

ASSOCIATION OF DIFFERENT CLASSES OF PEDIATRIC LUPUS NEPHRITIS WITH ACTIVITY AND CHRONICITY INDICES IN BANGLADESHI POPULATION

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ABSTRACT

Introduction: Lupus nephritis is one of the most common manifestations of SLE. Pediatric population, especially the adolescent age group have a higher chance of developing lupus nephritis. Aim of this study was to evaluate association of different histological classes of pediatric lupus nephritis with activity and chronicity indices in Bangladeshi population. **Materials and Methods:** This was a cross-sectional observational study conducted at the Department of Pathology, BSMMU. The study included the clinically diagnosed cases of pediatric lupus nephritis, from March 2021 to January 2023. Patients were enrolled by consecutive sampling after fulfilling the inclusion and exclusion criteria. Light microscopy findings of the formalin fixed paraffin-embedded renal biopsy specimens were evaluated and recorded according to the proposed ISN/RPS classification system of LN, 2018. Ethical measures were maintained throughout the study. The statistical analysis was carried out using the Statistical Package for Social Sciences version 22 for Windows (SPSS Inc., Chicago, Illinois, USA). Before starting this study, the research protocol was being approved by the IRB (Institutional Review Board) of BSMMU, Dhaka. **Results:** The mean age was 13.96 ± 3.09 years ranged from 6 to 18 years. 83.75% of patients were female and 16.25% were male. Class II LN was the most common histological class (32.5%), followed by class IV (27.5%). The active lesions are more frequently seen (60%) than the chronic lesions (12.5%). Among the histological features of activity index, the neutrophils/karyorrhexis was the most frequent that was present in 62 (77.5%) cases and it was most frequent in class IV LN (95.5%). Among the histological features of chronicity index, interstitial fibrosis was present in majority (30.0%) of cases, most frequently in class IV LN. Four of the activity indices and one of the chronicity indices were found significantly associated with

histological classes of LN. **Conclusion:** Class II LN was found to be the most common class of lupus nephritis according to the modified ISN/RPS 2018 classification system. Four of the activity indices and one of the chronicity indices were found statistically significant.

KEYWORDS: Lupus nephritis, Nephrotic syndrome, Activity index, Chronicity index.

BACKGROUND

Systemic lupus erythematosus (SLE) is a chronic relapsing autoimmune disorder characterized by presence of autoantibodies directed against self-nuclear antigens which affect multiple organs of the body e.g kidney, joints, skin, brain, heart, lungs, gastrointestinal tract, etc.^[1] Onset of SLE during childhood and adolescence period comprises about 15–20% of total SLE cases. Disease severity and multiorgan manifestations are more pronounced in the patients of this age group than the adult patient group.^[2] The rate of occurrence is more in the female children and adolescents due to hormonal changes.^[3] Lupus nephritis is one of the most common manifestations of SLE, occurring in about 20–75% of all SLE patients. This manifestation appears mostly within first 2 years of diagnosis of SLE.^[4] Lupus nephritis is one of the most prevalent secondary glomerulonephritis that can cause chronic renal failure.^[5] Chances of active lesions and proliferative glomerulonephritis are more in childhood lupus nephritis. Children, especially the adolescent age group have a higher chance of developing lupus nephritis. About 40–70% pediatric population diagnosed with SLE develop lupus nephritis which is 10–30% more than the adult counterpart.^[6] In Bangladesh, the onset of lupus nephritis is thought to be quite earlier as compared with the studies of some other countries.^[7] Because of the highly diverse histological findings and clinical presentation, a number of histological classifications have been made and tested to predict prognosis and therapeutic response of lupus nephritis.^[8] According to the Modification proposal 2018, six classes of lupus nephritis are identified based on light microscopic and immunofluorescence findings in a renal biopsy sample having minimum 10 glomeruli.^[9] In this proposal, the modified NIH semi-quantitative scoring system of the activity and chronicity indices is introduced. The subclasses A, A/C and C used in 2003 classification system are removed. Moreover, the definitions of the pathological findings are much more precise in new proposal. The modified classification system also includes the evaluation of tubulointerstitial lesions in the form of interstitial inflammation in activity index and interstitial fibrosis/tubular atrophy in chronicity index.^[9] In this scheme, vascular lesions, however, are not considered in the score. Activity and chronicity indices of new modified semi-quantitative scoring system also serve as a prognostic tool and guide the

clinicians to the treatment of lupus nephritis. For example, high chronicity index shows therapeutic resistance and higher renal failure rates. On the other hand, lesions with high activity index are more likely to respond to aggressive therapy and potentially reversible.^[10]

Early onset of lupus nephritis is becoming a matter of great concern in the recent years. The adolescent population are at risk of developing more severe form of lupus nephritis. The modified classification system eliminates the A, A/C and C subdivisions of class III and IV as these have no significant difference in terms of renal outcome. Moreover, it includes activity and chronicity indices that predict the progression of renal disease and treatment response. This study was aimed at evaluating association of different histological classes of pediatric lupus nephritis with activity and chronicity indices in Bangladeshi population.

METHODS

Study Design: Observational cross-sectional study.

Place of study: Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU).

Period of study: The study was conducted from March, 2021 to January, 2023.

Sampling method: Consecutive sampling method.

Study population: The study included clinically and biochemically diagnosed cases of LN, aged up to 18 years.

Study sample: Paraffin blocks, slides and clinical information of study population taken from BSMMU and Kidney Foundation Hospital Research Institute, Dhaka.

Sample size: In this study, the sample size was considered 80.

Inclusion criteria

1. Clinically and biochemically diagnosed cases of pediatric LN.
2. Renal tissue containing at least 10 glomeruli in light microscopic analysis.

Exclusion criteria

1. Renal biopsy sample received for light microscopic study only
2. LN cases already received long term (>6 months) immunosuppressant therapy/treatment.

3. The cases histologically diagnosed as 'Suggestive of lupus nephritis.

Data collection technique

This cross-sectional study was conducted in BSMMU, from March, 2021 to January, 2023 at the Department of Pathology, BSMMU, Dhaka. Permission was obtained from the Institutional review board (IRB), BSMMU. The study sample was the renal biopsy specimens clinically diagnosed as LN, in the Department of Pathology, BSMMU and Kidney Foundation Hospital and Research Institute, Dhaka. Samples were received in the lab from each patient. Samples were preserved in 10% formalin for light microscopy. Tissue in formalin was embedded in liquid paraffin and processed routinely. Then sections were cut thin (3-5 micrometers) with microtome and stained with hematoxylin and eosin, periodic acid Schiff (PAS), Masson's trichrome stain and methenamine silver stain. All steps of the study and the collected data of the patients were saved properly by using appropriate measures and maintaining confidentiality.

Histopathological Evaluation of Renal Biopsy Sections

Routine H&E, PAS, Masson Trichrome and silver stained sections of the renal biopsy sample were examined. Sections were examined for changes in four components: glomeruli, tubules, interstitium and blood vessels. The glomeruli were checked for cellularity, mesangium, basement membrane changes, segmental or global sclerosis, crescents, inflammatory cells, karyorrhectic debris, fibrinoid necrosis, hyaline thrombi, adhesion to Bowman's capsule and deposits. Epithelial changes and presence of various types of casts in tubules, interstitial inflammation, fibrosis, tubular atrophy and changes in the blood vessels was also noted. The histological diagnosis of each class was made according to modified ISN/RPS, 2018 classification system. Activity and chronicity indices were recorded from histological slides to grade the severity of individual histopathologic features on a 0 to 3+ scale. Histological findings were correlated with available clinical findings and laboratory investigation reports prior to making a final diagnosis.

Statistical analysis

All data of the patients were recorded methodically in a data sheet. Separate data collection sheet was used for each patient. After collection, all the required data were verified using the statistical package for social science version 22.0 for windows (SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (frequency and percentages) were used to summarize the patient's demographic and clinical characteristics and were presented in tables, figures and diagrams. Statistical significance was considered at 95% confidence level.

Continuous variables were expressed by mean with standard deviation (mean $\pm$ standard deviation) and range. In addition, categorical variables were expressed by frequencies and percentages. To test any association chi-square test was used. In all cases significance level were considered at P value < 0.05.

Ethical Considerations

Before starting this study, the research protocol was approved by the IRB (Institutional Review Board) of BSMMU, Dhaka. Precautions were taken to protect confidentiality of the participants. Informed written consent was obtained from the parents of the patients without any influence.

RESULTS

Distribution of cases according to demographic profile

Among 80 patients of LN, majority (67.5%) of the patients belonged to the adolescent age group (13- 18 years). The mean age was 13.96 ± 3.09 years ranged from 6 to 18 years. More than four fifth (83.75%) of patients were female and 13 patients (16.25%) were male. Table I shows the distribution of the study patients by demographic profile.

Table I: Distribution of the study patients by demographic profile (n=80).

	Frequency (n)	Percentage (%)
Age (years)		
Childhood (1-12 yrs)	26	32.5
Adolescence/ Teen age (13- 18yrs)	54	67.5
Mean ± SD	13.96 ± 3.09	
Min - max	6 - 18	
Gender		
Male	13	16.25
Female	67	83.75

Distribution of study patients into different classes of lupus nephritis according to revised ISN/RPS 2018 classification

Among 80 patients of LN, the most common was class II LN (32.5%), followed by class IV (27.5%) and the least common was class I and combined class IV+V (1.3% each). There were 14(17.5%) class III, 12(15%) class V and 4(5.0%) combined class III+V LN patients. No patient was diagnosed as LN class VI throughout the study period.

Table II: Distribution of study patients into different classes of lupus nephritis (n=80).

Histological classes	Frequency (n)	Percentage (%)
I	1	1.3
II	26	32.5
III	14	17.5
IV	22	27.5
V	12	15.0
III+V	4	5.0
IV+V	1	1.3

Distribution of different histological features of activity and chronicity indices in the study group

In this study, the highest total value of activity index was observed in class II LN and the value was 13. Among 22 cases of class IV LN, the AI had a wide range of value (4-13). The highest total value of chronicity indices was found in class III LN (4). No active or chronic lesions were present in class I LN.

Table III: Distribution of activity and chronicity indices in the study group (n=80).

Histological classes	Activity index (AI)	Chronicity index (CI)
	Mean $\pm$ SD (range)	Mean $\pm$ SD (range)
I	0.00 $\pm$ 0.00 (0.00 - 0.00)	0.00 $\pm$ 0.00 (0.00 - 0.00)
II	1.81 $\pm$ 1.60 (0.00 - 6.00)	0.23 $\pm$ 0.43 (0.00 - 1.00)
III	4.71 $\pm$ 1.73 (2.00 - 8.00)	1.00 $\pm$ 1.24 (0.00 - 4.00)
IV	8.00 $\pm$ 2.49 (4.00 - 13.00)	1.23 $\pm$ 0.97 (0.00 - 3.00)
V	1.33 $\pm$ 0.78 (0.00 - 3.00)	0.50 $\pm$ 0.90 (0.00 - 3.00)
III+V	4.50 $\pm$ 1.26 (3.00 - 6.00)	0.50 $\pm$ 0.58 (0.00 - 1.00)
IV+V	6.00 $\pm$ 0.0 (6.00 - 6.00)	1.0 0.0 (1.00 - 1.00)

Distribution of different histological features of activity and chronicity indices in the study group

Among the histological features of activity index, the neutrophils/karyorrhexis was the most frequent that was present in 62 (77.5%) cases, followed by interstitial inflammation in 59 (73.8%) cases and endocapillary hypercellularity in 48 (60.0%) cases.

Among the histological features of chronicity index, the most common finding was interstitial fibrosis (30.0%), followed by tubular atrophy (25%) and total glomerulosclerosis (11.2%). Fibrous crescents were found in a minority (2.5%) of cases.

Table IV: Distribution of different histological features of activity and chronicity indices in the study group (n=80).

Activity indices	Frequency (n)	Percentage (%)
Endocapillary hypercellularity	48	60.0
Neutrophils/karyorrhexis	62	77.5
Fibrinoid/necrosis	6	7.5
Hyaline deposits	15	18.8
Cellular/ fibrocellular crescents	17	21.3
Interstitial/inflammation	59	73.8
Chronicity indices		
Total glomerulosclerosis	9	11.2
Fibrous crescents	2	2.5
Tubular atrophy	20	25.0
Interstitial fibrosis	24	30.0

Association of different classes of LN with activity indices

In the present study, the most frequent distribution of different activity indices in different histological LN classes are: endocapillary hypercellularity in class IV (11.3%), neutrophils/karyorrhexis in class IV (95.5%), fibrinoid necrosis in class III (14.3%), hyaline deposits in class IV (31.8%), cellular/fibrocellular crescents in

class IV (54.5%) and interstitial inflammation in class IV (59.1%). Among the activity index parameters, the association endocapillary hypercellularity, neutrophils/karyorrhexis, cellular/fibrocellular crescents and interstitial inflammation with histomorphological classes of LN are statistically significant.

Table V: Association of different classes of LN with activity indices (n=80).

LN classes	Endocapillary hypercellularity (Present)	Neutrophils/karyorrhexis (Present)	Fibrinoid necrosis (Present)	Hyaline deposits (Present)	Cellular/fibrocellular crescents (Present)	Interstitial/Inflammation (Present)
I	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
II	4 (2.1)	16 (61.5)	2 (7.7)	2 (7.7)	1 (3.8)	3 (11.5)
III	15 (7.7)	13 (92.9)	4 (14.3)	3 (21.4)	3 (21.4)	3 (21.4)
IV	22 (11.3)	21 (95.5)	3 (9.1)	7 (31.8)	12 (54.5)	13 (59.1)
V	1 (0.5)	7 (58.3)	0 (0.0)	1 (8.3)	0 (0.0)	2 (16.7)
III+V	5 (2.1)	3 (75.0)	0 (0.0)	1 (50.0)	1 (25.0)	1 (50.0)
IV-V	1 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
p-value*	<0.001 (s)	0.045 (s)	0.872(ns)	0.204(ns)	0.001(s)	0.008 (s)

*Chi-Square test was done

s= significant (as p value is < 0.05)

ns=not significant (as p value is > 0.05)

Association of different classes of LN with chronicity indices

In the present study, the most frequent distribution of chronicity indices in different histological LN classes

are interstitial fibrosis in class IV (59.1%) and tubular atrophy in class III and IV (50% and 31.8% respectively). Among these indices, interstitial fibrosis was found statistically significant.

Table VI: Association of different classes of LN with chronicity indices (n=80).

LN classes	Total glomerulosclerosis (Present)	Fibrous crescents (Present)	Tubular atrophy (Present)	Interstitial fibrosis (Present)
I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
II	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)
III	2 (14.3)	1 (3.8)	7 (50.0)	3 (21.4)
IV	4 (18.2)	2 (7.1)	7 (31.8)	13 (59.1)
V	3 (25.0)	0 (0.0)	5 (19.2)	2 (16.7)
III+V	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)
IV-V	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
p-value*	0.538 (ns)	0.873 (ns)	0.150 (ns)	0.006 (s)

* Chi-Square test was done

s= significant (as p value is < 0.05)

ns= not significant (as p value is > 0.05)

DISCUSSION

Lupus nephritis is one of the serious complications of SLE. It occurs at a higher rate in the pediatric patients than the adult counterpart and renal involvement in this age group is also higher. As the clinical features cannot always predict the underlying histological severity, the renal biopsy plays a vital role in early diagnosis, histological classification and thus in the planning of early treatment guidelines in the patients with lupus nephritis. But not much studies were done on the clinical usefulness of this modified classification in the pediatric age group in Bangladesh.

In the present study, among 80 patients of LN, majority (67.5%) of the patients belonged to the adolescent age group (13 -18 years) and more than four fifth (83.75%) of patients were female. A study

done in a tertiary hospital of Bangladesh showed a mean age of 11.6 ± 3.9 years and 74.28% of them were female.^[11]

In the present study, according to ISN/RPS 2003 classification, the majority of the pediatric patients were diagnosed as class II LN (26; 32.5%), followed by class IV (22; 27.5%). This correlates with the results of a study done by Fahmi, Hameed and Fliah, 2017, where the most common histological lesion was class II (35.2%) of LN.^[12] However, few previous studies show different results in the distribution of LN classes in the pediatric age group, where they showed class IV LN as the most common class.^[11,13] All these studies lead to the observation that, the adult patients present with more aggressive form of LN than the pediatric group. In real terms, it can be said that, the classes of LN change in the course of time

and converted to more aggressive forms necessitating the renal biopsy to be done as early as possible and start the treatment accordingly.^[13]

In the present study, the mean activity index (AI) was 4.71 ± 1.73 in class III and 8.0 ± 2.49 in class IV LN. The highest range of AI is seen in class IV (3-14). The mean chronicity index (CI) was 1.0 ± 1.24 in class IV with the maximum range 0-4. In the study conducted by Islam *et al.*, 2021, the mean AI was 4.59 ± 2.06 in class III and 8.71 ± 3.41 in class IV LN and there was a mean CI of 2.74 ± 1.31 in class III LN, which is comparable to the present study.^[14] All these observations indicate that, class IV LN have more active lesions than the other classes and this group is more likely to respond to aggressive therapy and potentially reversible.^[10]

In the present study, regarding the activity and chronicity indices, active lesions were more frequently seen (60%) than the chronic lesions (12.5%). Among the histological features of activity indices, the neutrophils/karyorrhexis was the most frequent that was present in 62 (77.5%) cases, followed by interstitial inflammation in 59 (73.8%) cases and endocapillary hypercellularity in 48 (60.0%) cases. Among the histological features of chronicity indices, interstitial fibrosis was present in majority (30.0%) of cases, tubular atrophy in 20 (25%) cases and total glomerulosclerosis in 9 (11.2%) cases. Nearly similar findings are seen in a study done on the adult patients by Hashmi *et al.*, 2020, where active lesions were present in 66 (51.6%) patients which was more frequent than the chronic lesions (32.8%).^[5] But, they found endocapillary hypercellularity as the most common active lesion type (81.8%) and glomerular sclerosis as the most common chronic lesion type (69%).^[5]

In the present study, the most frequent distribution of activity indices in different histological LN classes are: fibrinoid necrosis in class III (14.3%) LN, while the endocapillary hypercellularity (11.3%), neutrophils/karyorrhexis (95.5%), hyaline deposits (31.8%), cellular/fibrocellular crescents (54.5%) and interstitial inflammation in class IV (59.1%) LN. The most frequent distribution of chronicity indices in different histological LN classes are: total glomerulosclerosis (18.2%), fibrous crescents (7.1%), tubular atrophy (31.8%) and interstitial fibrosis (59.1%) in class IV LN. Among the activity indices, the association endocapillary hypercellularity, neutrophils/karyorrhexis, cellular/fibrocellular crescents and interstitial inflammation with histological classes of LN are found statistically significant. The interstitial fibrosis among the chronicity indices is found statistically significant. This present study is similar with a study done by G

et al., 2022, in the aspect that, they found endocapillary hypercellularity, neutrophil infiltration, fibrinoid necrosis, hyaline deposits, and interstitial inflammation in a significantly higher proportion in the children in class III and IV lupus nephritis.^[14] They found the chronicity index significant for tubular atrophy and interstitial fibrosis, which partially corresponds to the present study. Basu *et al.*, 2020 conducted another study where they observed that, the endocapillary hypercellularity was statistically significant.^[4] All these studies indicate the presence of more active lesions in the pediatric lupus nephritis patients which demand for initiating an aggressive immunosuppressive therapy, as with the early initiation of therapy, the outcome would be better. Oni *et al.*, 2017 assessed the interobserver variability of the ISN/RPS 2003 classification criteria in children.^[2] They found a better agreement among the pathologists for the LN-activity scoring than the LN-chronicity scoring that supports the present study.

CONCLUSION

In this study, four of the activity indices and one of the chronicity indices were found significantly associated with histological classes of LN. Among the activity index parameters, the association endocapillary hypercellularity, neutrophils/karyorrhexis, cellular/fibrocellular crescents and interstitial inflammation with histomorphological classes of LN are statistically significant. Among chronicity indices, interstitial fibrosis was found statistically significant. This signifies that, active lesions are more common than the chronic lesions in the pediatric LN in Bangladeshi populations.

LIMITATIONS

This study was done on a limited population, not representing the large number of pediatric populations throughout the country.

RECOMMENDATIONS

Larger scale multicentric studies with detailed clinical information are recommended. Genetic testing will add further accuracy.

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