Journal of Immunology, Infection & Inflammatory Diseases

Research Article

Increased Level of Urinary Neutrophil Gelatinase Associated Lipocalin Protein in Patients of Lupus Nephritis

Sabrina Rasheed[1], Nadeem Afzal[1]*, Nauman Tarif[2], Faheem Shahzad[1] and Tafazul H Mahmood[3]

[1]Department of Immunology, University of Health Sciences Lahore, Pakistan
[2]Department of Nephrology, Fatima Memorial Hospital, Lahore, Pakistan
[3]Department of Rheumatology and Immunolgy, Shaikh Zayed Hospital, Lahore, Pakistan

This article was published in the following Scient Open Access Journal:
Journal of Immunology, Infection & Inflammatory Diseases

Received January 22, 2016; **Accepted** February 10, 2016; **Published** February 17, 2016

Abstract

Background: Systemic Lupus Erythematous (SLE) is an autoimmune disease characterized by the production of numerous auto antibodies that can affect multiple organs of the body. Most often SLE affect heart, joints, skin, lungs, blood vessels, liver, and kidneys. Lupus Nephritis (LN) is one of the common complications of SLE. Once renal tubules are damaged, they release a protein in urine i.e. Neutrophil gelatinase Associated Lipocalin (NGAL) which is as an indicator of an insult to kidney.

Objective: To determine the level of urine NGAL (μNGAL) of patients of SLE with and without nephritis.

Method: This was a comparative study, which included 164 subjects who were arranged in two groups. Group I included SLE patients without LN and Group II had SLE patients who had developed LN. There were 82 subjects in each group. Level of μNGAL was determined by ELISA technique and the data was analysed using SPSS 20.0.

Results: Highest median and IQR of μNGAL was observed in group II [2.45(1.85-3.70)] as compared to group I [1.41(1.13-1.95)] and on comparison statistically significant difference was observed ($p<0.001$). In both the groups there was no correlation with clinical manifestations i.e. photosensitivity, malar rash, oral ulcer, alopecia, arthralgia, serositis, and Raynaud's phenomenon.

Conclusion: Level of μNGAL was significantly increased in patients of LN as compared to SLE patients without nephritis.

Keywords: Systemic lupus erythematous, Lupus nephritis, Neutrophil gelatinase associated lipocalin

Introduction

Systemic Lupus Erythematous (SLE/Lupus) is prototypic multi organ autoimmune disorder [1]. In the USA, SLE has been documented as 2.0 to 7.6 cases per 100,000 persons per year[2] and its prevalence was 51 per 100,000 individuals [2,3]. In North America, South America, and Europe the estimated incidence of SLE was 2 to 8 per 100,000 per year [3]. Like other autoimmune disorders, SLE is more commonly seen in females as compared to males, and it is prevalence is about 9 times high in females [4]. The exact cause of SLE is not known, but multiple factors such as racial, genetic, hormonal, and environmental are associated with this disease [5,6]. Clinical manifestations of SLE can involve many organs and tissues e.g. lungs, kidneys, brain, skin etc [1].

Mortality rate due to Lupus Nephritis (LN) is very high and 5 and 10-year survival is 83%-92% and 74-84%, respectively [7]. According to the ACR criteria, 0.5 g of protein in the urine per 24 hours or more than 3+ score of dipstick confirms the diagnosis of LN. In SLE, if there is an increased level of serum creatinine and presence of blood and pus in urine in the absence of infection and menses indicate renal involvement [8,9]. Renal biopsy is a definitive way to assess the damage of renal tissue.

Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a protein of 25kD molecular weight and it belongs to the superfamily named lipocalin. Normal renal tubular cells release very low amount of NGAL but in response to injury many inflammatory cells, epithelial and endothelial cells rapidly release NGAL [10-12]. In ischaemic injuries of kidneys NGAL is accumulated at high concentration in cortical tubules of kidney, blood and urine [13]. As the kidney injury becomes severe, urinary and plasma level of NGAL increases significantly [14]. Once 50% or more kidney function is lost, only then serum creatinine starts to rise and it takes several hours to days to be detected but NGAL is an early biomarker of acute

***Corresponding Author:** Dr Nadeem Afzal, Professor and Head, Department of Immunology, University of Health Sciences, Lahore, Pakistan,
Tel: 92-321-4086-452; 92-42-99231304 extn – 343,
Email: immunology@uhs.edu.pk

kidney injury [13]. uNGAL has been documented for acute kidney injury in burns, cirrhosis, kidney surgery patients [15-17]. This study was designed to determine the level of uNGAL in patients of SLE with and without LN.

Patients and Methods

It was a cross sectional study that included 164 SLE patients and it was performed in the Department of Immunology, University of Health Sciences (UHS) Lahore. It was approved by the Ethical Review Committee and research boards of the University of Health Sciences Lahore and Sheikh Zayed Hospital, Lahore Pakistan. After getting an informed written consent, urine samples of the patients were collected from the Department of Immunology & Rheumatology Sheikh Zayed Hospital, Lahore. Study subjects were made into two groups; 82 subject in each i.e. Group-I had SLE patients without LN while Group-II had SLE patients of LN. These patients were diagnosed according to the criteria of American College of Rheumatology (ACR). These patients were positive for Anti-Nuclear Antibody (ANA) or anti double stranded DNA antibody (anti-ds DNA). Laboratory investigations like serum creatinine and 24 hours urinary protein of these subjects were recorded. The level of µNGAL was determined by enzyme linked immunosorbent assay (ELISA) (Glory Science Co., Ltd, China).

Data analysis

The data was entered and analysed using IBM SPSS (Statistical Package for Social Sciences) 20.0. Median and Inter Quartile Range (IQR) was used for quantitative variables e.g. age, disease, etc. Qualitative variables were expressed as frequencies and percentages e.g. gender, photosensitivity, etc. The data was not normally distributed therefore Mann-Whitney-U test was applied for comparison of µNGAL between the two groups. Chi-square test was applied to determine association of clinical manifestations of SLE. A *p*-value of ≤ 0.05 was considered as statistically significant.

Results

Demographic data

The current study comprised of 157 (95.7%) females and 7 (4.3%) males.The number of females was high in group-I 80 (97.6%) as compared to group-II 77 (93.9%) whereas the frequency of males was high in group-II 5 (6.1%) as compared to group-I 2 (2.4%) and on comparison it was not statistically significant. Gender distribution and its comparison is shown in Figure 1.

The median and Inter Quartile Range (IQR) of age of the patients was same in group-I and group-II and on comparison it was not statistically significant. The median and IQR of disease duration was high in group-II as compared to group-I and on comparison it was not statistically significant. The demographic data of the study subjects is summarized in Table 1.

µNGAL, 24 hours urinary protein, Serum creatinine

Median and IQR of µNGAL was high in group-II as compared to group-I and on comparison it was statistically significant (p<0.001). Median and IQR of 24 urinary protein was high in group-II as compared to group-I and on comparison it was statistically significant (p<0.001). Median and IQR of serum creatinine was high in group-II as compared to group-I and on comparison it was statistically significant (p<0.001). Level of µNGAL, 24 hours urinary protein, serum creatinine and their comparison between two groups is shown in Table 2.

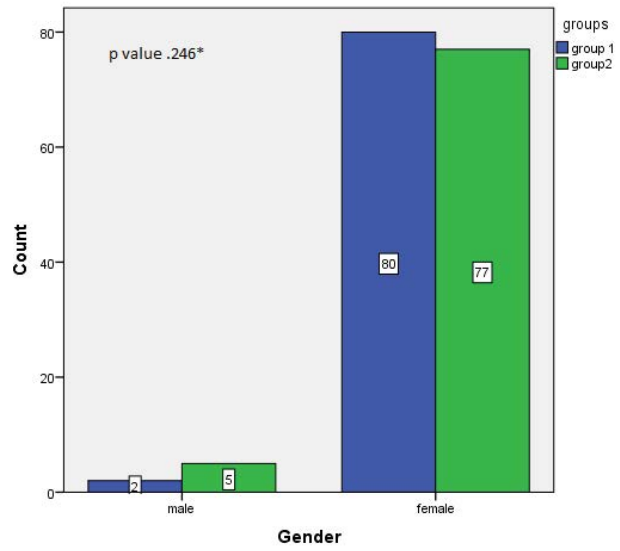

Figure 1: Gender distribution and its comparison in the studied population

Table 1: Median, IQR of age, disease duration and their comparison between two groups

Parameter	Group-I Median (IQR)	Group-II Median (IQR)	p value
Age (years)	30 (23-38.25)	30 (23-36)	0.874
Disease duration (months)	36 (24-60)	48 (24-96)	0.093

p≤0.05: statistically significant; IQR: Interquartile range

Table 2: Level of µNGAL, 24 hours urinary protein, serum creatinine and their comparison between two groups

Parameter	Group-I Median (IQR)	Group-II Median (IQR)	p value
µNGAL (µg/L)	1.41(1.13-1.95)	2.45(1.85-3.70)	<0.001*
24 hours urinary protein (mg/24hrs)	81.0(58.25-110.0)	317.50(220-580)	<0.001*
Serum creatinine (mg/dl)	0.7(0.6-0.8)	0.8(0.7-1.0)	<0.001*

*p ≤0.05 statistically significant; IQR: Interquartile range

ANA and dsDNA

Frequency of ANA was high in group-I as compared to group-II and on comparison it was not statistically significant. Frequency of dsDNA was high in group-I as compared to group-II and on comparison it was not statistically significant. Number, percentage and comparison of subjects with positive ANA and dsDNA test between two groups is shown in Table 3.

Clinical manifestations of SLE

Photosensitivity was observed in 72.6% and the subjects suffering from photosensitivity were more in group-II 61 (74.4%) as compared

Table 3: Number, percentage and comparison of subjects with positive ANA and dsDNA test between two groups

Parameter	Groups	n (%)	p value
ANA	group-I	67(81.7%)	0.099
	group-II	58(70.7%)	
dsDNA	group-I	79(96.3%)	0.304
	group-II	76(92.7%)	

n: number; %: percentage; p ≤0.05 statistically significant

Table 4: Number, percentage and comparison of clinical manifestations between two groups

Signs	Groups	n(%)	p-value
Photosensitivity	group-I	58(70%)	0.600
	group-II	61(74.4%)	
Malar rash	group-I	52(63.4%)	0.083
	group-II	41(50.0%)	
Oral ulcer	group-I	40(48.8%)	0.876
	group-II	41(50.0%)	
Alopecia	group-I	40(48.8%)	0.208
	group-II	32(39.0%)	
Artharalgia	group-I	40(48.8%)	0.348
	group-II	46(56.1%)	
Serositis	group-I	20(24.4%)	1.000
	group-II	20(24.4%)	
Raynaud's phenomenon	group-I	28(34.1%)	0.869
	group-II	27(32.9%)	
Proteinuria	group-I	44(53.7%)	<0.001*
	group-II	60(73.2%)	

n: number; %: percentage *p ≤0.05 statistically significant

to group-I 58 (70.7%) and on comparison it was not statistically significant. Malar rash was observed in 56.7% and the number was more in group-I 52 (63.4%) as compared to group-II 41 (50.0%) and on comparison it was not statistically significant. Oral ulcers were observed in 49.4% and the number was high in group-II 41 (50.0%) as compared to group-I 40 (48.8%) and on comparison it was not statistically significant. Alopecia was observed in 43.9% and the number was high in group-I 40 (48.8%) as compared to group-II 32 (39.0%) and on comparison it was not statistically significant.

Arthralgia was observed in 52.4% and the number was high in group-II 46 (56.1%) as compared to group-I 40 (48.8%) and on comparison it was not statistically significant. Serositis was observed in 24.4% and the number was same in group-I 20 (24.4%) and group-II 20 (24.4%) and on comparison it was not statistically significant. Raynaud's phenomenon was observed in 33.5% and the number was high in group-I 28 (34.1%) as compared to group-II 27 (32.9%) and on comparison it was not statistically significant.

Proteinuria was observed in 63.4% and the number was high in group-II 60 (73.2%) as compared to group-I 44 (53.7%) and on comparison it was statistically significant (p<0.001). Number, percentage and comparison of clinical manifestations between two groups is shown in Table 4.

Discussion

The current study comprised of 95.7% females and 4.3% males with the median age of 30 years which is in agreement with Leung et al. and Hochberg et al., who included 92.7% and 92% females and 7.3% and 8% males respectively [18,19]. Regarding the age of the subjects the current study is in agreement with Pitashny et al., who documented median age of the subjects as 35 years [20].

Median and IQR of μNGAL was high in group-II as compared to group-I and on comparison the difference was statistically significant (p<0.001). It is in agreement with Pitashny et al., who detected significantly high level of urinary lipocalin-2 in LN (median 17.1, IQR 10.3-45.4) as compared to patients without LN (median 11.2, IQR 3.1-20.3) [20]. The current study is also in agreement with Hammad et al., who documented increased level of μNGAL in patients of LN as compared to patients without LN (p<0.001) but Hammad et al., conducted the study in paediatric population [21].

Median and IQR of 24 hours urinary protein was high in group-II as compared to group-I and on comparison the difference was statistically significant (p<0.001). It is in agreement with Marques et al., who documented its increased level in LN patients but Marques et al., did not detect its level in SLE patients without nephritis [22].

Median and IQR of serum creatinine was high in group-II as compared to group-I and on comparison the difference was statistically significant (p<0.001). It is in concordance with Mok et al., who also reported elevated level of serum creatinine in LN patients [23].

Proteinuria was observed in 63.4% and its frequency was significantly high in group-II as compared to group-I. It is not in concordance with Mok et al., who reported it in 50% of patients [24]. Disagreement could be due to larger sample size (709 SLE patients) in the study of Mok et al., or due to ethnic differences as Mok et al., investigated Hong Kong population [24].

Conclusion

In this study, level of μNGAL was significantly increased in SLE patients with nephritis as compared to SLE patients without nephritis but these findings should be confirmed by studies on larger sample size.

Further suggestions: There should be studies on the determination of serum level of NGAL.

Limitations of the study: The study was performed on the small sample size and serum level of NGAL should also be determined.

Acknowledgments

The authors are grateful to University of Health Sciences Lahore and Miss Afia Shahid, Lab Manager, Department of Immunology UHS Lahore, for their technical and logistic support.

References

1. Miah T, Hoque M.A, Mahmood T, Tarafder BK. Clinical profile, management and outcome of lupus. *Mymensingh Med J.* 2008;17(2 Suppl):S6-S11.
2. Bongu A, Chang E, Ramsey-Goldman R. Can morbidity and mortality of SLE be improved? *Best Pract Res Clin Rheumatol.* 2002;16(2):313-332.
3. Bertsias G, Ioannidis JPA, Boletis J, et al. EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. *Ann Rheum Dis.* 2008;67:195-205.
4. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus*. 2006;15(5):308-318.
5. Cooper GS, Dooley MA, Treadwell EL, Clair EW, Parks CG, Gilkeson GS. Hormonal, environmental and infectious risk factors for developing systemic lupus erythematosus. *Arthritis Rheum.* 1998;41(10):1714-1724.
6. D'Cruz DP, Khamashta MA, Hughes GR. Systemic lupus erythematosus. *Lancet*. 2007;369(9561):587-596.
7. Mok CC. Prognostic factors in lupus nephritis. *Lupus*, 2005;14(1):39-44.
8. Christopher SL, Petri M, Astor BC, Fine D. Urine protein-to-creatinine ratio is a reliable measure of proteinuria in lupus nephritis. *J Rheumatol*. 2004;31:1557-1559.
9. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol.* 2004;15(2): 521-530.

10. Bolignano D, Donato V, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis.* 2008;52(3):595-605.

11. Rubinstein T, Pitashny M, Putterman C. The novel role of neutrophil gelatinase-B associated lipocalin (NGAL)/ Lipocalin-2 as a biomarker for lupus nephritis. *Autoimmun Rev.* 2008;7**(**3):229-234.

12. Ding H, He Y, Li K. et al. Urinary neutrophil gelatinaseassociatedlipocalin (NGAL) is an early biomarker for renal tubule interstitial injury in IgA nephropathy. *Clin Immunol.* 2007;123**(**2):227-234.

13. Mori K, Lee HT, Rapoport D, et al.. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest.* 2005;115:610-621.

14. Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol.* 2008;3:665-673.

15. White CE, Cohn S, Chung K, Barasch J. uNGAL a novel biomarker predicts acute kidney injury in truma and burn patients. *J of the American College of Surgeons*. 2011;213(3):S49.

16. Treeprasertsuk S, Wongkamjana A, Januvongvancih V, Sasipim Sallapant, Tiranathanagul K, Komolmit P. 590 Urine Neutrophil Gelatinase-Associated Lipocalin (UNGAL) Level: A Valid Diagnostic and Prognostic Marker for Acute Kidney Injury in Cirrhotic Patients. *Gastroentrology*. 2015;148(4): S-998.

17. Sprenkle PC, Wren J, Maschino AC, et al. Urine Neutrophil Gelatinase-Associated Lipocalin (uNGAL) as a Marker for Acute Kidney Injury in Kidney Surgery Patients. *J Urol.* 2013;190(1): 159-164.

18. Leung YY, Szeto CC, Tam LS, et al. Urine protein-to-creatinine ratio in an untimed urine collection is a reliable measure of proteinuria in lupus nephritis. *Rheumatology(Oxford).* 2007;46(4):649-652.

19. Hochberg MC, Boyde RE, Ahearn JM, et al. Systemic lupus erythematosus: A review of clinic-laboratory features and immunologic markers. *Medicine (Baltimore).* 1985;64(5):285-295.

20. Pitashny M, Schwartz N, Qing X, et al. Urinary Lipocalin-2 Is Associated With Renal Disease Activity in Human Lupus Nephritis. *Arthritis Rheum.* 2007;56**(**6):1894-1903.

21. Hammad A, Mosaad Y, Elhanbly S, et al. Urinary neutrophil gelatinase-associated lipocalin as a marker of severe lupus nephritis in children. *Lupus.* 2013; 22(5):486-491.

22. Marques MG, Cotovio P, Ferrer F, et al. Random spot urine protein/creatinine ratio: a reliable method for monitoring lupus nephritis. *Clin Kidney J.* 2013;6(6):590-594.

23. Mok CC, Tong KH, To CH, Siu YP, Au TC. Tacrolimus for induction therapy of diffuse proliferative lupus nephritis: An open-labeled pilot study. *Kidney Int*. 2005;68(2): 813-817.

24. Mok CC, Lau CS. Lupus in Hong Kong Chinese. *Lupus*. 2003;12(9):717-722.