Original Article

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Urinary Interleukin 6: A Prognostic Bio Marker in Lupus Nephritis

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Abstract

Background: Lupus nephritis is an frequent and potentially serious complication of SLE. It is an inflammatory disease of connective tissue and immune system, where we find organs and cells undergo damage initially mediated by tissue-restricting auto antibodies and immune complexes. Our aim was to evaluate the association of urinary interleukin 6 in lupus nephritis patients and validate its use as a prognostic marker. For this we took the complete demographic profiling of the participant patients along with all biochemical parameter were checked. For IL-6 analysis, we analyzed the whole immunological profile and correlated the level of urinary proteins, serum creatinine and urinary IL-6. The mean values of urinary IL-6 at presentation and after 6 months of treatment were calculated. Any change after 6 months was noted and correlation of Il-6 with disease activity scores was observed. Urinary IL-6 was found to be significantly high in patients of lupus nephritis than controls. Urinary IL-6 was higher in patients of severe form of lupus nephritis such as Class IV than other classes of lupus nephritis. Urinary IL-6 may be used as a prognostic marker of lupus nephritis. The patients who achieved complete remission had near normal levels of urinary IL-6 and patients with partial remission had decreased level of urinary IL-6 than at presentation after 6 months of induction phase treatment. But patients with no remission did not show any significant decrease in urinary IL-6 levels at 6 months. So, urinary Il-6 may be used to monitor the response to therapy.

Keywords: Urinary IL-6, Lupus nephritis, Bio marker, Urinary protein.

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Introduction

Systemic Lupus Erythematosus, commonly known as "Lupus or SLE", is an inflammatory disease of connective tissue and immune system. It is an immune system ailment wherein organs and cells experience harm at first interceded by tissue-restricting auto antibodies and immune complexes.[1] This is an chronic, multisystem, immune system condition which is portrayed by the nearness of auto antibodies to atomic material and immune system deposition in included tissues. While various methodologies have been made in unwinding the anticipation of this type of infection, it remains incompletely comprehended.[2] Renal involvement is one of the important prognostic factors for SLE. Lupus nephritis (LN) is present in 20-75% of patients depending on ethnicity and age. It is more common in younger individuals and individuals of African or Asian ancestry. End stage renal disease is observed among 25 to 30% of the LN patients.[1,2,3,4]Over the last few years there has been a growing interest in searching novel biomarkers which could predict future renal involvement or monitor renal function for early clinical identification, risk stratification and therapy adjustment.[5]

IL-6 is a pleiotropic cytokine expressed by antigen-presenting cells such as macrophages, B-cells, and dendritic cells and isalso produced by fibroblasts, mesangial cells, T cells, endothelial cells, epithelial cells, keratinocytes, and astrocytes.[6] Because of its purported role in the pathogenesis oflupus nephritis, urinary IL-6 has been a potential biomarker of interest. Urinary levels of IL-6 in 29 patients with active lupus nephritis were measured, and patients with WHO class IV nephritis on renal biopsy were found to have significantly higher IL-6 levels than other classes of nephritis ($p<0.01$).[7,8] So a study might be essential to clinically establish the correlation of LN and SLE with the novel biomarker. It can also be helpful to compare the efficacy between the classical and the newer parameter. So taking these factor into account here we evaluated the association of IL-6 in lupus nephritis patients to find a biomarkers in lupus nephritis 32 patients.

Subjects and Methods

Thirty two cases of lupus nephritis patients were included in the study**.** Patients with SLE with lupus nephritis diagnosed by history, biochemical and immunological tests and by light microscopy and immunofluorosecence of renal biopsy specimens were included in the study. The exclusion criteria was inability to obtain informed consent and SLE patients

without renal involvement. Complete demographic profiling, biochemical parameter, whole immunological profile and correlation of urinary proteins and urinary IL-6 were done The mean values of urinary IL-6 at presentation and after 6 months of treatment were calculated.

IL-6 was measured by Enzyme Amplified Sensitivity Immunoassay using human IL-6 EASIA kit (DIA Source Belgium). The procedure was done according to the manufactures direction. A calibration curve was plotted and IL-6 concontration in samples was determined by interpolation from the calibration curve. The use of the EASIA reader and sophisticated data reduction method result in high sensitivity in low range and in extended calibration range. The minimum detectable limit of human IL-6 was 2 pg/ml for this kit. The calculated intra-assay coefficient of variation was 4.2% and inter-assay variability was 5.4%.

The statistical data analysis was done through SPSS 21.0 Software. The association were obtained by student t-test. A p value <0.05 considered as significant.

Results

This study was conducted in the department of Nephrology, Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University (BHU) from January 2015 to June 2016 and during this period we enrolled 32 patients of SLE diagnosed based of Systemic Lupus International Collaborating Clinic (SLICC) criteria, who presented with renal involvement. They were compared to 20 healthy controls. All 32 patients underwent renal biopsy.

Table 1: Demographic profile of Patients and control

	Patients	Controls
Total number	32	20
Age (Mean±SD)	28.68 ± 9.28	27±9.3
Sex (M:F)	1:7	1:3
Patient presented in Pregnancy	1	
Class of LN in renal biopsy (II:III:IV:V)	2:6:19:5	
Outcome of Follow up CR:PR:NR : Death :Lost Follow up	16:6:3:2:5	
Cause of death	Sepsis:1, renal failure:1	

CR, Complete remission ; PR, Partial remission; NR, No remission.

Most common age group was affected 21-30 years (56.25 %) and out of this 94 % (n=17) and 6% (n=1) were female and male respectively. This was followed by 31-40 years (18.7 %) age group.

Table 2: Basic biochemical profiles of patients

Parameter	Range	Mean±SD
SBP (mmHg)	100-188	139.37 ±23.57
DBP (mmHg)	60-104	84.68 ± 11.26
Haemoglobin(gm/dL)	6.5-15.1	9.26± 1.95
WBC (per cumm)	18700-1420	7276.43± 5047.29
PLC (lac/Cumm)	0.24-3.38	1.535 ± 0.89
s. Creatinine (mg/dl)	0.6-7.5	2.53±1.92
s. Urea (mg/dl)	24-256	107.28±77.3
s. Sodium (mmol/L)	122-149	134.6±6.07
s. Potassium (mmol/L)	2.0-6.1	4.68±0.94
SGOT (IU/L)	21-100	40.34±17.4
SGPT (IU/L)	24-77	38.03±13.32
s. Albumin (gm/dl)	1.2-4	2.79±77.3
s. Globulin (gm/dl)	1.5-3.6	2.93±0.49
RBS (mg/dl)	80-154	116.9±17.5
24 hr Urinary Protein(mg)	575- 8500	2386 ± 1810

[Table-2] shows the mean and standard deviation of basic biochemical parameters, blood pressure and 24 hours urinary protein. The mean and standard deviation of SBP and DBP were 139.37 ±23.57mmHg & 84.68 ± 11.26mmHg respectively. The mean and standard deviation of haemoglobin, total leukocyte count, and platelets were 9.26± 1.95 gm/dl (6.5-15.1 gm/dl), 7276.43± 5047.29 per cumm (18700-1420cumm) and 1.535 ± 0.89 lac/dl (0.24-3.38 lac/dl) respectively. The mean and standard deviation of serum albumin, serum globulin , serum urea, serum creatinine and random blood sugar level were 2.79±77.3 gm/dl (1.2-4gm/dl), 2.93±0.49gm/dl (1.5-3.6gm/dl), 107.28±77.3 mg/dl (24-256mg/dl), 2.53±1.92mg/dl (0.6-7.5 mg/dl) and 116.9±17.5mg/dl (80-154mg/dl) respectively. The mean and standard deviation of serum sodium, potassium, SGOT and SGPT were134.6±6.07mmol/l (122-149 mmol/l), 4.68±0.94 mmol/l (2.0-6.1 mmol/l), IU/L40.34±17.4 (21-100IU/L) and 38.03±13.32 IU/L (24-77IU/L) respectively. 24 hour urinary protein was in range of 575- 8500 mg; mean and standard deviation was 2386 ± 1810 mg.

Table 3: Immunological profile of patients in different classes of LN

	ISN/RPS Class of LN				Total	
	II	III	IV	V	Cases	%
No. of cases	2	6	19	5	32	100
ANA (quantitative)	4.25± 3.88	4.4 ±1.1	5.31±2.98	3.7±2.11	4.82 ±2.61	
ANA (+)	2	6	18	5	31	96.8
Antids DNA (quantitative)	126.5 ±102.53	125.3±43.47	125.1±120.5	91±60	119.9±98.4	
Antids DNA (+)	2	6	13	4	25	78
C3 (quantitative)	110.5 ±92.63	63.5 ±30.38	52.94±28.56	59.2±27.94	59.5 ± 34.6	
C3 ↓	1	4	16	4	25	78
C4 (quantitative)	23 ±25.45	5.75±1.75	10.7±8.44	12.18±10.3	10.77 ± 9.55	
C4 ↓	1	6	13	3	23	72

The results of immunological study including complement levels of the cases of lupus nephritis are shown in Table-7. 31 (96.8%) out of 32 patients were positive for ANA, however only 25 (78%) out of 32 patients were positive for anti-dsDNA antibody. Mean C3 level was 59.5 ± 34.6mg/dl and mean C4 level was 10.77 ± 9.55 mg/dl in the patients. C3 was low in 25(78%) patients and C4 was low in 23(72%) out of 32 patients. One patient was positive for APLA and one patient was positive for anti-Smith antibody [Table 3].

Table 4: Distribution of cases according to ISN/RPS classification of LN according to histopathological findings on Renal Biopsy

IPS/ RPS Class of Lupus Nephritis	Number of Cases (n=32)	Percentage(%)
Class II	2	6.25
Class III	6	18.75
Class IV	19	59.4
Class V	5	15.6

All patients with evidence of lupus nephritis flare on history and investigation, underwent ultrasound guided percutaneous

renal biopsy. Immunoflouroscence was done in 15 pateints. Renal histology slides from these 32 patients were assessed according to ISN/RPS 2003 classification. 2 (6.25%) had Class II, 6 (18.75%) had Class III, 19(59.4%) had Class IV and 5 (15.6%) had Class V LN on histopathological finding [Table-4].

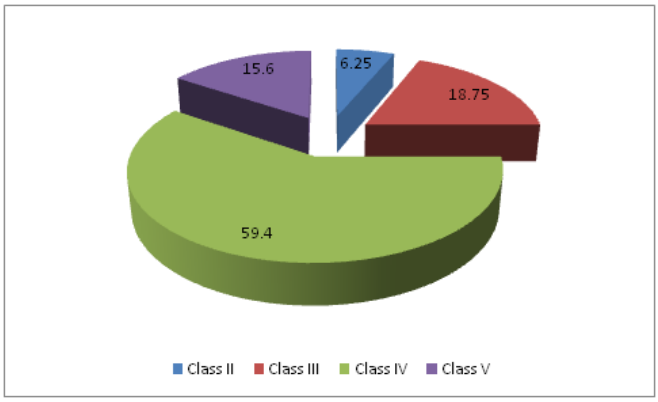

Figure 1:

Table 5: Urinary IL-6 values in Control group and in patients of Lupus Nephritis at flare

Group	Range	Mean ± SD	Z-value	p-value
Control	2.03-9.41	4.707 ± 2.282	6.021	<0.001
LN Patients	14.23-2478	301.582 ± 483.944		

Urinary interleukin levels were assessed in controls and all patients of lupus nephritis at presentation prior or shortly after performing renal biopsy. The mean urinary interleukin levels of control and cases of lupus nephritis at flare were 4.707 ± 2.282 and 301.582 ± 483.944 pg/ml respectively. These values between the control and cases were compared using non-parametric MANN-WHITNEY U-test which showed that the urinary IL-6 levels of patients of lupus nephritis at flare was significantly higher than the control group [Table.5].

Table 6: Correlation between 24 hour Urinary Protein and Urinary IL-6 at in patients of LN at presentation

24 hour Urinary Protein	No.of patients	Urinary IL-6 (Range)	Urinary IL-6 (Mean ± SD)	F-value	p-Value
≤1000mg	5	23.12-1051.00	328.815 ±418.096	0.226	0.799
1001-3500mg	20	28.76-2478.00	333.781 ±572.902		
>3500mg	7	14.23-522.85	190.131 ±188.653		

Patients were devided into 3 groups according to 24 hour urinary protein ≤1000mg, 1001-3500mg or >3500mg. Urinary IL-6 levels in these groups were calculated and compared using ANNOVA to see if there was any correlation between 24 hour urinary protein and urinary IL-6 in patients of LN at presentation. The p-value for this test was 0.799 which showed that it was not significant. So there was no correlation between 24 hour urinary protein and urinary IL-6 at in patients of LN at presentation [Table-6].

Table 7: Outcome of patients after 6 months induction treatment

	CR (%)	PR (%)	NR (%)	Death	Lost F/U
Total (n=32)	16 (50%)	6 (18.75%)	3 (9.3%)	2 (6.25%)	5 (15.6%)
Class II (n=2)	2 (100%)	-	-	-	-
Class III (n=6)	4 (66.67%)	1 (16.67%)	-	-	1(16.66%)
Class IV (n=19)	8(42.1%)	4 (21%)	3 (15.8%)	2 (10.5%)	2(10.5%)
Class V (n=5)	2 (40%)	1 (20%)	-	-	2 (40%)

[Table-7] show outcomes of patients of lupus nephritis after 6 months of induction phase of treatment. Out of the 32 patients of LN, 2 patients (6.25%) died, 5 patients (15.6%) lost follow up, 16 patients (50%) achieved complete remission, 6 patients (18.75%) achieved partial remission and 3 patients (9.37%) did not show any improvement after 6 months treatment. Of the 2 patients died, one died of sepsis and another due to renal failure. In Class II LN two out of two patients had complete remission. In Class III 4 patients (66.67%) out of 6 had complete remission and 1 patient (16.67%) had partial remission. In Class IV which is most severe type LN , only 8 patients (42.1%) out of 19 patients had complete remission, 4 (21%) had partial remission and 3 (15.8%) had no remission. In Class V LN, 2 (40%) out of 5 patients had complete remission, 1 (20%) had partial remission. One from Class III LN, two from Class IV LN and two from Class V LN patients lost follow up during study period.

Table 8: Urinary IL-6 after 6 months induction treatment in CR/PR/NR groups of Patients of LN

Group of patients Total (n=27)	Urinary IL-6 before start of treatment (Mean±SD) (pg/ml)	Urinary IL-6 after 6 months of Induction treatment(Mean±SD) (pg/ml)	Z-value	p-Value
Patients with CR(n=16)	101.152±77.546	10.73±2.778	3.516	<0.001
Patients with PR(n=6)	341.2133±218.677	45.33±14.065	2.201	0.028
Patients with NR(n=3)	1528.666±822.152	854.67±306.523	1.604	0.109

After 6 months of induction phase of treatment 16 patients had complete remission (CR), 6 patients had partial remission(PR) and 3 patients had no remission(NR). The patients were grouped into three groups according to their outcome. The mean values of urinary IL-6 at presentation and after 6 months of treatment were calculated. The values at presentation and after 6 months of treatment were compared for each group using WILCOXON SIGN test. P-value for CR group was <0.001 which implied there was a significant decrease in urinary IL-6 values after 6 months of treatment. The mean values were within normal limit of urinary IL-6. P-value was 0.028 for PR group which again showed that there was a significant decrease in urinary IL-6 values after 6 months of treatment but the values still higher than normal urinary IL-6. P-value for NR group was 0.109

which meant that there was no significant decrease in urinary IL-6 values after 6 months of treatment and the values were still very high [Table-8].

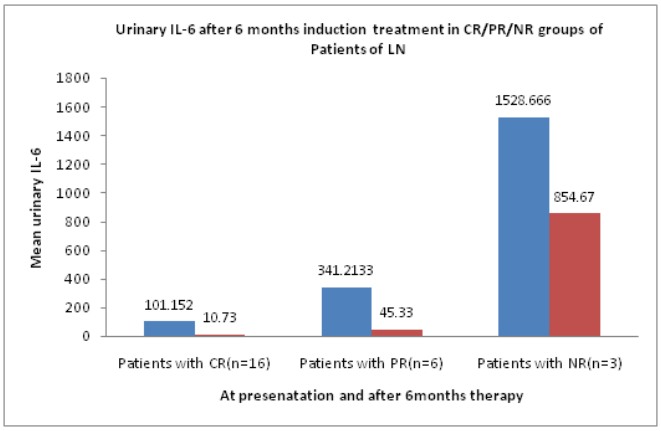

Figure 2:

Discussion

Lupus nephritis (LN) may influence the disease prognosis. A successful treatment of LN requires correct diagnosis, timely intervention and early treatment of any disease complication. Biomarkers which are able to mark disease prognosis at the earliest are still lacking. This study provided special reference to urinary IL-6 as a diagnostic and prognostic biomarker. During this period 32 patients of lupus nephritis were diagnosed and evaluated. 20 healthy persons were taken as controls. Of 32, 28 were females constituting a F:M ratio 7:1.The mean age of the patients presenting with lupus nephritis was found 28.68 ± 9.28 years. The various renal findings noted at presentation of patients were; proteinuria (100%), pedal edema (84%), hematuria (75%), hypertension (47%), and elevated serum creatinine (59.38%).Hematuria was present in 75% patients of all LN and was most commonly present in Class IV LN (100%) patients while none of the Class II LN patients had hematuria. Hypertension was present in 47% patients of lupus nephritis and was most commonly associated with Class III and Class IV LN.[9] Musculoskeletal system was the next commonly involved system after renal symptoms, involved in 56% patients presenting with lupus nephritis with SLE. Athralgia was most common symptom followed by joint stiffness, joint deformity were not noted[10] In this study we got the mean and standard deviation of haemoglobin was 9.26± 1.95 gm/dl (6.5-15.1 gm/dl) in our study. 81% patients of lupus nephritis had anaemia at presentation. 23 pateints had haemoglobin below 10gm% and 4 patients had severe anemia with haemoglobin below 7gm% with that leucopenia (TLC<4000/cmm) was found in 28% and thromobocytopenia (Platelet count < 1 lakh/cmm) also found in 28% of all lupus nephritis patients.[11] All patients (100%) were positive for ANA, however only 78% patients were positive for anti-ds DNA antibody, C3 was low in 78% patients and C4 was low in 72% of patients. Class IV was diagnosed on light microscopy of renal biopsy in 59.4% patients while 6.25% had Class II, 18.75% had Class III, 15.6% had Class V LN on histopathological finding.[12] Immunoflouroscence was done in 15 pateints. IF study in all 15 patients showed IgG, IgM, and C3 deposits in mesangium and GBM. All Class III and Class IV LN patients were given three doses of pulse methyl prednisone followed by oral predinsolone and either monthly pulses of injection cyclophosphamide in 15 patients or oral MMF in 9 patients. Three patients on MMF regimen did not show any remission after 3 months of treatment and their therapy was changed to monthly pulses of injection cyclophosphamide.[13] After 6 months of induction phase of treatment , 68.75% patients achieved some form of remission (complete remission: 50% , partial remission: 18.75%). 9.37% patients did not show any improvement after 6 months treatment. In Class IV which is most severe type LN, only 42.1% patients had complete remission. In Class II LN, which mild form of lupus nephritis two out of two patients had complete remission (100%). In Class III, 66.67% complete remission and in Class V LN, 40% patients had complete remission.

The mean urinary IL-6 levels, a biomarker of lupus nephritis flare, in patients of lupus nephritis at presentation was 301.582 ± 483.944 pg/ml and was significantly higher than the mean urinary IL-6 levels of controls 4.707 ± 2.282pg/ml. (p-value <0.001) The mean values of urinary IL-6 in different ISN/RPS classes of Lupus Nephritis patients based on histopatholgical finding in renal biopsy showed a statistically significant difference between different classes (p-value <0.005). Highest values of urinary IL-6 (475.126±580.90 pg/ml) was seen in Class-IV LN patients which is the most active form of LN and lowest values (28.28 ±7.30pg/ml) were seen in patients of Class-II LN. Class V LN patients had mean urinary IL-6 value of 93.038±195.69 pg/ml which was higher than the mean urinary IL-6 values for Class III LN patients. A significant correlation was found between serum creatinine and urinary IL-6 in patients of lupus nephritis. The patients with severe renal dysnction had highest levels of urinary IL-6 with a mean value 809.412 ±766.336 pg/ml while the patients with normal renal function had lowest levels of urinary IL-6 with a mean value 75.143±67.594 pg/ml. In our study, there was no correlation between 24 hour urinary protein and urinary IL-6 at in patients of LN at presentation. (F-value=0.226, p-value =0.799). After 6 months of induction phase of treatment patients with complete remission had a significant decrease in urinary IL-6 values than the values at presentation. The mean values was within normal range of urinary IL-6 in patients with complete remission at 6 months. The patients with partial remission again showed that there was a significant decrease in urinary IL-6 values after 6 months of treatment but the values still higher than the normal range of urinary IL-6. In patients with no remission, there was no significant decrease in urinary IL-6 values after 6 months of treatment and the values were still very high.

Conclusion

Urinary IL-6 may provide a simple noninvasive potential biomarker of disease activity of renal involvement in patients with SLE. Urinary IL-6 was found to be significantly high in patients of lupus nephritis than controls. Urinary IL-6 was higher in patients of severe form of lupus nephritis such as Class IV than other classes of lupus nephritis. Urinary IL-6 may be used as a prognostic marker of lupus nephritis. The patients who achieved complete remission had near normal

levels of urinary IL-6 and patients with partial remission had decreased level of urinary IL-6 than at presentation after 6 months of induction phase treatment. But patients with no remission did not show any significant decrease in urinary IL-6 levels at 6 months. So, urinary Il-6 may be used to monitor the response to therapy. One of our study limits was the low number of patients and shorter period of follow up . Prospective follow-up of SLE patients and further work is necessary to strengthen the findings of this study, validate the usefulness of urinary IL-6 as biomarker and its role as well as long term impact of high urinary levels in patients of lupus nephritis.

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