B-Cell Activating Factor (BAFF) and its receptors in Systemic Lupus Erythematosus, Rheumatoid Arthritis

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ABSTRACT

Objective: The aim of this study was to determine and compare the serum level of the BAFF and the level of its three receptors in patients with RA and SLE and also to correlate its serum level with disease activity indices and severity. Patients and methods: This study included 60 (RA (n=30), SLE (n=30)) Egyptian patients with collagen diseases from outpatient clinics of the Internal Medicine and Rheumatology Departments of Banha University Hospitals between December 2014 and December 2015 as well as 25 apparently healthy individuals who were either on the medical staff or were patient’s relatives that served as the healthy controls. All participants underwent history taking, clinical examination, laboratory and radiological investigations, and disease activity score estimation. The serum BAFF concentration, the serum TACI concentration, serum BAFFR concentration, serum BCMA concentration were determined with a quantitative sandwich enzyme immunoassay (ELISA) kits. Results: BAFF, BAFF-R, & TACI levels showed statistically significant increase in SLE & RA Groups as compared to control group (P<0.05) and BCMA level was statistically significant decrease in SLE & RA Groups as compared to control group (P<0.05). Further, BAFF, BAFF-R levels were statistically significantly increased in RA Group when compared to SLE Group. A significant positive correlation was found between the BAFF level and BAFF-R levels in both groups and SLE (r=0.854, p<0.001), RA (r=0.614, p<0.001), also between BAFF, BAFF-R and SLEDAI in SLE group (r=0.413, 0.419, p=0.023, 0.021) respectively, and between BAFF, BAFF-R and DAS28 in RA group (r=0.642, 0.430, p=0.001,0.018) respectively., the current study raveled that the best biomarker for diagnosis of RA &SLE were BAFF-R & BAFF as they had the Roc curve with more sensitivity and specificity. Conclusion: The BAFF serum levels and its three receptors levels are increased in patients with SLE and RA versus the controls. They also have a positive correlation with disease severity in SLE and RA, which suggests that BAFF may play a role in the pathogenesis and activity of these diseases. These results may pose the possibility that a human monoclonal antibody drug which selectively inhibits BAFF biological activity may be useful in the treatment of active resistant cases.

INTRODUCTION

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are chronic inflammatory rheumatic diseases, in which autoantibodies are part of the early disease manifestations. (Roba M. et al., 2015) A pathogenic involvement of B cells is well documented in SLE and implicated in RA. Systemic lupus erythematosus (SLE) is a complicated autoimmune disease. Although self-reactive B cells are considered culprits in autoimmune diseases both because they produce pathogenic autoantibodies and because they have multiple effector functions, including antigen uptake and transport (Pugh-Bernard, A.E., J.C. Cambier, 2006; Engel, P., et al., 2011), antigen presentation to T cells (Engel, P., et al., 2011; MacLennan, I.C., et al., 2003), production of...
cytokines, chemokines (Jacob, N and W. Stohl, 2010) and migration to sites of inflammation. (Bettelli, E., et al., 2007)

B cells have proven in recent years to be active participants in the development of this disease irrespective of autoantibody production. In light of this advancement, a central question surrounding the pathogenesis of the disease is whether intrinsic defects in SLE B cells play a role in triggering the immunological events that result in the onset of clinical disease. Although other immune cells play a role in SLE, B cells from SLE patients display signaling defects that may underlie the pathogenesis and explain the characteristic hyperactivity of B cells in active disease (El-Sayed, M., et al., 2008). The classic paradigm for rheumatoid arthritis (RA) pathogenesis holds that CD4+ T cells mediate joint damage both directly and by driving non-T effector cells to release inflammatory cytokines. In contrast, the new paradigm that is developing centers on an interaction of CD4+ T cells with B cells and the fact that autoreactive B cells can be driven by the T cells to produce immunoglobulin G (IgG) autoantibodies that may be directly involved in joint damage. It is well known that B cells are critical in the activation of CD4+ T cells. (Wei, F., Y. Chang, W. Wei, 2015)

BAFF is a B cell survival factor and a member of the TNF ligand superfamily, which is also commonly known as B lymphocyte stimulator (BLyS) or TNF superfamily member 13b (TNFSF13b). (Ramanujam, M., A. Davidson, 2004)

Under normal conditions, BAFF is expressed on the surface of cells as a homotrimer and can be cleaved by a furin protease. After cleavage, it is secreted from the cell membrane as a soluble, biologically active 17-kDa molecule. BAFF binds to three distinct receptors: transmembrane activator and calcium-modulator and cytophilin ligand interactor (TACI), B-cell maturation antigen (BCMA) and BAFF receptor (BAFF-R). (Codrina Ancuta,)

BAFF-R binds exclusively to BAFF, while BCMA and TACI can also bind to a second related TNF family ligand, a proliferation inducing ligand (APRIL). A small proportion of the circulating BAFF exists as multimers of 20 trimers bound to activated TACI. (Moura, R., et al., 2013)

BAFF plays a crucial role in B cell homeostasis and the regulation of B cell maturation. It can also regulate the function of T cells by providing costimulatory signals to T cells in conjunction with TCR. (Fang Wei, et al., 2005)

**Aim of the work:**

The aim of this study was to determine and compare the serum level of the BAFF and the level of its three receptors in patients with RA and SLE and also to correlate its serum level with disease activity indices and severity.

**Patients and methods:**

This study included 60 Egyptian patients with collagen diseases from outpatient clinics of the Rheumatology Department at Benha Teaching Hospital between December 2014 and December 2015 as well as 25 apparently healthy individuals who were either on the medical staff or were patient’s relatives that served as controls. The study was conducted according to the principles of the Helsinki Declaration and was approved by the local ethics committee of the Faculty of Medicine at Tanta University. Informed written consent was obtained from all subjects who participated in this study after explaining the aim and nature of the study.

The patients were subdivided into three groups according to clinical diagnosis. The SLE group included 30 patients who fulfilled at least four of the American College of Rheumatology (ACR) criteria for SLE diagnosis. (Hochberg, M.C., 1997) Assessment of disease activity was achieved using the SLE Disease Activity Index (SLEDAI) score (Urowitz, M.B., D.D. Gladman, 1998). Patients with evidence of pre-existing renal diseases, malignancy, concurrent infection, or a history of nephrotoxic drug use were excluded from this study.

The RA group included 30 patients who fulfilled the ACR criteria for classification of RA (Arnett, F.C., et al., 1988). The disease activity of RA was assessed with the Disease Activity Score 28 (DAS28). Patients were categorized as having severe disease activity (score >5.1), moderate disease activity (score 3.2–5.1), and low disease activity (score 2.6–3.2). A score of <2.6 signified remission. (Prevoo, M.L.L., et al., 1995)

Five milliliters of blood was obtained from all participants via venipuncture and placed into plain tubes for further investigation. A complete blood count was conducted and all patients were evaluated with regard to; the erythrocyte 8by using the Westergren method and expressed in mm/h. (David Gilmour and A.J. Sykes, 1951) The CRP levels were measured by the immunoturbidimetric method using spectrophotometer (Biosystems S.A., Barcelona, Spain), and a level of <6 mg/L was accepted as normal. The serum creatinine, TC, TG, HDL-C and LDL-C were assayed by enzymatic-colorimetric method using commercial assay kits (Spinreact. Spain). (Richmond, W., 1973 et al., Fossati, P., L. Prencipe, 1982; Gordon, T., W. Zidek, M. Amer, 1977; Friedewald, W., et al., 1972).
The serum BAFF concentration:

The serum TACI concentration, serum BAFFR concentration, serum BCMA concentration were determined with a quantitative sandwich enzyme immunoassay technique (Quantikine® Human BAFF Immunoassay, R&D Systems, Minneapolis, Minnesota, USA). (Omnikine™ Human TACI, Assay Biotechnology Company, Inc. Sunnyvale, CA, USA) (Bosterimmunoleader, Valley Ave, Pleasantton, CA, USA) for both BAFFR, BCMA. All kits were used according to the manufacturers' instructions. In brief polystyrene microplate wells were coated with a mouse monoclonal antibody against the different proteins, and after incubation with the serum, the assay was developed with a polyclonal second antibody against the different proteins conjugated to horseradish peroxidase color intensity was measured by an automated microplate reader at 450 nm (Star fax 2001, USA).

Statistical analysis of the data:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. ANOVA was used for comparing the different studied groups for normally distributed quantitative variables and post hoc test (LSD) was used to find the significant between each two groups. Kruskal Wallis test was used to compare different groups for abnormally distributed quantitative variables and Mann Whitney test was used for comparing each two groups. Pearson coefficient was used to correlate between quantitative variables. Receiver operating characteristic curve (ROC) was used to find the diagnostic performance and cutoffs of the marker. Significance of the obtained results was judged at the 5% level.

Result:

Table 1: Comparison between the three studied groups according to demographic data

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>Group SLE (n = 30)</th>
<th>RA Group (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45.7 ± 5.3</td>
<td>32.8 ± 7.2</td>
<td>41.1 ± 12.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (60.0%)</td>
<td>1 (3.3%)</td>
<td>3 (10.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>10 (40.0%)</td>
<td>29 (96.7%)</td>
<td>27 (90.0%)</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>6.50 (1.0 - 20.0)</td>
<td>7.0 (1.0 - 27.0)</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

Normally quantitative data was expressed in mean ± SD and was compared using F test (ANOVA) abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test

*: Statistically significant at p ≤ 0.05
a: Significant with control
b: Significant with group SLE

Table 2: Comparison between the three studied groups according to different studied parameters

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>Group SLE (n = 30)</th>
<th>RA Group (n = 30)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAFF-R</td>
<td>65.2±65.2</td>
<td>73.7±88.8</td>
<td>116.2±21.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCMA</td>
<td>323.0(202.0–1242.0)</td>
<td>225.0(125.0–472.0)</td>
<td>238.0(111.0–587.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>BAFF</td>
<td>651.4±100.2</td>
<td>740.9±167.1</td>
<td>984.0±194.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TACI</td>
<td>208.5±55.9</td>
<td>248.1±56.5</td>
<td>261.7±81.2</td>
<td>0.012</td>
</tr>
<tr>
<td>ESR</td>
<td>-</td>
<td>44.0(25.0–90.0)</td>
<td>40.0(7.0–90.0)</td>
<td>0.300</td>
</tr>
<tr>
<td>CRP</td>
<td>-</td>
<td>10.5(4.0–96.0)</td>
<td>12.0(4.0–96.0)</td>
<td>0.836</td>
</tr>
<tr>
<td>S.Creat.</td>
<td>-</td>
<td>0.95(0.5–2.4)</td>
<td>0.82(0.4–1.8)</td>
<td></td>
</tr>
</tbody>
</table>

Normally quantitative data was expressed in mean ± SD and was compared using F test (ANOVA) abnormally distributed data was expressed in median (Min. - Max.) and was compared using Mann Whitney test

*: Statistically significant at p ≤ 0.05
a: Significant with control
b: Significant with group SLE

BAFF, BAFF-R, & TACI levels showed statistically significant increase in SLE & RA Groups as compared to control group (P<0.05) and BCMA level was statistically significant decrease in SLE & RA Groups as compared to control group (P<0.05). Further, BAFF, BAFF-R levels were statistically significantly increased in RA Group when compared to SLE Group (Table 2).

Table 3: Correlation between the different studied parameters in each group

<table>
<thead>
<tr>
<th></th>
<th>Group SLE</th>
<th>Group RA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAFF-R</td>
<td>BAFF</td>
</tr>
<tr>
<td>BAFF-R</td>
<td>r</td>
<td>-0.084</td>
</tr>
<tr>
<td>Duration</td>
<td>r</td>
<td>0.657</td>
</tr>
<tr>
<td>ESR</td>
<td>r</td>
<td>0.163</td>
</tr>
<tr>
<td>CRP</td>
<td>r</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.101</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>0.571</td>
</tr>
</tbody>
</table>
A significant positive correlation was found between the BAFF level and BAFF-R levels in both groups and SLE \( (r=0.854, p<0.001) \), RA \( (r=0.614, p<0.001) \), also between BAFF, BAFF-R and SLEDAI in SLE group \( (r=0.413, 0.419, p<0.023, 0.021) \) respectively, and between BAFF, BAFF-R and DAS28 in RA group \( (r=0.642, 0.430, p<0.001,0.018) \) respectively.

**Table 4:** Agreement (sensitivity, specificity and accuracy) for BAFF-R, BCMA, BAFF and TACI with RA

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>p</th>
<th>95% C.I</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LL</th>
<th>UL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAFF-R</td>
<td>0.954*</td>
<td>&lt;0.001</td>
<td>0.894</td>
<td>1.0</td>
<td>&gt;94</td>
<td>86.67</td>
<td>100.0</td>
<td>100.0</td>
<td>93.2</td>
<td></td>
</tr>
<tr>
<td>BCMA</td>
<td>0.569</td>
<td>0.292</td>
<td>0.437</td>
<td>0.702</td>
<td>≤227</td>
<td>50.0</td>
<td>50.91</td>
<td>35.7</td>
<td>65.1</td>
<td></td>
</tr>
<tr>
<td>BAFF</td>
<td>0.905*</td>
<td>&lt;0.001</td>
<td>0.841</td>
<td>0.969</td>
<td>&gt;800</td>
<td>83.33</td>
<td>87.27</td>
<td>78.1</td>
<td>90.6</td>
<td></td>
</tr>
<tr>
<td>TACI</td>
<td>0.608</td>
<td>0.103</td>
<td>0.477</td>
<td>0.738</td>
<td>&gt;219</td>
<td>73.33</td>
<td>50.91</td>
<td>44.9</td>
<td>77.8</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1:** ROC curve for BAFF-R, BCMA, BAFF and TACI to diagnose RA
Discussion:

Recent advances in understanding the pathobiology of autoimmune diseases have highlighted the continuing crosstalk between activated immune cells, pro-inflammatory cytokines, and matrix-degrading mediators, promoting chronic inflammation & irreversible tissue damage. B cells are widely recognized as leading players in immune-mediated pathology based on their ability to produce not only different patterns of autoantibodies but also as independent antigen-presenting cells and by modulating the activation of T cells. (CodrinaAncuta,)

The current study emphasized the role of BAFF, a B-cell-activating factor, and BAFF receptors (BAFF-R, TACI, BCMA) in promoting B-cell homeostasis, proliferation, and survival under normal and autoimmune systemic disorders. The present study showed significant elevation of BAFF levels in RA cases in comparison with SLE and control cases. Additionally BAFF levels were significantly elevated in SLE subjects in comparison with healthy subjects.

This coincided with the study made by Wei F et al, who found that abnormal BAFF/BAFF receptor-signaling pathways were reported in several autoimmune disorders including SLE& RA. (Wei, F., Y. Chang, W. Wei, 2015) Moreover, Fang Wei et al, confirmed that the levels of BAFF increase in cases of autoimmune disease and are correlated with the level of disease activity. (Fang Wei, Yan Chang, Wei Wei, 2015)

Of interest, elevated BAFF levels were detected in synovial fluid, serum, and saliva in very early stages of RA, suggesting its involvement in cell-cell interactions network in the synovial microenvironment, as well as B-cell activation and the development of autoreactive B cells. (Moura, R.A., et al., 2012) RA exhibits a positive correlation between BAFF and disease activity, so that BAFF was recommended as a new index of RA activity (Leandro, M.J., G. Cambridge, 2013) and a new target for treatment of RA patients epically those who showed...
resistance to treatment with either traditional DEMARDS and/or anti-TNFα blockers. (Genovese, M.C., et al., 2013)

Moreover, the SLE patients with high levels of BAFF exhibited significantly higher levels of antidouble-strand DNA antibody in each of IgG, IgM and IgA classes. (Kouichi Hirayama & Miho Nagai, 2017)

Three distinct BAFF receptors typically expressed on B cells in different developmental stages are generally recognized: (i) BAFF receptor (BAFF-R); (ii) transmembrane activator and calcium modulator ligand interactor (TACI); and (iii) B-cell maturation antigen (BCMA). (Davidson, A., 2010) The current study showed significant elevation of BAFF-R levels in RA cases in comparison with SLE and control cases. Additionally, BAFF-R levels were significantly elevated in SLE subjects in comparison with healthy subjects. The current study showed positive correlation between BAFF-R & BAFF levels in RA & SLE cases. Moreover, the current study revealed that the best biomarker for diagnosis of RA & SLE were BAFF-R & BAFF as they had the ROC curve with more sensitivity and specificity.

This aligned with the study made by Woo YJ et al., who reported that BAFF-R is essentially engaged in naïve and memory B-cell populations, with the highest expression in follicular and marginal zone B-lymphocytes, and is upregulated by B-cell receptor on mature B cells and enables most of the BAFF-dependent actions. (Woo, Y.J., et al., 2011) Furthermore, the overexpression of BAFF receptors, as well as disturbed autocrine and paracrine BAFF network, seems to be related to inflammatory events and RA progression. (28) Additionally, the expression of BAFF-R on CD19+ cells (B cells) in active SLE patients was elevated in stable SLE patients. (Duan, J.H., et al., 2016)

Although BAFF-R stands as a specific receptor for both soluble and membrane-bound BAFF, TACI and BCMA can also bind to them. (Liu, Z., A. Davidson, 2011) This study showed significant elevation of TACI&decrease of BCMA levels in RA & SLE cases in comparison with control subjects. No significant difference between TACI& BCMA levels in RA & SLE cases.

This agreed with Torre D et al., who reported that TACI-excessive levels are detected during first steps of RA development. (De la Torre, et al., 2010) Also, elevated BAFF in serum and PBMCs and overexpression of TACI on B cells were demonstrated in SLE. TACI expression in CD19+ B cells was positively correlated positively with the SLEDAI score. (Barbosa, R.R., et al., 2014)

Tsuij et al. (2011) showed that TACI has dual functions, both promoting B-cell differentiation and limiting lymphoproliferation. Additionally, Coquery and Erickson (2012) showed that BCMA mRNA is expressed predominantly in terminally differentiating and immunoglobulin secreting B cell lines. (Coquery, C.M., L.D. Erickson, 2012) BCMA reduces the development of autoimmunity. Coquery and his team reported that in lupus-prone mice with BCMA deficiency, the activation status and neutrophil accumulation were found to be increased significantly in the spleen tissues. (Coquery, C.M., et al., 2014)

It was proved that BAFF-BAFF-R interface seems to be vital for the survival of B2 subpopulation from the transitional type 1 stage, with a minor input from TACI and without feedback from BCMA. (Pillai, S., H. Mattoo, A. Cariappa, 2011) BAFF enhances long-term B-cell survival primarily through NF-kB pathway &accounts for the survival of plasma cells expressing TACI and/or BCMA. (Wei, F., Y. Chang, W. Wei, 2015)

BAFF plays a prominent role in the pathogenesis of both RA and SLE. Several clinical trials have demonstrated the efficacy of the BAFF blockade as line of treatment of these diseases, suggesting that BAFF blocking agents should be valuable to the precision medicine linked to different markers of disease activity.

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