Aim of the work: To assess serum levels of B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) to determine their correlations with disease activity in pediatric systemic lupus erythematosus (pSLE) and juvenile idiopathic arthritis (JIA) patients.

Patients and methods: Twenty-nine pSLE patients and 33 JIA patients were recruited. SLE disease activity was assessed using the systemic lupus erythematosus disease activity index (SLEDAI), while the juvenile arthritis 27 joint disease activity score (JADAS-27) was calculated for JIA patients. Serum samples were assayed for BLyS and APRIL by the enzyme linked immunosorbent assay (ELISA).

Results: Serum BLyS and APRIL were elevated in both pSLE and JIA patients compared to controls. Serum BLyS levels correlated with both SLE and JIA disease activity ($p = 0.042$, $p = 0.019$, respectively) whereas serum APRIL levels correlated positively with JADAS-27 and inversely with SLEDAI ($p = 0.001$, $p = 0.02$, respectively). Elevated serum BLyS and APRIL were significantly associated with a lower incidence of nephritis ($p = 0.043$, $p = 0.016$, respectively), while elevated serum APRIL significantly associated with negative anti-dsDNA in pSLE patients ($p = 0.017$). In JIA patients, both serum BLyS and APRIL were significantly associated with the
1. Introduction

Emerging data from clinical trials are providing a critical insight regarding the role of B cells and autoantibodies in various autoimmune conditions. Deregulated B-cell function has been implicated in several autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis. B cells contribute to pathological immune responses through the secretion of cytokines, costimulation of T cells, antigen presentation, and the production of autoantibodies [1].

Given the crucial role of B cells in the pathogenesis of SLE and RA, it stands to reason that factors which promote B cell survival and/or function are also crucial [2]. B-lymphocyte stimulator (BLyS)—also called B cell-activating factor belonging to the tumor necrosis factor family (BAFF)—and a proliferation-inducing ligand (APRIL) are members of the tumor necrosis factor (TNF) family and are important regulators of B-cell maturation, survival, and function [3,4].

They regulate the size and composition of B-cell compartment and act as important driving factors for B-cell hyperplasia and autoantibody production in autoimmune processes [5].

BLyS and its homolog APRIL are widely expressed by many cell types, including hematopoietic and stromal cells [6]. There are two forms of BLyS, membrane-bound and soluble. The gene expression and the levels of BLyS are regulated by cytokines, basically interferon-γ and to a lesser extent interleukin-10 [7].

The binding of BLyS and APRIL to their receptors activates specific TNF receptor-associated factors (TRAFs), which regulate signal transduction in B cells [8,9].

Beside their well-known function as antibody secreting cells, an antibody independent role for B-cells in disease pathogenesis has been documented by experimental data as well as the promising results of B-cell depleting therapies in RA [10–12].

Juvenile idiopathic arthritis (JIA) is a heterogeneous condition gathering distinct forms of chronic arthritis of unknown etiology that begin before the age of sixteen and persist for at least six weeks [13].

SLE is a systemic autoimmune disease characterized by autoantibody production against self-antigens [14]. The role of BAFF (BLyS) in the pathogenesis and disease activity in SLE is well-known and the novel noticeable correlation with the damage index highlights the utility of BAFF as an indicator of disease damage and predictor of poor outcome [15]. The rationale for developing B-lymphocyte (BLyS) inhibitors has included successive investigations. B-cell stimulatory factors that can promote the loss of B-cell tolerance and drive autoantibody production are exciting new candidates [14].

This study was designed to assess the relationship of serum BLyS/APRIL levels to disease activity in pediatric pSLE and JIA patients.

2. Patients and methods

2.1. Study approval

This study was approved by the Ethics Committee of our institution (Benha faculty of medicine). Legal guardians of all subjects gave their written informed consent before participation in this study.

2.2. Participants

This study included 82 children recruited from the inpatient and outpatient clinics of the rheumatology and pediatric departments, faculty of medicine, Benha University Hospitals. Twenty-nine pediatric patients met the American College of Rheumatology (ACR) criteria for SLE classification [16], while 33 juvenile patients were diagnosed as juvenile idiopathic arthritis (JIA) according to the International League Against Rheumatism (ILAR) classification criteria [17]. Twenty age and gender matched children served as controls.

The following information was obtained: demographic data (age, gender, age at disease onset, duration of disease), JIA subtype, number of active joints, clinical manifestations of pediatric SLE (pSLE) patients at presentation, laboratory values and treatment data (biologics, concomitant DMARDs and corticosteroids including start and stop dates, as well as reasons for withdrawal).

pSLE patients were evaluated using the systemic lupus erythematosus disease activity index (SLEDAI) [18] while, nephritis was classified according to the World Health Organization (WHO) classification based on patients’ previous data [19]. JIA patients were evaluated using the juvenile arthritis 27 joints disease activity score (JADAS-27) [20].

2.3. Laboratory investigations

Complete blood cell count (CBC), erythrocyte sedimentation rate (ESR) by the Westergren method, complete urine analysis for casts and/or proteinuria, IgM rheumatoid factor (RF) done by Rose–Waaler test, antinuclear antibody (ANA) and double-stranded DNA antibodies (anti-dsDNA) done by indirect immunofluorescence on Hep-2 cells by Kallestad indirect fluorescent antibody (IFA) assays
Serum BLyS and APRIL as possible indicators of disease activity in pediatric systemic lupus

3. Results

3.1. Demographic and clinical characteristics of the study groups

The present study comprised 29 pSLE patients (females to males, 25:4 with median age 14.3 years) and 33 JIA patients (females to males, 23:10 with median age 12.2 years). JIA patients were further classified into 6 systemic-onset, 12 polyarticular and 15 oligoarticular subtypes. Nephritis was classed in 8 pSLE patients (27.58%), one patient was class II nephritis, 2 patients class were III, one was class IV and 3 were class V, while the nephritis class could not be determined in one patient. The median SLEDAI was 12 (range 6–28), while the median JADAS-27 of JIA patients was 15.1 (5–37) with no significant differences noted among different subtypes (p = 0.16).

Although the pSLE patients had shorter disease duration than JIA patients, no significant difference was noted between them (p < 0.05). Marked significant differences between pSLE and JIA patients were observed regarding certain medications, with more pSLE patients receiving corticosteroids (p < 0.001), azathioprine (p < 0.001) and cyclophosphamide (p = 0.013), while more JIA patients received methotrexate (p < 0.001). There were no differences regarding (NSAIDs) (p = 0.57) and hydroxychloroquine (p = 0.86) therapy. Only one polyarticular JIA patient was receiving etanercept (anti-tumor necrosis factor treatment). Characteristics of the studied patients are shown in Table 1.

3.2. BLyS and APRIL levels in pSLE

The median BLyS serum level was 1.14 ng/ml in pSLE patients (range 0.2–2.13) and 0.58 ng/ml in healthy controls (range 0.04–1.06), p < 0.001. Sera from patients with pSLE also showed a high median APRIL serum level of 11 ng/ml (range 0.11–205) compared to healthy controls (0.9 ng/ml, range 0.0–23.4), p = 0.046. Higher serum levels of both BLyS and APRIL were associated with lower incidence of renal involvement (p = 0.043 and p = 0.016, respectively). Meanwhile, higher serum APRIL levels associated absence of anti-dsDNA (p = 0.017), while serum BLyS did not (p = 0.197), (Table 2).

Only serum BLyS was significantly higher in patients receiving cytotoxic drugs (azathioprine, cyclophosphamide) (p = 0.017). On the other hand, neither high prednisone doses (> 1 mg/kg/day) nor gender significantly affect serum BLyS (p = 0.83, p = 0.23, respectively) or APRIL (p = 0.56, p = 0.25, respectively) levels, Table 2.

Serum BLyS levels correlated positively with disease activity (r = 0.38, p = 0.042). Inverse correlations between serum APRIL levels and disease activity (r = −0.429, p = 0.02) in pSLE patients were observed, Fig. 1(A and B). There were no significant correlations between serum BLyS, or APRIL levels with age, disease duration or blood leukocyte and lymphocyte counts (p < 0.05).

3.3. BLyS and APRIL levels in JIA disease

Serum BLyS levels were significantly higher in JIA patients (median 1.18 ng/ml, range 0.2–4.21) compared to controls (median 0.58 ng/ml, range 0.04–1.06), p < 0.001. The median serum BLyS level was significantly elevated in systemic onset, 3.52 ng/ml (range 1.23–4.21) compared to polyarticular onset, 1.25 ng/ml (range 0.45–4.12) and oligoarticular onset, 1.04 ng/ml (range 0.2–2.59) with p = 0.007. The median APRIL Level was 35.3 ng/ml (range 0.8–211) in sera of patients with JIA and 0.9 ng/ml (range 0.0–23.4) in sera of healthy controls (p < 0.001). A significantly elevated median APRIL level (p = 0.004) was found in sera of systemic onset patients (184.5 ng/ml, range 33.6–211) compared to polyarticular onset (41.75 ng/ml, range 3.5–201) and oligoarticular onset (23 ng/ml, range 0.8–100).

Both BLyS and APRIL levels were significantly elevated in sera of JIA patients with positive ANAs (p = 0.008, p < 0.001, respectively). Only serum BLyS was significantly higher in JIA patients with uveitis (p = 0.031) and APRIL was higher in sera of those with a positive RF (p = 0.035). There was a significant increase of serum BLyS levels in patients receiving DMARDs compared to other patients (p = 0.006). Also, there were no significant differences in serum levels of APRIL between JIA patients receiving and not DMARDs (p < 0.05). BLyS and APRIL levels among sera of JIA patients did not differ significantly with respect to gender or corticosteroid doses (prednisone ≥1 mg/kg/d), (p < 0.05), (Table 3).

Both BLyS and APRIL levels significantly correlated with disease activity (r = 0.497, p = 0.019 and r = 0.549, p = 0.001, respectively) in sera of JIA patients, Fig. 1 (C and D). Neither BLyS nor APRIL correlated with age, disease duration or thrombocyte count (p < 0.05). APRIL and BLyS serum levels correlated positively to each other in JIA patients but inversely in pSLE patients (r = 0.469, p = 0.006 and r = −0.403, p = 0.03, respectively), Fig. 1 (E and F).

4. Discussion

Autoreactive B cells are driven by self-antigens, but the factors that promote the loss of B cell tolerance and drive
autoantibody production are still unknown [21]. BLyS is considered as a fundamental survival factor for transitional and mature B cells, whereas APRIL mainly affects B1 cell activity, humoral responses and class switching of immunoglobulins [3].

Whereas many previous studies have evaluated the role of BLyS in adult populations with various immune based diseases [22–29], debated yet little information is available about serum APRIL levels in adult patients with SLE [29–31] and almost none on pSLE. Important differences do exist between pSLE and adult SLE and between JIA and adult RA [2]. Few studies have involved association of BLyS/APRIL in pediatric patients with autoimmune diseases [2,32], whereas one study assessed plasma BLyS protein and blood leukocyte BLyS mRNA in pSLE and JIA patients [2], another study [32] assessed BLyS/APRIL serum levels in JIA patients only.

One of the hallmarks of active disease in human SLE is the increased percentages of activated B cells and plasma cells in peripheral blood [33,34]. Concurring with previous studies, we demonstrated increased serum BLyS levels in pSLE patients compared to healthy controls with a significant correlation to disease activity (p = 0.042) [2].

In addition, similar previous findings in a study done on adult SLE patients reported a significant correlation between changes in circulating BLyS levels and changes in disease activity [26]. A better correlation of disease activity with blood leukocyte BLyS mRNA levels than with plasma BLyS protein levels was documented in another cross-sectional observational study [25]. Meanwhile, other studies did not find any association between BLyS serum levels and SLE activity in adult patients [22,23]. These disparities are not surprising as clinical manifestations of SLE are diverse and differences in the pathogenesis between adult and pSLE may be present.

Elevated APRIL levels were also observed in our pSLE patients compared to healthy controls with a limited inverse correlation to disease activity (p = 0.02). Similar results were obtained in previous studies in adult SLE patients [29–31], whereas a significant correlation between serum APRIL levels and musculoskeletal manifestations among patients with adult SLE when assessed by the BILAG index was reported in another study [30].

On comparing serum BLyS and APRIL levels with different clinical parameters, serological markers and management protocols, there was no association between serum BLyS levels and anti-dsDNA antibody while contradictory results were obtained with serum APRIL levels, whereas lower serum APRIL levels were found when anti-dsDNA antibody was present.

The direct link between anti-DNA antibodies and nephritis has been demonstrated [35,36], as well as its titers have been considered as a predictor of renal flare [37]. Moreover, Reichlin et al. [38] stated that both anti-dsDNA and anti-ribosomal P antibodies were associated with a higher prevalence of renal involvement in juvenile SLE.

Since these antibodies are strongly associated with nephritis in SLE this may explain the lower incidence of renal involvement in our patients with high serum BLyS and APRIL levels.
Davis et al. [39] reported an increased urinary excretion of BLyS in adult SLE patients, especially among those with clinically overt renal involvement. On the other hand, circulating soluble APRIL may not be the only form of APRIL relevant to B cell biology and/or B cell based autoimmunity [31]. In concordance with our results, Morel et al. [29], found an inverse correlation between serum APRIL and anti-dsDNA antibody titers, they did not report any correlation between serum BLyS and anti-dsDNA antibody titers. In other studies, serum BLyS correlated with anti-dsDNA antibody titers in adult SLE patients [22,40].

Interestingly, serum BLyS levels were higher in pSLE patients receiving cytotoxic drugs. This contradiction could be attributed to the fact that patients with more active disease receive more cytotoxic drugs. Adult RA or SLE patients treated with rituximab demonstrated reductions in B cell loads among

### Table 2
Comparison between BLyS and APRIL serum levels in different parameters of pSLE patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BLyS (ng/ml) median (range)</th>
<th>p</th>
<th>APRIL (ng/ml) median (range)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Females (25) Males (4)</td>
<td>0.23</td>
<td>10 (0.11–155)</td>
<td>0.25</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>+ ve (8) – ve (21)</td>
<td>0.043</td>
<td>5 (0.11–70)</td>
<td>0.016</td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>+ ve (26) – ve (3)</td>
<td>0.197</td>
<td>7.5 (0.11–205)</td>
<td>0.017</td>
</tr>
<tr>
<td>Cytotoxic agents</td>
<td>Yes (21) No (8)</td>
<td>0.017</td>
<td>11 (0.11–205)</td>
<td>0.96</td>
</tr>
<tr>
<td>Predisolone ≥1 mg/kg/d</td>
<td>Yes (3) No (26)</td>
<td>0.83</td>
<td>0.8 (0.8–40)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Anti-DNA, anti-double stranded DNA; BLyS, B lymphocyte stimulator; APRIL, a proliferation-inducing ligand.

Figure 1  (A and B) Correlation between B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) serum levels, and systemic lupus erythematosus disease activity index (SLEDAI); (C and D) correlation between BLyS and APRIL serum levels, and juvenile arthritis disease activity score (JADAS-27); (E and F) correlation between BLyS and APRIL serum levels in pediatric systemic lupus erythematosus (pSLE) and juvenile idiopathic arthritis (JIA) patients, respectively.
those under therapy that may have resulted in the elevated circulating BLyS protein levels [41]. The data presented here demonstrate that corticosteroid therapy has no significant role in BLyS/APRIL serum levels in pSLE patients concurring with previous reports [2].

In our JIA patients, elevated serum levels of both BLyS and APRIL were observed and significantly correlated with disease activity ($p = 0.019$, $p = 0.001$, respectively). Gheita et al. [32] found increased BAFF (BLyS) and APRIL serum levels in JIA patients associated with disease activity.

In contrast, no correlation has been reported between plasma BLyS protein concentration and disease activity, however, the Childhood Health Assessment Questionnaire (CHAQ) which is a measure of functional disability, was used to evaluate disease activity [2].

B-cell pathology plays an important role in early-onset JIA. B-cells function as amplifiers of chronic inflammation in the disease progress of JIA and might be a target of future therapies [42]. A profound decrease in circulating natural killer (NK) cells with coexisting hypergammaglobulinemia in JIA consistent with B-cell hyperactivity has been reported [43].

Thus, BLyS might play a crucial role in early activation of self-antigen-driven autoimmune B cells with autoimmune T cells further driving the switch for the production of pathogenic IgG autoantibodies [22].

In agreement with a previous report [32], we demonstrated elevated serum levels of BLyS and APRIL in systemic onset JIA patients compared to other types of onset. Furthermore, BLyS levels were significantly higher in JIA patients with uveitis. A primarily B-cell-infiltrative process of an enucleated eye globe has been observed in a 12-year-old patient with recurrent oligaarticular JIA [44].

Presence of RF was associated with higher APRIL levels in JIA. There was no comment in the results on BLyS or APRIL levels in RF positive or negative pSLE patients.

A significant correlation between BLyS and APRIL in serum of JIA patients was found in our study, in agreement with a previous report [32]. Similar to our results, Morel et al. [29] established an inverse correlation between BLyS and APRIL in serum of adult SLE patients, suggesting a protective role of APRIL.

In conclusion, serum BLyS showed elevated levels that correlated significantly with pSLE and JIA disease activity, accordingly anti-BLyS therapy might be of great benefit to offset disease flare. The inverse correlations observed between APRIL with both BLyS levels and disease activity in pSLE patients raise the possibility of being a down regulator of the disease process.

**Conflict of interest**

All the authors responsible for this work declare no conflict of interest.

**Acknowledgement**

We thank Professor Dr. Samia Abdel Moneim for her great effort in editing the manuscript, it is really appreciated.

**References**


**Table 3** Comparison between BLyS and APRIL serum levels in different clinical, laboratory and therapeutic variables of JIA patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BLyS (ng/ml) median (range)</th>
<th>p</th>
<th>APRIL (ng/ml) median (range)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Females (23)</td>
<td>1.31 (0.2–4.21)</td>
<td>0.32</td>
<td>27.5 (0.8–211)</td>
<td>0.27</td>
</tr>
<tr>
<td>Males (10)</td>
<td>2.13 (0.2–4.21)</td>
<td>0.031*</td>
<td>152 (0.8–211)</td>
<td>0.051</td>
</tr>
<tr>
<td>Uveitis +ve (13)</td>
<td>2.03 (0.2–4.21)</td>
<td>0.008*</td>
<td>142 (13.11–211)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Uveitis –ve (20)</td>
<td>1.32 (0.89–3.9)</td>
<td>0.48</td>
<td>100 (36–211)</td>
<td>0.035*</td>
</tr>
<tr>
<td>ANA +ve (15)</td>
<td>1.32 (0.8–211)</td>
<td>0.006*</td>
<td>47.50 (0.8–211)</td>
<td>0.07</td>
</tr>
<tr>
<td>ANA –ve (18)</td>
<td>1.32 (1.21–2.13)</td>
<td>0.57</td>
<td>36 (13.11–211)</td>
<td>0.66</td>
</tr>
<tr>
<td>RF +ve (18)</td>
<td>1.32 (0.8–211)</td>
<td>0.008*</td>
<td>142 (13.11–211)</td>
<td>0.07</td>
</tr>
<tr>
<td>RF –ve (28)</td>
<td>1.32 (0.2–4.21)</td>
<td>0.008*</td>
<td>142 (13.11–211)</td>
<td>0.07</td>
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<tr>
<td>Cytotoxic agents</td>
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<tr>
<td>Yes (23)</td>
<td>1.32 (0.8–211)</td>
<td>0.006*</td>
<td>47.50 (0.8–211)</td>
<td>0.07</td>
</tr>
<tr>
<td>No (10)</td>
<td>1.32 (1.21–2.13)</td>
<td>0.57</td>
<td>36 (13.11–211)</td>
<td>0.66</td>
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<tr>
<td>Prednisolone ≥1mg/kg/d</td>
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<tr>
<td>Yes (23)</td>
<td>1.32 (0.8–211)</td>
<td>0.006*</td>
<td>47.50 (0.8–211)</td>
<td>0.07</td>
</tr>
<tr>
<td>No (30)</td>
<td>1.32 (1.21–2.13)</td>
<td>0.57</td>
<td>36 (13.11–211)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

ANA, antinuclear antibody; RF, rheumatoid factor; BLyS, B lymphocyte stimulator; APRIL, a proliferation-inducing ligand.
Serum BLyS and APRIL as possible indicators of disease activity in pediatric systemic lupus erythematosus


