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Electron microscopy in the pathological diagnosis of pediatric nephro-immunological diseases

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Abstract

Background: Electron Microscopy (EM) can be essential in diagnosing and classifying immunological nephritis, which is vital for treatment and prognosis. However, Indian patients lack information on this topic. Hence, our study aimed to recognize the ultrastructural morphological changes in pediatric immune-mediated kidney disorders via EM.

Methods: In this prospective, longitudinal, observational study, 18 patients were enrolled. Patients were divided into three groups: lupus, familial Nephrotic Syndrome (NS), and non-familial Steroid-Resistant Nephrotic Syndrome (SRNS). A total of 15 patients were found to have lupus, whereas six patients had familial nephrotic syndrome, and 20 patients had non-familial SRNS. The EM findings and their contribution to the primary diagnosis were assigned into categories: 1) Essential, 2) Helpful, and 3) Auxiliary. One viable glomerulus (if available) and its accompanying tubule-interstitium were analyzed with transmission electron microscopy.

Results: The mean age of the patients was 7.65 years, with a minimum of 3 and a maximum of 13; most (75.6%) were girls. Podocyte effacement was present in 80.5% of cases, and subendothelial or subepithelial deposits in 49% of cases. EM was found to be essential for diagnosis in 34% of patients, important contributory information in 22%, and not contributory in 38% of cases.

Conclusion: EM helped in clinical diagnosis in the majority of the cases. This study thus encourages the inclusion of EM in the routine evaluation of all pediatric kidney biopsies.

Keywords: Electron Microscopy; Lupus Nephritis; Immunological Diseases; Kidney Biopsies; Pediatric

1. Introduction

In the pediatric population, kidney diseases are most common around the globe. In comparison to the adult population, pediatric nephro-immunological diseases like Lupus Nephritis, Familial Nephrotic Syndrome, and Steroid-resistant pediatric nephrotic syndrome are more prevalent in the pediatric population [1]. In addition, these diseases need urgent treatment. Hence, identifying the pathological pattern of kidney injury due to immunological kidney diseases in a biopsy specimen is essential, and it generally requires detailed examination. In our country, routine light microscopy (LM) and immunofluorescence and/or immunohistochemistry (IHC) have been an essential component of the diagnostic workup of medical renal biopsies.

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In Western countries, EM has been used for the pathological diagnosis of native kidney diseases since the 1960s~1970s [2,3]. Previous studies have reported that using EM was crucial to establishing a final diagnosis in 17–25% of cases, primarily in adult kidney biopsies [4-7]. Others concluded that 85% of adult kidney biopsies had an indication for EM for diagnostic confirmation [2]. It should be noted that the spectrum of paediatric kidney diseases differs from that of adults. To the best of our knowledge, only one study has assessed the diagnostic value of EM in kidney diseases from India, while the study included adult and paediatric patients' biopsy [8]. Hence, the contribution of EM to diagnosing paediatric kidney diseases has not yet been thoroughly evaluated. The current study aimed to recognize the morphological changes in paediatric nephro-immunological diseases via EM and thus help in clinical diagnosis.

2. Material and methods

This study was performed by prospective review of children admitted to the Pediatric ward and Intensive Care Unit (PICU) of Medical College and Hospital, Kolkata, aged 1 month to 12 years, over one year, between 2021 and 2022. Case records of all patients admitted to the pediatric ward and PICU were screened to identify those requiring renal biopsy. The biopsy samples were sent for electron microscopy with the parents' consent. General information, including sex, age, clinical manifestations, and complete laboratory data, was collected and analyzed. The ethics committee of XXXX approved this study.

2.1 LM examination

Kidney biopsy tissues were processed through standard protocols. Briefly, the specimens were fixed in a 4.5% buffered formaldehyde solution and embedded in paraffin. Deparaffinated sections 3 μm thick were used for histological staining with hematoxylin and eosin, Periodic acid–Schiff, methenamine silver, and one slide of Masson's trichrome.

2.2 IF examination

Direct IF was performed to determine immunoglobulins, complement components, and fibrinogen. Semiquantitative analysis with grades from 0 to 3+ (0, no staining; 1+, mild staining; 2+, moderate staining; and 3+, strong staining) was performed according to fluorescence intensity.

2.3 EM examination

Fresh kidney biopsy tissues were fixed in 3% buffered glutaraldehyde for 2 h and 1% osmium tetroxide for 1 h, followed by serial acetone dehydration and SPI-EPON 812 (SPI, West Chester, PA, USA) embedding. The ultrathin sections were stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (JEOL JEM; Model no. 1400plus; Make JEOL, Tokyo, Japan).

2.4 Evaluation of the role of EM in the final diagnosis of kidney biopsy

The EM findings and their contribution to the primary diagnosis were assigned into categories: 1) Essential, cases in which EM was essential to make the principal diagnosis or a significant secondary diagnosis, or if EM was necessary to resolve a differential diagnosis in cases where a firm preliminary diagnosis could not be fully substantiated; (2) important, cases in which EM did not alter the preliminary diagnosis but provided additional important information for the final diagnosis; (3) auxiliary, EM validated the primary Pediatr Nephrol diagnosis achieved with LM and IF. The role of EM in the diagnosis according to different pathological patterns and different age subgroups as well as clinical settings was analyzed.

2.5 Statistical Analysis

Categorical data were analyzed in terms of numbers, percentages, and ratios. Quantitative data was expressed as Mean (SD) and ratios. Quantitative continuous variables were compared between groups using Mann-Whitney's nonparametric tests if the variable had a non-normal distribution or unpaired t-test if the variable had a normal distribution. A p-value < 0.05 will be considered statistically significant for all tests.

3. Results

In this prospective, longitudinal, observational study, a total of 41 patients were recruited from the Pediatric Medicine Department, Medical College Kolkata. Patients were divided into three groups depending on their disease presentation. A total of 15 patients were found to have lupus nephritis, whereas 6 patients had familial nephrotic syndrome and 20 patients had SRNS. The demographic parameters of all patient groups are presented in Table 1. The mean age of the patients was 7.65±3.22 years, with a minimum of 3 and a maximum of 13, and most (75.6%) of them were girls. The

blood urea value is significantly higher (p-value <0.001) in the lupus nephritis group compared to the remaining groups. In contrast, 24-hour urine protein level and blood creatinine do not differ between the three groups.

Table 1 Demographic characteristics of pediatric patients

Parameters	**Lupus Nephritis (n-15)**	**Familial Nephrotic Syndrome (n-6)**	**SRNS (n-20)**	**p-value**
Age (mean ± S.D.)	9.11±3.24	9.66±1.21	6.8±3.15	0.119
Gender Boy Girl	 1 14	 3 3	 6 14	 0.081
Hematuria Pre No	 13 2	 5 1	 4 16	 **<0.001**
Hypertension Yes No	 12 3	 1 5	 16 4	 **0.007**
Blood Urea (mg/dl)	66.33±26.14	19.50±2.16	34.10±11.10	**<0.001**
Blood Creatinine (mg/dl)	0.85±0.39	0.60±0.10	0.59±0.17	0.119
Urine 24hr protein (mg)	321.16±317.64	99.50±1.05	1607.90±2647.30	0.225
C3 Normal Low	 2 13	 6 0	 20 0	 **<0.001**

SRNS- Steroid-resistant pediatric nephrotic syndrome

3.1 Electron microscopy, Light microscopy & IF diagnoses

Tubular atrophy (22%) was more predominant in total patients, followed by Focal segmental glomerular sclerosis (FSGS) (17%) and Interstitial fibrosis (12%) (Table 2).

Table 2 Role of LM, IF, and EM in pathological diagnosis of kidney biopsies in children

	Lupus Nephritis (n-15)	**Familial Nephrotic Syndrome (n-6)**	**SRNS (n-20)**	***p*-value**
Light Microscopy				
No changes	7	0	9	**0.037**
Tubular atrophy	1	3	5	
Interstitial fibrosis	1	3	1	
Presence of fibromuscular tissue	4	0	0	
Focal segmental glomerular sclerosis	2	0	5	
Immunofluorescence Microscopy				
Positive	13	0	3	**<0.001**
Negative	2	6	17	
Electron Microscopy				
Effacement of podocytes				
Diffuse	4	0	18	**0.014**

Focal	4	0	2	
Widespread	5	0	0	
Electron dense deposits				
Found	14	2	4	**<0.001**
Not-found	2	4	16	

SRNS- Steroid-resistant pediatric nephrotic syndrome

A representative photograph of the patient's kidney tissue is shown in Fig. 1. Among all patients, 39% were positive on immunofluorescence (Table 2).

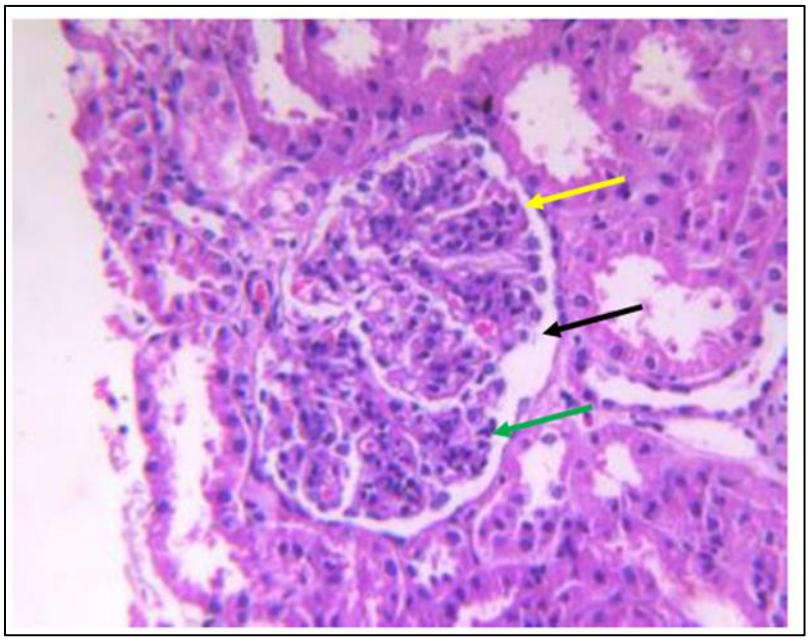

Figure 1 A representative photograph of HE-stained kidney biopsy under a light microscope. The photograph showed diffuse mesangial cell proliferation and matrix expansion (green arrow), lobular accentuation (yellow arrow), and endocapillary cellularity (black arrow).

As shown in Table 2 and Fig. 2, the value of EM in the final pathological diagnosis of the patient's kidney biopsies was evaluated. Our histological analysis revealed that Minimal change disease (MCD) was the most common, accounting for 44.0% of the cases, followed by lupus nephritis (LN) (28.0%), Alport syndrome (11%), and FSGS (11.0%). EM played an essential role in 34% of cases, including 39.0% of the cases of SRNS and 11.0% of the cases of Familial hematuria. In these cases, the diagnostic ultrastructural characteristics were sorted as EM showed critical diagnostic pathological features that were not visible with LM and IF (39%). Among all cases, electron-dense deposits were found in 49% of cases (Table 2).

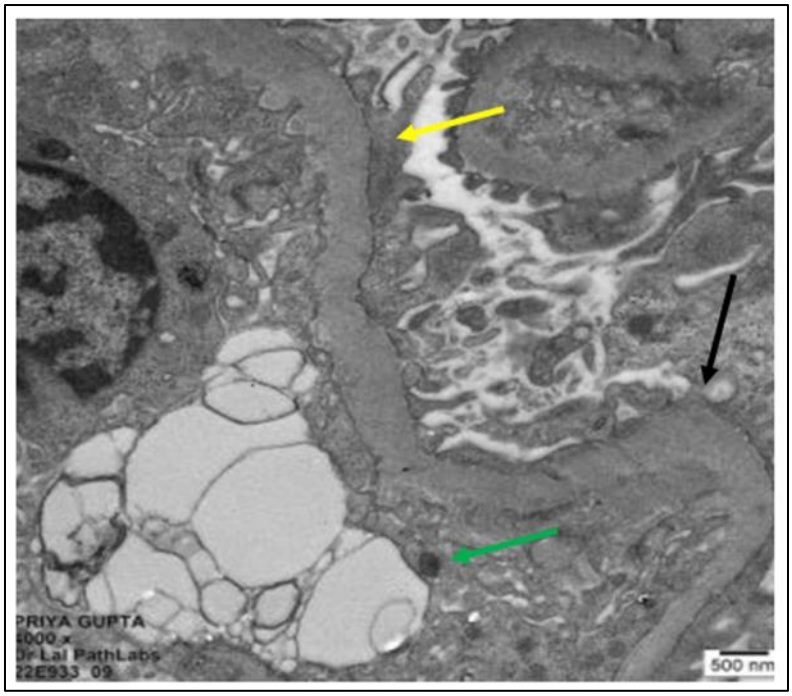

Figure 2 A representative photograph of Electron microscopy. The photograph showed focal effacement of the foot process (black arrow), and an area of normal foot process (yellow arrow). Subendothelial electron-dense deposits were also seen (green arrow) without a specific substructure.

The role of EM in IF-negative staining cases was evaluated and ultrastructural observation was crucial in 61% of IF-negative cases (Table 3).

Table 3 Role of EM in the final pathological diagnosis of IF-negative specimens

Patient ID	Primary diagnosis based on IF	Final pathological diagnosis by EM	Role of EM
P-1, P-6, P-8, P-12, P-11, P-16, P-28, P-36	Negative	Minimal change disease	Essential
P-2, P31	Negative	Crescentic GN	Auxiliary
P-5, P-7, P-22, P-33	Negative	Minimal change disease	Helpful
P-10, P24, P-31	Negative	Lupus nephritis (class II)	Helpful
P-13, P-25	Negative	Focal segmental glomerulosclerosis	Helpful
P-14, P-15, P-17, P-25, P-39, P-41	Negative	Alport's syndrome	Essential

4. Discussion

This study investigated pediatric patients with immune-mediated kidney disorders and concluded that EM was essential for the final diagnosis in 34% of cases, helpful in 22% of cases, and auxiliary in 5% of cases. Among all the cases, EM was considered to be valuable in the diagnosis of MCD, LN classification, and Alport syndrome. In addition, EM provided significant diagnostic information in IF-negative cases. Hence, the findings of our study emphasize the importance and necessity of EM for the diagnosis of immune-mediated kidney disorders in pediatric patients.

Our results revealed EM has diagnostic advantages over LM and IF by visualizing the precise distribution and morphology of electron-dense deposits and detecting an organized substructure within them. In most cases, diffuse effacement of podocyte foot processes is the diagnostic feature of MCD, while electron-dense deposits are characteristic of LN, and the Basket-weave appearance & criss-cross patterns are characteristic of Alport syndrome. A recent study conducted by Zhang and his colleagues on 890 pediatric patients supported our results [9].

Our data revealed that EM plays a crucial role in the diagnosis of 61% of IF-negative cases, the majority of which were MCD. In addition, EM detects electron-dense deposits and accurately localizes them. In our study, we identified electron-dense deposits in 49% of cases by EM. Electron-dense deposits were visualized in all the cases of LN. Earlier studies revealed that electron microscopy was "helpful" for patients with a final diagnosis of lupus nephritis [10]. Therefore, EM ultrastructural findings together with clinical manifestations provide an imperative indication for the classification of LN disease.

Interestingly, our data indicated that EM is more beneficial in the diagnosis of kidney biopsies in paediatric patients than in adults, as compared with the results of previous studies conducted mainly in adults (crucial in 17–25% and important in 21–42% of the cases) [6,7]. The reason may be the disease spectrum in adult patients, whereas the pediatric group has higher frequencies of podocytopathies and hereditary nephropathies, the diagnoses of which are dependent on EM.

Our study included pediatric patients strictly. To the best of the authors' knowledge, only one study was conducted earlier from India with a mixed age group (adults & pediatric) [8], and the results are in line. Hence, the routine use of this technique might be introduced in diagnostic kidney biopsies [11,12].

The limitations of the present study were its retrospective design and small sample size. Another limitation was the lack of patient follow-up. Further studies with larger sample sizes and patient follow-up are suggested to strengthen our results.

5. Conclusion

The results of the present study showed that, in the majority of cases, EM provided essential evidence for the final pathological diagnosis. Additionally, the appropriate use of EM can contribute to the early diagnosis of the disease. Early detection and subsequent treatment can lead to a better prognosis for pediatric immune-mediated kidney disorders, benefiting patients and physicians.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declared no conflicts of interest.

Statement of ethical approval

The study was approved by the Institute Ethics Committee of Medical College Kolkata, Kolkata (Ref. No. MC/KOL/IEC/NON-SPON/1315/05/2022 dated 17/05/2022). Parents of all the study participants provided written informed consent.

Statement of informed consent

The main text reported all the analyses performed for this article. Data of the article shall be shared on reasonable request to the corresponding author.

Author contributions

Conceptualization: R.K.M; Data curation: All authors; Formal analysis: D.D, S.K.R, R.K.M, B.B; Funding acquisition: N/A. Investigation: D.D, S.M, D.R, R.K.M; Methodology: D.D, S.M, S.K.R; Visualization: R.K.M, I.B. Writing-original draft: D.D, B.B. Writing, Review and editing: All authors.

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Supplementary Information

Table Demographic characteristics of pediatric patients

Parameters	**Lupus Nephritis (n-6)**	**Familial Nephrotic Syndrome (n-2)**	**Steroid-resistant pediatric nephrotic syndrome (n-10)**
Age (mean ± s.d.)	9.11±3.24	6.30±2.98	6.00±1.41
Gender			
Boy	0	1	3
Girl	6	1	7
Hematuria			
Pre	5	2	2
No	1	0	8
Hypertension			
Yes	6	0	8
No	0	2	2
Blood Urea (mg/dl)	89.66±75.65	20.00±2.82	34.10±11.10
Blood Creatinine (mg/dl)	0.85±0.39	0.65±0.07	0.59±0.17

Urine 24hr protein (mg)	321.16±317.64	99.50±0.70	1607.90±2647.30
C3			
Normal	1	2	10
Low	5	0	0