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B-cell disturbance in rheumatoid arthritis patients: Comparative study between treated and non-treated patients

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ABSTRACT

Alterations in B cell homeostasis and B cell activation markers, namely B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL), are described in rheumatoid arthritis (RA) patients. In the current study, 60 RA patients (30 receiving non-biologic disease modifying anti-rheumatic drugs (DMARDs) but have not received prior biological treatment and 30 treatment-naïve patients), and 30 healthy controls were enrolled. B cell count was determined by flow cytometry using the CD19-PE Kit (Immunotech, France). BAFF and APRIL blood concentration was measured using commercially available ELISA kit (Bosterbio, USA). B cell count diminished in RA patients compared to controls (p-value <0.05), however it was more pronounced in treated patients. Circulating BAFF levels increased in RA compared to healthy controls (p-value <0.05) with more increase in patients on treatment. Circulating APRIL levels were significantly lower (p-value <0.05) in treatment naïve rheumatoid arthritis patients (343.9 ± 21.7) than the control group (371.5 ± 24.3). Significant decrease in B cell count and increase in BAFF level were observed in RA patients on non-biologic DMARDs. Conversely, APRIL levels were not affected by the treatment.

KEYWORDS: disease modifying anti-rheumatic drugs (DMARDs), a proliferation-inducing ligand (APRIL), B-cell activating factor (BAFF), rheumatoid arthritis (RA)

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INTRODUCTION

Rheumatoid arthritis (RA) is a progressive, inflammatory disease, which is chronic and systemic in nature, primarily affecting the small joints of the hands and feet [1]. The role of B cells in RA is important and has been highlighted by the success of B cell-targeting therapy using the anti-CD20 monoclonal antibody rituximab. In addition to this B cell-specific biologic agent, other anti-B cell therapies are currently under development, especially therapies targeting cytokines implicated in B cell survival and/or differentiation [2].

B cell activation and maturation are controlled by soluble and membrane-bound B cell-activating factors, both belonging to the superfamily of tumor necrosis factor (TNF); the cytokine BAFF (B cell activating factor), and APRIL (a proliferation-inducing ligand) are also members of this superfamily [3]. Excessive BAFF and APRIL production has been described in several autoimmune diseases, mainly systemic lupus erythromatosis (SLE) and primary sjogren syndrome (SS), and it has also been described in RA. Thus, elevated BAFF levels contribute in breaking B cell tolerance, thus, promoting B cell autoimmunity [4].

In this context, the objective of our current study is to investigate the blood levels of BAFF and APRIL cytokines together with the measurement of circulating counts of B-cells in a group of patients with well-established RA receiving treatment with non-biologic DMARDs and another group with newly discovered RA who didn't receive any anti rheumatic drugs.

PATIENTS AND METHODS

Patients and controls

Sixty patients with RA, diagnosed according to 1987 American College of Rheumatolgy criteria, were recruited from department of rheumatology at El-Fayoum University Hospital. 30 patients with newly discovered RA, the other 30 with well-established RA receiving traditional DMARDs. 30 healthy controls were also included. Informed consent was obtained from all subjects. The protocol for this research project was approved by the ethics committee of Fayoum University.

Analysis of B cells

Absolute number of blood B cells was counted from fresh anticoagulated blood by flow cytometry (Epics XL, Beckman Coulter Inc., USA), using the CD19-PE Kit (Immunotech, France) according to the manufacturer's instructions.

BAFF and APRIL measurement

BAFF and APRIL blood concentrations were measured by sandwich enzyme-linked immune-sorbent assay using commercial kits (Boster Biological Technology Co., Ltd., CA) according to the manufacturer's recommendations.

Statistical analysis

Data analysis was performed using SPSS software, version 18 under windows 7.

RESULTS

Epidemiological characteristics and treatment strategies of study groups

Three groups were included in our study. Group 1 (G1) includes 30 well-established RA patients receiving DMARDs, group 2 (G2) includes 30 newly diagnosed RA patients receiving no treatment, and group 3 includes 30 healthy controls. Statistical analysis showed no significant difference between the study groups as regards age or sex distribution (Table 1), which confirms the proper matching between the study groups.

Low B-cell counts in patients with RA

B-cell count was significantly lower in patients with RA compared to control groups, with mean counts of 137.2 ± 60.5 in G1, and 179.3 ± 59.1 in G2 compared to 243.7 ± 22.5 in control group (Figure 1). Importantly, the well-established RA group receiving DMRADs revealed more reduction compared to newly diagnosed group receiving no treatment. This implicates a role for treatment and/or disease course in the further suppression of B-cell.

APRIL concentration is lowered in patients with newly diagnosed RA but not in well-established RA patients

Next we analyzed the concentration of blood circulating levels of APRIL in both patient groups. Compared to controls, G2 group expressed a significantly lower level of APRIL. G1 showed a mean concentration of 343.9 ± 21.7 pg/ml compared to control (mean = 371.5 ± 24.3 pg/ml) with p value <0.05. Notably, although well-established RA patients expressed lower levels of APRIL (mean = 359.3 pg/ml), the difference was not significant ($p > 0.05$) (Figure 2).

BAFF concentration is higher in both groups of patients with RA

In contrast to APRIL, BAFF concentration level was significantly higher in both patient groups compared to control. G1 showed a mean concentration of 1419.4 ± 417.3 pg/ml, G2 mean was 886.2 ± 258.4 while the mean of control was 444.4 ± 120.1 pg/ml) with p for both groups <0.05 (Figure 3).

Table 1. Comparing demographic characteristics of study groups.

Study group	Age (Years)		Sex		
	Mean ± SD	p-value	F	M	p-value
Well-established RA	43.6 ± 13.9	0.1	23 (33%)	7 (33%)	0.6
Newly discovered RA	46.1 ± 15.6		25 (36%)	5 (24%)	
Controls	38 ± 7.7		21 (30%)	9 (43%)	

Comparing study groups as regards their age and sex revealed no significant difference among them.

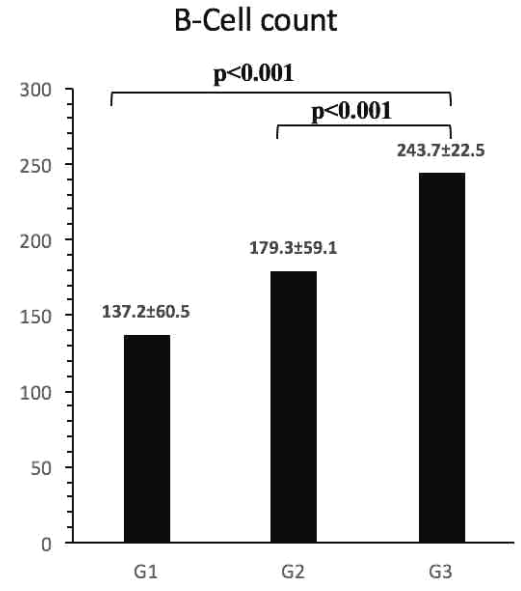

Figure 1. Mean counts of B-cells in different study groups. G1: well-established RA receiving DMRADS, G2: newly-diagnosed RA receiving no treatment, G3: control group. Data showed significantly lower B-cell counts in G1 (mean 137.2 ± 60.5, $p < 0.001$), and G2 (mean 179.3 ± 59.1, $p < 0.001$) compared to control (243.7 ± 22.5).

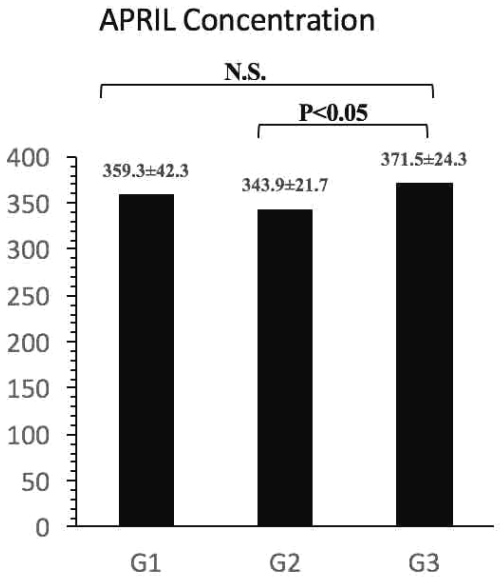

Figure 2. Mean concentration of APRIL among different study groups. G1: well-established RA receiving DMRADs, G2: newly-diagnosed RA receiving no treatment, G3: control group. Data showed significantly lower APRIL concentration in G2 (mean 343.9 ± 21.7 pg/ml, $p < 0.05$), and non-significant change in G1 (mean 359.3 ± 32.3 pg/ml, $p > 0.05$) compared to control (371.5 ± 24.3 pg/ml).

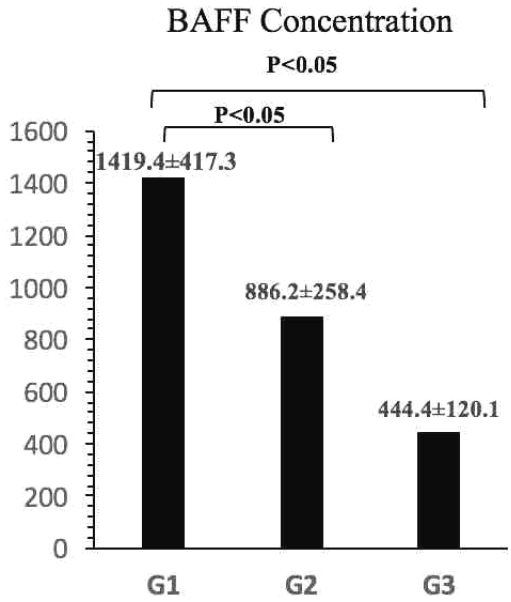

Figure 3. Mean concentration of BAFF among different study groups. G1: well-established RA receiving DMRADS, G2: newly-diagnosed RA receiving no treatment, G3: control group. Data showed significantly higher BAFF levels in G1 (mean 1419.9 ± 417.3 pg/ml, $p < 0.05$), and in G2 (mean 886.2 ± 258.4 pg/ml, $p < 0.05$) compared to control (444.4 ± 120.1).

DISCUSSION

Rheumatoid arthritis is a chronic, progressive inflammatory systemic disease, affecting primarily the small joints of the hands and feet symmetrically and characterized by joint destruction, progressive disability, and premature death [1].

The hypotheses most ideally accepted propose that RA results from abnormal immunological response to unidentified triggering agents (possibly a virus) in a genetically susceptible individual. Humoral and cell-mediated mechanisms are both involved. Moreover, circulating immune complexes are present in both blood and synovial fluids of patients with rheumatoid arthritis [5].

The involvement of B cell in systemic autoimmune disease has emerged as a new concept over the past ten years, leading to new avenues for innovative therapeutic strategies. In fact, B cells play a number of critical roles in inducing and perpetuating autoimmune reactions. They exert different functions during the immune response, including presentation of antigens, release of cytokines, cooperation with

and activation of T cells, production of auto-antibodies and they may act also as regulatory B cells [6].

In our study B cell count was measured in 60 patients with RA and 30 healthy controls. The RA patients were divided into 2 groups. The first group (G1) was thirty patients with well-established RA and receiving treatment with non-biologic DMARDs. The second group (G2) was the other thirty with newly discovered RA who didn't receive any anti-rheumatic drugs.

In our study there is statistically significant difference (p-value <0.05) between different study groups as regards to B cell count with low mean among well-established RA group (137.2 ± 60.5) and high mean among controls (243.7 ± 22.5).

The B cell count diminished in the two groups of RA patients below its normal value range which may be due to migration to target organ i.e. synovium or alternatively to lymphoid tissue as has been previously reported [7, 8], however it is more pronounced among well-established RA which may be explained by the effect of treatment.

In concordance with our study Gaugler *et al*. (2013) found that peripheral B cells are disturbed in RA but not in ankylosing spondylitis (AS), with the decrease in circulating B and T cells being significant between RA and healthy controls only (all post-hoc Dunn tests: $p < 0.0001$). Previous work evaluating B cell have yielded other results. Fedele *et al*. (2014) reported that B lymphocyte frequency is different according to RA disease duration; very early rheumatoid arthritis (VERA) and early rheumatoid arthritis (ERA) patients showed similar percentages of CD19+ cells (VERA: 10.1 ± 4.5%; ERA: 9.8 ± 4.2%), gated on total lymphocytes, with controls (9.5 ± 2.6%) but significantly higher percentages compared to LSRA (long standing rheumatoid arthritis) patients (6.9 ± 4.2%, $p = 0.002$ vs VERA and $p = 0.001$ vs ERA) [9, 10].

B cell maturation and activation are under the control of soluble and membrane-bound B cell-activating factors that belong to the tumor necrosis factor superfamily: the cytokine BAFF has emerged as a crucial factor that modulates B cell survival, activation, maturation, tolerance and homeostasis and the cytokine APRIL is another member of this superfamily that has similar functions to BAFF [11]. BAFF role in autoimmunity is now well understood and illustrated by the clinical efficacy of anti-BAFF therapies in SLE [4]. APRIL shares similar functions with BAFF, but the precise biological role of APRIL in immune cell development and regulation requires further clarification [12].

In our present study BAFF and APRIL were measured in the same groups of RA as well as healthy controls. As regards BAFF concentration there was a statistically significant difference (p-value <0.05) between the different study groups with high mean among well-established rheumatoid arthritis group (1419.4 ± 417.3 pg/ml) and low mean among controls (444.4 ± 120.1 pg/ml). This means that BAFF concentration is increased in the two groups of RA, either well established or newly discovered, but with more increase in the well-established group. The findings of our study are supported by that of Cheema *et al*. (2001) and Nakajima *et al*. (2007) who described elevated serum and tissue levels in patients with RA [13, 14]. Similarly, and in agreement with that, Bosello *et al*. (2008) found the serum BAFF level elevated in RA patients and associated it with autoantibody production. Further studies have showed an association with higher disease activity [15].

Gaugler *et al*. (2013) measured the concentration of BAFF and APRIL in the serum of RA, AS patients and healthy controls. No difference in serum BAFF concentrations was observed between the three groups (RA vs AS vs HC: 1100.5 ± 62.5 vs 904.9 ± 29.4 vs 939.2 ± 30.3 pg/ml, $p > 0.05$). This may be due to different patient characteristics (age, disease duration) and presumably, depending on the treatment they received [9]. It has been described that cytokines, such as IL-10, IFN-α and IFN-γ may increase BAFF expression in various cell types [12, 3], while high dose corticosteroid treatment inhibits its expression [16]. Biological treatments, such as rituximab, induce BAFF, while TNF-α blockade does not affect BAFF levels [17].

Our study found that APRIL concentration was significantly lower (p-value <0.05) in newly discovered rheumatoid arthritis patients (343.9 ± 21.7) than the control group (371.5 ± 24.3). On the other hand, there is no statistically significant difference (p-value >0.05) between the well-established rheumatoid arthritis group and controls.

This means that APRIL concentration is slightly decreased in the two groups of RA but decreased more in the newly discovered group.

The results of our study are in agreement with Koyama *et al*. (2005) who studied 48 patients with SLE, 21 patients with RA and 41 healthy controls. They found that APRIL level was elevated in SLE patients. However, in RA patients it was not higher than those in normal control [18]. Moreover, analysis of an experimental model for rheumatoid arthritis revealed elevated BAFF levels, but normal or low APRIL levels [19].

Previous works measuring APRIL concentration revealed other results. Moura *et al*. (2010) reported that VERA patients, at baseline and even after methotrexate (MTX) treatment have higher levels of both APRIL ($p < 0.05$) and BAFF ($p < 0.001$) as compared with established RA and controls. This difference in results could be attributed to the inclusion criteria of the patients; while Moura studied untreated VERA patients with <6 weeks of disease duration, we did not consider the disease duration in the newly discovered RA. Some of our patients may have a disease duration of a year or more but because of misdiagnosis they did not receive any anti-rheumatic drugs [20].

In disagreement with our study Vallerskog *et al*. (2006) and Gaugler *et al*. (2013) found increased APRIL levels in RA compared to healthy controls [21, 9]. These discrepancies may be explained by the patient's characteristics, the disease duration, and the disease activity.

Hua *et al*. (2016) found that the IL-10 production of B-cell was greater with APRIL than with BAFF or control medium, in a dose-dependent manner. TACI expression was greater in IL-10-producing B cells (B10) than non-IL-10-producing B cells whereas BAFF-R expression was lower. In conclusion APRIL but not BAFF promotes IL-10 production by CpG-activated B cells and enhances the regulatory role of B cells on T cells. B10 cells in RA patients are responsive to APRIL, which suggests a possible therapeutic application of APRIL to expand B10 cells [22]. This could also explain the difference of clinical efficacy observed between belimumab and atacicept in RA. The factor APRIL may decrease or remain normal in patient with RA in contrast to BAFF. Furthermore, APRIL may have a protective role according to Hua *et al*. (2016) through promoting IL-10 production and this aspect seems to be promising in future treatment research.

CONCLUSION

In conclusion, the contribution of B lymphocyte to RA pathogenesis goes beyond autoantibody production. Disturbances in B cell homeostasis in RA are not only due to the use of anti-rheumatic drugs but closely related to the disease process itself.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing financial interests.

ABBREVIATIONS

AS	:	Ankylosing Spondylitis
APRIL	:	A Proliferation-Inducing Ligand
BAFF	:	B-Cell Activating Factor
DMARDs	:	Disease-Modifying Antirheumatic Drugs
ERA	:	Early Rheumatoid Arthritis
MTX	:	Methotrexate
RA	:	Rheumatoid Arthritis
SS	:	Sjogren Syndrome
SLE	:	Systemic Lupus Erythematosus
VERA	:	Very Early Rheumatoid Arthritis

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