ORIGINAL ARTICLE

Correlation of Urinary Neutrophil Gelatinase Associated Lipocalin with Routine Biomarkers used to Detect Kidney Injury in Systemic Lupus Erythematosus Patients with and without Nephritis

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Abstract:

Systemic lupus erythematosus is associated with various complications with kidney involvement Lupus Nephritis(LN) being the most common. The routine biomarkers for the diagnosis of Lupus Nephritis have low specificity and sensitivity leading to delayed diagnosis of the condition thus increasing the rate of mortality and morbidity. Hence there is need of a new biomarker showing correlation with the old biomarkers indicating the severity of renal damage. This search for a new marker to detect kidney involvement in patients with lupus induced nephritis can help to diagnose patients with kidney involvement at an early stage leading to timely initiation of therapy and decrease the rate of mortality and morbidity in these patients.

Objective: This study was designed to find out correlation of Neutrophil gelatinase associated lipocalin (uNGAL) with the biomarkers routinely used to measure kidney injury in patients with SLE and therefore finding the efficacy of NGAL in detection of renal involvement in these patients.

Methods: It is a cross sectional study conducted in Deptt. of Biochemistry and Chemical Pathology, Sheikh Zayed Hospital Lahore from June 2015 to June 2016 Eighty Four (n=84) SLE patients were selected for this study from Sheikh Zayed Hospital Lahore. These cases were divided into two equal groups (n=42) A and B. Group A comprised of SLE cases without kidney involvement and group B had cases with biopsy proven lupus nephritis. Urinary NGAL, serum and urinary creatinine and micro-albuminuria were measured in both groups. Correlation of NGAL with serum and urinary creatinine, albumin creatinine ratio and creatinine clearance was calculated using Pearson's correlation and scatter plots were drawn for both groups.

Results: This study revealed that uNGAL correlated positively with creatinine and ACR (albumin creatinine ratio) and correlated in a negative manner with Creatinine clearance of the patients. However, the new biomarker showed no correlation with creatinine measured in urine.

Conclusion: Our study indicated that the new bio-marker i.e. uNGAL had a significant correlation with renal disease activity of SLE patients and thus can be used to assess the status of renal involvement in lupus patients.

Key words: neutrophil gelatinase associated lipocalin, systemic lupus erythmatosus, lupus nephritis, albumin creatinine ratio, biomarker

Introduction

Systemic lupus erythematosus is an autoimmune disease involving multiple organs manifesting a variable clinical course. SLE is diagnosed on the basis of characteristic clinical findings on joints, Central Nervous System , skin and kidneys along with serological markers.[1] American college of rheumatology established the basis for diagnosis

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of SLE on the presence of any four out of eleven criteria and this is recently revised in 2015.[2] Most common non-specific symptoms at the onset of disease activity are fever , generalized fatigue and joint pain and these are followed by joint swelling and butterfly rash most particularly in women of child bearing age.[3]

In the year 1998, a study was conducted in northern areas of Pakistan and the prevalence of Lupus was found to be 0.5/100 in the residing population.[4] In 2002, a study was conducted in Germany and the prevalence was found to be 36.7/100 000 and the ratio of women to men was 4:1.[5]

Lupus nephritis is a commonly found severe manifestation of SLE and exerts a negative impact on long term patient survival.[6] Individuals with lupus induced nephritis from different ethnicities and races show a considerable difference in disease severity, response towards therapy, clinical outcome of the disease and prevalence rate.[6] Asian patients with systemic lupus erythmatosus show more severe renal disease and higher rate of renal involvement (50-60%) as compared to Caucasians (30-38%).[7-9] Therefore we can state that lupus nephritis is a major concern among Asian patients with SLE because of higher incidence and severity of renal involvement.[10]

International society of nephrology has classified LN in four major histological classes.[11] Lupus induced nephritis, no doubt is a serious yet treatable condition and thus it is important for the practitioners to diagnose it at an initial stage.[12] In contrast to the early stage of lupus where only 30 percent of the affected individuals show abnormality in renal function, almost 60-80 percent patients show deranged kidney functions later in the disease course.[13]

The biochemical tests which are present nowadays to measure the intensity of renal involvement in SLE do not show much sensitivity to diagnose the disease at an early stage and as a result of this the initiation of therapy is delayed leading to permanent kidney damage.[14,15] Hence, there is a need to look for a biomarker which can help to identify flare as well as have a good correlation with the routine biomarkers used to identify kidney injury due to LN. Also, the new marker for the diagnosis of lupus nephritis should be easy to interpret and assay and should be readily available.[16]

Neutrophil gelatinase associated lipocalin has proved to be a diagnostic biomarker for acute and chronic kidney ailments.[17] Many studies have been carried out in the past in which the role of NGAL in determining kidney injury in patients with lupus nephritis has been highlighted.[14,18-20] In these studies the level of NGAL was raised in patients of lupus nephritis.

This study was designed to determine urinary NGAL levels and to find out the correlation of NGAL with the routinely used biomarkers used to detect kidney injury in SLE patients with kidney involvement. This correlation can be helpful in determining that NGAL was raised due to kidney involvement and not due to other systemic causes. uNGAL correlation with kidney injury biomarkers in patients with SLE can add clinically relevant information in the management and treatment of patients with lupus nephritis.

Methods

This was a cross sectional study completed over a period of one year i.e. from January 2015 to January 2016. The patients were selected from Nephrology unit and Medical unit (both indoor and OPD) of Sheikh Zayed hospital Lahore.

A total of eighty four adult subjects, both male and female were selected who were diagnosed cases of systemic lupus erythematosus (according to ACR criteria) and were divided into two groups A&B with equal number of cases(n=42).[2] Sample size in each group (A&B) was estimated by using 95% confidence level and 80% power of test. Group A (n=42) comprised of SLE patients without kidney involvement and group B (n=42)was comprised of patients with lupus induced nephritis. A written informed consent was taken from all participants. Cases with 'evidence of rhabdomyolysis, malignancy, UTI or pregnancy were excluded.

'A 3 ml of blood sample and 5 ml of urine sample were taken and kept frozen for later analysis. Laboratory investigations included tests[21-23] namely creatinine(serum and urinary usng Jaffe's method, utrine albumin(quantitative turbidimetric method), Ungal (urinary NGAL level was performed using ELISA. The assay was performed as per company protocols), albumin and creatinineratio (ACR calculated using formula),creatinine clearance (calculated by CG formula; Cockcroft and Gault)

GPE, biochemical results and past history was also recorded on a Performa. Data analysis was done using SPSS 20.0.Correlation of uNGAL with serum and urinary creatinine, Albumin creatinine ratio and creatinine clearance was calculated using Pearson's correlation coefficient (r) and p-value was calculated for both groups. p vaule <0.05 was considered significant. Scatter plots were drawn showing correlation between different variables.

Results

Total number of study subjects was 84 divided into two groups. Serum and urinary creatinine, Urinary albumin and Urinary NGAL were measured for both groups. The cases without nephritis had lower uNGAL levels as compared to cases with nephritis (p value <0.001)

The correlation of Urinary NGAL levels with other markers of kidney injury used in the study was found by Pearson coefficient of correlation. uNGAL correlated in a positive manner with serum creatinine and Albumin creatinine ratio in group A and B. However, uNGAL correlated in a negative manner with estimated creatinine clearance in both groups. On the other hand, no correlation was seen between uNGAL and urinary creatinine either in group A or B (Table.1.)

Table 1: *Correlation of Urinary NGAL with creatinine, creatinine clearance and albumin creatinine ratio*

		Overall	Group A	Group B
Serum Creatinine (mg/dl)	r	0.717**	0.159	0.551**
	p-value	< 0.001	0.314	< 0.001
spot Urinary Creatinine (mg/dl)	r	-0.082	-0.137	-0.234
	p-value	0.458	0.387	0.136
Creatinine Clearance (ml/min)	r	-0.696**	-0.122	-0.551**
	p-value	< 0.001	0.440	< 0.001
Albumin/creatinine Ratio(mg/g)	r	0.775**	0.129	0.816**
	p-value	< 0.001	0.415	< 0.001

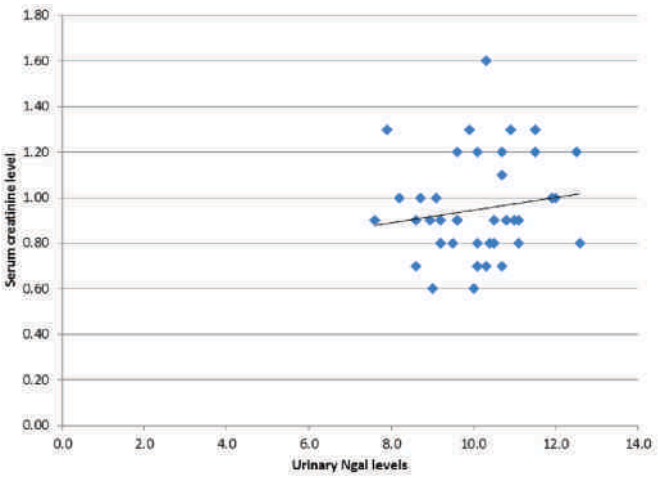

Figure.1: *Scatter plot presenting correlation between Urinary NGAL and serum creatinine for cases in group A*

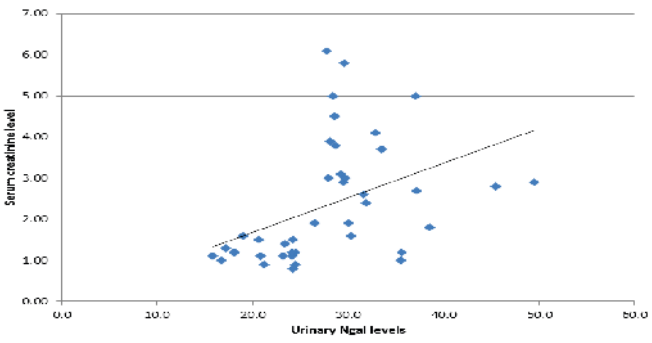

Figure.2: *Scatter plot presenting correlation between Urinary NGAL and serum creatinine for cases in group B*

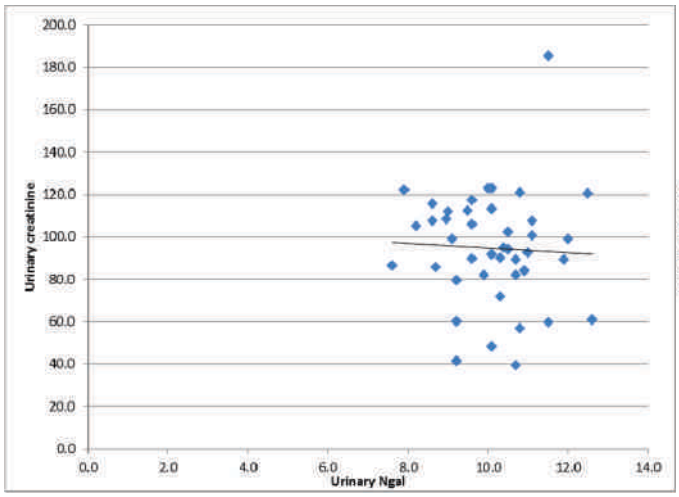

Figure.3: *Scatter plot presenting correlation between Urinary NGAL and urinary creatinine for cases in group A*

Another scatter plot showing no significant correlation between uNGAL and urinary creatinine for lupus patients with nephritis (r= -0.234, p=0.136) (Fig.4) Figure 3 & 4 show that urinary NGAL does not correlate with urinary creatinine with any of the groups in our study.

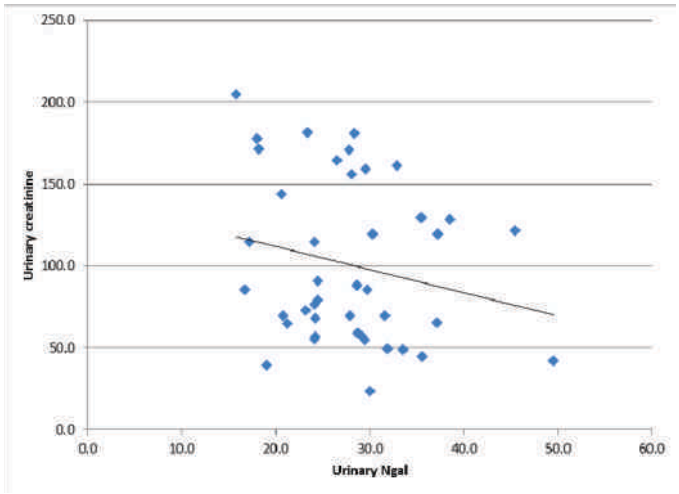

Figure.4: *Scatter plot presenting correlation between Urinary NGAL and urinary creatinine for cases in group B*

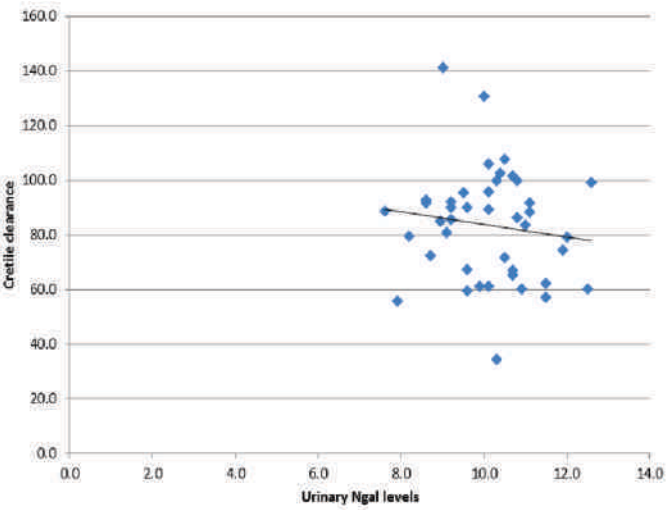

Figure.5: *Scatter plot presenting correlation between Urinary NGAL and creatinine clearance for cases in group A*

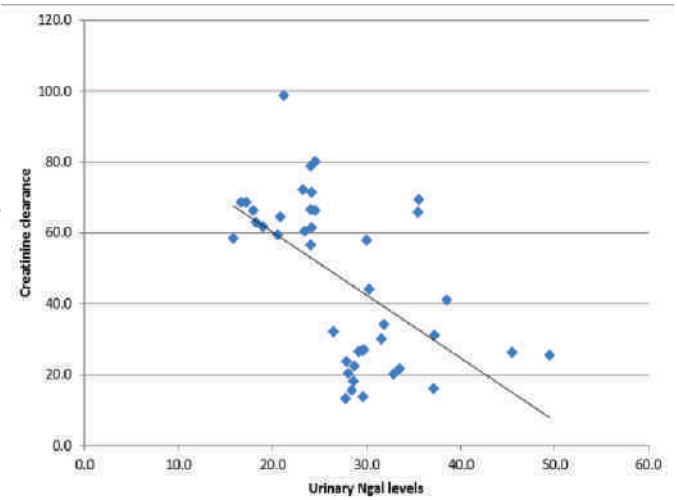

Figure.6: *Scatter plot presenting correlation between Urinary NGAL and creatinine clearance for cases in group B*

Discussion

Systemic lupus erythematosus is an auto-immune chronic inflammatory disease and lupus nephritis is one of its common complications.[24] Patients with SLE have diverse degrees of renal involvement ranging from asymptomatic protein uria or hematuria to RPGN(rapidly progressive glomerulonephritis), nephrotic syndrome or end stage renal failure.[24] Forty to seventy percent of all SLE patients present with renal involvement which is a major cause of mortality and morbidity.[1]

The routine biomarkers used for the detection of kidney injury in lupus nephritis patients have low sensitivity and specificity leading to delayed diagnosis and treatment. All these factors lead to the clinicians to look out for a new biomarker with better diagnostic capabilities and having good correlation with the routinely used biomarkers. Neutrophil Gelatinase associated lipocalin or Lipocalin 2 has emerged as a new promising biomarker for the diagnosis of kidney injury. NGAL is present in neutrophils where it is bound with gelatin and usually expressed by injured epithelia e.g. injured renal epithelial cells.[25-27] Even before the rise in serum creatinine after kidney damage, the injured tubular cells release NGAL which can be found in urine.[28]

Several studies have been carried out in which the role of NGAL in AKI was studied, but its role in the diagnosis of LN along with its correlation with the routinely used bio markers used to asses kidney function in LN is not very clear.[18] This study was designed to find out the correlation of a new biomarker i.e. NGAL with old renal biomarkers used routinely to assess kidney damage in patients with lupus induced nephritis.

In the current study, it was revealed that urinary lipocalin 2 has a significant correlation with the routine biomarkers used to detect lupus nephritis and can give us an idea about kidney involvement in patient with SLE. Our study is in close agreement with the study conducted by Pitashney and his colleagues who revealed that urinary lipocalin 2 had a significant correlation with renal components of SLEDIA(SLE disease activity index).[19] In 2015 another study was conducted in Egypt to find out the relation of NGAL with renal disease activity and it was found that there was a positive correlation of NGAL with renal SLEDAI.[29]

In our study, the correlation of NGAL with other kidney injury markers can also be useful to monitor the effectiveness of therapy in these patients. Sabah Alharazy along with other colleagues while evaluating the role of NGAL for monitoring therapy in LN found a positive correlation of NGAL with urinary protein and serum creatinine and negative correlation with GFR.[30] Similar results were found in the study conducted by Torres et al in which urinary NGAL showed a positive correlation with estimated GFR and protein creatinine ratio.[31] Elewa et al conducted a study on fifty five subjects and noticed a significant correlation between urinary NGAL and SLEDAI (renal index) and concluded that NGAL can act as a reliable predictor of worsening of kidney disease and can be used to monitor treatment.[32]

In our study, this new biological marker under discussion showed a positive correlation with urine albumin in both group A and B. Davide Bolignano along with his coworkers also found in his study that uNGAL was correlated in a positive manner with urinary protein and it is directly related with the severity of renal disease in patients with proteinuria.[33] This correlation of NGAL with urinary protein also strengthen the fact that increased levels of NGAL are mainly due to increased production by the injured renal epithelia and not due to other systemic causes. Koura and Galal conducted a study in 2011. They found that children and adult subjects with lupus induced nephritis had a higher level of NGAL as compared to their healthy counterparts and NGAL was directly related with the renal involvement in patients with SLE.

This significant correlation of NGAL with other indicators of renal disease activity in lupus nephritis suggest the fact that it can be used as a valuable diagnostic marker for lupus nephritis giving a clear picture of renal involvement in the disease. NGAL can also be used as a marker for predicting deterioration in renal disease activity and

monitoring therapy response in SLE patients as clear from its correlation with other kidney injury markers.

Conclusion

In our study, we found a significant correlation between NGAL and other biomarkers which are used to detect kidney injury in patients with lupus induced nephritis. This may confirm the association between urinary NGAL and renal dysfunction. Our study supported the fact that NGAL can be used as a diagnostic biomarker for lupus nephritis and is significantly correlated with various other parameters. We suggest that in future, studies should be carried out showing correlation between uNGAL and kidney biopsy findings.

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