SHORT PAPER

Elevated Levels of Interleukin-23 in Sera of Patients with Pemphigus Vulgaris

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ABSTRACT

Background: Pemphigus vulgaris, a chronic mucocutaneous disease, is the most prevalent type of pemphigus which manifests with development of bullae and erosions on skin and mucosal membranes. **Objectives:** To investigate the potential role of IL-23 in pemphigus vulgaris. **Methods:** In this study, 30 patients with pemphigus vulgaris and 30 healthy individuals were selected according to inclusion and exclusion criteria. Measurement of IL-23 serum levels in blood samples was conducted by ELISA. Data was analyzed using Student's *t-test* for comparison of IL-23 levels between the two groups. **Results**: Mean serum levels of IL-23 in patients with pemphigus and healthy controls were 25.1 ± 4.2 and 17.9 ± 4.7 pg/ml, respectively ($p<0.05$). **Conclusions**: Based on the findings of this study, serum levels of IL-23 were higher in patients with pemphigus in comparison to healthy individuals with no clinical significance.

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Keywords: Autoimmune Disease, Interleukin-23, Pemphigus Vulgaris

INTRODUCTION

Pemphigus vulgaris is a life threatening autoimmune disease and with an unknown etiology. Its prevalence is increasing and is more prevalent during the fifth and sixth decades. Predisposing factors for development of intraepithelial lesions in pemphigus vulgaris is the attachment of IgG auto-antibodies to intra-membranous glycoprotein connector molecules in desmosomes (DSG1 and DSG3). Lack of this connection due to antigen-antibody reaction leads to loose intercellular attachment and finally detachment of epithelial cells and development of blisters and desquamation (1,2).

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Presence of eosinophils and neutrophils in combination with mononuclear cells in the epithelium, dermis, and epidermis is a distinct histological feature of bullous autoimmune diseases. Eosinophilic spongiosis and neutrophilic spongiosis have been noted in primary studies on pemphigus vulgaris (3-5).

Interleukin-23 is a pro-inflammatory, heterodimeric cytokine containing a distinct p19 subunit and a p40 subunit shared with IL-12. IL-23 plays a crucial role in the inflammatory response of infections. This cytokine causes an elevation of some of matrix methaloproteinases; it also increases angiogenesis and reduces the infiltration of CD8+ lymphocytes (6). It has recently been shown that this cytokine plays a role in the development of neoplastic tumors (7-9). This cytokine, in combination with IL-6 and TGF-β1, leads to differentiation of Th17 cells which stimulate the production of inflammatory cytokines, such as IL-1, IL-6, TNF-α, and chemokines. In an experimental study conducted on rats by Kikly et al. it was indicated that the deficiency of production of IL-23 subunits or their receptors decreases the severity of disease signs and inflammatory reactions, demonstrating the role of IL-23 in inflammation process (10). Nguyen et al. studied animal and human specimens and reported that in autoimmune Sjögren's syndrome, Th17/IL-23 pathway was up-regulated (6).

With respect to the importance of pemphigus as a life-threatening autoimmune disease the potential role of IL-23 in pemphigus vulgaris was evaluated in the present study.

MATERIALS AND METHODS

Subjects. In this descriptive-analytical study, simple random sampling method was employed because of limitations in accessing patients. The subjects were patients with pemphigus vulgaris diagnosed based on clinical-pathological signs, referring to Sina Educational and Treatment Center at Tabriz University of Medical Sciences. Blood samples were taken during the active phase of the condition before treatment was instituted. PV was diagnosed by the immunofluorescence test. Patients with congenital and acquired immune deficiencies like AIDS, chemotherapy patients, injective drug addicts, and pregnant women were excluded from this study. Healthy control individuals were selected randomly from people referring to Tabriz Faculty of Dentistry. Thirty PV patients among which 12 were new cases and 30 healthy age/sex matched individuals entered to study. After chart completion and necessary examinations, 5-mL blood samples were taken from each subject.

ELISA Assay. IL-23 serum levels were measured by the ELISA technique (e-Bioscience, UK). To this end, first anti-interleukin-23 antibody was coated on certain plate wells. Then patients' isolated sera were added to the wells on it and after incubation, biotin-marked antibody was added and incubated again. Subsequently, enzymatic substrate was added and color changes were compared with standard charts (optical density charts) and recorded as IL-23 serum levels.

Statistical Analysis. Data was first reported by descriptive statistics. Kolmogorov-Smirnov analysis was used for evaluation of data distribution. Student's *t-test* was utilized for comparison of IL-23 between the two groups. Statistical significance was defined at $p<0.05$ in this study.

RESULTS

The demographic data of the patients are presented in Table 1.

Table 1. Demographic data of the subjects based on groups and IL-23.

Variable	Patient (n=30)	Control (n=30)	Statistics
Gender n(%)			
female	15 (50)	15 (50)	χ^2=0.00, df=1,
male	15 (50)	15 (50)	p>0.05
Age n (%)			
20-29 (year)	7 (23.4)	7 (23.4)	
30-39 (year)	20 (66.7)	21(70)	
40-50 (year)	3 (9.9)	2 (6.6)	t= -0.3, df=58,
Mean	33.2	33.6	p>0.05
SD	5.3	5.1	
min-max	22-42	23-43	
IL-23			
Mean	25.1	17.8	t= 7.47, df=56.7,
SD pg/ml	4.5	4	p=0.009

The mean serum levels of IL-23 in pemphigus vulgaris patients and healthy individuals were 25.1 (±4.2) and 17.9 (±4.7), respectively. In both the pemphigus and control groups, male-to-female ratio was 1:1. Mean age of pemphigus vulgaris patients and healthy individuals were 33.2 (5) and 33.6 (5), respectively. Student's *t-test* revealed no significant differences in the age of participants between the two groups. Therefore, age and gender factors were matched. Kolmogorov-Smirnov analysis indicated normal distribution of data (p=0.894). Therefore, Student's *t-test* was used for comparisons. Based on Student's *t-test*, the mean serum level of IL-23 was higher in patients with pemphigus vulgaris (p=0.009) (Figure 1).

DISCUSSION

Pemphigus is an autoimmune disease in which antibodies with unknown mechanisms are produced against surface glycoproteins (desmoglins 1 and 3) of epithelial cells incorporating desmosomes. Pemphigus vulgaris is the most prevalent type of the disease. Oral manifestations are first sings of disease in most cases. While anti-desmoglin antibody attaches to its target antigen, attachment function of desmosomes is vanished and acantholysis develops (1,11).

Various studies have addressed the presence of different antibodies in patients with pemphigus. In an experimental study conducted on rats by Kikly et al., it was indicated

that the deficiency of IL-23 subunits or their receptors decreases disease severity and inflammatory reactions, demonstrating the role of IL-23 in inflammatory process (10).

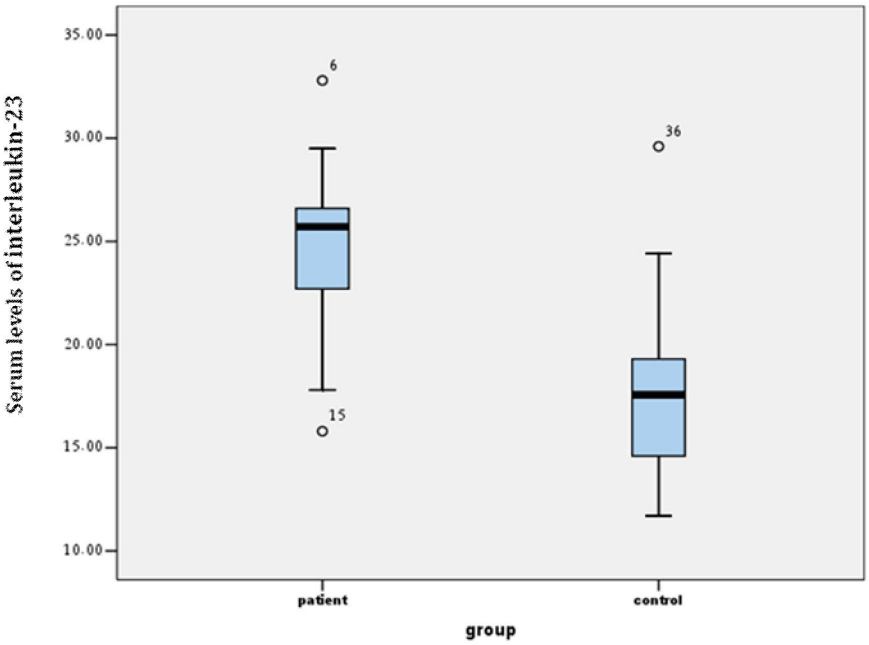

Figure 1. Box-plot of IL-23 level in sera of the two groups.

Toto et al. evaluated the role of IL-10 and CD28+ cells in the development of pemphigus in an experimental model. They suggested that CD28-deficient mice are 5 times more sensitive to the development of PV than wild-type mice. They also confirmed the potential role of IL-10 in pemphigus vulgaris (12). Feliciani et al. reported that complement C3 subunit is pivotal in pemphigus vulgaris acantholysis and that complement activation is elevated by IL-1α and TNF-α (13). Dass et al. indicated a novel organ-specific role for IL-23 in the pathophysiology of graft-versus-host disease (GvHD) and showed that IL-23 can induce tissue-specific pathology within the context of a systemic inflammatory disorder. Furthermore, they also defined IL-23 as a potential therapeutic target for the prevention of this life-threatening disorder (14). Ciccia et al. showed the role of IL-23 in autoimmune gut inflammation (Crohn's disease) (15). Nguyen et al. investigated the Th17/IL-23 system status in Sjögren's syndrome. They reported that the Th17/IL-23 system is up-regulated in patients and mice with Sjogren's syndrome at the time of disease, which coincided with the elevation of IL-17 and IL-23 levels (6). Costa et al. investigated the serum levels of several immunoglobolins in autoimmune disease and reported increased expression of IL-17 in sera and in target tissues of patients with various autoimmune diseases. They also indicated that in animal models IL-23 is involved in the development of autoimmune diabetes. In humans, IL-23 seems to cause multi-organ inflammation, contributing to rheumatoid arthritis, inflammatory bowel disease and celiac disease manifestations (16). It can be concluded that some autoimmune diseases are ascribed to the response of IL-17/23. It seems that Th17 differentiation is related to TGF-β, IL-6, IL-21, IL-1β, and IL-23 signaling

pathways. IL-23, a cytokine from IL-12 family, induces the inflammatory responses of Th17 (16,17). In the investigation carried out by Wenting et al., the IL-23 level increased at dermal lesions of patients with pemphigus (18). The results of the present study coincide with previous studies and showed that elevation of the serum levels of IL-23 in patients with pemphigus vulgaris could be attributed to the role of this interleukin in inflammatory and autoimmune diseases. In a study by Arakawa et al. the specimens from skin lesions of pemphigus vulgaris patients contained IL-17 producing cells; however, IL-17-positive cells were undetectable in healthy control skin samples (19).

Under the limitations of this study, serum levels of IL-23 in patients with pemphigus vulgaris were higher than healthy individuals, which indicate the potential role of this cytokine in the pathogenesis of pemphigus vulgaris.

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