ORIGINAL ARTICLE

PREDICTION OF RENAL FLARE IN ADULT PATIENTS WITH LUPUS INDUCED NEPHRITIS

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Background: The most common complication of SLE is lupus nephritis (LN) causing high morbidity and mortality. The routine biomarkers used for the diagnosis of LN do not have the ability to predict the worsening in renal disease activity. Thus, there is need of a new biomarker leading to detection of flare in LN. The objective of this study was to assess the role of urinary neutrophil gelatinase associated lipocalin (uNGAL) as a predictor of renal flare in patients with lupus nephritis. **Methods:** Including a total of 84 subjects, 42 cases were lupus patients without renal involvement and 42 cases were lupus patients with nephritis (24 active nephritis and 18 inactive nephritis). The diagnosis of lupus nephritis was established on the basis of renal biopsy. uNGAL was estimated in both groups. **Results**: This study revealed that the nephritis group had increased levels of uNGAL as compared to systemic erythematosus patients without having lupus nephritis (p-value <0.05). Patients with active nephritis had increased uNGAL levels as compared to patients with inactive nephritis. **Conclusion**: From the findings in our study, it can be stated that uNGAL can prove to be a noninvasive, reliable and sensitive biomarker to predict flare in cases of lupus nephritis.

Keywords: Neutrophil Gelatinase Associated Lipocalin; Systemic lupus erythematosus; Lupus nephritis; Biomarker; Renal Disease Activity; Flare

Citation: Batool H, Naseem T, Shoukat. Prediction of renal flare in adult patients with lupus induced nephritis. J Ayub Med Coll Abbottabad 2021;33(1):9–13.

INTRODUCTION

Renal involvement is a major area of concern in patients with systemic lupus erythematosus. Kidney disease occurs in almost 40–75% of individuals suffering from SLE and has an unpredictable disease course and morbidity pattern.[1] The clinical management of lupus induced nephritis is still a great challenge for clinicians and nephrologists because of its heterogeneous classification and varying disease course.[2] In spite of an initial therapy, almost 25% of the patients of lupus nephritis will eventually progress to renal damage later in their disease course. This progression towards end stage renal disease is only because of the inability of clinicians to recognize remissions and relapses early enough to establish a proper treatment.[3]

The mainstay of treatment for lupus nephritis has been corticosteroids, azathioprine, cyclophosphamide and, more recently, mycophenolate.[4] lupus nephritis is categorized into different classes depending upon the immune complex renal lesions.[5] The disease course of lupus nephritis is very diverse; ranging from symptomless mild proteinuria to rapidly progressing nephritis or nephrotic syndrome. It is of utmost importance for practicing nephrologists to diagnose lupus nephritis at an early stage because it is usually treatable during its early phase.[6] During early stages of lupus nephritis only about 30% of the affected population has deranged renal function tests or abnormal urine examination findings. However, the percentage of affected population with lupus nephritis having abnormal laboratory findings is increased to almost 80% in the in the later course of the disease.[7]

Despite of the importance of early diagnosis of lupus nephritis, the renal biomarkers currently being used for the measurement of disease severity are not very sensitive. As a result of this poor sensitivity, treatment of lupus nephritis cannot be initiated at early stages of the disease. In all these circumstances, renal biopsy remains the only ideal choice to be used as a “gold standard” to diagnose lupus nephritis and measure the renal disease progression in SLE patients.[8,9] Serial renal biopsies are usually carried out by the nephrologists to measure the disease activity which are invasive and not without complications. The laboratory markers which are being used for decades for the diagnosis of lupus nephritis cannot anticipate flares in the disease or predict the histopathlological findings of the involved kidney. The treatment can only be started once this renal damage becomes permanent.[10] Therefore; it is of crucial importance to search for new biomarkers with enhanced predictive powers to reduce the morbidity and mortality in lupus nephritis patients.

During recent times, significant efforts have been made to find out an improved biomarker for lupus induced nephritis with superior predictive powers. More emphasis has been made to look for a urinary biomarker because urinary sample is easy to obtain, non-invasive and carry no risk as compared to serial renal biopsies. Furthermore, kidney function is more closely related to urinary biomarker as compared to its serum counterpart. Lipocalin 2 or neutrophil gelatinase associated lipocalin (uNGAL) is a glycosylated protein representing one of the secondary neutrophil protein having a molecular weight of 25 kDa.[11]

Along with various other clinical roles, lipocalin 2 has emerged as one of the most promising biomarkers in the diagnostic field of acute and chronic renal ailments in the recent years. Davide Bolignano and his coworkers concluded in their study that before any visible rise in serum creatinine levels, massive amount of NGAL was released by renal tubular cells after and episode of kidney injury.[12] Mori and Nakao concluded in their study that after an episode of kidney injury (either due to nephrotoxins or ischemia), there is an increased accumulation of NGAL in urine, blood and renal tubular cells and this increased level was due to production of NGAL by inflamed but still vital renal tubular cells.[13]. Also, in many chronic renal ailments such as autoimmune glomerulonephritis and polycystic kidney disease, lipocalin 2 plays a significant role in predicting renal disease activity. Along with this, NGAL can also be used to reflect kidney damage and progression of disease in chronic kidney ailments.[12]

The importance of urinary lipocalin 2 in predicting relapses and remissions in lupus nephritis is still not clear.[14] The serial measurement of urinary and plasma NGAL may prove to be very beneficial in predicting worsening of renal disease activity in patients of SLE.[15] The current study was designed to determine urinary NGAL level in cases of lupus nephritis with active and inactive disease to study its role in prediction of renal flares in lupus nephritis. It would be very beneficial if one could detect the degree of disease progression early in its course and start prompt treatment.

MATERIAL AND METHODS

This cross-sectional analytical study was conducted in the Department of Biochemistry and Chemical Pathology, Sheikh Zayed Hospital Lahore from 1st May 2015 to 30th April 2016. Cases were selected from the Department of Rheumatology and Nephrology, Sheikh Zayed Hospital, Lahore. A total of 84 diagnosed cases of SLE, both adult male and adult female, were selected and divided into two groups; Group A (42 cases without lupus nephritis) and Group B (42 cases with lupus nephritis). Patients having evidence of rhabdomyolysis, pregnancy or UTI were excluded from the study. A written consent was taken from all the study subjects. Renal biopsy was used to label patients as a case of LN.in group "B" two categories of cases were included, i.e., patients with active nephritis (n=24) and patients with inactive phase of lupus nephritis (n=18). The status of active or inactive renal disease was assessed using renal component of SLEDAI (SLE disease activity index) which include four features, i.e., proteinuria, pyuria, hematuria and presence or absence of urinary casts. Each of these items in the renal component of SLEDAI score four (4) points and the total score of renal SLEDAI ranges from 0–16. Active lupus nephritis is labeled when renal SLEDAI score is ≥ 4 out of 16 and inactive lupus nephritis is declared when total score is 0 or <4 out of 16.[16] The personal and medical history along with the biochemical findings of the study subjects were recorded on a pre-designed *Proforma*. Urine samples were taken and kept frozen for later analysis.

Urinary NGAL was estimated by ELISA using Glory science co., Ltd USA's rapid ELISA kit. The data was analyzed using SPSS 20.0 and *p*-value <0.05 was considered significant.

RESULTS

This study was conducted with 84 SLE cases, out of which 42 cases had lupus nephritis (Group A) and 42 were without Lupus induced nephritis (Group B). The data was tested for normality by using Shapiro Wilks test so that, appropriate statistical test could be applied for comparison. The groups were gender matched and age matched. The mean urinary NGAL value in group B was significantly higher as compare to group A with *p*-value <0.001 (Table-1.). It was found that in group-B, patients with inactive lupus nephritis (n=18) had lower NGAL levels (mean 21.3±3.1 ng/ml) as compared to patients with active lupus nephritis (n=24) (mean 32.6±5.7 ng/ml).

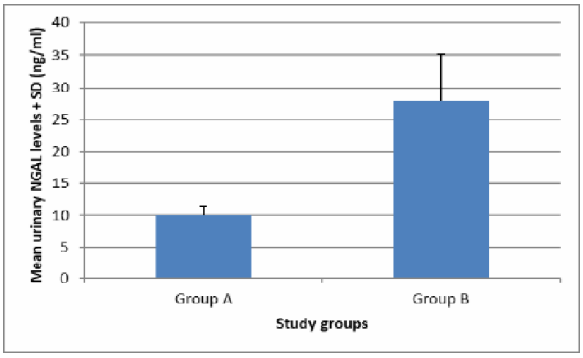

Figure-1: Bar diagram presenting mean urinary NGAL levels with SD as bars for cases in two groups

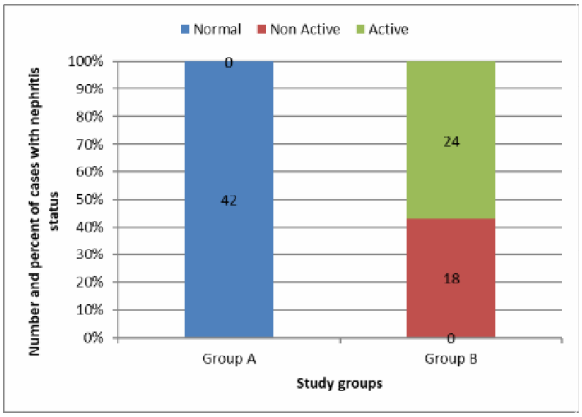

Figure-2: Component bar diagram presenting nephritis status of cases in two groups

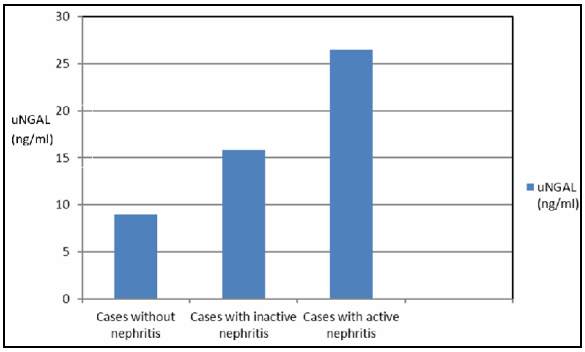

Figure-3: Bar diagram presenting mean uNGAL levels as bars for cases without nephritis, with inactive nephritis and with active nephritis.

Table-1: Comparison of urinary NGAL level for cases in two groups

	Urinary NGAL (ng/l)			
	Mean	SD	Minimum	Maximum
Group A	10.1	1.2	7.6	12.6
Group B	27.8	7.4	15.8	49.5
Comparison	t= -15.34		p-value < 0.001	

Table-2: Comparison of nephritis status for cases in two groups

Group	Nephritis					
	Normal		Non-Active		Active	
	N	%	N	%	n	%
Group A	42	100.0	0	0.0	0	0.0
Group B	0	0.0	18	42.9	24	57.1

Chi-square = 84.0. *p*-value <0.001

Table-3: Comparison of Mean and SD of uNGAL in study cases

	Mean	SD
uNGAL in cases without nephritis(ng/ml)	10.1	±1.2
uNGAL in cases with inactive nephritis(ng/ml)*	21.3	±3.1
uNGAL in cases with active nephritis(ng/ml)**	32.6	±5.7

*p-value <0.001. **p-value <0.001

DISCUSSION

SLE is a multisystem connective tissue disease and kidney involvement is a major cause of mortality and morbidity in such patients. Almost 50% of the cases diagnosed with SLE have the evidence of kidney involvement. However, during the early stages of the disease there are no visible sign and symptoms in the affected cases.[17] Loss of protein in the urine and frequent urination leading to edema of the lower extremities are often the first noticeable signs of lupus induced nephritis.[17]

Lupus nephritis usually presents late in the course of the disease and it is very difficult to pick up clinical signs initially. Therefore, it is very essential to find out a new biomarker with better predictive powers as compared to the routinely used laboratory tests so that lupus nephritis can be diagnosed at its early stage or the flare can be picked up early and treatment can be started accordingly.[18] The routine laboratory tests used for the prediction of renal flare in the patient of lupus nephritis e.g. urine analysis for the presence or absence of hematuria, casts, pyuria and serum urea & creatinine levels have low specificity and precision.[17, 19]Subsequently, the only choice left with the practicing nephrologists is serial renal biopsies which are invasive in nature and not without complications.

In this study we estimated urinary levels of NGAL in lupus nephritis patients (both active and inactive disease phases) to find out the role of this new urinary biomarker in predicting flare in these patients. The cases in our study were divided into two groups both containing equal number of cases (n=42). One group was labeled as group-A (SLE patients without nephritis) and the other was labeled as group B (SLE patients with nephritis). Furthermore, group B had two types of cases, i.e., with active disease phase(n=24) and with inactive disease activity (n=18).

The cases in the group with lupus nephritis were either in the active phase of the disease or inactive phase. In the current study it was found that lupus patients with inactive phase of the renal disease had lower NGAL values (mean 21.3±3.1 ng/ml) as compared to the cases with active phase of renal disease (mean 32.6±5.7 ng/ml, Table-3, fig.3). Similar findings were presented by Alharazy *et al* who proved that uNGAL levels were significantly lower in lupus nephritis patients who were in the remission phase as compared to those with active disease activity.[20] Tamar Rubinstein and his colleagues stated that patients with active renal disease activity had considerably higher levels of NGAL as compared with the lupus patients in whom kidney was not involved.[21] Also Cortes and Torres

found that during a renal flare in patients with systemic lupus erythematosus, uNGAL levels was significantly increased.[22] Our study is in close agreement with Suzuki *et al*, who conducted a study in pediatric SLE patients and found that uNGAL levels were raised significantly in the urine samples of the patients at the time of renal flare relative to the samples taken three months earlier. [23]

Till date, only four clinical studies have been conducted on the usefulness of lipocalin 2 as a biomarker for the diagnosis of LN or for the prediction of its flare or relapse. Three of these studies were performed on pediatric patients and only one was conducted on adult population. In 2008, eighty-five pediatric patients with SLE were selected for a study and it was found that levels of NGAL were increased in these cases during episodes of relapse.[23] Hinze et al. conducted another longitudinal study on pediatric patients in which it became evident that uNGAL levels could predict the episode of relapse in LN.[10]

In group B of our study two types of lupus nephritis patients were included; one with active renal disease and one with inactive renal disease. Patients with inactive status of the disease had lower urinary NGAL levels as compared to the patients with active disease but still higher than the patients in group A (SLE patients without renal disease). This finding shows the positive relationship of NGAL with worsening of the renal disease activity. From this we can also conclude that an undiagnosed LN patient in the remission phase (with normal RFTs) can also be diagnosed with the help of NGAL. NGAL levels can be used as an alternative to renal biopsy for the diagnosis of patients in remission and can predict the impending relapse as levels of uNGAL increase with the worsening of kidney disease.

uNGAL can be added to the routine panel of investigations for lupus nephritis patients and can help to pick the disease early in its course. Urinary NGAL is directly related with the kidney function and can be extremely helpful in the early therapeutical management of the patients with lupus nephritis.

CONCLUSION

Biomarkers used currently by the nephrologists and pathologists are not capable of early detection of lupus nephritis and cannot predict the forthcoming flare in these patients. The patients with lupus nephritis have two phases of the renal disease activity, i.e., relapse and remission. In our study it was evident that patients with active phase of LN had higher urinary levels of NGAL as compared to the patients with inactive disease activity. Therefore, it can be suggested that NGAL can be used as a reliable diagnostic and predictive marker of flare in lupus induced nephritis and in the future can ultimately replace the invasive method of diagnosis of LN, i.e., renal biopsy.

AUTHORS' CONTRIBUTION

HB: Principal Author, write up, sample collection, analysis and literature review. TN: Study design, supervision, literature review. MS: Data collection and analysis

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Submitted: October 10, 2017	*Revised: January 12, 2020*	*Accepted: January 12, 2020*

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