL-17) is one of the specific cytokines of T helper cells. IL-17 plays an important role in delayed-type reaction and recruits neutrophils and monocytes to the inflammation area. The other important role of IL-17 is its effect on T helper 17 cells, a certain type of CD4+ cells, leading to its key role in autoimmune vesiculobullous diseases. The present study investigated the levels of IL-17 in serum among pemphigus patients in comparison with healthy subjects.

Materials and methods:
In this case-control study, blood samples (3 ml) were collected from 43 patients with pemphigus and 36 healthy subjects. Centrifuge process was done on the clotted blood samples and the plasma was collected and preserved in −20ºC. The level of IL-17 was determined through sandwich enzyme-linked immunosorbent assay (ELISA) method. Mann-Whitney, t-, and chi-square tests, and also the linear regression model (P < 0.05), were used for data analysis.

Results:
No difference existed between case and control groups regarding serum levels of IL-17 (P = 0.143). The regression model showed no significant linear correlation between gender and blood levels of IL-17 (P = 0.164). However, blood level of IL-17 was reversely correlated to age (β = −0.0236, B = −0.029, P = 0.036).

Conclusion:
Serum level of IL-17 could not be considered as a good marker to differentiate pemphigus from the healthy subjects.

Key words:
•Interleukin-17 •Pemphigus •Serum

Introduction

One of the relatively rare autoimmune bullous diseases involving the skin and mucous epithelia is pemphigus.[1] Several variants of this disorder have been known,[2] the most prevalent of which is pemphigus vulgaris (PV).[3] The annual incidence of PV has been reported to be 1/100,000 in Iran, with a mean age of onset of 42 years, a female to male ratio of 1.5/1, and a death rate of about 6%.[4]

In PV, autoantibodies are produced against such desmosome components of epithelium as cadherin and desmoglein (Dsg), especially Dsg3.[2] These glyco-protein molecules are found in a membrane in desmosome and strengthen intercellular connections. Lack of these connections due to antibody-antigen reactions leads to weakness of intercellular connections, and finally separation of the cells, which, in turn, results in desquamation and blister formation.[5] Since Dsg3 is expressed mainly in oral epithelium,[6] the first manifestation of the disease is mucosal involvement among 74% of the cases.[7] Moreover, 80%–90% of the patients show oral manifestations during their disease period, and oral lesions are the first signs of the disease in 60% of the cases.[5]

To diagnose the disease, biopsy of skin and/or mucous in addition to histological and biological investigations, is necessary.[8] Suprabasal acantholysis and blister formation are in favor of PV with a high probability. However, the definite diagnosis of PV will be made when deposition of IgG is observed in intercellular spaces of epidermis.[9] Corticosteroids, especially prednisone, are prescribed locally to treat mild to moderate cases of PV. In severe cases, systemic corticosteroids are used.[2]

Auto-reactive T cells play a role in induction of antibody production. It has been hypothesized that , cytokines have an essential role in the disease pathogenesis. It has been emphasized that PV is a Th2-mediated disease, confirmed by many studies demonstrating an increase in Th2-type cytokines. Recently, the studies have focused on the role of the recently discovered Th17 subset in occurrence of PV.[10]

Interleukin 17 (IL-17) is a protein that exists in endothelial, peripheral T and B cells, fibroblasts, lung, myelomonocytic cells, 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 induces tissue destruction and delayed inflammatory response, production of IL-17 induced by IL-23. These cytokines that acting synergistically with TNF-α and IL-1, are secreted in response to extra cellular pathogens and destroy their cellular matrix.[11-13].

Due to the reactions of these cytokines with several molecules, many roles have been defined for them. However, their most important role is contribution to pre-inflammatory processes. 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.[14-16]

Regarding the important role of inflammatory cytokines such as IL-17 in the immune response of autoimmune diseases mentioned above, we decided to more accurately study the role of this cytokine in the pathogenesis of PV.

Materials and Methods

This case-control study was done on 43 PV patients referred to Razi Hospital, Tehran University of Medical Sciences (TUMS), Tehran, as a case group, and 36 healthy subjects referred to Oral and Maxillofacial Diseases Department, International Campus of School of Dentistry, TUMS. Inclusion criteria for the case group were biopsy-confirmed PV and presence of PV lesions on skin and in mouth. The exclusion criteria included presence of any systemic diseases such as hepatitis, diabetes, hypertension, cardiovascular diseases, pregnancy; presence of periodontal disease; taking immuno-suppressor drugs during past month; and being under diet therapy during past three months.

Three ml of blood sample was obtained from each subject. Centrifuge process was done on the clotted blood samples, and the plasma was collected and preserved in −20ºC. The level of IL-17 was determined through sandwich enzyme-linked immunosorbent assay (ELISA) method using specific kits for IL-17.

The concentration of IL-17 (picogram/milliliter)

was calculated based on standard curves from OD of standard wells. Abcam's IL-17 (Interleukin-17) Human in vitro ELISA kit is designed for the assessment of IL-17 in supernatants, buffered solutions, serum, plasma, and other body fluids. A monoclonal antibody specific for IL-17 was coated onto the wells of the provided microtiter strips. Samples, including standards of known IL-17 concentrations, control specimens or unknowns were pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for IL-17 were simultaneously incubated.

After washing, the enzyme Streptavidin-HRP that binds the biotinylated antibody was added, incubated, and washed. A tetramethyl benzidine (TMB) substrate solution was added, which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of IL-17 present in the samples.

Statistical analysis

SPSS version 22 served for data gathering. Data analysis was done using chi-square test, t-test, and Mann-Whitney test, and linear regression model.

Results

The mean age of 43 PV patients (24 men and 19 women) was 36.46 ± 9.0 years. The mean age of 36 healthy subjects (18 men and 18 women) was 36.19 ± 8.61 years. There is no significance differences between the two groups. The age distribution in the case group also was not significantly different from that in control group (P = 0.606).

Table 1 shows the mean of IL-17 serum levels in case and control groups. Although the mean serum level of IL-17 in the case group was higher than that in control group, the difference was not significant.

The linear regression analysis with age, gender, and PV as independent variables revealed no linear correlation between gender and mean serum level of IL-17 (P = 0.574), as well as between PV and mean serum level of IL-17 (P = 0.16). However, age was reversely correlated with mean serum level of IL-17 ($\beta = -0.0236$, B = -0.029, P = 0.036).

Table 1. Mean serum level of IL-17 among 43 patients with pemphigus vulgaris (case group) and 36 healthy subjects (control group).

Study group	N	Mean serum level of IL-17 (pg/ml**)	SD*	Minimum	Maximum
Case	43	0.537	1.425	0	0.78
Control	36	0.216	0.172	0	0.62

*Standard deviation, ** Picogram/milliliter

Discussion

With regard to the role of IL-17 in tissue inflammation related to PV and its possible role in occurrence of PV lesion, the present study compared serum level of IL-17 in PV patients with that in healthy subjects. No significant difference existed between case and control groups regarding serum level of IL-17, although it was higher in the case group.

Since we found no similar study in the literature, our results have been compared with studies on IL-17 and other diseases. Al-Samadi et al.[17] investigated the eventual presence and function of IL-17C in cultured human oral keratinocytes and control biopsies compared to recurrent aphthous ulcer (RAU) lesions. They found that interleukin IL-17C highly expressed in epithelial cells in RAU lesions, where it seems to stimulate oral keratinocytes to produce pro-inflammatory cytokines.

Caproni et al.[18] measured the serum levels of IL-17 among 20 patients with psoriasis before and after treatment using ELISA technique. According to their results, the level of IL-17 among patients was higher than that among healthy controls, and its level decreased after completion of treatment. Both PV and psoriasis have inflammatory and autoimmune nature. However, the higher level of IL-17 in serum of PV patients compared to that of healthy subjects in our study was not significant.

Differences in the results of the studies on psoriasis and PV can be explained by the differences in pathogenesis. There is evidence suggesting pathogenic process of psoriasis is driven by

autoantigen.[19] The reason for this comparison is the lack of similar study in this field.

Shaker and Hassan studied the serum level of IL-17 among 20 patients with lichen planus and compared it with that among 20 healthy subjects. In their study, the level of IL-17 in serum of the patients was higher than that of healthy subjects.[20] Again, the difference between their results and ours might come from the difference in sample size. In a study by Mortazavi et al., the mean level of IL-17 of PV patients was higher than in the controls, but the difference was not significant.[21] The findings of this research are compatible with our study.

Studies of Sinha's group showed a higher level of IL-17A in the active phase of PV.[10,22] The findings of the current study were similar to this research because our study was in the active phase of PV.

According to our research, gender and PV as independent variables revealed no correlation between gender and mean serum level of IL-17 as well as between PV and mean serum level of IL-17, because of random selection and because men participated more than women. The other notable finding in our study was the reverse correlation between age and serum level of IL-17, which might be due to decrease in capability of the immune system as a consequence of aging. The importance of this finding is the correlation between the immune system and IL-17, because of decreasing of levels of both; on the other hand, the immune system and PV have a reverse correlation.

Conclusion

Based on our results, the higher level of IL-17 in serum of PV patients compared to that in serum of healthy subjects was not significant, which may be due to small sample size. Thus, further studies on this subject with bigger sample sizes are recommended.

Conflict of Interest

Authors declare no conflicts of interest.

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Refrences

1.Veldman C, Feliciani C. Pemphigus: a complex T cell–dependent autoimmune disorder leading to acantholysis. Clin Rev Allergy Immunol. 2008;34(3):313-20.

2.Scully C, Challacombe SJ. Pemphigus vulgaris: update on ethipathogensis, oral mainfestatins, and management. Crit Rev Oral Biol Med. 2002;13(5):397-408.

3.Thorat MS, Raju A, Pradeep AR. Pemphigus vulgaris: effects on periodontal health. J oral Sci. 2010;52(3):449-54.

4.Chams-Davatchi C, Valikhani M, Daneshpazhooh M, Esmaili N, et al. Pemphigus: Analiysis of 1209 cases. Int J Dermatol. 2005;44(6):470-6.

5.Burket LW, Greenberg MS, Glick M. Vesicular and Bullous Lesion. In: Custance P. Burket's Oral Medicine. 11th ed. Bc Decker, 2008:62-3.

6.Shirakata Y, Amagai M, Hanakawa Y, Nishikawa T, et al. Lack of mucosal involvement in pemphigus foliaceus may be due to low expression of desmoglein 1. J Invest Dermatol. 1998;110(1):76-8.

7.Asilian A, Yoosefi A, Faghini G. Pemphiguse vulagaris in Iran: epidemiology and clinical profile. Skinmed. 2006;5(2):69-71.

8.Harman KE, Gratian MJ, Seed PT, Bhogal BS, et al. Diagnosis of pemphigus by ELISA: a critical evaluation of two ELISAs for the detection of antibodies to the major pemphigus antigens, desmoglein 1 and 3. Clin Exp Dermatol. 2000;25(3):236-40.

9.Harman KE, Albert S, Black MM; British Association of Dermatologists. Guidelines for the management of pemphigus vulagaris. Br J Dermatol. 2003;149(5):926-37.

10.Giordano CN, Sinha AA. Cytokine networks in Pemphigus vulgaris: An integrated viewpoint. Autoimmunity. 2012;45(6):427-39.

11.Beissert S, Werfel T, Frieling U, Böhm M, et al. A comparison of oral methylprednisolone plus azathioprine or mycophenolate mofetil for the treatment of pemphigus. Arch Dermatol. 2006 ;142(11):1447-54.

12.Margolis DJ. A randomized trial and the treatment of pemphigus vulgaris. J Invest Dermatol. 2010;130(8):1964-6.

13.Chiricozzi A, Guttman-Yassky E, Suárez-Fariñas M, Nograles KE, Tian S, Cardinale I, et al. Integrative responses to IL-17 and TNF-α in human keratinocytes account for key inflammatory pathogenic circuits in psoriasis. J invest Dermatol. 2011; 131(3): 677-87.

14.Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity. 2004;21(4):467-76.

15.Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. J Leukoç Biol. 2002;71(1):1-8.

16.Kawaguchi M, Adachi M, Oda N, Kokubu F, et al. IL-17 cytokine family. J Allergy clin Immunol. 2004; 114(6): 1265-73.

17.Al-Samadi A, Kouri VP, Salem A, Ainola M, et al. IL-17C and its receptor IL-17RA/IL-17RE identify human oral epithelial cell as an inflammatory cell in recurrent aphthous ulcer. J Oral Pathol Med. 2014;43(2):117-24.

18.Caproni M1, Antiga E, Melani L, Volpi W, Del Bianco E, Fabbri P. Serum levels of IL-17 and IL-22 are reduced by etanercept, but not by acitretin, in patients with psoriasis: a randomized-controlled trial. J Clin Immunol. 2009;29(2): 210-4.

19.Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol. 2004;135(1):1-8.

20.Shaker O, Hassan AS. Possible role of interleukin-17 in the pathogenesis of lichen planus. Br J Dermatol. 2012;166(6):1367–8.

21.Mortazavi H, Esmaili N, Khezri S, Khamesipour A, Vasheghani Farahani I, Daneshpazhooh M, et al. The Effect of Conventional Immunosuppressive Therapy on Cytokine Serum Levels in Pemphigus Vulgaris Patients. Iran J Allergy Asthma Immunol 2014; 13(3):174-183.

22.Sinha AA. Constructing immunoprofiles to deconstruct disease complexity in pemphigus. Autoimmunity 2012; 45(1):36-43.