

# Investigation of serum APRIL and BAFF levels in pemphigus vulgaris patients in Southern Iran

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**Background:** Pemphigus vulgaris, the most common form of pemphigus, is due to the production of auto-antibodies directed against adhesion molecules (desmoglein 1 and 3) that belong to the cadherin family. B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) may play a significant role in autoimmune diseases such as pemphigus vulgaris. Only a few studies have been done on the level of APRIL and BAFF in PV patients but none have investigated both levels; therefore, determination of both proliferation inducing ligands (BAFF and APRIL) in pemphigus vulgaris is of significant importance and was considered as the goal of the present study.

**Methods:** This analysis included 22 patients with PV and 22 sex and age matched healthy controls. None of the patients had previously been treated with corticosteroids or any immunosuppressive drugs in the previous three months. The BAFF and APRIL levels were evaluated in patient and control groups by use of ELISA method.

**Results:** Comparison of the serum levels of BAFF and APRIL between patients and controls was done using Mann-Whitney U test. The BAFF levels in our 22 patients and control sera were undetectable. On the other hand, concentrations of APRIL in 22 cases were 2.09+/-4.94 and 0.85+/-2.01 in the control group.

**Conclusion:** BAFF levels were undetectable in cases and the difference in APRIL levels between patient and control groups was not significant (p=0.28). So, it can be concluded that although BAFF and APRIL may play a role in autoimmune diseases, their role in pemphigus vulgaris is doubtful.

**Keywords:** pemphigus vulgaris, autoimmune bullous disease, B- cell activation factor, tumor necrosis factor, APRIL, proliferation-inducing ligand.

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## INTRODUCTION

Normally, the immune system can distinguish between "self" and "non-self" and only attacks those tissues that recognizes as "non self." This is usually, but not always, the desired response. Autoimmune diseases generate from an overactive immune response of the body against substances and tissues normally present in the body. In other words, the body actually attacks its own

cells. In fact, the immune system mistakes some parts of the body as a pathogen and attacks it <sup>1</sup>. Pemphigus vulgaris (PV) is one of the diseases categorized as an autoimmune disease. Pemphigus is the general name for a group of chronic bullous diseases. Pemphigus used to be a general term that included most bullous eruptions of the skin, but by improvement of diagnostic tests, bullous diseases have been reclassified more specifically. PV is the most common form of pemphigus. PV incidence

varies from 0.5-3.2 cases per 100,000 and is higher in patients of Ashkenazi Jewish descent and those of Mediterranean origin. PV is a potentially life-threatening autoimmune mucocutaneous disease with a mortality rate of approximately 5-15%. PV is mediated by circulating auto antibodies directed against keratinocyte cell surface molecules <sup>2</sup>.

In recent studies, B cells have been accounted responsible for clinical manifestations of some autoimmune diseases. B cells require signals from multiple sources for their development from precursor cells, and differentiation into effector cells. B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) may play a significant role in autoimmune diseases. BAFF has been identified as a critical regulator of B cell development and differentiation. BAFF, APRIL and their receptors play important immunological roles, especially in the B cell arm of the immune system. Defects in the production of BAFF and/or expression of its receptors have been associated with a diverse array of human immunopathologies characterised by perturbed B cell function and behaviour, including autoimmunity, malignancy, and immunodeficiency <sup>3,4</sup>.

Tangye et al, worked on BAFF and APRIL in human B cell disorders in 2006 <sup>3</sup>. This review noted that in previous studies, BAFF levels had a significant correlation with the activity of systemic lupus, rheumatoid arthritis, Wegener's disease and B-cell related malignancies. Also, Bossen et al, in 2006, reviewed BAFF, APRIL and their receptors, structure, function and signaling <sup>4</sup>. Some studies have also been carried out specifically in autoimmune diseases. Among them, Zhu et al, conducted a research on the effects of BAFF and BAFF-R-Fc fusion protein in immune thrombocytopenia in 2009 <sup>5</sup>. In this study, the effects of recombinant human BAFF (rhBAFF) and BAFF-R-Fc fusion protein (BR3-Fc) on B cells, T cells, platelets, secretion of interferon gamma (IFN-gamma), and interleukin-4 (IL-4) were measured by flow cytometry and ELISA. Patients with an active disease had higher levels of plasma BAFF and BAFF mRNA than patients in remission and controls <sup>5</sup>. In the same year, another study was performed in the field of dermatological diseases by Jee et al, on B cell-activating factor level in children with atopic dermatitis <sup>6</sup>. In this study, levels of serum BAFF, a proliferation-inducing

ligand (APRIL), total serum IgE level, and total eosinophil count were measured in 245 children. Serum BAFF level in children with atopic dermatitis was significantly higher than in healthy controls but serum APRIL level was not different between the groups. In this study, serum BAFF level significantly correlated with total serum IgE level and total eosinophil count <sup>6</sup>. However, the results of another study showed elevated APRIL levels in atopic dermatitis patients while BAFF levels remained in the normal range <sup>7</sup>.

Interestingly, a few studies have also been carried out on bullous diseases, specially PV. In one study that was conducted by Asashima et al, increased levels of BAFF in bullous pemphigoid but not in PV were shown <sup>8</sup>. This study did not include APRIL levels. Another study that was conducted in Tokyo University indicated increased levels of BAFF and APRIL in bullous pemphigoid but did not investigate these levels in PV <sup>9</sup>. As stated, only a few studies have been done on this particular subject but none have investigated both BAFF and APRIL levels in PV. Also, no study on this subject has been carried out in Iran. Therefore, comparison of both proliferation inducing ligands (BAFF and APRIL) levels between PV and normal controls is important and was considered as the goal of the present study.

## PATIENTS AND METHODS

This analysis included 22 patients with PV (10 males and 12 females) who were visited at Shahid Faghihi Hospital. As the main goal of the study was to determine the APRIL and BAFF levels in new patients and also to eliminate the possible effect of corticosteroids on these serum levels, these sera were obtained when the patients first presented and were diagnosed with PV or known cases of PV who discontinued medication and came with relapse of the disease and were taken prior to treatment. None of the patients had previously been treated with corticosteroids or any immunosuppressive drugs in the previous three months. Control sera were collected from 22 age and sex matched healthy donors who were visited at Shahid Faghihi Laboratory for routine checkup.

In the present study, diagnosis of PV was based on the following criteria: characteristic clinical findings of mucous membrane ulcerations and/

or multiple fragile vesiculobullous lesions of the skin; histological evidence of suprabasal cleft and intraepithelial acantholysis with many infiltrating polymorphonuclear cells, especially eosinophils, along the basal membrane and within the blister cavity and the presence of auto antibodies specific to Dsg3 in ELISA.

Patients and healthy volunteers gave consent to participate in this study and all the possible risks and benefits of participation were clearly explained to them.

Five milliliters of fresh venous blood samples were obtained from patients and centrifuged shortly after clot formation; then, sera were stored at -70°C for further analysis. This procedure was similarly done for the control group. In both groups, a detailed questionnaire was completed reviewing any other systemic diseases or consumption of any drugs in cases and controls. Serum BAFF levels were measured by ELISA method using BMS2007INST human BAFF kit (Bender MedSystems) according to the manufacturer’s protocol. Similarly, serum APRIL levels were calculated by the use of BMS2008TEN kit.

Comparison of serum levels of BAFF and APRIL between patients and controls was done using Mann-Whitney U test. P value <0.05 was considered significant.

**RESULTS**

Serum APRIL and BAFF levels were measured by ELISA in 22 patients and controls, each group containing 12 females and 10 males. Regarding patients’ age in the case group, the mean age at

disease presentation was 43.45+/- 3.01 with a peak in the fourth decade. APRIL levels (mean +/- SD) in cases and controls were 2.09+/-4.94 and 0.85+/-2.01, respectively (Figure 1). Statistical analysis revealed no significant difference between the two groups (p=0.28). Regarding BAFF levels, sera levels in both cases and controls were almost immeasurable. APRIL levels in patients after sex stratification is demonstrated in Figure 2. In this study, 12 female and 10 male patients were included. Distribution of APRIL levels in both genders of controls is also demonstrated in Figure 3. APRIL levels were higher in the male sex in both groups (for male and female patients: mean=3.5 and 0.84, respectively, for male and female controls: mean=1.4 and 0.34, respectively). Since we considered P value<0.05 as significant, the difference in APRIL levels between male and female patients was not significant (p= 0.15 and 0.18 for patients and control, respectively). As a result, it can be concluded that although APRIL levels were higher in male patients and controls, the difference was not consequential.

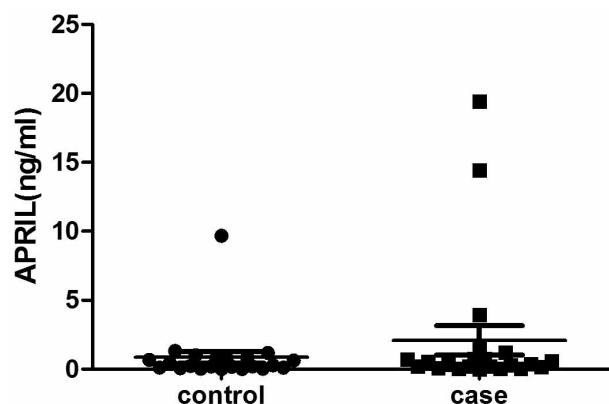


Figure 1. APRIL levels Patients and Control

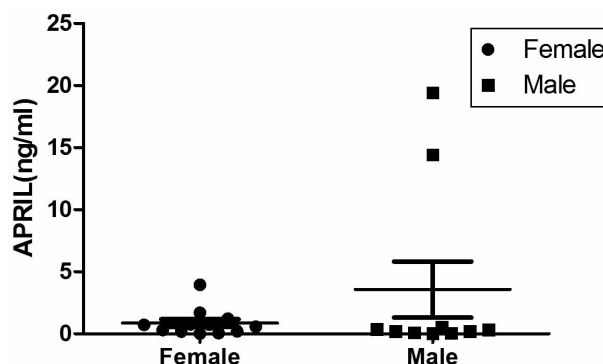


Figure 2. APRIL levels demonstrated by sex in patients.

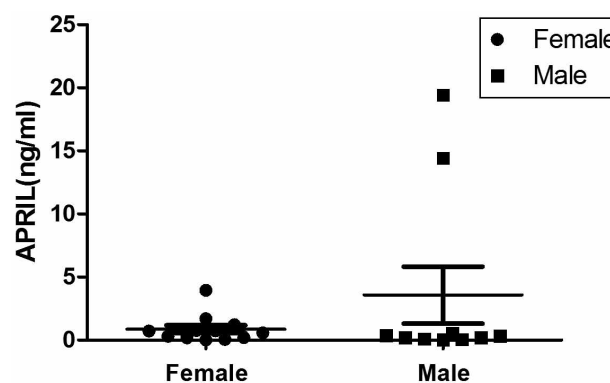


Figure 3. APRIL levels demonstrated by sex difference in controls

## DISCUSSION

Since there are limited studies on pemphigus vulgaris to determine BAFF or APRIL levels and the originality of the topic, this study was designed. Also, no study has measured both BAFF and APRIL levels in such patients. Measuring these factors may help with future treatment innovations.

As explained in the results section, BAFF levels in our 22 patients' and controls' sera were immeasurable. We have also checked the method and the laboratory kit quality to assess BAFF levels and found no possibility of technique errors. Therefore, it seems the BAFF level was normally very low in both patients and control that we could not find measurable amounts. On the other hand, while the concentration of APRIL in 22 cases were higher than 22 controls (2.09+/-4.94 and 0.85+/-2.01, respectively), the difference did not reach a significant level (p=0.28).

A study done by Watanabe et al <sup>9</sup>, showed increased serum APRIL levels in patients with bullous pemphigoid but no other study has been done on pemphigus vulgaris. As mentioned before, in our study, serum APRIL levels were elevated in patients but not to a significant level. We found no increase in BAFF level in our assay that is similar to Asashima study <sup>8</sup>.

We compared APRIL levels in cases and controls between the sexes to determine possible gender differences (Figure 2,3). Although APRIL levels were higher in the male sex in both groups (for male and female patients: mean=3.5 and 0.84, respectively, for male and female controls: mean= 1.4 and 0.34, respectively), P value was not significant in either of the groups (0.15 and 0.18, respectively).

It can be concluded that although BAFF and APRIL may play a role in autoimmune diseases, their role in pemphigus vulgaris is doubtful. We noted that gender differences, as explained, did not affect the levels of APRIL and BAFF. The sex ratio between our patients was 1.2 with a female predominance. Disease prevalence was also higher in the female gender in our study which was

consistent with other studies in this regard <sup>10,11</sup>. The mean age at first disease presentation was 43.45+/- 3.01 with a peak in the fourth decade, showing that the disease is more prevalent in middle-aged patients in Southern Iran. This conclusion is consistent with the epidemiology that was considered for the disease in other studies <sup>2</sup>.

Based on our results, we recommend that this study be conducted on a larger sample size in different ethnic parts of Iran and different types of bullos diseases to achieve a better perspective.

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