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Original article

Serum neutrophil gelatinase-associated lipocalin as a biomarker of disease activity in pediatric lupus nephritis

Background: *Neutrophil gelatinase-associated lipocalin (NGAL) is expressed in immature neutrophil precursors and in epithelial cells during both inflammation and neoplastic transformation. A recent prospective pediatric study demonstrated that concentrations of NGAL in urine and plasma represent novel, sensitive, and specific biomarkers for early identification of acute kidney injury following cardiac surgery.*
Objective: *To assess the relationship of serum NGAL levels with disease activity in pediatric systemic lupus erythematosus (SLE) with special emphasis on lupus nephritis.*
Methods: *The study included 30 children and adolescents with pediatric SLE with a mean age of 16.48±3.524 years. Patients were clinically and laboratory evaluated and categorized into those with nephritis and those without nephritis. Activity was assessed using SLEDAI score, NGAL levels were measured in the sera of included patients and were compared to those of 20 matched controls using ELISA.*
Results: *Serum NGAL was significantly higher in SLE patients in comparison to the controls ($z=-5.962$, $p<0.001$). Furthermore serum NGAL was significantly higher in SLE patients with nephritis and in those without nephritis in comparison to the controls ($p<0.001$ in both). Serum NGAL was higher in SLE patients with nephritis in comparison to those without nephritis, yet the results are borderline regarding statistical significance ($p=0.05$). Levels of serum NGAL correlated significantly with disease activity as assessed by SLE disease activity index (SLEDAI) ($r=0.485$, $p< 0.01$). There was a significant correlation between serum NGAL and urinary protein to creatinine ratio, 24hr urinary protein and BUN of SLE patients.*
Conclusion: *Our results suggest that serum NGAL represents a novel biomarker for disease activity in pediatric SLE patients, and a marker of severity of renal involvement.*

Keywords: SLE, NGAL, SLEDAI, lupus nephritis.

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INTRODUCTION

Renal involvement is one of the main determinants of poor prognosis of systemic lupus erythematosus (SLE) and is more frequently encountered in children than in adults with SLE[1]. Currently available renal biomarkers, *i.e.* measures of the degree of SLE renal disease activity and severity, are too insensitive to allow for early identification of patients with active SLE nephritis, prohibiting timely initiation of therapy to avoid permanent renal damage[2]. Randomized clinical trials in SLE are hindered by the lack of high-quality biomarkers to verify the effects of therapies within a short period of time[3].

Neutrophil gelatinase-associated lipocalin (NGAL) is a member of the lipocalin family of proteins that has been extensively studied in acute kidney injury[4]. NGAL is one of the most robustly expressed proteins in the kidney following ischemic or nephrotoxic injury in both animals[5] and humans[6]. Importantly, a prospective pediatric study demonstrated that concentrations of NGAL in urine and plasma represent novel, sensitive, and specific biomarkers for early identification of acute kidney injury following cardiac surgery[7].

Given the role NGAL plays in kidney injury, we were stimulated to investigate its serum levels in a group of children and adolescents with SLE in relation to lupus nephritis, and disease activity.

METHODS

Study population

This case control study was conducted on 30 children and adolescents fulfilling the American College of Rheumatology Classification Criteria for

SLE[1] prior to the age of 16 years. Their ages ranged from 5 to 21 years with a mean age ± (SD): 16.48 ±3.524 [median=14.5 years]. They were recruited on one of their routine visits to the Pediatric Allergy and Immunology Clinic, Children's Hospital, Ain Shams University.

A sample of 20 age and sex matched healthy children were studied as controls. Their ages ranged from 9 to 19 years with a mean age ± (SD): 14.8 ±3.381 [median=15.5 years]. They were obtained from the out patient clinic of the same hospital.

Methods

The medical records were reviewed to screen for pre- existing renal disease in patients and to obtain pediatric SLE-specific information. Review of system information and the results of routine laboratory testing at the time of the study visits were recorded. Relevant demographic data of all participants were obtained.

All patients and controls were subjected to the following:

1. **Clinical history taking and examination** stressing on disease activity in SLE patients measured by SLE disease activity index (SLEDAI)[8], and data about lupus nephritis:
   - Presence or absence.
   - Clinical presentations.
   - Laboratory evidence.
2. **Laboratory testing (for patients only):**
   a. Serum creatinine and BUN using synchron CX7 autoanalyzer.
   b. Urinalysis, urinary protein to creatinine ratio, 24 hours protein in urine using synchron CX7 autoanalyzer.
   c. CBC on coulter counter.
   d. ESR by Westergen method.
   e. Anti-double stranded DNA antibodies were assessed employing indirect immuno-fluorescent test (IMMCO Diagnostics, USA).
   f. Complement 3 (C3) assay using turbidimetery (Turbiquant C3, Behringwerke Diagnostics-Marburg, Germany).

Quantitative measurement of serum NGAL

This was done by enzyme-linked immunosorbent assay (ELISA) using (Human Lipocalin-2/NGAL ELISA, Biovendor, Czech Republic, that specifically detects human NGAL).

Test Principle

In the BioVendor Human Lipocalin-2/NGAL ELISA, the Standards, Quality controls and samples were incubated in microtiter wells pre-coated with polyclonal anti-human lipocalin-2 antibody. After incubation and a washing, biotin-labeled polyclonal anti-human lipocalin-2 antibody was added and incubated with captured lipocalin-2. After another washing, the streptavidin-HRP conjugate was added. After incubation and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution, and absorbance of the resulting yellow product was measured spectrophotometrically at 450nm. The absorbance was proportional to the concentration of lipocalin-2. A standard curves was constructed by plotting absorbance values against concentrations of standards, and the concentrations of unknown samples were determined using this standard curve.

Statistical analysis

Analysis of the data was done on IBM computer system using SPSS version 15 (Statistical program for social science group USA). Data were presented as mean±SD for parametric data in addition to median and interquartile range (IQR) (the difference between 75^{th} and 25^{th} percentiles) for non parametric data.

Comparison between groups was done using Mann Whitney test for non parametric variables. Spearman correlation co-efficient rank test was used to rank different variables against each other in linear correlation. For all tests (probability) P values < 0.05 was considered significant.

RESULTS

In the studied group, 22 patients had nephritis (2 males and 20 females) with a frequency of 73% and 8 patients had SLE without nephritis (1 male and 7 females).

Serum NGAL was significantly higher in SLE patients [mean±SD: 271±126.480; median: 255 ng/ml] in comparison to the controls [mean ±SD:62.40 ±24.442; median: 75 ng/ml], (z= -5.962, p<0.001). It was significantly higher in SLE patients with nephritis [M±SD: 295.91±125.941; median: 277.50 ng/ml], and in patients without nephritis [mean ±SD: 202.50±106.670; median: 172.50 ng/ml] in comparison to the controls (z= -5.568, p<0.001 and z= -4.121, p<0.001) respectively.

Serum NGAL was higher in SLE patients with nephritis in comparison to those without nephritis, with borderline statistical significance (z= -1.958, p= 0.05) (Fig 1).

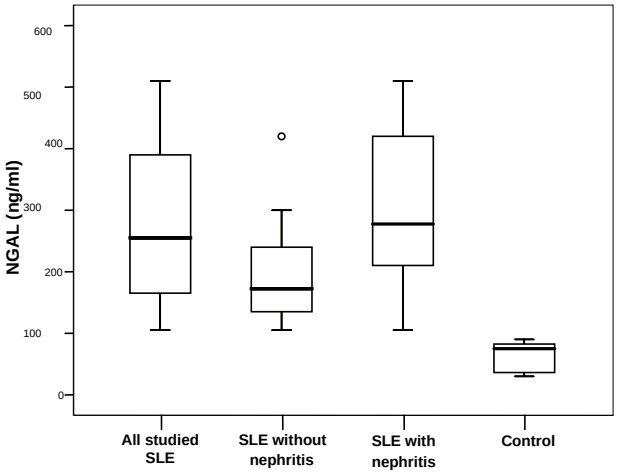
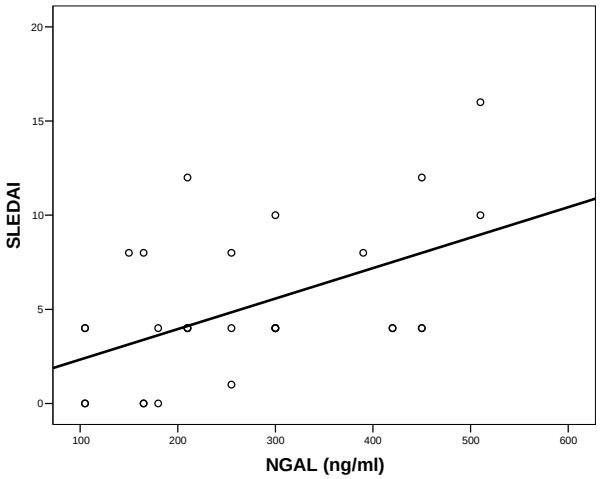


Figure 1. Serum NGAL in the studied patients and controls.



r =0.485 p = 0.007

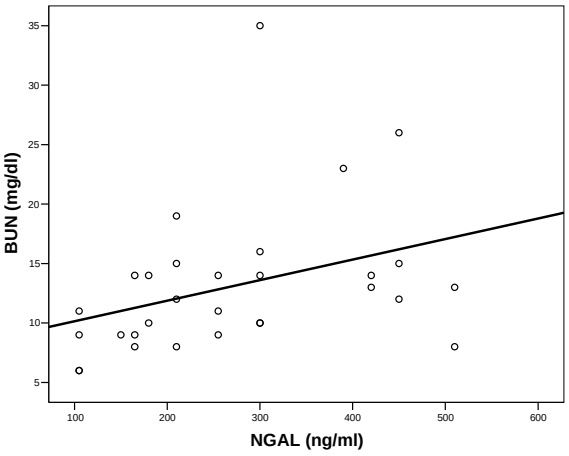
Figure 2. Correlation between serum NGAL and SLEDAI in SLE patients.

There was a significant correlation between serum NGAL and SLEDAI in SLE patients as a group, (r= 0.485, p<0.01) while there was no correlation between serum NGAL and age and disease duration (Fig 2).

There was a significant correlation between serum NGAL and age and SLEDAI of SLE patients without nephritis (r= -0.839, p<0.01 and r=0.803, p<0.05 respectively), while there was no correlation between serum NGAL and disease duration in the same group.

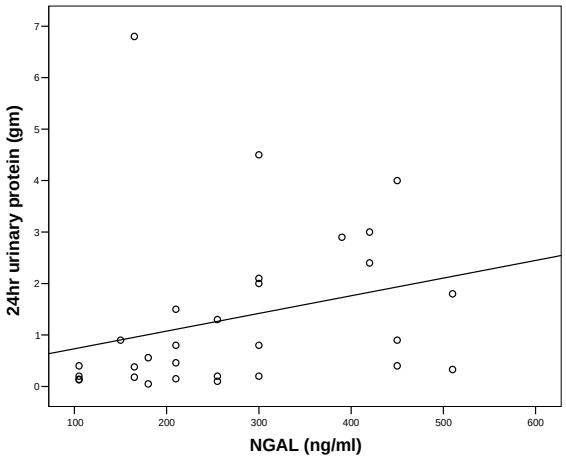
There was a significant correlation between serum NGAL and prot/creat in urine, 24hr urinary protein and BUN (r= 0.367, p< 0.05; r=0.466, p< 0.01 and r= 0.470, p< 0.01 respectively), while there was no significant correlation between serum NGAL and the other laboratory data of SLE patients (Figs 3 and 4).

There was a significant correlation between serum NGAL and disease duration in SLE patients with nephritis (r= 0.481, p<0.05) , while there was no correlation between serum NGAL and age and SLEDAI in those patients.



r = 0.466 p = 0.009

Figure 3. Correlation between serum NGAL and BUN in SLE patients.



r = 0.486 p = 0.009

Figure 4. Correlation between serum NGAL and 24hrs urinary protein in SLE patients.

DISCUSSION

Renal involvement is one of the main determinants of poor prognosis of SLE and more frequently encountered in children than in adults with SLE[1]. In the present cross sectional study on pediatric SLE patients we sought to investigate the relation of serum NGAL to disease activity with special emphasis on renal involvement.

The results showed that serum NGAL was significantly higher in SLE patients in comparison to the controls as it is one of the substances expressed in immature neutrophil precursors and in epithelial cells during both inflammation and neoplastic transformation. When we compared serum NGAL in the two groups of pediatric SLE patients (patients with nephritis and those without nephritis) we found that serum NGAL was higher in SLE patients with nephritis in comparison to those without nephritis with borderline significance. In 2005, Mishra and his colleagues[7] measured plasma and urinary NGAL in children who developed acute kidney injury after cardiac surgery necessitating extra-corporeal cardio-pulmonary bypass. A significant rise in the average plasma NGAL levels was seen as early as 2 hours post-operatively, urinary NGAL levels showed similar results although sensitivity and specificity of the test were somewhat higher than for plasma NGAL in their study, which means that NGAL can be considered as an early biomarker for acute kidney injury.

In a previous study by Suzuki and his colleagues[9], plasma concentration of NGAL fluctuated widely in pediatric SLE patients, and there was no significant increase with renal disease activity change while there was marked increase in urinary NGAL with worsening renal disease activity in pediatric patients with lupus nephritis. Another study showed that urinary NGAL was significantly higher in adult lupus patients with active nephritis than in those without nephritis[10].

Serum NGAL as a marker of renal injury has also been studied in patients subjected to percutaneous coronary interventions (PCI) with coronary angiography [11]. In this study the chief risk for the kidneys is thought to be the radiographic contrast agents used in this procedure. A significant (but modest) rise in serum NGAL was seen in the first sample taken at two hours after PCI; this peaked at four hours, while the rise in urine NGAL peaked at eight hours. Multivariate analysis was performed with serum creatinine, which did not rise significantly during the 48 hours of the study, as well as with serum cystatin C, which peaked at 24 hours. None of the patients developed acute renal dysfunction. This is an interesting study of potential subclinical renal injury from the contrast agent, in which NGAL was clearly the earliest responding marker. Another study showed that serum NGAL was significantly increased in critically ill children with acute kidney injury compared with those without acute kidney injury[12].

In our study, we found that there was a significant correlation between serum NGAL and SLEDAI in all investigated patients, this means that disease activity in SLE patients affects serum NGAL level. Our results were in agreement with other studies in pediatric SLE, the most recent was that of Suzuki and his colleagues[9] where they found that children with SLE had higher plasma NGAL during activity.

As regard disease duration we found that there was a significant correlation between serum NGAL and disease duration in SLE patients with nephritis, this means that serum NGAL may have a role in chronic kidney disease, this is supported by a previous study of Mitsnefes and his colleagues[13] which showed that children with chronic kidney disease in stages 2-4 had serum NGAL levels that correlated significantly, if not highly so, with glomerular filtration rate (GFR), and at lower values of GFR, serum NGAL level correlated rather better than cystatin C level.

The present study revealed a significant positive correlation between serum NGAL and protein/creatinine ratio, 24 hr urinary protein and BUN. The correlation of serum NGAL and proteinuria, which is an important parameter for disease activity in lupus nephritis, was supported by a previous study that suggested that NGAL might be expressed by the damaged tubule to induce re-epithelialisation[14], Further support for this notion derives from the identification of NGAL as a regulator of epithelial morphogenesis in cultured kidney tubule cells[15], and as an iron-transporting protein that is complementary to transferrin during nephrogenesis[16].

The current study did not find significant correlation between serum NGAL and other laboratory parameters including (Hb, WBCs, platelets, ESR, creatinine clearance, serum creatinine , antiDNA and C3).

In a previous study in adult SLE by Pitashny and his colleagues[10] there was no correlation between urinary NGAL and Hb, WBCs, platelets. They could not find any correlation between the levels of urinary NGAL and the levels of complement or anti-dsDNA antibodies in adult patients. Although these are commonly used indicators of renal disease activity in clinical

practice[17], the association between these parameters and disease risk is imperfect[18]. The absence of a correlation between NGAL and anti-dsDNA antibodies is, however, somewhat surprising in light of in vitro studies in which pathogenic anti-dsDNA antibodies up-regulated the expression of NGAL in mesangial cells[19].

One possible explanation may be the relative lack of sensitivity of the anti-dsDNA assays for specific detection of nephritogenic anti-dsDNA antibodies that induce renal disease and up-regulate lipocalin-2 expression in the kidneys. Similarly, although the role of complement in LN pathogenesis has been well described[20], in the present study increased NGAL was not associated with complement consumption.

Previous studies have indicated that other forms of renal injury, including ischemia, nephrotoxic drugs, or infection, can also up-regulate lipocalin-2 expression in the kidneys[21]. Therefore, elevations in serum NGAL are not specific to nephritis in SLE. Nevertheless, this does not detract from a potentially valuable role for NGAL in lupus patients as suggested by the results of our study, in the initial diagnosis of kidney involvement, or in subsequent monitoring of disease activity.

Our results suggest that serum NGAL represents a novel biomarker for disease activity in pediatric SLE patients as evident from its correlation with SLEDAI, and a marker for severity of renal involvement as obvious from its correlation with protein excretion and BUN.

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