LETTER TO THE EDITOR
Iran J AllergyAsthmaImmunol
February 2020; 19(1):102-105.
Doi:

Serum Levels of Interleukin 18 and Interleukin 10 in Iranian Patients with Bullous Pemphigoid and their Correlation with BP180-NC16a and BP230

Nafisseh Esmaili[1,2], Hossein Mortazavi[1,2], Mahshid Sadat Ansari[1], Maryam Nasimi[1], Yasaman Ardalan-rad[1], and Amir Teimourpour[3]

[1] Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2] Autoimmune Bullous Research Center, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Received: 19 May 2019; Received in revised form: 27 August 2019; Accepted: 29 August 2019

TO THE EDITOR

Significance of interleukin (IL)-10 in the pathogenesis of the autoimmune process and bullous pemphigoid (BP) is under investigation.[1] Activation of inflammasomes, an innate immune signaling complex, leads to IL-18 and IL-1β production. A recent study showed an increased level of IL-18 in blister fluid and serum of BP patients.[2]

Since in different studies and ethnicities, different cytokine responses have been reported,[3,4] we aimed to evaluate serum levels of IL-10 and IL-18 in first admitted untreated Iranian BP patients versus BP patients who were under treatment and compare it with healthy controls. In other words, we aimed to show the effect of therapy on cytokine levels. In addition, we used the BP180 and BP230 ELISA method for the diagnosis of BP and compared the levels of cytokines with BP180 and BP230 levels. We selected IL-10 and IL-18 because pieces of evidence showed that these two cytokines are involved in the pathogenesis of autoimmune diseases.[1,5]

Keywords: Bullous pemphigoid; Cytokines; Enzyme-linked immunosorbent assay; Interleukin-10; Interleukin-18

Corresponding Author: Yasaman Ardalan-rad, MD; Department of Dermatology, Razi Hospital, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 8827 2626, Fax: (+98 21) 6615 5050, E-mail: yasamanardalan@yahoo.com

activity. So, IL-18 binding protein (IL-18BP) which is an inhibitor of IL-18, might be used as an adjuvant to therapy of BP.

Eighty individuals entered the study in three groups, including 39 healthy controls (27 male and 12 female), 16 first admitted untreated BP patients (8 male and 8 female), and 25 BP patients who were under treatment with systemic agents (14 male and 11 female). The control group consisted of healthy volunteers without the established autoimmune disorder. In BP patients who were under treatment, the therapy was started with prednisolone 0.5 to 1 mg/kg/day and azathioprine1 to 2mg/kg/day, and then according to the treatment response, the doses were adjusted.

Blood sera were obtained from patients and healthy controls, then stored at -70°C.Cytokine measurement was performed by using the ELISA method. For the measurement of IL-10 and IL-18, the following kits (IBL International GmbH, Flughafenstraße, Hamburg, Germany) were used.

The diagnosis of BP was confirmed by histopathology, direct immunofluorescent, BP180, and BP230ELISA findings. Serum levels of immunoglobulin G (IgG) against BP180 and BP230 were measured with anti-BP180-NC16A-4X and anti-BP230-CF ELISA kits, respectively (EUROIMMUN, Medizinische Labordiagnostika AG, Germany).

The project was approved by Tehran University of Medical Sciences (TUMS) deputy of research (No. 97-03-101-39853), and by the ethics committee of TUMS

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

(IR.TUMS.MEDICINE.REC.1397.797).

For statistical analysis, the Mann Whitney test, Kruskal Wallis test, Bonferroni adjustment, Chi-square test, and Spearman correlation coefficient were used.

The mean age of first admitted untreated BP patients, BP patients under treatment and control group were accordingly 66.13, 65.52 and 58.67 years.

There were statistically significant differences in the level of IL-18 between three groups (p=0.008) (Table1). Based on the pairwise comparison, there were significant differences between healthy controls *vs.* first admitted untreated BP patients (p=0.009), and BP patients who were under treatment *vs.*first admitted untreated BP patients (p=0.028), but there was no significant difference between the control group and BP patients who were under treatment (p=0.857).

Regarding IL-10 levels, based on post hoc test, there was only a significant difference between healthy controls and first admitted untreated BP patients (p=0.015).

When comparing untreated BP patients (new cases) with patients being under treatment (treated), non-significant decreased levels of IL-18 and increased levels of IL-10 were observed (Table 1).

With regard to the level of BP180, there were significant differences between the control group *vs.* BP patients being under treatment and first admitted untreated BP patients (p<0.0001). Regarding the levels of BP230, there was a significant difference between the control group and first admitted untreated BP patients (p=0.026) (Table 1).

A significant weak correlation was found between IL-18 (rho=0.252, p=0.024), IL-10 (rho=0.37, p=0.001) levels, and Anti-BP180. Besides, a weak correlation between IL-18 (rho=0.298, p=0.007) and Anti-BP230 was observed.

Table 1. Serum levels of IL-18, IL-10, Anti-BP180 and 230 in three groups including healthy controls (Control), first admitted untreated BP patients (New Case) and BP patients under treatment (Treated)

Cytokine & ELISA	Groups	N	Mean	SD	Median	Minimum	Maximum	*p*-value*
IL-18(pg/ml)	Control	39	222.46	255.35	78	78	998	
	New case	16	599.88	519.47	520	78	1684	0.008
	Treated	25	249.20	284.16	78	78	1068	
IL-10 (pg/ml)	Control	39	3.18	1.10	3.00	3.00	9.90	
	New case	16	3.88	1.91	3.00	3.00	9.80	0.015
	Treated	25	4.30	5.27	3.00	3.00	29.10	
Anti-BP180 (U/ml)	Control	39	4.21	6.55	1.40	1.00	33.00	
	New case	16	170.33	147.88	150.50	3.60	423.00	<0.001
	Treated	25	81.09	113.20	25.00	1.00	376.00	
Anti-BP320 (U/ml)	Control	39	4.41	6.76	2.00	1.00	38.00	
	New case	16	72.21	114.52	12.60	1.00	356.00	0.022
	Treated	25	37.98	66.06	5.20	1.00	242.00	

*Kruskal Wallis test

In previous studies, the role of cytokines in the pathogenesis and severity of pemphigus and BP, as the most prevalent autoimmune bullous diseases, have been studied.[6,7]In our study, the mean serum level of IL-10 in first admitted untreated BP patients was significantly higher than its level in healthy controls (p=0.015). In the study of D'Auria et al increased serum levels of TNF-α, IL-6, and IL-10 as modulators of Th2 response was described.[8] Whereas in Ahmed's group study, only 2 of 15 BP patients had detectable serum levels of IL-10, Liu et al showed that in BP patients, the number of IL-10 producing B cells was increased.[9] Antiga et al demonstrated that there was an insignificant difference between the serum level of IL-10 in BP patients and healthy control groups.[1]

In the present study, the mean and maximum serum levels of IL-10 in BP patients who were under treatment were higher than their levels in first admitted untreated BP patients and the control group. However, there was no difference between the median and minimum levels of IL-10 (Table 1). In Antiga et al study, serum levels of IL-10 were increased after corticosteroid therapy. Increased number of IL-10 producing cells following corticosteroid therapy may be responsible for increased serum levels of IL-10.[1,9] In the present study, the higher levels of IL-10 in BP

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

patients who were under treatment might be due to the anti-inflammatory effect of corticosteroid therapy.

In the present study, the level of IL-18 (mean and median) in first admitted untreated BP patients was significantly higher than the control group (p=0.009). Moreover, its level in BP patients being under treatment was higher than the control group (Table 1).

IL-18 is a member of the IL-1 family cytokines. Macrophages, keratinocytes, and osteoblasts are major sources of IL-18.[10] Through interferon-gamma production, IL-18 may be involved in autoimmunity and increased levels of IL-18 have been reported in the autoimmune process. In addition, IL-18 contributes to innate immunity.[10] Therefore, IL-18 may be involved in the pathogenesis of autoimmune diseases such as BP.[11] The important finding of our study is the higher level of IL-18 in first admitted untreated BP patients in comparison with both the control group and BP patients who were under treatment. This finding suggests the important role of IL-18 in the disease activity and the point that IL-18 and innate immunity might be involved in the pathogenesis of BP. The lower level of IL-18 in BP patients being under treatment in comparison with treated patients shows the effect of therapy on IL-18 level (Table 1). Recently, IL-18 binding protein (IL-18BP) which is an inhibitor of IL-18 is discovered, therefore, it might be used as an adjuvant to therapy of BP.[5,11]

Regarding BP180 and BP230 titers in patients and healthy controls, the levels of auto-antibodies in BP patients were higher than the control group and these levels diminished after therapy. Our results were in accordance with previous studies.[12] We also found a weak significant correlation of BP180 with IL-18 levels. This finding is in accordance with previous report and correlation of IL-18 levels with the activity of BP.[2] Recent studies elucidated the critical role of BP180 in autoimmunity of BP, therefore the correlation of IL-18 with BP180 in our study shows the important role of IL-18 in the pathogenesis of BP.[13,14]

IL-18, innate immunity[15,] and IL-10 might be involved in the pathogenesis of BP. IL-18 binding protein (IL-18BP) could be used as part of BP therapy and for this purpose, further studies are needed.

ACKNOWLEDGEMENTS

This research has been supported by Tehran University of Medical Sciences and Health Services grant 97-03-101-39853.

REFERNCES

1. Antiga E, Quaglino P, Volpi W, Pierini I, Del Bianco E, Bianchi B, et al. Regulatory T cells in skin lesions and blood of patients with bullous pemphigoid. J Eur Acad Dermatol Venereol 2014;28(2):222-30.
2. Fang H, Shao S, Cao T, Lei J, Dang E, Zhang J, et al. Increased expression of NLRP3 inflammasome components and interleukin-18 in patients with bullous pemphigoid. J Dermatol Sci 2016;83(2):116-23.
3. Tucker P, Pfefferbaum B, Nitiéma P, Khan Q, Aggarwal R, Walling EEJC. Possible link of Interleukin-6 and Interleukin-2 with psychiatric diagnosis, ethnicity, disaster or BMI. Cytokine2017;96:247-52.
4. Kowalski E, Kneibner D, Kridin K, Amber K. Serum and blister fluid levels of cytokines and chemokines in pemphigus and bullous pemphigoid. Autoimmun Rev 2019, 18(5):526-34.
5. Boraschi D, Dinarello CAJEcn. IL-18 in autoimmunity. Eur Cytokine Netw2006;17(4):224-52.
6. Mortazavi H, Esmaili N, Khezri S, Khamesipour A, Farahani IV, 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):174-83.
7. Genovese G, Di Zenzo G, Cozzani E, Berti E, Cugno M, Marzano AVJFii. New insights into the pathogenesis of bullous pemphigoid: 2019 update. Front Immunol2019;10:1506.
8. D'Auria L, Mussi A, Bonifati C, Mastroianni A, Giacalone B, Ameglio F. Increased serum IL-6, TNF-alpha and IL-10 levels in patients with bullous pemphigoid: relationships with disease activity. J Eur Acad Dermatol Venereol 1999;12(1):11-5.
9. Liu Z, Dang E, Li B, Qiao H, Jin L, Zhang J, et al. Dysfunction of CD19+ CD24 hi CD27+ B regulatory cells in patients with bullous pemphigoid. Sci Rep 2018;8(1):703.
10. Rao CV. Immunology: Alpha Science International Limited; 2016.
11. Esmailbeig M, Ghaderi AJEcn. Interleukin-18: a regulator of cancer and autoimmune diseases. Eur Cytokine Netw2017;28(4):127-40.
12. Esmaili N, Mortazavi H, Kamyab-Hesari K, Aghazadeh N, Daneshpazhooh M, Khani S, et al. Diagnostic accuracy of BP 180 NC 16a and BP 230-C3 ELISA in serum and saliva of patients with bullous pemphigoid. Clin Exp Dermatol 2015;40(3):324-30.
13. Liu Y, Li L, Xia YJFii. BP180 is critical in the

autoimmunity of bullous pemphigoid. Front Immunol2017;8:1752.

14. Nakama K, Koga H, Ishii N, Ohata C, Hashimoto T, Nakama T. Clinical and Immunological Profiles of 14 Patients With Bullous Pemphigoid Without IgG Autoantibodies to the BP180 NC16A Domain. JAMA Dermatol 2018;154(3):347-50.
15. Sun X-K, Chen J-F, Shen HJAodr. Immunohistochemical study of toll-like receptors 2, 4, and 9 expressions in pemphigus and bullous pemphigoid lesions. Arch Dermatol Res2016;308(6):429-36.