Plasma Osteopontin and Interleukin-18 Levels in Patients with Systemic Lupus Erythematosus: Association with Disease Activity and Lupus Nephritis

OMAR M. HERDAN, M.D.*; ESSAM A. ABDA, M.D.** and OMNIA A. MOHAMMED, M.D.***
The Departments of Internal Medicine*, Rheumatology & Rehabilitation** and Clinical Pathology***, Faculty of Medicine, Assiut University

Abstract

Dysregulation of cytokine production from activated T lymphocytes might play an important role in the pathogenesis and flare of SLE. Studies have reported that interleukins IL-18, IL10, IL-12, tumor necrosis factor (TNF)-alpha, osteopontin and interferon (IFN) gamma levels are elevated in patients with SLE, and that some of them correlated with disease activity.

Lupus nephritis (LN) occurs in more than one-third of patients with systemic lupus erythematosus. Its pathogenesis is mostly attributable to the glomerular deposition of immune complexes and overproduction of T helper-(Th-) 1 cytokines. Thus, the complex network of cytokines may contribute to the pathogenesis and activation of SLE, and an imbalance of Th1 and Th2 immune responses has been suggested to be one possible mechanism by which lupus nephritis develops and flares.

Study Design: A cross-sectional one.

Objective: The aim of our present study was to analyze the possible correlation between the plasma concentrations of OPN and IL-18 and disease activity in patients with systemic lupus erythematosus with or without renal disease. We also investigated the correlation between plasma IL-18 and OPN concentrations to further confirm the association of OPN with disease activity.

Subjects and Methods: It included 55 patients having SLE, divided into 2 groups, 30 RSLE group (28 women, 2 men, with mean age at diagnosis of 35.07±4.62yr.) and 25 SLE group (23 women, 2 men, with mean age at diagnosis of 35.84±4.74yr.), in addition to 30 age and sex matched healthy volunteers (28 women, 2 men, with mean age 36.74±4.79yr.). We measured the plasma concentration of OPN, and the plasma proinflammatory IL-18 concentration in 55 SLE patients with or without renal disease (RSLE group and SLE group, respectively) and in 30 sex-and age-matched controls using an enzyme linked immunosorbent assay. The disease activity was evaluated with the SLE disease activity index (SLEDAI).

Results: Plasma OPN and IL-18 concentrations were significantly higher in RSLE and SLE patients than in the controls (p=<0.000 and <0.001 respectively). Plasma OPN concentrations were significantly higher in RSLE patients than in the SLE patients (p<0.05). Plasma OPN concentration correlated positively and significantly with SLE disease activity index in RSLE patient groups (r=0.287; p=0.02). Plasma IL-18 level was positively and significantly correlated with SLEDAI score in combined SLE patient groups and in both groups with or without renal disease (r=1.55, p<0.05, r=0.287, p<0.05, r=0.146, p<0.05 respectively). In RSLE patients, plasma OPN concentration showed a significant positive correlation with proinflammatory cytokine IL-18 concentration (r=0.302; p=0.01).

Conclusion: This study confirmed that the circulating IL-18 and OPN concentrations were significantly elevated in SLE patients and correlated with the SLEDAI score in addition to their association with lupus nephritis. This suggests a crucial role for Th1 cytokines in the inflammatory processes and tissue damage in SLE disease. Both cytokines may act as potential marker for both lupus nephritis and monitoring SLE disease activity.

Key Words: SLE – Osteopontin – IL-18 – SLEDAI.

Introduction

SYSTEMIC lupus erythematosus (SLE) is a prototypic systemic autoimmune disease characterized by various immunological abnormalities, including dysregulated activation of both T and B-lymphocytes with the production of a large quantity of auto-reactive antibodies [1,2]. The aetiology and pathogenic mechanism of this immunological disorder have not been clearly elucidated. Aberrant production and imbalance of T-helper (Th) cell cytokines have been implicated in the pathogenesis of autoimmunity [3].

IL-18 is a novel proinflammatory Th-1 cytokine produced by various cell types including Kupffer cells, activated macrophages, keratinocytes, intestinal epithelial cells, osteoblasts and adrenal cortex cells [4]. IL-18 is a member of the IL-1 family...
and has a synergistic effect with IL-12 on the activation of natural killer cells and cytotoxic T lymphocytes [5].

Recent reports indicated that circulating concentrations of the proinflammatory Th1 cytokine IL-18 were elevated in SLE patients [6], and this elevation is correlated with disease activity [7] or clinical manifestations [8,9].

Previous findings demonstrated that the plasma concentration of proinflammatory cytokine interleukin (IL)-18 was significantly elevated in SLE patients compared with controls [10].

Plasma IL-12 concentrations in SLE patients were significantly higher than those of normal subjects [11]. The combination of IL-12 and IL-18 is very critical for the induction of the innate immune response and inflammatory reaction in SLE [4]. A positive correlation has been found between the plasma IL-18/IL-4 ratio and SLE disease activity index (SLEDAI), suggesting an imbalanced cytokine profile with Th1 predominance [6].

The ratio of intracellular interferon (IFN)-gamma/IL-4 staining of Th cells did not support a predominance of Th2 in SLE, in contrast, there was a significant Th1 predominance among SLE patients with WHO class IV lupus nephritis and diffuse proliferative glomerulonephritis [12].

IL-18 promotes proliferation and IFN-gamma production by Th1, CD8+, and NK cells in mice and in humans [13]. IL-18 is expressed in several human diseases including rheumatoid arthritis [14] and inflammatory bowel disease [15].

MRL/lpr mice treated with IL-18 or a combination of IL-18 and IL-12 developed accelerated proteinuria, glomerulonephritis and vasculitis compared with controls. These effects were accompanied by enhanced production of anti-dsDNA Abs and proinflammatory cytokines. Furthermore, IL-18-treated mice, but not mice treated with IL-12 plus IL-18 developed a “butterfly” facial rash with inflammation and increased apoptosis. These results suggest that IL-18 is an important mediator of lupus disease and therefore a potential target for therapeutic intervention in this and other related diseases [16].

Osteopontin (OPN, early T-lymphocyte activation protein 1, Eta-1) is a secreted phosphorylated glycoprotein expressed in mineralized tissues (bone and teeth) and damaged renal tissues [17]. During inflammation, OPN is expressed by cells of both innate and adaptive immunity, such as natural killer (NK) cells, activated T cells, macrophages and resident fibroblasts [18,19].

OPN is also called early T-lymphocyte activation protein1 (Eta-1) because of its early production upon cell activation, and has been shown to enhance Th1 but inhibit Th2 response [20].

The in vitro production of OPN is modulated by IL-2, transforming growth factor-β, epidermal growth factor and platelet-derived growth factor [18]. Among multiple receptors for OPN, CD44 is the most characterized receptor that appears to mediate the cell chemotaxis and attachment [21].

OPN plays various biological roles for host defense, bone formation, osteoclast activation and wound healing [20]. Its cytokine activities include the stimulation of macrophage and T-cell migration [20,22], protection against herpes viruses and bacterial infections through the activation of the Th1 response [20], and induction of Th1-cell-mediated autoimmunity [17].

Increased plasma concentration, protein expression and local production of OPN have been observed in many different diseases such as sepsis, multiple sclerosis, autoimmune/lymphoproliferative syndrome, rheumatoid arthritis and renal tissue of SLE patients [23,24].

However, the circulating level of OPN in SLE patients and its correlation with disease severity has not been well defined [9]. Therefore, the aim of our present study was to estimate plasma concentrations of OPN and IL-18 in 55 SLE patients, and to analyze the possible correlation between the plasma concentration of OPN and IL-18 and disease activity in SLE patients with or without renal disease. We also investigated the correlation between plasma IL-18 and OPN concentrations to further confirm the association of OPN with disease activity.

Material and Methods

55 patients (51 women, 4 men) with a diagnosis of SLE admitted in the Internal Medicine and Rheumatology and Rehabilitation Departments of Assiut University Hospitals were enrolled in this study over a period of 2 years from the first of August 2008 to the end of July 2010. All patients were considered eligible for inclusion in the analysis if they fulfilled the following criteria: (1) at least four of the revised American College of
Rheumatology (ACR) criteria for the diagnosis of SLE [25]; (2) age more than 16 years at the time of diagnosis of SLE. They were subdivided into 2 groups: 30 SLE patients experiencing renal disease (RSLE group, "lupus nephritis") which characterized by continuous proteinuria over 0.5g/24h [25] and 25 SLE patients without renal disease (SLE group). 30 age and sex matched healthy volunteers (28 women, 2 men) were recruited as controls (NC group). Patients and controls gave written informed consent before entering the study and the study protocol was approved by the local Ethics Committee of Faculty of Medicine, Assiut University Hospital. None of the control subjects had clinical evidence of any connective tissue or renal disease.

Renal SLEDAI score consisted of the 4 kidney-related parameters of the SLEDAI: Hematuria, pyuria, proteinuria, and urinary casts. Each item in the renal SLEDAI is assigned 4 points. Thus scores for renal SLEDAI can range from 0 (inactive renal disease) to a maximum of 16. Patients were classified into either the group of patients without lupus nephritis (LN) at the time of study (Renal SLEDAI score of 0) or the group of patients with LN that has a renal SLEDAI score of \( \geq 8 \) or when proteinuria was the renal-related criterion, a renal SLEDAI score of 4 [26].

Exclusion criteria:

Patients with diabetes mellitus, hypertension, chronic kidney disease, positive HCV antibodies, positive HbsAg, any malignant disease that can affect kidney (e.g. lymphoma), any chronic disease affecting kidney, urinary tract infection, and those who were pregnant or taking any medication that induces nephrosis were excluded.

Clinical evaluation and Laboratory investigations:

To all patients careful medical history was taken and thorough clinical examination was performed, stressing on disease activity which was carried out according to SLE disease activity index (SLEDAI) [27].

All patients underwent the following laboratory investigations: Complete blood picture done by Cell-Dyn 3700. Erythrocyte sedimentation rate. Serum glucose, blood urea, serum creatinine, and liver function tests measured by using auto analyzes Cobas integra 800. Creatinine clearance also was calculated. Protein estimation in 24 hour urine by using dye binding method (CBBG-250) [28]. Complete urine analysis by means of Accu-Tell reagent strips and microscopic examination of urine. Complements C3 and C4 done by "NL" BINDARIDTM radial immunodiffusion kit [29]. Anti-ds DNA done by Enzyme Immunoassay kit MK017 BiNDAZYMDTM Human [30]. Osteopontin determination in EDTA plasma by quantitative ELISA technique which is solid phase sandwich enzyme linked immunosorbert assay using the Human Osteopontin Assay kit-IBL. Code No. 27158 [31]. Interleukin-18 measured in EDTA plasma by using the Invitrogen Human interleukin-18 (Hu-IL-18) ELISA kit which is solid phase sandwich immunosorbent assay, catalog KHC0181 [32].

Statistical analysis:

The statistical analysis was performed with SPSS/Windows statistical software (version 17.0), Chicago. USA. Data were expressed as mean, standard deviation (SD), number, and percentage. Using Mann-Whitney to determine significance for numeric variable. Using Chi-square to determine significance for non-parametric variable. Using Person’s correlation for numeric variable in the same group. \( p >0.05 \) non-significant, \( p <0.05 \) significant, \( p <0.01 \) moderate significance and \( p <0.001 \) highly significant.

Results

Clinical, Laboratory and treatment data of the study groups were summarized in Table (1). The proportions of the RSLE group and SLE group with active disease were 83.3% and 48% respectively.

Table (2) and Figs. (1&2) showed that plasma osteopontin and interleukin-18 levels were significantly higher in RSLE and SLE patients than in controls. In addition Table (2) revealed that plasma OPN concentrations were significantly higher in RSLE patients than in the SLE patients. Meanwhile, there was no significant difference in IL-18 concentrations between RSLE and SLE patients.

Table (3) revealed that the plasma IL-18 concentration was significantly and positively correlated with that of osteopontin (OPN) in RSLE patients \( (r=0.302, p <0.05) \).

Table (4) showed that there was a significant positive correlation between OPN concentration and SLEDAI score in patients with renal disease (RSLE), \( (r=0.287, p <0.05) \). Plasma IL-18 level was found to be positively correlated with SLEDAI score in combined SLE patient groups and in both groups with or without renal disease \( (p <0.05) \).

Total SLEDAI score in renal patients (RSLE group) was high when compared with that of pa-
Patients without renal disease (SLE group), \((p<0.05)\) as shown in Table (5).

OPN and plasma IL-18 concentrations were significantly positively correlated with renal SLE-DAI score in RSLE patients \((r=0.276, r=0.249, p<0.05\) respectively) as shown in Table (6).

Significant positive correlation was found between OPN concentration and 24 hour urinary protein in RSLE patients \((r=0.458, p=0.018)\). Also there was a significant positive correlation between IL-18 concentration and 24 hour urinary protein in RSLE patients \((r=0.351, p=0.040)\).

![Fig. (1): Plasma concentration of IL-18 in RSLE and SLE patients and healthy control.](image1)

![Fig. (2): Plasma concentration of OPN in RSLE and SLE patients and healthy control.](image2)

Table (1): Characteristics of renal systemic lupus and systemic lupus patients and healthy control (NC).

<table>
<thead>
<tr>
<th>Variables</th>
<th>RSLE</th>
<th>SLE</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>30</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Age (Yr.)</td>
<td>36.74 ± 4.79</td>
<td>35.84 ± 4.74</td>
<td>36.74 ± 4.79</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>35.07 ± 4.62</td>
<td>35.84 ± 4.74</td>
<td>36.74 ± 4.79</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2 (6.67%)</td>
<td>2 (8%)</td>
<td>2 (6.67%)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (93.33%)</td>
<td>23 (92%)</td>
<td>28 (93.33%)</td>
</tr>
<tr>
<td>Disease duration (Yr.)</td>
<td>3.52 ± 2.70</td>
<td>3.37 ± 2.86</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>15.84 ± 6.93</td>
<td>9.33 ± 6.43</td>
<td>–</td>
</tr>
<tr>
<td>* SLEDAI score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>520.41 ± 231.72</td>
<td>731.63 ± 521.60</td>
<td>–</td>
</tr>
<tr>
<td>C3 (Complement 3) mg/dL</td>
<td>175.63 ± 95.42</td>
<td>194.90 ± 150.02</td>
<td>–</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>588.70 ± 176.35</td>
<td>538.70 ± 123.35</td>
<td>–</td>
</tr>
<tr>
<td>* Anti-ds DNA IU/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>1475.03 ± 334.23</td>
<td>94.26 ± 6.66</td>
<td>85.62 ± 15.30</td>
</tr>
<tr>
<td>¶ 24 hour protein in urine (Mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>27.96 ± 12.65</td>
<td>5.00 ± 0.00</td>
<td>–</td>
</tr>
<tr>
<td>Treatment with prednisolone:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>27</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>Daily dose (mg)</td>
<td>27.96 ± 12.65</td>
<td>5.00 ± 0.00</td>
<td>–</td>
</tr>
<tr>
<td>Treatment with hydroxychloroquine:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>8</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>Daily dose (mg)</td>
<td>200.00 ± 0.00</td>
<td>216.67 ± 57.76</td>
<td>–</td>
</tr>
<tr>
<td>¶ 40.450mg.</td>
<td>928.48 ± 547.87mg</td>
<td>928.48 ± 547.87mg</td>
<td>–</td>
</tr>
</tbody>
</table>

* Systemic lupus erythematosus disease activity index score.
* Anti-ds DNA, anti-body to native DNA.
¶ Proteinuria in all patients with systemic lupus erythematosus (Renal and non-renal) was 928.48 ± 547.87mg.
Table (2): Comparative analysis of plasma osteopontin and interleukin-18 concentration of RSLE, SLE patients and Controls.

<table>
<thead>
<tr>
<th></th>
<th>Plasma OPN (Ng/ml)</th>
<th>Plasma IL-18 (Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±S.D</td>
<td><em>p</em>-value</td>
</tr>
<tr>
<td>RSLE patients (n=30)</td>
<td>79.14±45.18</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Vs. Controls (n=30)</td>
<td>14.20±1.40</td>
<td></td>
</tr>
<tr>
<td>SLE patients (n=25)</td>
<td>70.14±79.00</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Vs. Controls (n=30)</td>
<td>14.20±1.40</td>
<td></td>
</tr>
<tr>
<td>RSLE patients (n=30)</td>
<td>79.14±45.18</td>
<td>0.04</td>
</tr>
<tr>
<td>SLE patients (n=25)</td>
<td>70.14±79.00</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.05

Cut off point level of OPN in all systemic lupus patients was 17Ng/ml.
Cut off point level of IL-18 in all systemic lupus patients was 160.75Pg./ml.
Cut off level was calculated according to the following formula: (S.D × 2+Mean) of the control, this was done according to the statistical point of view.

Table (3): Correlation of plasma levels of osteopontin and interleukin-18 in RSLE and SEL patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>r</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>.014</td>
<td>.955</td>
</tr>
<tr>
<td>RSLE</td>
<td>.302</td>
<td>0.01*</td>
</tr>
<tr>
<td>SLE+RSLE</td>
<td>.178</td>
<td>.227</td>
</tr>
</tbody>
</table>

Osteopontin:
- SLE: 0.235, 0.332
- RSLE: 0.287, 0.015*
- SLE+RSLE: 0.082, 0.582

IL-18:
- SLE: 0.155, 0.025*
- RSLE: 0.287, 0.015*
- SLE+RSLE: 0.146, 0.023*

*p<0.05 is significant.

Table (4): Correlations of plasma levels of osteopontin and interleukin-18 with SLEDAI score in RSLE and SEL patients.

<table>
<thead>
<tr>
<th>Patients with lupus nephritis (RSLE) (N = 30)</th>
<th>Patients without lupus nephritis (SLE) (N = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Patients</td>
</tr>
<tr>
<td>Total SLEDAI score</td>
<td>(Mean±SD)</td>
</tr>
<tr>
<td>Renal SLEDAI score</td>
<td>(Mean±SD)</td>
</tr>
<tr>
<td>Renal SLEDAI score</td>
<td>(Mean±SD)</td>
</tr>
</tbody>
</table>

Table (5): Comparative analysis of disease activity scores in the studied patients.

<table>
<thead>
<tr>
<th>Item</th>
<th>Patients with lupus nephritis (RSLE) (N = 30)</th>
<th>Patients without lupus nephritis (SLE) (N = 25)</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal SLEDAI score</td>
<td>0.276</td>
<td>.249</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03*</td>
<td>0.04*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 is significant.
Discussion

It has been suggested that SLE is a Th2-polarized disease because of its production of autoantibodies specific for self-antigens [33]. However, other studies have demonstrated that serum cytokines for Th1 response including IL-12 [11], TNF-alpha [34] and IFN-gamma [35] were also significantly higher in SLE patients.

Previous studies have reported different results for the correlation of the Th1/Th2 ratio with SLE disease activity. An in vitro study showed a positive and significant correlation of the ratio with SLEDAI using in vitro-stimulated peripheral blood mononuclear cells [3], while another reported a negative correlation of the ratio between IFN-gamma/IL-10-secreting cells and disease activity by enzyme-linked immunospot analysis of freshly isolated peripheral blood mononuclear cells [36].

Animal experiments using autoimmune mice found that the ratio of Th1/Th2 cytokine mRNA expression of IL-2 and IL-4, IFN-gamma and IL-10 in polymorph nuclear neutrophils and peripheral blood mononuclear cells exhibited a reciprocal relationship with disease severity [37]. However, several clinical studies have indicated that elevation in Th1 cytokines, including IL-12 [11], TNF-alpha [34], and IFN-gamma [35], can mediate the inflammatory processes that lead to irreversible organ damage, such as renal failure in SLE [38].

The proinflammatory cytokine TNF-alpha was found to remain elevated throughout the course of disease [39]; suggesting it has a significant role in the inflammatory process [38]. Previous studies have demonstrated that SLE patients exhibited significantly higher plasma concentrations of proinflammatory cytokine IL-12, IL-17 and IL-18, and Th2 cytokine IL-4 [6].

Using MRL/lpr mice with spontaneous lupus-like autoimmune disease, it was shown that daily injection of IL-18 or IL-18 plus IL-12 resulted in accelerated proteinuria, glomerulonephritis and raised levels of proinflammatory cytokines [16].

Therefore, IL-18 is suggested to be an important mediator of lupus-like disease including lupus nephritis. SLE patients with diffuse proliferative lupus nephritis also showed the predominance of Th1 immune response and the peripheral blood Th1 to Th2 ratio could be useful as a parameter that reflects the renal histological activity [23].

Our present results demonstrated that plasma IL-18 levels were higher in SLE patients compared with controls and the elevation correlated positively with SLEDAI in SLE patients with renal disease (Tables 2, 4 & Fig. 1), and this was in line with Wong et al. [6] who reported significantly elevated IL-18 concentration in SLE patients compared with control, also Wong et al. [40] stated that previous studies have also demonstrated that elevated production of inflammatory mediators such as IL-18, nitric oxide, soluble thrombomodulin and soluble vascular cell adhesion molecule-1 is associated with renal disease in SLE patients, and the elevation of IL-18 was correlated with disease activity in SLE patients with renal impairment.

Additionally, in this study the plasma IL-18 concentrations in RSLE and SLE patients did not show any correlations with the dosages of prednisolone, hydroxychloroquine, and azathioprine. In addition, Esfandiari et al. [16] stated that IL-18 accelerates spontaneous autoimmune lupus disease with characteristic glomerulonephritis and vasculitis as well as Chan et al. [41] reported that patients with active lupus nephritis have a predominant Th1 type of T-lymphocyte activation.

To further support IL-18 in lupus nephritis Fantuzz et al. [42] stated that IL-18 is overexpressed in LN and IL-12-induced IFN-gamma production is dependent of IL-18. Thus, IL-18 is crucial in priming Th1 differentiation and may be an important mediator in the pathogenesis of LN.

Faust et al. [43] mentioned that, in support of this hypothesis, enhanced renal expression of IL-18 has been found in nephritic lupus-prone MRL/lpr mice, whereas daily injections of IL-18 in these mice resulted in an increase of circulating proinflammatory cytokines, accelerated proteinuria and aggravation of nephritis. Moreover, higher serum levels of IL-18 have been detected in MRL/lpr mice as compared to wild type MRL mice [16].

Finally, the in vitro production of IL-18 by peripheral mononuclear cells is up-regulated in lupus patients with nephritis when compared to those without evidence of renal disease [40]. In an attempt to further elucidate the role of IL-18 for the development of LN, Calvani et al. [8] investigated its circulating levels in a large cohort of lupus patients, and found that the mean value of IL-18 in SLE patients was more than twice that of normal controls and that its levels were significantly increased in patients with LN compared to those without renal involvement. Importantly, serum IL-18 levels were not influenced by organ involvement other than renal. This suggests that the high levels
of this cytokine in sera of SLE patients are primarily attributable to the occurrence of LN.

In further support of this notion Calvania et al. [44] found a remarkable association between IL-18 overproduction and the degree of glomerular inflammation as demonstrated by its in situ expression in kidney biopsy specimens. These results further indicated that IL-18 played an inflammatory role in glomerulonephritis of SLE patients and altogether, they suggest that Th1 predominance and its cytokines (including IL-18) is important in SLE disease and lupus nephritis development.

OPN is a cytokine that has been newly implicated in autoimmunity and other inflammatory processes [17]. OPN is one type of functional proteins in the extracellular matrix, which is characterized by a series of biological functions including cell adhesion, cytokine expression, cell migration, signal transduction, regulation of immunologic activity, and inhibition of cellular apoptosis. As a member of Th1 cytokines, OPN participates in inflammatory and immunologic reaction [45-48].

In the immune system, OPN is expressed by many different cell types, including macrophages, neutrophils, dendritic cells, NK cells, and T and B lymphocytes; it is up-regulated in response to injury and inflammation in every organ examined; for example, cardiac tissue, kidney, lung, bone, brain, the gastrointestinal tract, joints, liver, adipose tissue [49] and most tumors [50].

OPN has been identified as a biomarker for various types of cancers and inflammatory diseases [51,52]. Excessive or dysregulated OPN expression has been linked to the pathogenesis of autoimmune disorders such as systemic lupus erythematosus [53].

In this study we investigated the circulating level of OPN and its correlation with plasma IL-18 concentration and SLE disease activity. The results of our present study indicated that the plasma concentration of cytokine OPN was significantly increased in SLE patients compared with controls, and correlated positively with SLEDAI (Tables 2,4 & Fig. 2). Similar finding was observed by Wong et al. [9].

In addition, our results revealed that the plasma OPN concentration showed a significant positive correlation with proinflammatory cytokine IL-18 concentration in RSLE patients (Table 3), and the plasma OPN and IL-18 concentrations in both RSLE and SLE patients did not show any correlation with the dosages of prednisolone, hydroxychloroquine and azathioprine.

Li et al. [54] also found that OPN and its mRNA expression are increased in peripheral blood mononuclear cells of SLE patients, while more obvious findings exist in lymphocyte too. Iizuka et al. [55] observed that over expression of OPN leads to enhanced B cell which causes increased anti-dsDNA antibodies. The significant correlation between OPN and IL-18 in RSLE patients (Table 3) is interesting, especially since the RSLE patients were more medicated than the SLE group.

There could be several possible reasons for this but the absence of such a correlation in the SLE group may suggest that cells in the kidney are either producing both cytokines or have an inductive relationship and this is in accord with Wüthrich et al. [56] who stated that osteopontin has been found in glomeruli of MRL/lpr mice, as well as Hofmann et al. [57] stated that in renal biopsies from patients with diffuse proliferative glomerulonephritis, we noted the co-localization of OPN with macrophage marker CD68.

In conjunction with the inflammatory activities of IL-18, such as the induction of Th1 cytokine IFN-gamma and activation of T cells, natural killer cells and cytotoxic T lymphocytes [4], OPN can enhance the Th1-mediated inflammatory process, activation of natural killer and T cells, and macrophage migration in the exacerbation of SLE.

Acting together with other proinflammatory cytokines, including IL-1 and TNF-alpha, OPN may be an important cytokine for initiating and perpetuating the Th1 immune response and renal derangement in SLE. The reported data showed that the change of OPN serum level is related to the activity of SLE, degree of renal damage and course of the disease itself [58]. In fact, OPN has been shown, at least partly, to account for SLE nephritis, probably through the predominance of the Th1-type response in both peripheral and renal tissues [48].

Conclusion:

This study confirmed the association between the production of OPN and IL-18 and SLE disease activity and lupus nephritis. This suggests a crucial role for the Th1 cytokines in the inflammatory processes and tissue damage in SLE disease. In addition excess of IL-18 and OPN within nephritic glomeruli provides considerable evidence for the potential pathogenic role of these cytokines in LN. In view of these results, OPN and IL-18 may re-
present or serve as potential or novel markers for lupus nephritis and monitoring of SLE disease activity and their measurements may be helpful in the assessment of patients.

References


