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ORIGINAL ARTICLE

Assessment of Serum Neutrophil Gelatinase Associated Lipocalin (NGAL) levels in patients of Systemic Lupus Erythematosus (SLE) with and without Lupus Nephritis (LN)

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ABSTRACT

Background: Lupus nephritis (LN) can occur as a complication of SLE causing further distress to patient. NGAL levels can help in foreseeing kidney injury in such afflicted. Thus, the study aimed to estimate serum NGAL levels in SLE patients with and without Lupus Nephritis.

Methods: A cross sectional study was conducted at immunology Department, University of Health Sciences (UHS) Lahore. Purposive sampling technique was used to collect samples of patients diagnosed with SLE with and without Lupus Nephritis LN.

Results: Study showed statistically significant results (p=0.00) with mean ±SD value of NGAL measured in patients of SLE with LN & without Lupus Nephritis LN.

Conclusion: The study indicated that NGAL level was observed to be significantly raised in the patients of SLE with lupus nephritis as compare to the patients of SLE without lupus nephritis.

Keywords: Acute kidney injury, Anti-double stranded DNA antibody, Chronic kidney disease, Lupus nephritis

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multi-organ disorder of autoimmune origin that has been known to be caused by the dysregulation of hormonal, infectious, environmental and genetic factors[1]. It affects joints, kidneys and skin, manifesting mainly as blood disorders, oral ulcers, arthritis, photosensitivity, renal pathology, malar rash among others[2]. These form the diagnostic criteria along with laboratory confirmation of anti-double stranded DNA antibodies (anti-dsDNA), antinuclear antibodies (ANA) & anti-Smith (Sm) antibodies[3]. It is prevalent among people of African descent and infrequently seen in Caucasians[12]. In Asia, Chinese communities are more frequently affected than those of subcontinent[4] and are more likely to develop renal issues and lethal complications[5]. Although, data regarding prevalence of SLE in Pakistani population is scanty, a study depicted that it mostly affects women around the age of 30 years, which is concordant with worldwide statistics[6]. Also, Pakistani patients tend to show less hematological and cutaneous manifestations along with renal involvement[10].

Lupus Nephritis (LN) is one of the fatal complications of SLE which can further deteriorate the quality of life and is also associated with considerable debilitation in SLE affectees[3]. Early detection can help in management of renal issues however, definitive diagnosis and prognosis can be ascertained through renal biopsy, which is an invasive procedure and may not be indicated for many patients.

NGAL is a glycoprotein that plays role in transporting iron to and from the cells, programmed cell death, and differentiation of tissues. It is normally produced in minute quantity in the kidneys, but levels are increased in conditions like infection, ischemia, and inflammation[7]. Serum NGAL has proven to be an indicator of ongoing kidney injury and can point towards the onset of renal impairment in lupus cases[8]. It can potentially be tested in lieu of biopsy, so as to predict probability of occurrence of LN in future as previous study has shown that urinary neutrophil gelatinase-associated lipocalin (UNGAL) was raised in patients of Lupus Nephritis[9].

Since most past research has focused on estimating and predicting AKI through UNGAL[9,11] the aim of the study to determine the levels of serum NGAL in the serum of SLE patients with & without Lupus Nephritis.

MATERIAL AND METHODS

A cross sectional comparative study was undertaken at the Immunology Department, University of Health Sciences (UHS) Lahore. Samples collection was done from Rheumatology department Sheikh Zaid Hospital, Lahore by purposive sampling technique. Samples were included of adult patients comprising both genders (Between 18-65 years of age) diagnosed with SLE (ACR criteria 2012) with LN and without LN. The groundwork was done during January 2015 to December 2015 after approval from the Ethical Review Committee (ERC) and Advanced Study and Review Board of UHS Lahore and ERC of Sheikh Zaid Hospital, Lahore. Patients were divided into 2 groups:

Group I comprised of 31 SLE patients without LN.

Group II comprised of 29 SLE patients with LN.

Sample size (n = 25) was calculated by the following formula:

$$n_1 = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2(\sigma 12 + \sigma 22)}{(\mu 1 - \mu 2)^2}$$

α = Desired Level of Significance = 5%
1-β =1power of study = 90%
μ1=1mean of controls
μ2=1mean of cases
σ1=1Standard deviation of controls
σ2=1Standard deviation of cases (15)
n= 1Sample Size = 25

Serum NGAL level was measured by ELISA method and determined by commercially available ELISA kit (Bioassay Technology Laboratory, China).

Sample Preparation: Three (3ml) of blood from anterior cubital vein of each subject was obtained in sterile container after written informed consent. Serum was separated by centrifugation and stored at - 80°C. The standard solutions of reagent were diluted by using following method

Table showing the dilutions of standard solution

Standard No.	Concentration ng/ml	Dilution
05	1600	120 µl standard diluent+120 µl original standard
04	800	120 µl standard 5 + 120 µl standard diluent
03	400	120 µl standard 4 + 120 µl standard diluent
02	200	120 µl standard 3 + 120 µl standard diluent
01	100	120 µl standard 2 + 120 µl standard diluent

Chemicals were brought to the temperature of 25° (room temperature) prior to use. After setting the strips in the frames,

50µl standard solution was poured to the standard well. Forty (40) µl test serum was added to sample wells then 10µl anti-NGAL antibody was dropped to sample wells, after that 50µl streptavidin-HRP was added to sample wells, blank control and standard wells. The sealer was applied to cover the plate and incubation was done for 60 minutes at 37°C. After uncovering the plate, it was washed 5 times with wash buffer. It was soaked with 0.35 ml wash buffer for 30 seconds for each wash. The well plate was then dried onto paper towels. In the next step 50µl substrate solution A was added to each well which was followed by addition of 50µl substrate solution B to each well. The plate received a second incubation after being covered with a fresh sealer for 10 minutes at 37°C and in the dark. Each well received 50 microliters of stop solution, which quickly caused the blue colour to turn yellow. Within 30 minutes of injecting the stop solution, the optical density (OD) of each well was measured using a microplate reader at 450 nm. (Liu et al., 2005). The results were calculated after obtaining readings of each standard & sample. The standard curve of absorbance was made by using ELISA reader's computer software (BD USA). The detection range of the kit was 10ng/ml – 3000ng/ml and the sensitivity of the kit was 5.01ng/ml.

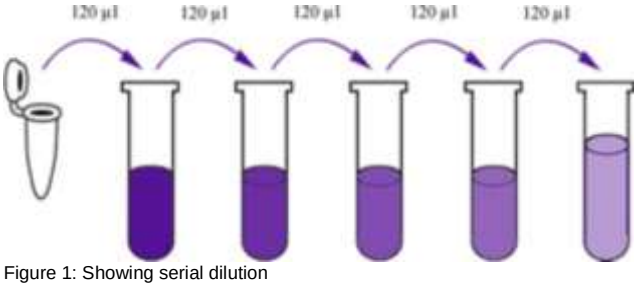

Figure 1: Showing serial dilution

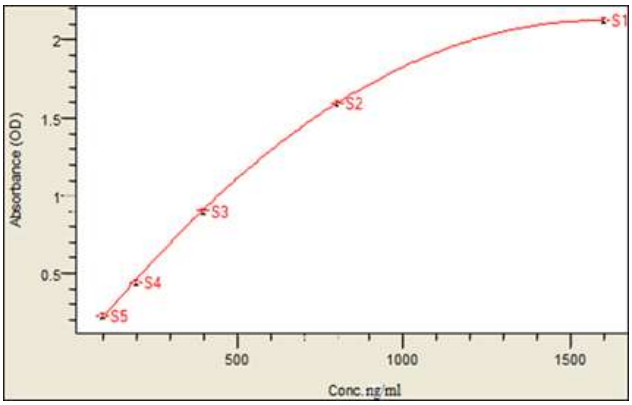

Figure 2: Standard curve showing the concentration of NGAL (ng/ml) on the X-axis and absorbance (OD) at 450nm on the Y-axis.

Statistical Analysis: SPSS 20.0 software was used to analyze the data. Mean ±SD was given for quantitative variables like serum NGAL. Frequencies & percentages were given for qualitative variables like gender.

Two independent `t` test was applied to compare serum NGAL levels using cases and controls. A p-value of ≤ 0.05 was taken as significant statistically.

RESULTS

Sixty serum samples were drawn from patients of SLE which were clinically diagnosed according to ACR criteria (2012). Among the selected patients, 31 were Lupus patients with Nephritis and 29 were SLE patients without LN. Among SLE patients with nephritis 28 were women & 3 were men while among SLE patients without nephritis, there were 28 females and 1 male. Mean age of female patients was 27.09 years & it was 22 years for male patients. The laboratory parameters for the diagnosis of SLE were ANA, C3 and C4 levels (Table 1).

Table 1: Comparison of Mean ± S.D of diagnostic laboratory parameters between two groups

Characteristic	SLE with nephritis	SLE without nephritis	p value
ANA	Positive	Positive	
C3 (mg/dl) Mean±SD	1.45±0.67	1.55±0.68	0.57
C4 (mg/dl) Mean±SD	1.51±0.67	1.62±0.67	0.55

*p ≤ 0.05☐statistically significant

Additional to the above mentioned parameters, further renal function tests including serum creatinine, blood urea nitrogen (BUN), and 24hr urinary proteins levels were also noted (Table 2). Comparison of mean ± S.D between both groups is also noted (Table 2, 3).

Table 2: Comparison of Mean ± S.D of renal parameters between two groups

	Mean ± S.D		
	SLE with nephritis	SLE without nephritis	p- value
Creatinine (mg/dl)	1.96±0.17	1.03±0.18	0.00☐
Urinary proteins (mg/24hrs)	1.00±0.00	1.96±0.18	0.00☐
BUN (mg/dl)	1.19±0.40	1.10±0.30	0.33

*p ≤ 0.05 = statistically significant

Table 3: Comparison of Mean ±SD of serum NGAL between two groups

Groups	NGAL Mean ±SD(IU/ml)	p- value
SLE with lupus nephritis	475.74±190.70	
SLE without lupus nephritis	12.00±10.02	0.00*

*p ≤ 0.05 = statistically significant

The correlation analysis of different parameters in the study was made (Table 4) and Table 5)

Table 4: Correlation analysis of renal parameters in patients with LN

Variables		Dependent variables			
		Creatinine	Urinary proteins	BUN	NGAL
Creatinine	R	1	0.22	-0.28	-0.01
	P	----	0.22	0.12	0.91
Urinary proteins	R	0.22	1.00	0.62	0.03
	P	0.22	-----	0.00	0.83
BUN	R	-0.28	0.62	1.00	0.11
	P	0.12	0.00	----	0.53
NGAL	R	-0.01	0.03	0.11	1.00
	P	0.91	0.83	0.53	----

Table 4 Correlation analysis. R: Correlation Coefficient,(+) positive correlation, (-) negative correlation, 0-0.3 weak correlation, 0.4-0.6 intermediate correlation, 0.7-0.9 strong correlation.

Table 5: Correlation analysis of renal parameters inpatients without LN

Variables		Dependent variables			
		Creatinine	Urinary proteins	BUN	NGAL
Creatinine	R	1	0.22	0.27	0.01
	P	----	0.24	0.14	0.94
Urinary proteins	R	0.22	1	0.58	-0.14
	P	0.24	----	0.00	0.44
BUN	R	0.27	0.58	1	-0.12
	P	0.14	0.00	-----	0.51
NGA=-L	R	0.01	-0.14	-0.12	1
	P	0.94	0.44	0.51	------

Table 5 Correlation analysis. R: Correlation Coefficient, (+) positive correlation, (-) negative correlation, 0-0.3 weak correlation, 0.4-0.6 intermediate correlation, 0.7-0.9 strong correlation.

DISCUSSION

In the present study, mean ±SD value of NGAL was measured in patients of SLE with LN & without LN. Comparison of the two

groups showed a statistically significant difference (p=0.00). These results are in accordance with the study conducted by Nakhjavani et al., (2018)[14] on Iranian patients.

As far as other renal disorders are concerned, the current study is in accordance with Bolignano, Xiang and Bolignano [16,17,18], who documented increased level of serum NGAL in patients with chronic kidney disease (CKD) e.g. in polycystic kidney disease, IgA nephropathy, dysplasia, obstruction, LN and glomerulonephritis. The current study is in accordance with Devarajan[19] who suggested raised level of NGAL in acute kidney injury (AKI) or acute renal failure. However, scientist suggested that NGAL is not a reliable predictor of kidney damage in multiple diseases that leads to AKI as this is mainly due to the inability to specifically measure NGAL released by the tubular cells, unpredictable release and complex nature of the molecule [20].

The present study is not in accordance with the research findings of Rhee et al., (2015)[21] who have found that serum NGAL level alone cannot be used to find out the renal damage in persons suffering from IgA nephropathy.

This study is consistent with findings of Zhao et al., (2010)[27] who documented significant increase in the serum creatinine level among SLE patients with nephritis. Similar findings were also reported by Koyama et al., 2005)[25], as they suggested significantly high serum creatinine level in patients with LN than in patients without nephritis

The 24hr urinary protein levels were noted in both the groups. Mean±SD of SLE patients with LN was 1.0±0.00 mg/24hrs and that of SLE patients without LN was 1.96±0.18mg/24hrs,their comparison showed significant difference (p=0.00).

Present study is in accordance with Houssiau et al., (2012)[22], they suggested that 24hr urinary protein measurement should be done as part of a complete initial evaluation of the patient with SLE and possible LN.

BUN was noted in both the groups. Mean± SD of patients with LN was 1.19±0.40mg/dl and that of without LN was 1.10±0.30mg/dl on comparison there was no documentable difference between these two (p= 0.33).These findings are not in accordance with the study conducted by Satirapoj et al.,[9] they reported that patients of Lupus with kidney disease had high BUN level as compared to those patients who had no kidney damage.

In a study conducted by Caregaro et al., (1994)[24] serum creatinine was correlated with GFR but no correlation was seen among creatinine level in serum and plasma NGAL in patients suffering with AKI due to liver cirrhosis. These findings goes with the findings of current study but reason of AKI in this study is SLE. Beier et al., (2011)[23] showed in their study that an increase in the level of BUN in patients with normal creatinine proved fatal in patients of renal ailments.

Current study showed that among the SLE patients without nephritis serum creatinine had weak positive correlation with 24hr urinary proteins, BUN and NGAL, on the other hand, serum NGAL had weak negative correlation with 24hr urinary proteins and BUN. In a prospective study conducted by (de Nicola et al. 2011)[26] patients with stable CKD, transient azotemia, AKI and normal kidney function were selected, and the plasma NGAL levels of these patients were evaluated.

This study indicates that NGAL level is more suitable in predicting AKI, which is in accordance with current study, than in diagnosing CKD.

CONCLUSION

Serum NGAL level was significantly raised in the patients of SLE with lupus nephritis as compared to the patients of SLE without lupus nephritis.

Recommendations: Further studies with larger sample size and involving multiple healthcare centers should be conducted to validate the diagnostic and prognostic value of serum NGAL.

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