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RESEARCH

# Spectrum of Anemia in Indian children with Nephrotic Syndrome: a prospective observational study

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## Abstract

We aimed to estimate the prevalence of anemia in children with nephrotic syndrome (NS), determine its etiology, and correlate severity with disease duration and response to steroids. This was a prospective cohort study carried from 15th July 2019-14th July 2021 at the pediatric nephrology clinic, of a teaching hospital in India. We screened children aged 3 months-18 years with NS for eligibility. We excluded those suffering from chronic kidney disease and, on haematinics. All children underwent investigations for evaluation of nephrotic syndrome and anemia. To define the clinical phenotype of nephrotic syndrome, the patients were classified as infrequent relapsers, frequent relapsers, steroid dependent and steroid resistant NS as per ISPN guidelines. Children were followed up at least for a period of one year to define their response to steroids. A total of 125 children were finally analysed for all treatment outcomes. Of 125, 37 (30%) children presented with the first episode of NS. Remaining 88 were follow up cases of NS. Of 125 children, 41 (33%) were found to be anemic as per the WHO criteria. Iron deficiency anemia was found in 21 (51%) children. Steroid resistance was twice more prevalent in the anemic group compared to the non-anemic group, 7.3% vs 4.8% respectively, however this difference was not statistically significant, $p=0.65$. Anemic group had a trend of higher no. of children receiving antihypertensives compared to non-anemics (38 (93%) vs. 67 (80%), $p=0.07$.

*Conclusion*: Iron deficiency anemia was the commonest cause of anemia and, anemia and need for anti-hypertensives to attain BP control and adequate proteinuria often coexisted in children suffering from nephrotic syndrome.

**What is Known:**
- *Anemia is a significant complication in children suffering from nephrotic syndrome.*
- *Cause of anemia in nephrotic syndrome is multifactorial.*

**What is New:**
- *Iron deficiency anemia was the most common cause of anemia in Indian children with nephrotic syndrome.*
- *Anemia and need for anti-hypertensives to attain adequate BP control and proteinuria often coexisted in children with nephrotic syndrome.*

**Keywords** Steroid sensitive nephrotic syndrome · Steroid resistant nephrotic syndrome · Erythropoietin · Anemia · Iron deficiency

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## Introduction

Nephrotic syndrome (NS), characterized by significant proteinuria (> 40 mg/m$^2$/hr), hypoalbuminemia and edema, has a worldwide incidence of 1.2–16.9/100,000 children. Its incidence in India is 9–10/100,000 children which is higher as compared to western countries [1, 2]. Although exact data and prevalence of anemia in NS is lacking, it is postulated to be a significant complication.

Among the various etiologies of anemia, iron deficiency is the most prevalent in pediatric patients with NS. It has been attributed to increased urinary losses of iron bound to transferrin in patients with NS [3, 4]. Brown et al. reported increased urinary losses of iron in six of nine patients with NS but only two developed iron deficiency anemia, and iron supplementation resulted in resolution of the anemia. Notably, these two patients had longstanding membranous nephropathy. Thus, duration of the renal disease may be a crucial factor in the pathogenesis of iron deficiency anemia in NS [5]. EPO (molecular weight 30.4 kDa), along with albumin is lost in urine in NS. Thus, EPO deficiency is likely to contribute to anemia in nephrotic patients [6, 7]. It is known that angiotensin converting enzyme inhibitors (ACE-I) may cause anemia by lowering circulating levels of EPO [8]. Vitamin $B_{12}$ deficiency causes impaired DNA synthesis and premature death of hematopoietic cells leading to megaloblastic anemia. Significant urinary losses of transcobalamin and vitamin B12 in children with NS along with decreased serum levels of vitamin B12 have been reported. Folate deficiency can occur secondary to vitamin B12 deficiency due to trapping of folate in its methyl form which renders it unavailable as coenzyme for crucial reactions [6, 9]. In difficult-to-treat NS, ceruloplasmin, a copper carrier protein in plasma, is lost in urine and thus results in copper deficiency. Copper deficiency leads to ineffective erythropoiesis and hypochromic microcytic anemia [3, 10]. As ACE-Is are commonly prescribed in NS to limit proteinuria and hypertension, their role in causation and/or exacerbation of anemia in patients with steroid responsive NS and normal renal functions needs to be ascertained.

Anemia is thus multifactorial in patients with NS. Side effects of immunosuppressive/modulating treatment, renal losses of EPO, iron binding proteins, vitamin B12 binding transcobalamin, or copper binding ceruloplasmin or preexisting iron deficiency all may contribute. Expectedly, patients with longer lasting proteinuria, steroid-resistant forms and where anemia was the first manifestation before the diagnosis, are likely to suffer from greater degree of anemia. Moreover, prevalence of anemia due to NS per se will be difficult to assess in countries with widely prevalent nutritional anemia. Nevertheless, morbidity of the primary illness may be limited by supplementation of the specific deficient factor to reduce anemia [11]. Hence, we planned to estimate the prevalence of anemia in children with NS, determine its etiology and correlate the degree of anemia with disease duration and response to steroids.

## Methods

This was a prospective cohort study carried over two years (15th July 2019-14th July 2021) at Pediatric Nephrology Clinic (PNC), of a university teaching hospital, in northern India. The study was approved by the Institute's Ethics Committee (letter No. GMCH/IEC/2019/364 dated 02.07.2019).

## Participants

We screened all children aged 3 months-18 years with NS, both new-onset and those on follow-up, attending our PNC for eligibility. NS was defined as generalized edema, nephrotic range proteinuria, hypercholesterolemia, and hypoalbuminemia [1, 2]. We excluded children with chronic kidney disease and/or who were already receiving haematinics. All eligible children were enrolled after obtaining written and informed parental consent.

## Study protocol

Patients' demographic details, clinical details about the onset, nature, duration of illness, steroid responsiveness, complications, treatment history were recorded in a case record form. Estimated glomerular filtration rate (eGFR) was calculated using Schwartz formula [12].

At enrollment, all children underwent workup for NS and investigations for anemia. Anemia workup included complete blood count (CBC), peripheral blood film (PBF) examination, red blood cell (RBC) indices, serum iron studies [i.e., serum iron, serum ferritin, transferrin saturation and total iron binding capacity (TIBC)], serum vitamin $B_{12}$ & folate levels, serum copper levels and serum EPO levels. For hemoglobin estimation and CBC examination, 2 mL EDTA blood sample was processed by Coulter (LH 780 Hematology Analyzer, Beckman Coulter, Inc., Miami, FL). For morphology, RBC indices were analysed using coulter method and RBC morphology on PBF was manually visualized. For serum iron studies, 3 mL blood was centrifuged and the serum was processed

using Ferrozine/Magnesium carbonate method (Iron & TIBC kits, Coral Clinical Systems®, Goa, India). Serum ferritin levels were measured by ELISA (ADVIA Centaur®, Siemens Healthcare Diagnostics, US Pats). Serum Vitamin $B_{12}$, and folate levels were measured by direct chemiluminescent immunoassay (ADVIA Centaur®, Siemens Healthcare Diagnostics, US Pats). Serum EPO levels were measured by ELISA immunoassay (Biomerical EPO ELISA kit, CA, USA) and serum copper levels were measured using inductively coupled plasma-atomic emission spectrophotometry (JY 2000@2, HORIBA Jobin– Yvon, France). The reference normal laboratory values are listed in Supplementary Table 1.

Children with NS who had anemia as per WHO criteria [Supplementary table 2] were labelled as anemics and those without anemia were labelled as non-anemics. Severity of anemia was defined as per age and gender by WHO criteria [3, 13]. For etiology, results from iron studies, B12, folate, EPO and copper levels were analysed. Iron deficiency anemia was defined as low hemoglobin levels as per reference standard in the presence of low serum ferritin levels. In situations where ferritin levels were equivocal, low transferrin saturation was used [14]. Vitamin B12 deficiency was defined as vitamin B12 levels < 200 pg/mL [14, 15]. Folate, EPO and copper deficiency were defined as decreased serum levels of the index substrate as per standard laboratory reference values (Supplementary table 1). To define the severity of nephrotic syndrome, study participants were classified as infrequent relapsers, frequent relapsers, steroid dependent, and steroid resistant NS as per ISPN guidelines [1]. All children were followed up at least for a period of one year on outpatient basis to define their response to steroids. Renal biopsy was performed when indicated as per KDIGO and ISPN guidelines [1, 12].

**Table 1** Characteristics of anemia in nephrotic syndrome

| **Severity of anemia** | N = 41 |
|---|---|
| Mild *n (%)* | 16 (39) |
| Moderate *n (%)* | 23 (56) |
| Severe *n (%)* | 2 (4.9) |
| **Morphology of anemia** | |
| Macrocytic *n (%)* | 0 (0) |
| Microcytic *n (%)* | 25 (61) |
| Normocytic *n (%)* | 16 (39) |
| Anisopoikilocytosis *n (%)* | 2 (4.8) |
| Fragmented *n (%)* | 0 (0) |
| ***Etiology of anemia*** | |
| Iron deficiency *n (%)* | 21 (51) |
| B12 deficiency *n (%)* | 19 (46) |
| Folate deficiency *n (%)* | 12 (29) |
| EPO deficiency *n (%)* | 3 (7.3) |

#Erythropoietin (EPO)

**Statistical analysis** Descriptive statistics was used to define the baseline variables. The continuous variables between anemics and non-anemics were compared by student 't' test or Mann Whitney-U test, wherever applicable. Categorical variables between these two groups were compared by chi-square test with Yates correction or Fisher Exact test, wherever applicable. Correlation of GFR and duration of disease, with degree of anemia was noted by Pearson or Spearman correlation. Association of steroid responsiveness and use of ACE-Is with degree of anemia was studied by using chi-square test. Data was entered in excel spreadsheet and the analysis was done in SPSS v22 (IBM, New York).

**Sample Size** Approximately one fourth of all children with NS have anemia as per the existing literature [3]. Assuming anemia prevalence of 0.25 among children with NS, desired width of 95% confidence interval as 15%, α-error of 0.05 and power of 80%, we required to enroll 125 children with NS in the study.

## Results

We screened 134 children aged 3 months to 18 years with NS, who attended PNC during the study period. Of them, 5 denied consent and remaining 129 children were enrolled. Of 129 children, two children expired and two were lost to follow up. A total of 125 children were finally analysed for the outcomes. Our cohort had 82 (66%) males with male: female ratio of 1.9:1. The median age of the children was 72 (IQR: 39, 120) months. Out of 125, 37 (30%) children presented with first episode of NS. Remaining 88 children were follow up cases of NS, out of which 34% (43/125) were infrequent relapsers, 20% (25/125) were frequent relapsers, 10% (13/125) had steroid dependent NS and 5.6% (7/125) had steroid resistant NS. At enrolment, 115 (92%) children were steroid responsive, and 100 (80%) children were in remission. There was no significant difference in the proportion of children who were in remission between anemics and non-anemics (78% vs 81% respectively, $p = 0.49$). Renal biopsy was performed in 28 of 125 (22%) children, of whom 9 had minimal change disease (MCD), 13 had focal segmental glomerulosclerosis (FSGS), two children each had collapsing glomerulopathy and diffuse mesangial sclerosis, and one child each had C3 glomerulopathy and membranous glomerulonephritis.

Of 125 study children, 41 were found to be anemic as per the WHO criteria [13]. Thus, the prevalence of anemia in our cohort was 33% (95% CI 24.7, 41.8). Of these 41 children, 23 (56%) children had moderate anemia while severe anemia

was found in only 2 (4.9%) children (Table 1). Majority of the anemic children, 25 (61%) had predominantly microcytic picture on peripheral blood film, remaining 39% had normocytic normochromic picture while none had macrocytes or fragmented cells. Iron deficiency anemia was found in 21 out of 41 (51%) children making it the most common etiology of anemia in our cohort. Anemia due to other deficiencies like $B_{12}$, folate and EPO were found in 46%, 29% and 7.3% children, respectively (Table 1).

Of the 21 children with iron deficiency anemia, 14 (67%) children had microcytic hypochromic picture on PBF. Rest had normocytic normochromic picture. Reduced ferritin and transferrin saturation levels were found in 17/21(81%) and 11/21(52%) children, respectively. TIBC was raised in only 2 patients. High EPO levels were found in 9/21(43%) children. Vitamin $B_{12}$ deficiency was found in 19 out of 41 (46%) children with anemia with 58% (11/19) patients having normocytic normochromic picture. None had macrocytes on PBF. Folate deficiency was found in total 12 out of 41 (29%) patients, 3 out of 41 (7.3%) children with anemia had low EPO levels out of which 2 had concomitant iron deficiency also (Table 1). Multiple micronutrient deficiencies were observed in 12 (29% out of 41) anemic patients: 7 (17%) had concomitant iron and B12 deficiency, 3 (7.3%) had concomitant iron and folate deficiency, 2 (4.9%) had concomitant iron, B12 as well as folate deficiency.

The anemic (n-41) and non-anemic group (n = 84) did not differ significantly in terms of age, gender, anthropometric parameters, duration of illness, blood pressure records, total and differential leucocyte counts, reticulocyte count and platelet count (Table 2). In the anemic group, 22% children suffered from steroid dependent or steroid resistant nephrotic syndrome compared to 13% in the non-anemic group, p = 0.3, Table 2. Steroid resistance/non-responsiveness was more prevalent in the anemic group compared to the non-anemic group 7.3% vs 4.8% respectively, however this difference was not statistically significant, $p = 0.65$). Median eGFR was 94.9 (IQR: 74.5, 141.5) mL/min/1.73m$^2$ in the anemic group vs.101.5 (IQR: 77.9, 118.3) ml/min/1.73m$^2$ in the non-anemic group, $p = 0.93$. Median proteinuria was 2.3 (0.8, 3.3) g/24 h in the anemic group and 1.4 (0.6, 3.3) g/24 h in the non-anemic group, $p = 0.1$. The red blood cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) were significantly different between the anemic and non-anemic groups, $p < 0.001$. Renal function tests, iron studies, serum B12, folate and copper levels did not show significant difference between the two groups. Levels of serum EPO were significantly higher [14.9 (6.6, 42.1) IU/ml] in the anemic group vs. 8.6 (4.1, 15.2) IU/ml in the non-anemic group, $p <$ of 0.001(Table 3).

**Table 2** Comparison of demographic and clinical characteristics in nephrotic syndrome

| Characteristics | Anemic (n = 41) | Non anemic (n = 84) | P value |
|---|---|---|---|
| Males *n (%)* | 26 (63) | 56 (67) | 0.84 |
| Age *months #* | 72.0 (33.0,144.5) | 75.0 (48.0, 120.0) | 0.67 |
| Weight *kg #* | 17.9 (12.3, 27.4) | 19.6 (15.0, 27.7) | 0.33 |
| BMI *kg/m2 #* | 16.5 (15.2, 19.1) | 16.3 (14.5, 18.1) | 0.39 |
| Systolic Blood Pressure *mmHg #* | 100 (90, 107) | 100 (90, 100) | 0.47 |
| Diastolic Blood Pressure *mmHg #* | 60 (60, 70) | 60 (60, 70) | 0.43 |
| *Duration of illness months #* | 18.0 (5.5, 46.5) | 23.0 (2.1, 48.0) | 0.80 |
| *Clinical diagnosis* | | | 0.65 |
| First episode nephrotic syndrome *n (%)* | 13 (32) | 24 (29) | |
| Infrequently relapsing nephrotic syndrome *n (%)* | 11 (27) | 32 (38) | |
| Frequently relapsing nephrotic syndrome *n (%)* | 8 (20) | 17 (20) | |
| Steroid dependent nephrotic syndrome *n (%)* | 6 (15) | 7 (8.3) | |
| Steroid resistant nephrotic syndrome *n (%)* | 3 (7.3) | 4 (4.8) | |
| *Status at the time of enrolment* | | | 0.49 |
| Remission *n (%)* | 32 (78) | 68 (81) | |
| Relapse *n (%)* | 9 (22) | 14 (17) | |
| Not known *n (%)* | 0 (0) | 02 (2.4) | |
| *Response to steroid* | | | 0.36 |
| Yes *n (%)* | 37 (90) | 78 (93) | |
| Not known *n (%)* | 0 (0) | 2 (2.4) | |

#Median (IQR)

Body mass index (BMI)

**Table 3** Comparison of laboratory parameters in nephrotic syndrome

| Parameter | Anemic (n = 41) | Non-anemic (n = 84) | P value |
|---|---|---|---|
| eGFR *ml/min/1.73m$^2$ #* | 94.9 (74.5, 141.5) | 101.5 (77.9, 118.3) | 0.93 |
| Proteinura *g/24 h #* | 2.3 (0.8, 3.3) | 1.4 (0.6, 3.3) | 0.10 |
| WBC × *10$^9$/L #* | 12.4 (8.2, 15.8) | 11.6 (9.2, 15.2) | 0.68 |
| Platelet count × *10$^9$/L #* | 400.0 (234.5, 569.0) | 396.5 (319.0, 528.5) | 0.99 |
| Mean Corpuscular Volume *fL #* | 74.0 (65.9, 82.6) | 81.2 (77.1, 86.2) | 0.00 |
| Mean Corpuscular Hemoglobin *pg #* | 23.3 (20.0, 26.6) | 26.0 (24.7, 27.6) | 0.00 |
| MCHC *g/L#* | 31.1 (30.0, 32.2) | 32.1 (31.6, 32.7) | 0.00 |
| Serum creatinine *mg/dL#* | 0.4 (0.3, 0.6) | 0.5 (0.4, 0.6) | 0.47 |
| Serum Iron *µg/dL#* | 43.0 (37.5, 82.0) | 57.0 (37.3, 86.0) | 0.24 |
| TIBC *µg/dL#* | 300.0 (160.5, 346.5) | 300.0 (214.0–375.0) | 0.32 |
| UIBC *µg/dL#* | 233.0 (133.0, 300.0) | 214.0 ( 150.3, 286.0) | 0.85 |
| Transferrin saturation *%#* | 24 (13.3, 33.4) | 21 (14.0, 33.3) | 0.89 |
| Serum Ferritin *ng/ml#* | 14.0 (7.4, 38.6) | 21.7 (11.3, 43.5) | 0.18 |
| Serum EPO *IU/ml#* | 14.9 (6.6, 42.1) | 8.6 (4.1, 15.2) | 0.00 |
| Serum Vit B12 *pg/ml#* | 240.0 (122.0, 299.5) | 231.5 (138.0, 293.8) | 0.78 |
| Serum Folate *ng/ml#* | 7.8 (5.1, 12.4) | 7.5 (5.6, 12.1) | 0.89 |
| Serum Copper *ug/dl#* | 104.0 (69.8, 148.9) | 90.2 (68.1, 112.5) | 0.17 |

*TLC* Total Leucocyte Count, *MCHC* Mean Corpuscular Hemoglobin Concentration, *RDW* Red Cell Distribution Width, *TIBC* Total Iron Binding Capacity, *UIBC* Unsaturated Iron Binding Capacity, *EPO* Erythropoietin

*Mean (SD), #Median (IQR)

All the 125 children in the cohort received steroid therapy. Thirteen children received cyclosporine, 6 received Tacrolimus (TAC), 27 received levamisole, 5 received Mycophenolate Mofetil (MMF) and 7 received Cyclophosphamide. Proportion of children receiving Cyclophosphamide therapy was found to be higher among anemic (12%) as compared to non-anemic group (2.4%) with $p = 0.038$. In this cohort of 125 nephrotic children, 105 (84%) children had hypertension during any time in their course of followup (including periods of relapse). Anemic group had a trend of higher no. of children receiving ACE-inhibitors (ACE-I) compared to non-anemics (38 (93%) vs. 67 (80%), $p = 0.07$. Persisting hypertension was found in 5 patients (4 anemic 1 non anemic) for which they received amlodipine. Use of amlodipine was found to be significantly associated with presence of anemia, $p = 0.04$. Among the other complications, 4 children had AKI, 6 had UTI, 3 had sepsis, 5 had rickets, 1 developed cyclophosphamide induced febrile neutropenia, 1 had cataract, 2 had fungal infection, 2 had cortical sino venous thrombosis and 6 had cushingoid features, with none of these complications having significant association with presence of anemia. The renal biopsy findings were not significantly different between the anemic and non-anemic groups, $p = 0.55$.

The median duration of illness was 18.0 (5.5,46.5) months in the anemic group and 23.0 (2.1,48.0) months in the non-anemic group with no significance of duration of illness on occurrence of anemia, $p = 0.80$ (Table 4).

## Discussion

In a cohort of 125 children of nephrotic syndrome, the prevalence of anemia was observed to be 33%. The most common cause of anemia among these children was iron deficiency

**Table 4** Correlation of severity of anemia in nephrotic syndrome

| Characteristics | Mild anemia (n = 16) | Moderate anemia (n = 23) | Severe anemia (n = 2) | Value of significance |
|---|---|---|---|---|
| **Duration of illness** *months #* | 35.0 (12.8, 57.5) | 12.0 (3.0, 28.0) | 18.5 (13.0, -) | 0.29 |
| **Responsive to steroids** *n (%)* | 13 (81) | 22 (96) | 2 (100) | 0.29 |

#Median (IQR)

anemia which accounted for more than half of all anemic children, followed by vitamin B12 and folate deficiency. Multiple micronutrient deficiencies were observed in 29% of all anemic children. Intuitively, EPO concentration was significantly increased in anemic children compared to non-anemic children. There was a trend of increased use of antihypertensives in anemic children compared to non-anemic children with nephrotic syndrome.

The prevalence of anemia was 33% in children with NS. Our figures were lesser compared to a study by Feinstein et al. who found anemia in 59% of 32 nephrotic children [7]. This was probably due to duration of proteinuria not being long-lasting (Table 2) and fewer (only 5.6%) steroid resistant cases in our cohort. Thus, a significant proportion with new-onset NS would dilute the true prevalence of anemia as duration of active disease is an important determinant of anemia in NS. The anemia rates were however comparable to the national prevalence rates in otherwise 'healthy' children (Supplementary table 3), with 80% of cohort being in remission, which may be due to stringent follow up of participants in the PNC clinic. As reported previously, we did not find any significant correlation between eGFR and level of proteinuria with presence of anemia or iron deficiency [7]. Duration of disease was comparable in NS children with and without anemia. The predominant picture found on PBF of anemic children was microcytic hypochromic with low MCV, MCH and MCHC and high RDW. Microcytic hypochromic picture can be found in iron deficiency, EPO deficiency as well as copper deficiency. We had B12 deficiency in 46% children but we did not find macrocytes in any. This could be possibly because of mixed etiology of the anemia. In our study, we could measure serum copper levels in only 67 of 125 patients. Out of these, 8 were found to be copper deficient of which 7 were either first episode or infrequent relapser. Like Feinstein et al., we also found that levels of serum EPO were significantly higher in the anemic group as compared to the non-anemic group reflecting compensatory mechanism in the anemics [7].

Both cyclophosphamide and MMF have been linked to severe anemia because of bone marrow suppression [3]. We found significant association of anemia with cyclophosphamide but did not find any correlation between MMF therapy and occurrence of anemia among the nephrotics. This was probably because most children in our cohort were on MMF for less than 6 months at the time of enrolment. It is likely that were the duration of disease modifying agents longer, more side effects would be apparent. There was a trend that proportion of anemics on ACE-I was higher than proportion of non anemics receiving ACE-I. Also, persisting hypertension was seen in anemics though it did not show statistical significance due to low numbers. This highlights difficult-to-treat proteinuria in nephrotic children who were anemic. Use of ACE-I has been shown to cause anemia by lowering circulating levels of EPO [8]. We did not find any significant association between use of ACE-I and occurrence of anemia. Moreover, most children had high EPO levels which is expected, as its an acute phase reactant and in general increased in all anemic patients. Comparison is made difficult due to the treatment influencing anemia.

Strengths of this study are the adequate sample size, comparison of anemic nephrotics with non-anemic nephrotics who were comparable in demographic details and a detailed categorization of anemia. The limitations of our study are that we could not measure the urinary losses like of iron, transferrin, cobalamin, EPO, and copper due to financial constraints. Likewise, we could not check serum copper levels in all the patients. There could be other causes of anemia like vitamin A and riboflavin deficiency which may contribute to anemia but were not specifically investigated. However, we did a detailed examination and tried to rule out these micronutrient deficiencies clinically. To conclude iron deficiency was the most common etiology of anemia and, anemia and need for anti-hypertensives to attain BP control and adequate proteinuria often coexisted in children suffering from nephrotic syndrome.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00431-023-05150-6.

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**Authors contribution** VM designed the study, clinically managed the patients, and edited the first draft. AS did literature search, enrolled patients, and wrote the first draft. AT did hematological investigations including EPO levels. SDC did renal biopsy and gave intellectual input. SJ did the biochemical analysis. All authors approved the final manuscript.

**Availability of data and material** The authors declare that the data is transparent. The original data has been entered in Microsoft Excel and can be made available in case the journal wishes.

**Code availability** Not applicable.

## Declarations

**Ethics approval** The study was approved by Institute Ethics Committee, Government Medical College, Chandigarh, India (IEC Regd No. ECR/658/Inst/PB/2014/RR-2017) vide letter No. GMCH/IEC/2019/364 dated 05.07.2019.

**Ethics Accordance** The study was conducted on ethical guidelines for biomedical research on human subjects as given in the "Declaration of Helsinki" (modified 2000) and by Central Ethics Committee on Human Research (CECHR) of Indian Council of Medical Research (ICMR), New Delhi, India.

**Consent to participate** The authors affirm that informed consent was properly documented from the parent/ primary caregiver.

**Consent for publication** The authors affirm that proper consent for publication was taken from the participants.

**Conflict of interest** The authors have no conflict of interest to declare.

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