ORIGINAL ARTICLE

Urinary and serum neutrophil gelatinase-associated lipocalin as a biomarker in Egyptian systemic lupus erythematosus patients: Relation to lupus nephritis and disease activity

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Abstract  Background: Neutrophil gelatinase-associated lipocalin (NGAL) is an excellent structural biomarker for the early diagnosis of acute kidney injury, prognosis, dialysis requirement and mortality in several common clinical scenarios.

Aim of the work: The aim of this work is to detect the levels of both urinary and serum NGAL in SLE patients with and without lupus nephritis (LN) and to correlate their levels with renal biopsy class and disease activity.

Patients and methods: The study included 35 SLE patients; 22 with LN and 13 without as well as 30 matched controls. The SLE Disease Activity Index (SLEDAI) was assessed and the renal biopsy class determined. Urinary and serum levels of NGAL were assessed by ELISA.

Results: The 35 patients had a median age of 30 years and disease duration of 4 years. They were 31 females and 4 males. The SLE patients had an elevated urinary NGAL (UNGAL) (median 19 ng/ml, IQR 8–87) as compared to controls (median 2 ng/ml, IQR 1–18.3) ($p < 0.006$). Levels of UNGAL were higher in patients with LN than those without ($p < 0.023$). In patients with LN, serum levels of NGAL were not significantly different from controls ($p = 0.6$). The UNGAL level significantly correlated with the renal score of SLEDAI ($r = 0.54$, $p = 0.001$) but serum NGAL level did not ($r = 0.25$, $p = 0.15$). UNGAL significantly correlated with grade III and IV.
1. Introduction

SLE is a chronic inflammatory disease of unknown etiology which can affect the skin, joints, kidneys, lungs, nervous system, serous membranes and/or other organs of the body. Main features of the disease are immunologic abnormalities; the disease is characterized by the production of auto-antibodies. The course of SLE is variable and may be associated by periods of remissions and relapses [1] and could remarkably affect the quality of life in those patients [2]. Other factors have been implicated in the pathogenesis of SLE including cytokine imbalance [3] and gene polymorphisms [4].

One of the most severe manifestations of SLE is lupus nephritis (LN), which remains a serious cause of morbidity and mortality, either secondary to kidney disease or to immunosuppressive drug toxicity [5]. In studies on Egyptian SLE patients, LN was frequently reported and assessed in relation to many biomarkers for apoptosis [6], adipocytokines [7], cartilage degradation [8], oxidative stress [9] and nephritogenic autoantibodies [10]. Novel markers of renal involvement including urinary neutrophil gelatinase-associated lipocalin (UNGAL) have also been assessed in SLE patients [11].

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25 kDa protein of the lipocalin superfamily. This protein is secreted by immune cells, hepatocytes and renal tubular cells in several pathological conditions. NGAL has recently generated great interest as an early marker of renal injury. However, like many other endogenous biomarkers it is produced by several cell types and it exists in several molecular forms. Different pathological conditions may be involved in the production of this molecule [12]. A prominent role of UNGAL was suggested as a potential biomarker of lupus nephritis that could serially forecast renal disease activity in SLE patients [13].

The aim of the present study was to assess the serum and urinary NGAL in Egyptian SLE patients with and without renal involvement and study their relation to other biochemical parameters, renal biopsy class and disease activity. Detecting its sensitivity, specificity and predictive values for LN in SLE patients was considered.

2. Patients and methods

The present study was carried out on 35 SLE patients; 31 females and 4 males with an age interquartile range (IQR) of 24–37 years (median 30 years). All patients fulfilled the systemic lupus international collaborating clinics (SLICC) classification criteria for SLE [14]. They were selected from the outpatient clinics of the Rheumatology and Rehabilitation and Internal Medicine Departments as well as the Nephrology Department of the Urology Center (Mansoura University Hospitals). Patients were grouped according to the presence or absence of active renal disease or a renal SLE Disease Activity Index (SLEDAI) score of ≥4 and considered with or without LN respectively. Thirty healthy subjects were included as a control group. This study was approved by the Ethics Committee of Faculty of Medicine Mansoura University. Written informed consent was obtained from all participants.

Exclusion criteria: Patients suffering of any of the following were excluded: Breast tumors, inflammatory bowel diseases, polycystic kidneys, acute kidney injury (AKI), post-renal transplantation, chronic heart diseases as there is an increase in the level of NGAL in these conditions.

All patients were subjected to full history taking and clinical examination. Disease activity in the SLE patient was detected by the SLEDAI [15]. Renal activity was assessed using renal SLEDAI which includes four renal elements in total SLEDAI score namely proteinuria, hematuria, pyuria and urinary casts. Each item in the renal SLEDAI is scored 4 points; the renal SLEDAI score ranged from 0 to 16. Renal biopsy was performed to assess the grade of LN for patients with persistent hypertension, rising creatinine levels, persistent hematuria, proteinuria, casts. The world health organization (WHO) classification of LN [16] was used to define the histopathological lesions.

Assessment of serum and urinary NGAL by ELISA: Urine and serum samples were concomitantly taken from all patients immediately after diagnosis. Serum NGAL was measured using a serum separator tube (SST) and samples were allowed to clot for 30 min before centrifugation for 15 min at 1000g. Serum was removed and immediately assayed or aliquot and sample stored at ≤−20 °C. Repeated freeze-thaw cycles were avoided. For urinary NGAL aseptically collected morning urine sample (mid-stream) was collected voided directly into a sterile container. It was then centrifuged to remove particulate matter, immediately assayed or aliquot and stored at ≤−20 °C. Repeated freeze-thaw cycles were avoided. Human NGAL was measured using the quantitative sandwich enzyme immunoassay technique using Human Lipocalin-2/NGAL Immunoassay [Quantikine, R&D system, Minneapolis, USA]. A monoclonal antibody specific for Lipocalin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Lipocalin-2 present in the samples bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Lipocalin-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Lipocalin-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical analysis: The clinical and laboratory data were tabulated, coded then analyzed using the computer program ‘statistical package for social science’ (SPSS) version 17.0.
Descriptive statistics were calculated for the anthropometric measurements and laboratory data in the form of: Mean, Standard deviation (±SD) and interquartile range (IQR). The significance of difference was assessed using Mann–Whitney U-test for continuous non-parametric data. To assess correlation between some investigated parameters, Spearman rho test was used. To detect the sensitivity, specificity, positive predictive value, negative predictive value of UNGAL excretion a conventional receiver operating characteristic (ROC) curve was used.

### 3. Results

Thirty-five SLE patients; 31 females and 4 males (F:M 7.8:1) with an age IQR of 24–37 years (median 30 years) and disease duration of 1–6 years (median 4 years). Clinical manifestations and laboratory data of the SLE patients with and without LN are presented in Tables 1 and 2. The median age of patients with LN (n = 22) was 27 years (IQR 23–27 years) and those without (n = 13) was 32 years (IQR 29.5–40.5 years). In patients with LN they were 20 females and 2 males (F:M 7.8:1).
10:1) while in patients without they were 11 females and 2 males (F:M 5.5:1). In patients with LN the median disease duration was 3.5 years (IQR 1.75–6 years) and in those without LN was 5 years (IQR 0.75–7 years). Renal biopsy was done to 14/22 (63.6%) of patients with LN; 3 (21.4%) had class II LN, 6 (42.9%) had class III and 5 patients (35.7%) had class IV LN.

A comparison of the urinary and serum NGAL between SLE patients and control is presented in Figs. 1 and 2 respectively. The urinary NGAL level in SLE patients was significantly increased (median 19 ng/ml, IQR 8–87) compared to that of the controls (median 2 ng/ml, IQR 1–18.3) ($p = 0.006$). There was no significant difference between serum NGAL levels in SLE patients (median 25 ng/ml, IQR 5–102) and control (median 20 ng/ml, IQR 14.4–35.6) ($p = 0.6$).

The UNGAL level significantly correlated with the total SLEDAI ($r = 0.54$, $p = 0.001$). A stronger correlation was found between UNGAL and the renal SLEDAI ($r = 0.63$, $p = 0.0001$). In LN patients, no association was found between UNGAL and the total SLEDAI ($r = 0.32$, $p = 0.14$) but a very strong correlation was found between UNGAL and renal SLEDAI ($r = 0.9$, $p = 0.0001$). In patients without LN, no significant correlation was found with the total or renal SLEDAI. The serum NGAL did not show any significant correlation with the total SLEDAI ($r = 0.25$, $p = 0.15$) or renal SLEDAI ($r = 0.4$, $p = 0.3$). In patients with LN, there was no significant correlation between serum NGAL and total SLEDAI ($r = 0.16$, $p = 0.47$) but a significant correlation was found with the renal SLEDAI ($r = 0.48$, $p = 0.04$). In patients without LN there was no significant correlation between serum NGAL and total or renal SLEDAI.

Correlations between urinary NGAL with laboratory parameters in the SLE patients with and without LN are presented in Table 3. Correlation between urinary and serum NGAL with renal biopsy showed that UNGAL significantly correlated with the grade of renal biopsy ($r = 0.67$, $p = 0.009$). No significant correlation was found between serum NGAL and renal biopsy grades in patients with LN ($r = 0.09$, $p = 0.77$). A significant correlation was found between urinary and serum NGAL in SLE patients ($p = 0.001$) and in those with ($p = 0.03$) and those without (0.024) LN.

The cut-off value, sensitivity and specificity of UNGAL in SLE patients are presented in Fig. 3. At a cutoff value of 18 ng/ml, the sensitivity of UNGAL levels for the diagnosis of LN was 85.7%, with a specificity of 80%. The area under the ROC curve (AUC) was 0.93.

4. Discussion

Despite the extensive work done to reveal the underlying mechanisms responsible for the pathogenesis of SLE, few biomarkers have been remarkably involved. The lack of reliable, specific biomarkers not only delays clinical management of SLE but also halts development of new therapeutic agent [17]. LN is considered a real challenge in the management of SLE patients because of the difficulty in diagnosing its onset and detecting relapses before serious renal damage has occurred. NGAL/Lipocalin-2 has been identified to have a role in the pathogenesis of several disease states in different organ systems, especially in kidney diseases. Lipocalin-2 may have a protective role in the context of renal insults through the induction or prevention of apoptosis by an iron-transport dependent mechanism [18].

In the present study it has been shown that UNGAL is an excellent marker of LN activity. The SLE patients have a significantly higher level of UNGAL compared to the controls.
Patients with active LN have significantly higher UNGAL levels when compared to patients without LN or to control. However, no significant difference was found between patients without LN and controls. These findings are similar to those of other studies [19–22] reporting that UNGAL, but not serum NGAL, is considered a high quality biomarker for SLE renal disease. In contrast to our results a wonderful role of plasma NGAL as a biomarker in LN has been suggested explaining that kidney injury results in an increase in NGAL expression in distant organs especially the liver and lung and the over expressed NGAL protein may be a distinct systemic pool. Also any kidney injury will affect glomerular filtration rate and decrease renal clearance of NGAL leading to its further accumulation in systemic circulation [23]. The present results are in accordance to those of Pitashny et al. [19] who found a weak correlation of UNGAL with total SLEDAI in SLE patients and a significant one with renal SLEDAI in SLE patients especially those with LN. In contrast to our results [24] no correlation was found between UNGAL and SLEDAI score in LN patients suggesting that its excretion is related to renal damage rather than to systemic or immune response occurring in the kidney [24] and was not correlated to renal activity [25]. The increase in UNGAL levels in LN may result from increased glomerular protein loss, disturbed reabsorption in the proximal nephron segment and increased intrarenal production [26]. Furthermore, the glomerulus may represent a source of NGAL [26,27]. In our results serum NGAL significantly correlated with the renal SLEDAI in SLE patients with LN. These results go with the results of others [23,28] suggesting that serum NGAL correlated with SLEDAI in LN patients indicating that disease activity affects NGAL plasma level which might be expressed by damaged tubules to induce re-epithelialization. This was further supported by the identification of NGAL as a regulator of epithelial morphogenesis in cultured kidney tubules [29]. In a study on Egyptian SLE patients, there was a significant increase of UNGAL and a noticeable correlation between UNGAL and laboratory parameters of renal disease activity as well as with renal SLEDAI suggesting UNGAL as a significant predictor for renal flare [13]. In another study on Egyptian SLE patients, there was a significant increase in serum UNGAL levels and significantly correlated with the renal biopsy class of LN and was considered an important marker [11].

**Urinary NGAL** did not correlate with the hematologic laboratory findings in the SLE patients. This was supported by the findings of another study [20]. However, a significant correlation was present between UNGAL with hemoglobin and RBC in LN patients. This was not previously reported in studies on SLE, however a significant correlation was reported between NGAL and anemia in several other systemic diseases and chronic inflammatory states [12]. In this study, serum NGAL significantly correlated with RBCs and hemoglobin. Similarly, this was reported by another study [19]. NGAL

![Figure 3](image_url)

**Figure 3** Receiver operating characteristic (ROC) curve of sensitivity and specificity of urinary neutrophil gelatinase-associated lipocalin (UNGAL) for detection of lupus nephritis. At a cutoff value of 18 ng/ml the sensitivity was 85.7% and specificity 80%. The area under the ROC curve (AUC) was 0.93.

### Table 3  Correlation between urinary Neutrophil gelatinase associated lipocalin (UNGAL) and laboratory parameters in the systemic lupus erythematosus (SLE) patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Urinary NGAL in SLE patients</th>
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<tbody>
<tr>
<td></td>
<td>All patients (n = 35)</td>
</tr>
<tr>
<td>RBCs (×10⁶/mm³)</td>
<td>−0.34 (0.06)</td>
</tr>
<tr>
<td>Hemoglobin (gm/dl)</td>
<td>−0.28 (0.12)</td>
</tr>
<tr>
<td>WBCs (×10³/mm³)</td>
<td>0.04 (0.85)</td>
</tr>
<tr>
<td>Platelets (×10³/mm³)</td>
<td>0.01 (0.96)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.26 (0.21)</td>
</tr>
<tr>
<td>ESR (mm/1st h)</td>
<td>0.16 (0.45)</td>
</tr>
<tr>
<td>Proteinuria (g/day)</td>
<td>0.5* (0.01)</td>
</tr>
<tr>
<td>ANA positivity (%)</td>
<td>−0.09 (0.59)</td>
</tr>
<tr>
<td>Anti-ds DNA (IU/ml)</td>
<td>−0.29 (0.2)</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>0.01 (0.96)</td>
</tr>
<tr>
<td>C4 (mg/dl)</td>
<td>0.03 (0.86)</td>
</tr>
</tbody>
</table>


* Significant at p < 0.05.
represents a key factor in the regulation of RBC growth [30] and the ability to suppress erythropoiesis [31]. In patients with chronic kidney disease (CKD), anemia is caused from the progressive reduction of the endogenous production of erythropoietin. These patients present with high serum and urine NGAL, making this molecule a promising marker not only of CKD progression but also a wonderful tool in the management of iron deficiency [32].

There was a significant correlation between UNGAL and 24-h urinary protein in all SLE patients but not in those with LN which is in agreement with the findings of another study on children with LN [21]. There was no significant correlation between UNGAL levels and serum creatinine which supports previous findings [20] that mentioned that the presence of lipocalin-2 may have a stronger association with acute disease activity than with the degree of renal insufficiency. In the present study no significant correlation was found between serum NGAL and 24 h urine protein which is in disharmony to the results of other studies [23,33,34].

In the current study, there were no correlations between the UNGAL and complement level or anti-dsDNA antibodies. In disagreement, it has been reported that pathogenic anti-dsDNA antibodies up-regulate the expression of NGAL in mesangial cells [35]. A possible explanation found that the levels of anti-dsDNA antibodies may decrease concurrently with acute SLE flares due to increased tissue deposition demonstrating a complex relationship [36]. It has further been recently postulated that UNGAL predicts renal flare in patients with a history of biopsy-proven lupus nephritis with a higher sensitivity and specificity than anti-dsDNA antibody titers [37].

Urinary NGAL showed significant correlation with the classes of renal biopsy in the patients with LN and at a cutoff value of (18 ng/ml), the sensitivity was 85.7% and specificity 80%. This is in harmony with the finding of another study [21] that found UNGAL considerable in detection of class III and IV LN. At a cutoff value of 20 ng/mg creatinine, the sensitivity of UNGAL levels for the diagnosis of LN was 57%, with a specificity of 83%.

Our results showed a significant correlation between urinary and serum NGAL in SLE patients. On the contrary, no significant correlation was found in 2 previous studies [11,14] explaining that extra renal sources of NGAL are not responsible for increased urinary levels in LN and that the enhanced local production results from renal tubular cells or increased NGAL leakage from glomerular capillaries. Interestingly, it has recently been shown that UNGAL had the best sensitivity and specificity as a biomarker for lupus nephritis compared to other urinary markers including transforming growth factor-β, monocyte chemotactrant protein-1 and interleukin 17 [38].

In conclusion, UNGAL significantly correlates with measures of LN and disease activity and can be considered as a potential and powerful marker for lupus nephritis in Egyptian SLE patients. Further longitudinal larger scale studies, including more patients and control are recommended to investigate its role in response to therapy.

Conflict of interest

None.

References


