Serum BAFF Levels In Patients With Systemic Lupus Erythematosus, Psoriasis, And Alopecia Areata

*Neveen Salah Seif Eldin1, Shereen Bendary El Sayed, 2 Rasha Ahmed Reda Nasr 2

1 Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, Cairo- Egypt.
2 Department of Medical Microbiology and Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

E-mail for Correspondence : drnevins@hotmail.com Tel: (202) 22585577; Fax: +(202) 22585577

Abstract: Serum B cell activating factor (BAFF) level in SLE patients with and without lupus nephritis, psoriasis patients before and after treatment, and Alopecia Areata (AA) patients was estimated, compared to controls, and correlated with disease activity. SLE patients were grouped into 2 groups: Group (A) with lupus nephritis and Group (B) without nephritis. Disease activity in SLE patients was assessed by SLEDAI score, and clinical severity of Psoriatic patients was estimated by using the PASI score before and after treatment. Serum BAFF measurement was done by ELISA for patients and controls. In this study, serum BAFF levels were significantly elevated in SLE patients compared to controls. Those with lupus nephritis had significantly higher levels of BAFF compared to patients without nephritis. Also BAFF levels correlated highly significant with SLEDAI score. In psoriatic patients, BAFF levels were significantly higher than controls before treatment, and it showed a strong positive correlation with PASI score before and after treatment. In AA patients, BAFF levels were significantly higher than controls. BAFF levels also were significantly increased in multilesional AA group compared with other groups. Our conclusion was that BAFF appears to be an important marker involved in the pathogenesis of SLE, Psoriasis and AA.

Key words: BAFF, SLE, Psoriasis, Alopecia

INTRODUCTION

B lymphocytes have multiple functions that contribute to the pathogenesis of autoimmunity. They produce autoantibodies that mediate tissue injury, function as antigen-presenting cells that present epitopes of self antigens to autoreactive T cells, and produce soluble mediators involved in the organization of lymphoid tissue and in the initiation and perpetuation of inflammatory processes (Lipsky 2001). B lymphocytes can also migrate to inflamed sites, where they act as local effector cells in some autoimmune diseases (Cassese et al; 2001).

The discovery of the B cell activating factor belonging to the tumor necrosis factor (TNF) family (BAFF), which is also known as B-lymphocyte stimulator (BLyS), has been a major breakthrough in the understanding of the pathophysiology of autoimmune diseases (Binard et al; 2008). BAFF is expressed by monocytes, macrophages and dendritic cells (DCs) and at lower levels by T cells. Human follicular DCs are also a source of BAFF. Nonlymphoid cell types also produce BAFF, for example airway and salivary gland epithelial cells, fibroblast-like synoviocytes, astrocytes, vascular cell adhesion molecule 1-positive stromal bone marrow cells and osteoclasts (Mackay et al; 2007).

BAFF exerts its effects by binding to 3 different types of B-cell membrane receptors: B-cell maturation antigen (BCMA), BAFF-receptor (BAFF-R) (BR3) and transmembrane activator and cyclophilin ligand interactor (TACI) (Gross et al; 2000). Hundred percent of B cells express BAFF-R (or BR3), which is considered as the most important one mediating most of the BAFF effects on B cells (Rauch et al; 2009). Docking of BAFF with its receptors activates phospholipase C- y2 and subsequently the NF-kB pathways in the absence of B cell receptor (BCR) activation (Hatada et al; 2003, Doreau et al; 2009).
This results in the inhibition of B cell apoptosis and hence prolonged B lymphocyte survival (Tieng and Peeva 2008; Yap and Lai 2010).

Breakdown of the regulation of BAFF expression results in excessive BAFF production, impairing B cell tolerance and leading to autoimmune phenomena (Krivosikova et al; 2009). BAFF levels are elevated in the serum of patients with various autoimmune diseases, and it is considered an important driving factor for B cell autoantibody production (Zhang et al; 2005).

Overexpression of BAFF is considered to be a characteristic feature of systemic autoimmune disease particularly systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and primary systemic sclerosis (SS) (Matsushita et al; 2007, Fawzy et al; 2011; de Zubiria Salgado and Herrera-Diaz 2012). Several authors stated that in SLE patients serum levels of BAFF were significantly increased, and were correlated with IgG levels and antidouble-stranded DNA (anti-dsDNA) titers and disease activity index score (Petri et al 2008, Thorn et al 2010). Selective blockade of BAFF prevents and treats SLE nephritis in mice (Ramanujam et al; 2010).

Psoriasis is a chronic inflammatory skin disease which is variable in severity and characterized by type I cytokine pattern (Meier and Sheth, 2009). BAFF is elevated in serum of patients with psoriasis in association with disease activity while its mechanism is still unknown (Samoud El-Kissi et al; 2008). In addition, in a study done by Pongratz et al; 2010, they concluded that serum BAFF levels correlated with disease activity (DAS28) in psoriatic arthritis (PsA) patients.

Alopecia areata (AA) is regarded as a tissue-specific autoimmune disease of hair follicles. CD8+ T cells are considered to act as the effector cells with help from CD4+ T cells (Alexis et al; 2004). Various autoantibodies have also been found in the sera of AA patients, suggesting that B cells are excessively activated in AA (Kuwano et al; 2007).

The aim of the present study was to estimate serum BAFF levels in SLE patients with and without lupus nephritis, psoriasis patients before and after treatment, and in different types of Alopecia Areata patients, and compare the results to those of controls. Also to correlate serum BAFF levels with the laboratory investigations, and disease activity in these diseases, trying to verify the role of BAFF in these autoimmune diseases and be able to find new modalities of treatment.

**Patients and Methods**

This study is a case-control pilot study which was carried out on 40 SLE patients, 40 psoriasis patients, and 20 Alopecia Areata patients. Patients were collected from the outpatient clinic of the Dermatology, Venereology and Andrology department, Ain Shams University, Cairo, Egypt. Most SLE patients were referred from the Rheumatology department.

**SLE patients**

SLE patients comprised 40 patients (32 females and 8 males), fulfilling at least four of the American college of rheumatology (ACR) revised Criteria for diagnoses of SLE (Hochberg 1997). Mean age of patients was (42.2 ±12 years), range: “17 to 52 years”, and mean disease duration was (8.95 ± 5.42 years), range “5 to13 years”. For all patients the following was done: Full history especially age, duration of the disease, urinary symptoms, SLE manifestations as joint and muscle pains, skin rash, photosensitivity, hair loss, Raynaud's phenomenon, CNS symptoms including seizures, symptoms of hypertension as vomiting, headache, blurred vision and history of medication received by the patient. Thorough clinical examination was performed to each patient including: blood pressure measurement, joint affection, chest and heart examination, abdominal examination for hepatosplenomegaly and CNS examination especially consciousness level, and motor and sensory systems. Thorough dermatological examination was done also to determine malar rash, any skin rash, oral ulcers, hair and nail changes, pigmentary changes and others. Clinical disease activity at the time of the study was assessed according to the systemic lupus erythematosus disease activity index (SLEDAI) (Bombardier et al; 1992).

SLE patients were categorized according to renal involvement as reported by Weening et al; 2004, into 2 groups:

Group (A): patients having clinical manifestations and biopsy proven lupus nephritis. They were 20 patients; 12 females and 8 males, mean age (35.3 ±7.21 years), ranging from 17 to 50 years. They were having clinical evidence of renal involvement in the form of persistent proteinuria (more than 0.5 gm/day or more than 3+ by dipstick test) and/or cellular casts in urine (red blood cells, haemoglobin, granular, tubular or mixed).

Group (B): Patients with no clinical or laboratory evidence of renal involvement. They were 20 female patients, mean age (30.5 ±8.8 years), range “20 to 52 years of age. These patients had normal urine analysis, serum creatinine and creatinine clearance.
Psoriasis patients

Psoriasis patients were 40 patients, 24 female and 16 males. All patients were diagnosed clinically. Histopathological diagnosis was done for suspected cases only. Mean age of patients ± SD was (40.60 ± 10.09 years); ranging from 29 to 67 years of age; mean disease duration (8.5 ± 1.2 years). Clinical severity of symptoms in the patients was estimated using the PASI (Psoriasis Area and Severity Index) score (Fredricksson and Petterson,1978). PASI score ranging from 0 to 72 with higher scores indicating greater disease severity. PASI score was done before and after treatment. A 50% reduction in the PASI score after treatment was our clinically significant endpoint in the assessment of psoriasis according to Carlin et al; 2004. Patients were classified into 3 groups according to their PASI scores before treatment: group 1: eleven patients (27.5%); PASI < 30; group 2: twenty patients (50%) PASI “30 to 50”; and group 3: nine patients (22.5%) PASI “50 to 72”.

Treatment for these patients was either phototherapy, in the form of PUVA (Psoralens and UVA), or methotrexate depending on the disease severity and the laboratory investigations of the patients. Complete blood picture and liver and kidney function tests were done for each patient in addition to ocular examination for those taking PUVA. Patients received phototherapy 3 times weekly; 8-Methoxypsoralen was given orally in a dose of "0.3 to 0.6 mg/kg" 2 hours before exposure to UVA. The initial dose of light depended on the skin type of the patient and was usually 1 joule/ cm² which can be increased by "0.5 to 1 joule/ cm²" every 2-3 sessions. Methotrexate was given systemically in a dose of "0.2 to 0.4mg/kg per week". All patients were given vaseline as an emollient.

Alopecia areata patients

Forty age and sex matched apparently healthy individuals served as controls, 28 male and 12 female. Their ages ranged from 12 to 42 years with a mean value of (28.60 ± 8.27 years), with no dermatological, systemic, or autoimmune diseases. An informed consent was obtained from all patients and controls before enrollment. The study protocol was approved by the ethics committee of Ain Shams University.

Laboratory Tests

For every patient and control the following laboratory tests were done: Routine laboratory investigations including: Complete blood picture by Coulter counter (Coulter Microdiff 18, Fullerton, CA, USA), erythrocyte sedimentation rate estimation by Westergren method, ANA assessment by indirect immunofluorescence supplied by the IMMCO Diagnostics (USA), serum anti double stranded DNA (dsDNA) antibodies were measured using ELISA technique (ORGENTEC) (Carl-Zeiss-StraBe 49, 55129 Mainz, Postfach 100352 55134 Mainz), and complement level assessment using the turbidimeter supplied by Behring Diagnostics (Germany) was assessed. Renal function tests were also done including serum creatinine, blood urea, creatinine clearance and routine microscopic urine analysis for presence of pyuria, hematuria and casts, albuminuria by Dipsticks. Twenty four hours urine was used to test for the presence of proteins in the urine.

Blood samples for BAFF measurement were collected from patients and controls once. As for the psoriasis patients, blood samples were taken twice before beginning treatment and at the end of the study period when reaching 50% reduction in PASI Score. Two milliliters of venous blood were collected aseptically, transferred into a clean dry tube, then allowed to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Serum was removed and stored at –20°C. The measurement of BAFF in serum was done by enzyme-linked immunosorbant assay (ELISA) using Quantikine ®Human sTNF-R1 Immunoassay Kit, Catalog Number DBLYS0 (R & D systems, Minneapolis, MN, USA) as recommended by the manufacturer. A standard curve was generated for each set of samples assayed and BAFF concentrations were expressed in pg/ml according to a standard curve. The minimum detectable dose (MDD) was 3.38 pg/mL.

Statistical Analysis

Clinical features were reported by descriptive analysis. Data analyses were performed by IBM computer using SPSS windows (V 6.2) package as follows: Descriptive data were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA test); paired and unpaired student t-test. Pearson’s correlation coefficient was used for the correlation analysis of. Differences were considered statistically significant at \( P<.05 \).
Table 1. Comparison of BAFF Levels (pg/ml) among studied Groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>1201.00</td>
<td>1985.00</td>
<td>1524.6500</td>
<td>271.81655</td>
<td>0.000*</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>897.00</td>
<td>1203.00</td>
<td>1077.6500</td>
<td>88.79561</td>
<td>0.000*</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>590.00</td>
<td>1305.00</td>
<td>993.8000</td>
<td>297.90632</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>430.00</td>
<td>943.00</td>
<td>831.3000</td>
<td>128.65871</td>
<td></td>
</tr>
</tbody>
</table>

* ANOVA TEST
  * Significant P value <0.05
  * test showed high significance between groups

Figure 1. Comparison between BAFF levels in SLE patients and controls

RESULTS

BAFF serum levels among the SLE, psoriasis, alopecia areata patients and controls are summarized in table (1)

SLE patients

Mean Serum levels of BAFF was significantly higher in SLE patients than normal controls (1525± 272 pg/ml vs. 831±129 pg/ml) (P<.05) (Figure 1).

SLE patients with lupus nephritis had significantly higher levels of BAFF (P<.05), compared to patients without renal impairment and to healthy controls (1767± 146 pg/ml vs 1283 ± 68 pg/ml and 831±129 pg/ml respectively) (Figure 2).

Disease activity as assessed by SLEDAI score correlated highly significantly with the BAFF levels (P<.001); increased BAFF levels were associated with an increase in SLEDAI score.

Serum BAFF levels in SLE patients had no statistically significant correlation with the duration of the disease, age of patients, C3, ANA and anti-dsDNA antibodies (P>.05), while it correlated significantly with ESR.
Psoriasis patients

The mean ± SD level of PASI score for patients before treatment was (45 ± 16), ranging from a minimum of 24 and a maximum of 72. Serum BAFF levels in psoriatic patients before treatment was significantly higher than that of healthy controls (1078 ± 89 pg/ml vs. 831 ± 129 pg/ml) (P < 0.05).

Serum BAFF levels in the group 3 PASI (50-72) was significantly higher than that in group 1 PASI (<30, P < 0.05) or group 2 PASI (30 to 50, P < 0.05), indicating that BAFF levels increased in accordance with disease activity. Thus, serum BAFF levels showed a strong positive correlation with the severity of PsV before treatment as established by the PASI score (data not shown).

After reaching a (50%) reduction in patient's PASI score, the levels of serum BAFF decreased from "1078 ± 89 pg/ml before treatment to 897 ± 44 pg/ml" after treatment. There was a significant difference between BAFF levels before and after treatment (P < 0.05) (Figure 3).

Another significant difference was found between PASI score before and after treatment (P < 0.05), with mean levels of "45 ± 16 before treatment dropping to 18 ± 8" after treatment. There was a strong positive correlation between PASI score and BAFF levels after treatment (P < 0.05) (Figure 3).

Alopecia Areata patients

Serum BAFF levels in AA patients were significantly higher than that of healthy control subjects (994 ± 298 pg/ml vs. 831 ± 129 pg/ml) (P < 0.05) (table 1).

Fifty percent of AA patients were ANA +ve and (50%) were ANA –ve. Mean ± SD of serum BAFF level in ANA +ve patients were significantly higher than ANA –ve patients (1259 ± 53 pg/ml vs 728 ± 143 pg/ml) (P < 0.05) (Figure 4).

Serum BAFF levels were significantly increased in poly group (poly; 1205 pg/ml compared with other groups (mono; 1100 pg/ml P < 0.05; AT/AU 950 pg/ml P < 0.05 and controls; 831; P < 0.05). In addition, there was no significant correlation between disease duration and serum BAFF levels.

By comparing mean BAFF levels in all study groups (SLE, psoriasis, AA, and controls), the highest level was among SLE patients (1525 ± 272 pg/ml), followed by psoriasis patients (1078 ± 89 pg/ml), followed by AA patients (994 ± 298 pg/ml) and controls (833 ± 129 pg/ml). There was a significant difference between all groups (P < 0.05) by using ANOVA test (table 1). By using independent T test, there was a significant difference between all groups except psoriasis versus
Figure 3. PASI and BAFF levels in psoriasis patients before and after treatment.

Figure 4. Mean BAFF levels in alopecia groups with positive and negative ANA showing a significant difference between them.
Alopecia (Figure 5).

**DISCUSSION**

B-cell activation factor from the tumor necrosis factor family (BAFF) is a key surviving factor during B-cell maturation. Excessive BAFF production corrupts B-cell tolerance and leads to autoimmunity. Elevated serum BAFF levels have been detected in some patients suffering from various autoimmune conditions (Mackay et al; 2007 and Mariette, 2012).

In this study, serum BAFF levels were significantly elevated in SLE patients compared to controls. Several authors (Morimoto et al; 2007, Binard et al; 2008; Fawzy et al; 2011; and Mariette 2012) confirmed our results. They stated that serum levels of BAFF were elevated in patients with SLE. Moreover, SLE patients with lupus nephritis had significantly higher levels of BAFF compared to patients without renal impairment and to healthy controls. These results were in agreement with several authors; Neusser et al; 2011, found on microdissected renal biopsies of lupus nephritis patients that BAFF mRNA levels were significantly increased in the glomeruli of patients with class III and IV nephritis when compared to pre-transplant living donor kidneys. In addition, it was stated that BAFF correlates with the amount of proteinuria and anti-DNA levels in a high percentage of patients and in mice models (de Zubiria Salgado and Herrera-Diaz 2012). In transgenic murine models with BAFF over expression, there is an induced lupus-like syndrome with lupus nephritis (Groom et al; 2007). Moreover, another study stated that blockade of BAFF is a promising therapeutic approach for SLE, especially in patients with kidney involvement (Morimoto et al; 2007). Another study reported that early inhibition of BAFF-R and transmembrane activator and cyclophilin ligand interactor (TACI) in mice who have...
increased levels of circulating BAFF led to improved mortality, decreased renal inflammation and decreased glomerular immune complex deposition in addition to improved anti-DNA antibody levels (Blanco et al; 2012). On the contrary Petri et al. 2008, stated that glomerulonephritis may increase BAFF excretion in urine, thereby resulting in lower plasma BAFF levels when renal disease contributes to the SLEDAI score.

In the present study, BAFF levels correlated highly significantly with the SLE disease activity (SLEDAI score). These results agree with several authors (Becker-Merok et al; 2006, Zhao et al; 2010 and Fawzy et al; 2011). Gladman et al. 2000, reported a decrease in BAFF levels with improvement in SLE disease activity, whereas increases in BAFF levels were associated with worsening of SLE disease activity indicative of mild-to-moderate flare and this finding supports the rationale for investigation of BAFF antagonism as a potential therapeutic approach in SLE. Moreover, Petri et al. 2008, stated that BAFF levels predicted increases in SLE disease activity, supporting the notion that elevated BAFF has a role as a biomarker for current and/or future SLE disease activity.

The results of this study could not find a significant relationship between elevated BAFF levels and positive expression of either ANA or anti-dsDNA antibodies. This is in accordance with the studies of Fawzy et al; 2011 and Becker-Merok et al; 2006. On the contrary, several studies reported a significant correlation between serum BAFF levels and anti-dsDNA antibodies (Cheema et al; 2001; Binard et al; 2008; Petri et al; 2008).

In our studied psoriatic patients, serum BAFF levels were significantly higher than that of healthy control subjects before starting any treatment. Serum BAFF levels in group 3 PASI (50-72) were significantly higher than that in group 1 PASI (<30) or group 2 PASI (30-50), indicating that BAFF levels increased in accordance with disease activity. Therefore, serum BAFF levels showed a strong positive correlation with the severity of PsV before treatment as established by the PASI score. Our results were in agreement with the study of Samoud El-Kissi et al; 2008, who found a significant elevation of BAFF in PsV patients and also a significant correlation between BAFF levels and PASI score indicating a role for BAFF in the pathogenesis of psoriasis.

All patients received treatment according to their clinical condition and disease severity, in the form of phototherapy or methotrexate. After reaching a 50% reduction in patient's PASI score, the levels of serum BAFF decreased from (1078 ± 89 pg/ml) before treatment to (897 ± 44 pg/ml) after treatment. There was a highly significant difference between BAFF levels before and after treatment. To the best of our knowledge, there was no published work on the effect of therapy on serum BAFF levels in psoriasis and hence, this is the first published study. These data further add evidence for the important role of BAFF in the pathogenesis of PsV. Another highly significant difference was found between PASI score before and after treatment, dropping from (45± 16) before treatment to (18 ± 8) after treatment. There was also a strong positive correlation between PASI score and BAFF levels after treatment. Thus, serum BAFF levels seem to monitor disease resolution and response to therapy in PsV patients.

As regards Apecia Areata patients, serum BAFF levels were significantly higher than that of healthy control subjects. These results were in agreement with Kuwano et al; 2008, who reported extremely high serum BAFF levels in AA patients compared to normal or negative disease controls. IFN-γ production is elevated in lesions of AA and IFN-γ stimulates BAFF production (Mackay et al; 2003).

Fifty percent of AA patients were ANA +ve and (50%) were ANA –ve. Serum BAFF levels in ANA +ve patients were significantly higher than ANA –ve patients. On the contrary, Kuwano et al; 2008, found no significant relationship between serum BAFF levels and autoantibodies found in AA. In accordance with our results, Kuwano et al; 2008, also reported that there was no significant correlation between the disease duration and serum BAFF levels. In this study, serum BAFF levels also were significantly increased in poly (multilesional) group compared with other groups (poly; 1205 pg/ml; mono;1100 pg/ml; AT/AU 950 pg/ml and controls; 831 pg/ml). These findings were also reported by Kuwano et al; 2008, suggesting that BAFF correlates with the development of multiple lesions of AA. They reported that the reason of the difference between poly group and AT/AU group has remained unresolved. They also added that, this peculiar elevation of BAFF in certain types of AA suggests the specific regulation and role of BAFF in the AA development. Their conclusion was that serum BAFF levels are elevated in AA, regulated in a specific manner and appear to play an important role in the development of AA. Therefore, the inhibition of BAFF may be a reasonable and specific strategy for the therapy of AA.

By comparing mean BAFF levels in all study groups (SLE, psoriasis, AA, and controls), the highest levels were among SLE patients followed by psoriasis patients, followed by AA patients and controls. There was a significant difference between all groups by using ANOVA test. By using independent T test, there was a significant difference between all groups except psoriasis versus Alopecia. In agreement to the current results, a study reported that SLE patients had the highest median serum BAFF levels among the study groups supporting the great role of BAFF in SLE patients (Fawzy et al; 2011). The US Food and Drug Administration has approved a drug specifically for the treatment of SLE (Dubey et al; 2011). This approval was considered an important milestone for SLE, but also, more broadly, in the field of systemic autoimmune diseases (Chiche et al; 2012). Belimumab (Benlysta) is the first of a new class of immunomodulators with a specific inhibition of soluble BAFF (Dennis, 2012). Specific binding of belimumab with soluble BAFF prevents its
interaction with its three receptors and indirectly decreases B-cell survival and production of autoantibodies. BLyS is BR3’s only ligand and the interaction of BAFF and BR3 is necessary for survival of naïve B cells and mature primary B cells. This enables belimumab to have a greater effect on early B cells, such as naïve B cells, and a lesser effect on memory and plasma B cells (Chiche et al; 2012). In contrast to other drugs under development, belimumab does not neutralize membrane-bound BLyS (Dennis, 2012; Fairfax et al: 2012). However, as a substantial percentage of SLE patients do not respond to belimumab, thus further research is needed to better characterize the pathogenetic mechanisms of SLE, identify additional therapeutic targets, and develop effective and nontoxic novel agents against these targets (Stohl and Hilbert 2012).

Conclusion

BAFF appears to be an important marker involved in the pathogenesis of several autoimmune diseases as SLE, Psoriasis and AA, in which elevated levels of autoantibodies contribute to disease pathology. Conventional therapies in autoimmune diseases are not target-specific and can lead to significant toxicity; therefore, more specific therapies are needed for the treatment of these autoimmune diseases, and the understanding of the BAFF system offers the opportunity to improve theses therapeutic approaches.

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REFERENCES


