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RESEARCH ARTICLE

Predictive Role of Plasma Neutrophil Gelatinase-associated Lipocalin and IL-18 in Lupus nephritis

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Lupus nephritis is complication of systemic lupus erythematosus. Laboratory markers have limited specificity and sensitivity for predicting the development of lupus nephritis. Therefore, utilizing, accurate, and non-invasive biomarkers for distinction between flare of nephritis and chronic kidney damage seems to be necessary. Promising biomarkers have been identified, such as Neutrophil Gelatinase -associated Lipocalin and interleukin-18.

Objective: We have therefore undertaken to clarify the role of plasma Neutrophil Gelatinase -associated Lipocalin and interleukin-18 levels in detection of nephritis in patients with systemic lupus erythematosus.

Methods: This study enrolled 40 patients with systemic lupus erythematosus and 40 apparently healthy persons as control. The patients were subdivided into two subgroups each one included 20 patients. Subgroup 1: with nephritis.Subgroup 2: without nephritis. All groups were subjected to measurements of, anti-double stranded DNA antibody, 24 hours urinary protein,plasma Neutrophil Gelatinase -associated Lipocalin and interleukin-18.

Results: We found a highly significant rise in plasma concentrations of Neutrophil Gelatinase -associated Lipocalin ($P < 0.01$) and interleukin-18 ($P<0.01$) in patients with nephritis compared to those without and a highly significant increase of plasma Neutrophil Gelatinase -associated Lipocalin ($P<0.01$) and interleukin-18 ($P<0.01$) in all patients compared to control group. There were a positive correlation between Neutrophil Gelatinase -associated Lipocalin and interleukin-18 in group1 ($P < 0.01$, $P = 0.856$), and group 2 ($P < 0.01$, $P = 0.979$). **Conclusions:** we propose that in lupus nephritis the elevated plasma Neutrophil Gelatinase -associated Lipocalin and interleukin-18 may serve as markers of induction or maintaining of nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune and inflammatory disease [1]. its pathogenesis involve a multitude of cells and molecules that participate in apoptosis, innate and adaptive immune responses [2] which together cause a wide spectrum of major clinical manifestations that may affect any organ [3] including the joints, skin, brain, heart, lungs, blood vessels and kidneys [4].

Lupus nephritis (LN) is a common and serious complication in SLE patients, which can be seen in up to 60% of all SLE patients. Furthermore, 10–15% of nephritis patients' progress to end-stage renal disease (ESRD) requiring hemodialysis, and the 5-year survival of nephritis patients is stalled at 82%, whereas 5-year survival for those

without nephritis is 92% [5]. Proteinuria is the most frequently observed abnormality in LN. Although the precise etiology of LN is not entirely known, several factors have been proposed in the initiation and progression of LN. Two important factors that are suggested to be involved in that are apoptosis imbalance and overproduction of several cytokines like IL-18 [6].

Current laboratory markers for LN are unsatisfactory. They lack sensitivity and specificity for differentiating renal activity, damage in LN and the significant kidney damage can occur before renal function is impaired [7]. So that, the gold standard to confirm the diagnosis of LN is renal biopsy [8], and the serial renal biopsies may be ideal in close monitoring of its progression [9]. However, it is invasive and has risks of hemorrhage and infection [8]. Therefore; non-invasive biomarkers, sensitive to facilitate early detection of LN, specific for activity of LN, and able to guide initiation of therapies seems to be necessary [10]. Urinary and plasma NGAL, IL-18, cystatin C, and IL- 6 have been shown to be early detector biomarkers of acute kidney injury after cardiopulmonary bypass surgery and with contrast-induced kidney injury [11].

NGAL is a member of lipocalin family, widely expressed in tubular mesangial, neutrophil, and epithelial cells [12]. That functions as a carrier for cellular iron transport, apoptosis, and tissue differentiation [13]. In previous studies they found that, in systemic injection of pathogenic anti-DNA antibodies promoted significant NGAL overexpression by kidney cells and tissue [12] while administration of exogenous NGAL to wild-type mice exacerbated the histological injury and worsened survival, thus conclusively establishing a central role for NGAL in the pathogenesis of antibody-induced nephritis [12].

IL-18 is a T helper-1 related cytokine, produced by antigen presenting cells [14] and causing severe inflammatory disorders in autoimmune diseases [15] by stimulating the production of inflammatory cytokines such as IFN-γ, TNF-α and IL-1β [14]. Accumulating evidence indicates that serum IL-18 levels are significantly elevated in SLE [16]. Additionally, other studies have reported enhanced IL-18 renal expression in murine models of LN [14]. Therefore, the aim of the work is to clarify the role of both NGAL and IL-18 levels in detection of renal affection in patients with SLE.

1. PATIENTS AND METHODS

Total of (40) patients with SLEwere carried on this study; all of them fulfilled at least 4 of the 11(ACR) criteria for the diagnosis of SLE. Their age ranged between (17-42) years with mean ±SD (27.93 ±7.49) years. Which presenting to the internal medicine department of Al Zahraa hospital between January 2014 and October 2014. Patients with hepatic disease, cardiac disease or diabetes mellitus were excluded. Other 40 apparently healthy persons of matching age who served as healthy control group of this study for baseline normal values assessment, their ages ranged between (19-40) years with mean ±SD of (25.73 ±4.33). All patients and healthy control before inclusion in the study signed consent.Also approval of the ethical committee of faculty of medicine, AL-Azhar University was obtained before the start of the study.

All subjects participating in the study were classified into the following:

Group (1): include 20 SLE patients with lupus nephritis. Their age ranged between (17-40) years with mean ±SD of (28.40±7.47).

Group (2): include 20 SLE patients without lupus nephritis. Their age ranged between (19-42) years with mean ±SD of (27.45±7.67).

Group (3): include 40 apparently healthy persons as control group, their ages ranged between (19-40) years with mean ±SD of (25.73 ±4.33).

All studied participants were subjected to the following:

1- Comprehensive adult history was taken and comprehensive physical examination was done.

2- Laboratory investigations:-

a) Complete blood picture.
b) ESR.
c) Renal function tests (serum creatinine, blood urea nitrogen).
d) Complement (C 3) & (C4).
e) Anti-double stranded DNA antibody.
f) Urine analysis & urinary protein to creatinine ratio.
g) 24 hours urinary protein.
h) Renal biopsy.
i) Plasma NGAL.
j) Plasma IL-18.

Five ml of venous blood samples were collected from each subject participating in the study and divided into parts: The first one (1.6 ml of blood) was on 0.4 ml citrate for ESR determination using Westergren's method.The second one was plain tube (1.4 ml of blood) was left to clot then centrifuged at 1000 xg for 15 minutes and the separated serum was stored at -20ºC for determination of serum creatinine, anti-ds DNA, anti-ANA, C3 and C4. The third one (2.0 ml of blood) was on EDTA tube for determination of plasma NGAL and IL-18. Keep freshly collected blood on ice. Within 20 minutes after blood sampling, separate plasma by centrifugation: 1500 xg at 4°C for 15 min. Remove plasma and transfer to fresh polypropylene tube. Be careful to not disturb white cells in the buffy coat. Recentrifuge the transferred plasma in order to avoid contamination with white blood cells: 1500 xg at 4°C for 15 min. Samples should be assayed immediately after collection or can be stored at -20°C.

Serum creatinine was performed o automated auto analyzer Hitachi 912(Roche-Hitachi, Japan) using colorimetric techniques.

Anti-ds DNA and anti-ANA were determined using indirect immuno fluorescence assay on mouth kidney and stomach slides and crithidialuciliae slides (ImmcoDiagnosticsInc, 640 Ellicott street, New York, USA) The slides were analyzed with Nikon epi fluorescence microscope (Nikon Inc., USA). Complement (C_3) 3 and C4 was determined by single radial immune diffusion plates (Diffuplates, Biocientifica, New Delhi, India).

Complete urine analysis was done to detect the presence of active urinary sediment; (protinuria, pyuria, RBCs or RBCs casts, granular cast), 24 hours of urine collection was used for calculating 24 hour urinary protein by ponceau S-TCA method[17].
Urine and plasma samples were taken for 24 hour creatinine clearance using US/P formula. Where U is the concentration of creatinine in urine in mg/dl, S is the concentration of creatinine in plasma in mg/dl and V is the volume of urine produced per minute [18]. Urinary creatinine was determined on Hitachi 912(Roche-Hitachi, Japan) using colorimetric techniques.

Plasma NGAL was determined using sandwich ELISA technique [19] and the kit was supplied from Bio Vander Gmb (ImNeuenheimer Feld 583, D-69120,Heidelberg, Germany) and plasma IL-18 was determined also using sandwich ELISA technique [20] and the kit was supplied from MBL (MBL International15A Constitution Way, Woburn, USA).

2. STATISTICAL ANALYSIS:

The data were collected, revised, coded and entered to the statistical package for social science (SPSS) version 17 using Chi-square test and/or Fisher, independent t-test, Mann-Whitney test. Pearson and Spearman correlation coefficient.

3. RESULTS.

4.1 BASELINE PATIENT CHARACTERISTICS:

Table 1 summarizes the characteristics of the 40 SLE patients included in the study. Their mean ± SD of age was (27.93 ±7.49) years and all were females (100%). Among them, 26 patients (75%) had dermatological manifestations. 21 patients (52.5%) had arthralgia, 19 patients (47.5%) had serositis. three patients (7.5%) had vasculities. Two patients (5%) had fever. 20 patients (50%) had LN, four patients (10%) had hematological manifestation and all patients had ds-DNA test positive. In addition, we demonstrated that the levels of plasma NGAL, plasma IL -18, Anti-ds DNA and ESR in blood samples from all patients and control groups were detectable. Among them, only plasma NGAL, plasma IL-18 and ESR levels in all patients were significantly higher (146.71±74.47), (313.29±105.30) and (76.40 ±39.51) respectively, than those in healthy group (62.60 ±17.55), (87.87±17.67) and (14.15±4.94) respectively, all had the same P value ($P < 0.01$).

4.2 COMPARATIVE STUDY:

The results of the current study had revealed that, there were highly significant elevation of the plasma NGAL level and plasma IL-18 in patients with LN (213.27± 43.19), (382.83 ± 93.90) respectively, when compared to the patients without LN (80.14±13.78), (243.76 ±61.35) respectively, all had same P <0 .01). There was a significant decrease in creatinine clearance in group1 (59.63±16.99) than group 2 (73.52±23.80; $P < 0.05$) but there was a highly significant increase in urinary 24 hour protein in group1 (2.14±1.60) than group 2 (0.24 ± 0.14; $P < 0.01$). In

addition, there was a significant increase in ESR in-group 1 (92.00 ± 39.89) when compared to group 2 (60.80±33.18), p <0.05 (see table 2).

There were highly significant increases in plasma NGAL, plasma IL-18 and ESR levels in patients who had LN (213.27±43.19), (382.83±93.90) and (92.00±39.89) respectively, when compared to a healthy group (62.60 ±17.55), (87.87±17.67) and (14.15 ± 4.94), respectively, all had the same P < 0.01 (see table 2).

As showed in table 4, there was a highly significant increase in plasma NGAL, IL-18 and ESR (213.27±43.19), (243.76 ±61.35) and (60.80±33.18) respectively, when compared to control group (62.60 ±17.55), (87.87±17.67) and (14.15± 4.94) respectively, with P<0.01 for all of them.

4.3ASSOCIATION OF PLASMA LEVELS OF NGAL AND IL-18 IN BOTH GROUP 1 AND 2 PATIENTS

Furthermore, correlation analysis was used to investigate the relationships of plasma NGAL and plasma IL-18 in-group 1 and in-group 2. As presented in table 5, there was a strong significant positive correlation (r = 0.82, P<0.01) between plasma NGAL and plasma IL-18 levels in group 1 patients (figure 3). In addition, there was a strong positive correlation between NGAL and SIEDAI score (r= 0.753, P <0.01) in the same group (figure 5). However, There was a significant positive correlation between plasma NGAL and 24 hours urinary proteins in-group 1 patients (r=-0.449; P <0.05) (figure 4).

However, we found a strong significant positive correlation between plasma levels of IL-18 and 24 hours urinary protein in patients with LN (r=0.655, P< 0.01) (table 6). There was also a strong positive correlation between these two parameters in patients without LN (r =656, P <0.01) (table 8). Furthermore, there was a strong significant positive correlation between IL-18 levels and SLEDAI score in patients with LN (r = 0.856, P< 0.01) (table 6) but no significant correlation between the same parameter in patients without LN (r=-0.205,P>0 .05) (table 8).

As shown in table 7, there was a highly significant positive correlation between plasma NGAL and plasma IL-18 in SLE patients without nephritis as a group, (r= 0.979, P<0.01).While there was no correlation between plasma NGAL and SLEDAI (r= -0.187, P<0.01) in the same group. On the other hand, there was a strong significant correlation between plasma NGAL and 24 hr urinary protein in SLE patients without nephritis (r= 0.703, P<0.01).There was no significant correlation between plasma NGAL and SLIEDAI (r = -0.187,P>0 .05).

Table 1.Demographic and clinical characteristics data of all SLE patients and healthy controls.

Characteristics	SLE patients (n = 40)	Healthy controls (n = 40)	P	Significant
Age (years)	(27.93 ±7.49)	(25.73 ± 4.33)	>0.05	NS
Sex (female/male) n.	40 females (100%)	40 females (100%)	-	-
Arthralgia / n (%)	21 (52.5%)	-	-	-
Renal diseases /n (%)	20 (50%)	-	-	-
Serosities n/%	19 (47.5%)	-	-	-
Neurological disorder n. (%)	2 (5%)	-	-	-
Dermatological n /%5656	26 (75%)	-	-	-
Hematological disorder n/%	4 (10%)	-	-	-
Vasculities n/%	3(7.5%)	-	-	-
Fever n (%)	2 (5%)	-	-	-
Anti-ds-DNA antibody/ n (%)	All positive	-	-	-
ESR (mm/h)	(76.40 ±39.51)	(14.15±4.94)	<0.01	HS
Plasma IL-18 (pg/ml)	(313.29±105.30)	(87.87±17.67)	<0.01	HS
Plasma NGAL (ng/ml)	(146.71±74.47)	(62.60 ±17.55)	<0.01	HS

Values are expressed as mean ± standard deviation. Anti-ds-DNA antibody, anti-double stranded DNA antibody; ESR, erythrocyte sedimentation rate; SLE, systemic lupus erythematosus; NGAL: Neutrophil Gelatinase -associated Lipocalin

Table 2. Comparison between group 1 (SLE with LN) and group 2 (SLE without LN)

Parameter	Group 1/ n (20)	Group 2 n(20)	p value	significance
ESR (mm/h)	(92.00±39.89)	(60.80±33.18)	<0.05	S

Creatinine clearance (ml/minute)	(59.63±16.99)	(73.52±23.80)	<0.05	S
proteins/24h/mg (mg/day)	(2.14±1.60)	(0.24±0.14)	<0.01	HS
Plasma IL-18(pg/ml)	(382.83±93.90)	(243.76 ±61.35)	<0.01	HS
Plasma NGAL(ng/ml)	(213.27±43.19)	(80.14±13.78)	<0.01	HS

NGAL: Neutrophil Gelatinase -associated Lipocalin

Table 3.Comparison between group 1 (SLE with LN) and healthy group 3

parameter	group1	Group 3	p value	significance
ESR (mm/h)	(92.00±39.89)	(14.15±4.94)	<0.01	HS
Plasma IL-18(pg/ml)	(382.83±93.90)	(87.87±17.67)	<0.01	HS
Plasma NGAL(ng/ml)	(213.27±43.19)	(62.60 ±17.55)	<0.01	HS

NGAL: Neutrophil Gelatinase -associated Lipocalin

Table 4.Comparison between group 2 (SLE with LN) and healthy group (3)

parameter	Group 2	Group 3	p value	significance
ESR	(60.80±33.18)	(14.15±4.94)	<0.01	HS
Plasma IL-18(pg/ml)	(243.76 ±61.35)	(87.87±17.67)	<0.01	HS
Plasma NGAL(ng/ml)	(213.27±43.19)	(62.60 ±17.55)	<0.01	HS

NGAL: Neutrophil Gelatinase -associated Lipocalin

Table 5.Correlation between plasma NGAL and other parameters in-group 1

Group 1	r	p	significance
proteins/24h/mg	0.449	<0.05	S
Plasma IL-18(pg/ml)	0.82	<0.01	HS
SLEDAI	0.753	<0.01	HS

SLEDAI:SLE disease activity index.

Table 6. Correlation between plasma IL-18 and all laboratory parameters in group1

Group 1	r	p	significance
proteins/24h/mg	0.655	<0.01	HS
SLEDAI	0.856	<0.01	HS

SLEDAI: SLE disease activity index.

Table 7.Correlation between plasma NGAL and other parameters in-group 2

Group 2	r	p	significance
Proteins/24h/gm.	0.703	<0.01	HS

Plasma IL-18(pg/ml)	0.979	<0.01	HS
SLEDAI	-0.187	>0.05	NS

SLEDAI:SLE disease activity index.

Table 8.Correlation between plasma IL-18 and other parameters in-group 2

Group 2	r	P	significance
Proteins / 24 h/mg	0.656	<0.01	HS
SLEDAI score	-0.205	>0.05	NS

SLEDAI:SLE disease activity index.

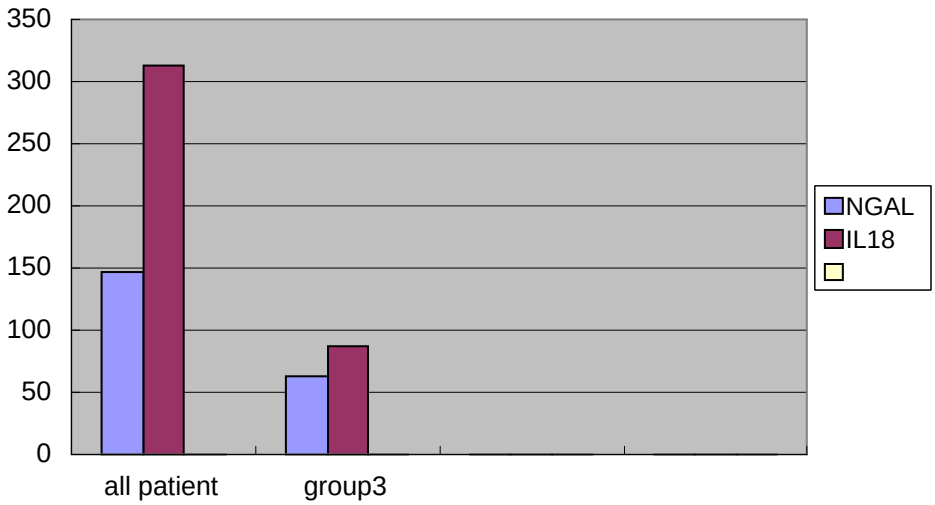

Figure 1.Comparison of plasma NGAL and plasma IL18 between all patients and control group.

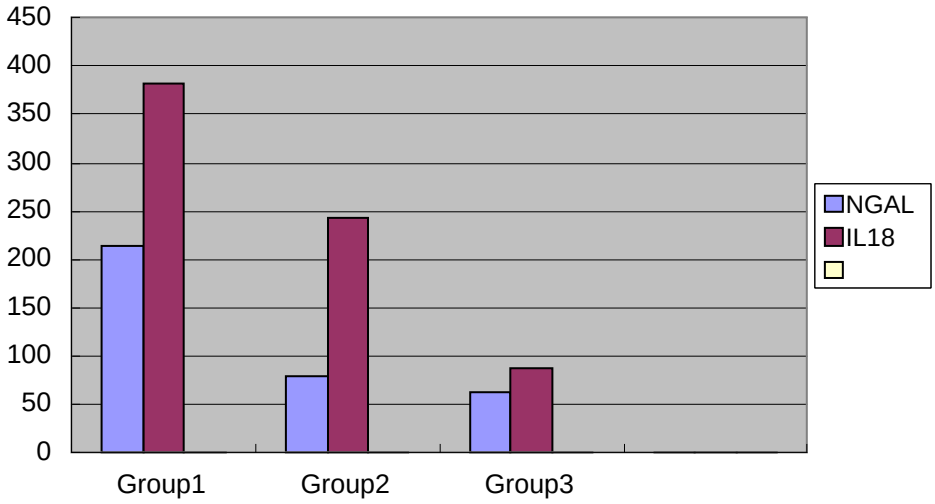

Figure 2.Comparison of plasma NGAL and plasma IL18 between groups 1, group 2, and group 3.

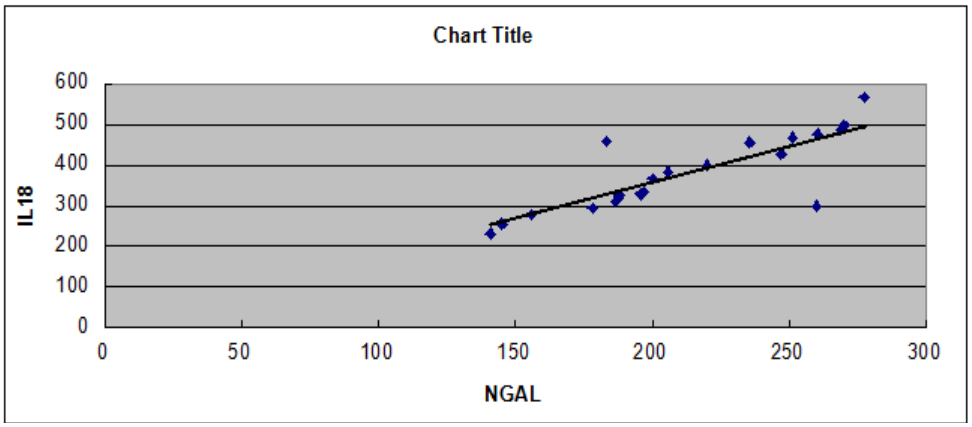

Figure 3.Correlation between plasma NGAL and IL18 in-group 1

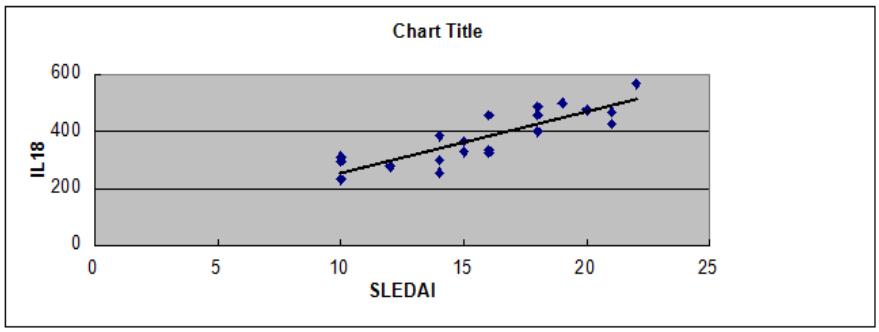

Figure 4.Correlation between IL18 and SLEDAI score in group 1

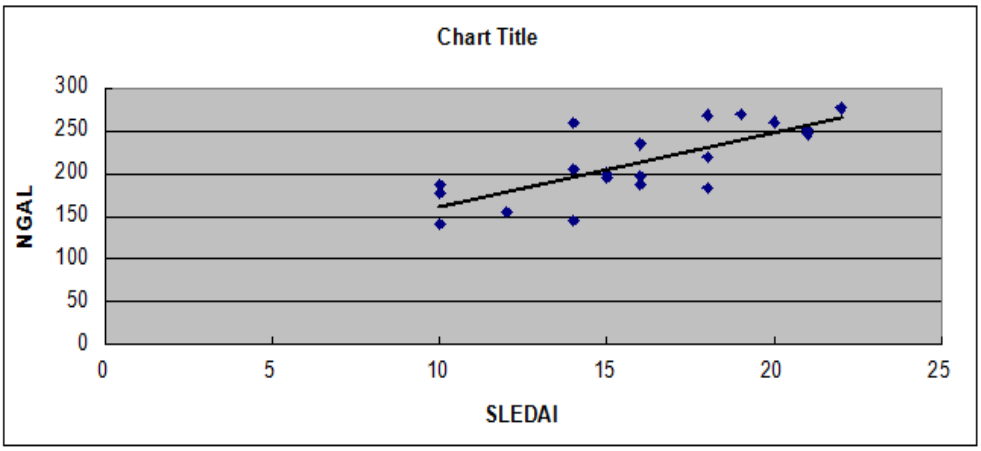

Figure 5.Correlation between plasma NGAL and SLEDAI score in group1

4. DISCUSSION

SLE is a chronic autoimmune inflammatory disease with a variable clinical course and characterized by periods of remissions and relapses. The renal involvement is one of the main determinants of poor prognosis of it. The pathogenesis of LN is a complex process and the medical therapy for LN depends on the severity of the disease. Thus, finding reliable biomarkers for LN will help to evaluate disease activity, identify patients at risk for kidney damage and facilitate early diagnosis and intervention to improve favorable outcomes [21].

In our study, we found that, the plasma NGAL level was increased in all SLE patients when compared to control group and statistically was highly significant ($P < 0.01$) as it is one of substances expressed in immature neutrophil precursor and epithelial cell during inflammation. Furthermore, plasma NGAL showed highly significant increase in lupus nephritis patients in comparison to SLE without nephritis ($P < 0.01$). Our result was supported by Suzuki and his colleague, who studied 85 pediatric SLE patients and reported that both plasma and urine NGAL level were significantly higher in patients with SLE than healthy control [22]. Hinze et al 2009 found that plasma NGAL increased significantly by 26% as early as 3 months prior to worsening of lupus nephritis. Also Pitashny et al 2007 studied 70 adult SLE patients and demonstrated that urinary NGAL levels were significantly higher in lupus nephritis than in non-renal SLE.

This suggested a promising role of plasma NGAL as a biomarker in lupus nephritis. Several mechanisms may be postulated, kidney injury result in dramatically increase in NGAL messenger RNA expression in distant organs, especially the liver and lung and over expressed NGAL protein that may constitute a distinct systemic pool, additional contribution to systemic pool may be derived from NGAL released from neutrophils and macrophages. Furthermore, any decrease in glomerular filtration resulting from kidney injury would be expected to decrease the renal clearance of NGAL with subsequent accumulation in the systemic circulation [23]

In our study, we found that there was a significant positive correlation between plasma NGAL and SLEDAI in LN patients and no significant correlation in patients without LN. This means thatdisease activity in SLE patients affects plasmaNGAL level. Our results were in agreement withSuzuki et al 2009, who studied pediatric SLE and foundthat children with SLE had higher plasma NGALduring SLE activity.

Our study also revealed a significant positive correlation between plasma NGAL, and 24 h urinary protein in all SLE patients and in LN group. Consistent with our results, the studies done by both El-Gamal et al., 2011[24] and Bolignano et al., 2008[25]. There results verified, that measurements of the plasma NGAL levels could reflect the activity of LN supporting the other laboratory tests for assessment of renal function. Also suggested that NGAL might be expressed by the damaged tubule to induce re-epithelialization [26], further support for this notion was derived from the identification of NGAL as a regulator of epithelial morphogenesis in cultured kidney tubules [27]. On the other hand, a study done by Rubinstein et al 2010 found no correlation between urinary NGAL and proteinuria.

In the current study, our findings showed that the plasma IL-18 levels were significantly elevated in patients with SLE as a group and as active and inactive subgroups in comparison to the control group ($P < 0.01$ in all). Moreover, there was a significant difference in plasma IL-18 level between patients of active and inactive disease. These findings were in agreement with Omar and his colleagues' 2011 study where they found that the circulating concentrations of the IL-18 were elevated in SLE patients**,** and this elevation was correlated with disease activity and over expressed in LN. Thus, IL-18 is crucial in priming Th 1 differentiation and may be an important mediator in the pathogenesis of LN [28]. Furthermore, the present results approved previous studies showing that plasma level of IL18 was significantly higher in patients with lupus nephritis than those without nephritis which in agreement with[29][30][31]. This suggests the possible role of IL18 in the pathogenesis of SLE, in particular lupus nephritis.

We found that the level of plasma IL-18 was significantly correlated with SLEDAI in patients with LN but not in lupus without nephritis. This further indicates that IL-18 plays an inflammatory role in glomerulonephritis of SLE patients. This result is in concordance with that of Wong and his colleague [31].

Plasma IL18 of patients with LN showed a significant correlation with and 24 h urinary protein these results verified that measurement of the plasma level of IL18 could reflect the activity of LN supporting the other laboratory tests for assessment of renal function. Consistent with our results a significant correlation was demonstrated between plasma levels of IL-18 and 24h urinary protein in lupus nephritis and all SLE patients [32]. On the other hand, another study revealed that there was no significant correlation between IL18 levels with and 24 h urinary protein in SLE patients[33].

5. CONCLUTION

We conclude that plasma NGAL and plasma IL-18 can be used as biomarkers for SLE and LN.

6. RECOMMENDATION

We recommend adding these two parameters to routine investigation of SLE and particularly LN will help in assessing disease activity, flare and follow up of LN to prevent delay in appropriate treatment. Further study is needed on large number of patients to verify the relation between these parameters and renal biopsy in order to be used as non-invasive tests.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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