



Primary membranous nephropathy in children and adolescents: a single-centre report from South Asia

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Abstract

Background Unlike adults, primary membranous nephropathy (PMN) comprises only 1–2% of childhood nephrotic syndrome. The clinical behaviour of PMN in children is not explicit and we report upon clinical presentation and outcome.

Methods This prospective study includes children and adolescents (<20 years) with biopsy-proven PMN without secondary causes. Anti-PLA2R assessment: before and after completing therapy. Outcome: percentage of patients achieving remission.

Results Study cohort included 48 (M:F ratio 1.1:1) patients and median age 17 (IQR 15–18) years, with 35 (72.9%) PLA2R related. Median interval from symptom onset to presentation was 5 months, where median proteinuria, serum albumin and creatinine were 4.9 g/day, 2.1 g/dL and 0.63 mg/dL, respectively. Forty-seven patients received immunosuppressive therapy, with various agents used as first-line therapy: cyclical CYC/GC (53.1%), CNI/GC (21.3%), rituximab (14.9%), prednisolone alone (4.3%), azathioprine (4.3%) and mycophenolate mofetil (2.1%). Median follow-up was 29 (14, 59) months. At 6 months, 11 (24.4%) and 17 (37.7%) had complete remission (CR) or partial remission (PR), while at last follow-up (median 29 months), 20 (45.4%) and 14 (31.8%) had CR and PR respectively. No significant differences in outcome were observed with different agents. A total of 60% patients treated with rituximab as first line/for relapsing disease, and all cases with resistant disease receiving rituximab had CR or PR at last follow-up. PLA2R antibody presence was associated with clinical outcome.

Conclusions Three-quarters of PMN in children and adolescents is PLA2R related and two-thirds respond to immunosuppressive therapy. Rituximab is a promising agent to manage PMN in children. Anti-PLA2R is associated with clinical outcomes.

Keywords Children · Adolescents · Paediatric membranous nephropathy · PLA2R · THSD7A · Rituximab · Outcomes

Raja Ramachandran and Saurabh Nayak have equal contribution as the 1st author

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Introduction

Primary membranous nephropathy (PMN) is an autoimmune disease characterized by diffuse thickening of the glomerular basement membrane on light microscopy, granular immunoglobulin deposits on immunofluorescence and subepithelial electron-dense deposits on electron microscopy [1]. PMN is an uncommon cause of paediatric and adolescent nephrotic syndrome [2]. Membranous nephropathy (MN) secondary to chronic hepatitis B infections and systemic lupus erythematosus is frequently seen in the first decade of life and currently classified as secondary MN [3, 4]. Patients with PMN have autoantibodies to M-type phospholipase A2 receptor (PLA2R), thrombospondin 7A domain (THSD7A) and bovine serum albumin (BSA) [2, 5, 6]. Autoantibodies to antigens mentioned above help in differentiating primary from secondary MN [7].

Three-fourths of adult PMN patients have antibodies to PLA2R antigen, which shows a good association with clinical activity [8]. Nearly half of the PMN cases in children are PLA2R related [9, 10]. Reporting of antibodies to BSA and neutral endopeptidase in paediatric PMN is inconsistent and limited to a geographical location [6, 11]. The existing literature on autoantibodies and their relationship to clinical outcome in paediatric/adolescent PMN are limited to three small case series (less than 20 patients) [12–14]. Two-thirds to three-fourths of adolescents with PLA2R-related PMN respond to immunosuppressive therapy [14].

Cyclical cyclophosphamide and steroids, calcineurin inhibitors and rituximab are first-line therapies for the management of adult PMN [15, 16]. The current literature on the clinical outcome of immunosuppressive treatment in paediatric/adolescent PMN is scanty. There is no data on the clinical outcome in patients with relapsing or resistant PMN in the adolescent population. In the current study, we report the clinicopathological, serological and therapeutic outcome of paediatric/adolescent PMN.

Methods

Study design

The present prospective (cohort) study is a clinicopathological description of paediatric and adolescent patients enrolled in the primary MN registry.

Setting and participants

The study was carried out in the Department of Nephrology and Histopathology, Nehru hospital, Postgraduate Institute of Medical Education and Research, Chandigarh, from December 2011 until October 2019. Patients aged 1–20 years, who had demonstrable changes of MN on kidney biopsy, were included. Essentially light microscopy should show thickened glomerular basement membrane with or without characteristic spikes and immunofluorescence showing finely granular immune-complex deposits of IgG type. Excluded were patients with systemic lupus erythematosus-related MN and chronic hepatitis B/C-associated MN. All the patients provided written informed consent (from parents if < 18 years of age). Institute Ethics Committee approved the study (PGI/IEC/2014/241), which was conducted as per the Declaration of Helsinki. The decision regarding immunosuppressive agents was at the discretion of the treating nephrologists, who chose the immunosuppressive therapy as per the patient's characteristics and clinical wisdom. Patients were followed every month for the first year and then quarterly, for proteinuria, serum albumin and serum creatinine. Serum anti-PLA2R (by ELISA) and THSD7A (by indirect

immunofluorescence) testing were performed at baseline, 6 and 12 months of starting immunosuppressive agents. Similarly, testing for autoantibodies was also performed prior to and after completion of second-line immunosuppressive agents. Plasma samples were stored in an ultralow temperature freezer ($-80\text{ }^{\circ}\text{C}$) and tested at a later date.

PLA2R and THSD7A glomerular staining procedure

Immunohistochemistry was done on paraffin sections by dewaxing slides in xylene/alcohol followed by rinsing in water. Freshly prepared 0.3% hydrogen peroxide was added for 20 min, followed by washing in PBS (pH 7.2). Antigen retrieval was done in citrate buffer (pH 6.0) using PT Link (Dako) at $98\text{--}99\text{ }^{\circ}\text{C}$. Slides were incubated with anti-PLA2R1 (HPA012657) or anti-THSD7A (HPA000923) antibodies (Sigma Aldrich) for 1 hour and biotinylated secondary antibody (Dako) for 30 min at room temperature. Di-amino benzidine solution was added for 1 min followed by haematoxylin stain and after mounting with dextreneptylate xylene, brown colour positivity on membranes was scored as mild (1+), moderate (2+) or intense (3+).

Definitions

Nephrotic syndrome was defined by proteinuria $\geq 3.5\text{ g/day}$ or $\geq 1.5\text{ g/day}$ along with a serum albumin $< 2.5\text{ g/dL}$, oedema and hyperlipidaemia [17]. Complete remission (CR) was the reduction of proteinuria to $< 0.3\text{ g/day}$ with normal serum albumin ($\geq 3.5\text{ g/dl}$) and serum creatinine. Partial remission (PR) was the reduction of proteinuria to $0.3\text{--}3.5\text{ g/day}$ and stable serum creatinine (change in serum creatinine $< 25\%$) or a decrease in proteinuria $> 50\%$ from baseline with stable serum creatinine (change in serum creatinine $< 25\%$) and serum albumin $> 3.5\text{ g/dL}$ [18]. Relapse was defined as nephrotic range proteinuria after achieving remission. All patients with circulating anti-PLA2R antibodies and/or enhanced staining for PLA2R in the glomeruli were classified as having PLA2R-related PMN. Clinico-serological miscorrelation is clinical remission with the persistence of anti-PLA2R ($> 14\text{ RU/ml}$) or the presence of serological remission ($< 20\text{ RU/ml}$) but with resistant disease.

Statistical analysis

Data are expressed as numbers, percentages, mean and standard deviation or median and interquartile range, as appropriate. Parametric data are expressed as mean and standard deviation and non-parametric data as median and interquartile range (IQR). For clinico-serological association, a chi-square test was performed. For inferential statistical analysis, multivariate Cox regression with proportional hazards assumption was utilized and hazard ratios (HRs) with associated 95%

confidence intervals were calculated. Statistical analysis was performed using IBM SPSS 24.0 Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. and Graph Pad Prism 8 (San Diego, CA 92108) and a *p*-value of <0.05 was considered significant.

Results

Patient characteristics

The study cohort included 48 patients with a median age of 17 years (IQR 15–18). Figure 1 depicts the study flow diagram indicating enrollment and inclusion in final analysis. The study included 25 (52%) males and 23 (48%) females. The median duration of illness prior to initiation of

immunosuppressants was 5 (IQR 3–8) months. The median proteinuria, serum creatinine and serum albumin were 4.9 g/day (3.5, 7.4), 2.1 mg/dL (1.8, 3.12) and 0.63 g/dL (0.5, 0.82), respectively. Forty-four (91.6%) had nephrotic syndrome, three patients had sub-nephrotic proteinuria and one patient had sub-nephrotic proteinuria with low serum albumin. Four patients (8.3%) were ≤ 10 years of age. Thirty-five (72.9%) patients had PLA2R-related MN. Enhanced staining of PLA2R in the glomeruli and autoantibodies in serum (at baseline or at 6/12 months) were seen in 33 (68.8%) and 32 (66.7%) patients, respectively. Two patients had detectable antibodies in serum in the absence of positive glomerular staining. Yet, another two patients with strong glomerular staining of PLA2R and undetectable serum anti-PLA2R levels at baseline turned seropositive at 6-month testing. Two patients had kidney dysfunction at presentation, one had a serum

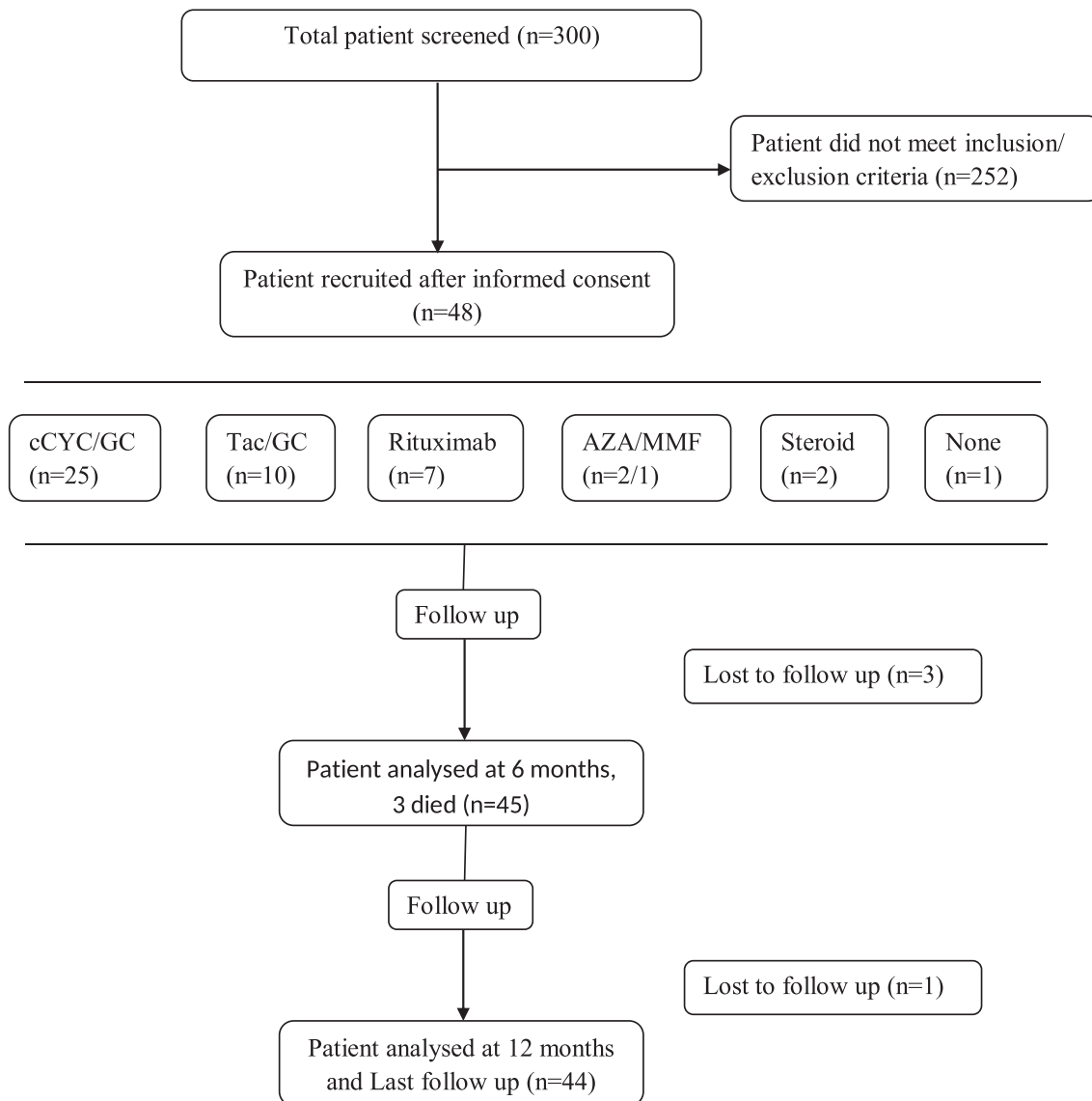


Fig. 1 Study flow chart

creatinine of 1.7 mg/dl and the other patient had 7.7 mg/dl. None of the PLA2R-negative patients had staining/serology suggestive of THSD7A-related MN. The baseline laboratory and clinical parameters are displayed in Table 1. Supplemental Table 1 comprises individual patient details from baseline parameters to clinical response at last follow-up.

Therapy and outcomes

Twelve patients received at least 3 months of antiproteinuric therapy. Forty-seven patients received immunosuppressive therapy, one had spontaneous remission. All the patients were started/continued on ACE inhibitor/ARB therapy. Twenty five (53.1%), 10 (21.3%), 7 (14.9%), 2 (4.3%), 2 (4.3%) and one (2.1%) patients were given cyclical cyclophosphamide/glucocorticoids (cCYC/GC), calcineurin inhibitor/glucocorticoids (CNI/GC), rituximab, azathioprine/steroids, steroids alone and mycophenolate mofetil/steroids respectively as first-line immunosuppressive agents. Serum albumin, creatinine and proteinuria are shown in Fig. 2 a–c. Tacrolimus (Tac) was the CNI used in all patients who underwent CNI/GC therapy. At the end of 6 months of follow-up, only 45 patients were analyzed as three patients were lost to follow-up (two were non-complaint to therapy and progressed to stage 5 chronic kidney disease, both reported use of complementary and alternative medicines from a local spiritual healer and reported once they had advanced kidney failure, another patient declined to report after the first visit). Of the remaining 45 cases, at 6 months of follow-up, 11 (24.4%), 17 (37.8%), 3 (6.7%) and 14 (31.1%) were in CR and PR, had died and were resistant to therapy, respectively. At the end of 12 months, one additional patient was lost to follow-up. Of the remaining 44 cases, 15 (34.1%), 16 (36.4%), 1 (7.3%), 9 (15.9%)

and 3 (6.8%) had CR, PR, relapse, resistant disease and death respectively. Figure 3 depicts response rate with various therapies. At the last follow-up, 20 (43.5%) and 14 (30.4%) patients were in CR and PR, respectively. There was no significant difference in the clinical outcomes between the therapies administered (Fig. 4). The clinical details and outcome of various first-line immunosuppressive agents are mentioned in Table 2.

A Cox proportional hazard model (regression) included only those patients who were given cCYC/GC, Tac/GC and rituximab as a first-line agent ($n = 38$). Considering cCYC/GC as a reference therapy, response to therapy at 12 months was not significantly associated with the choice of immunosuppressive agent in the proportional hazard model of Cox regression. Age less than 10 years and baseline serum albumin > 3 g/dL were associated with a favourable prognosis (Table 3). Ten patients were excluded from multivariate analysis (4 lost to follow-up, two on steroids, 2 MMF, 1 AZA and 1 no treatment) due to inadequate data or small numbers ($n < 5$) on immunosuppressive therapy.

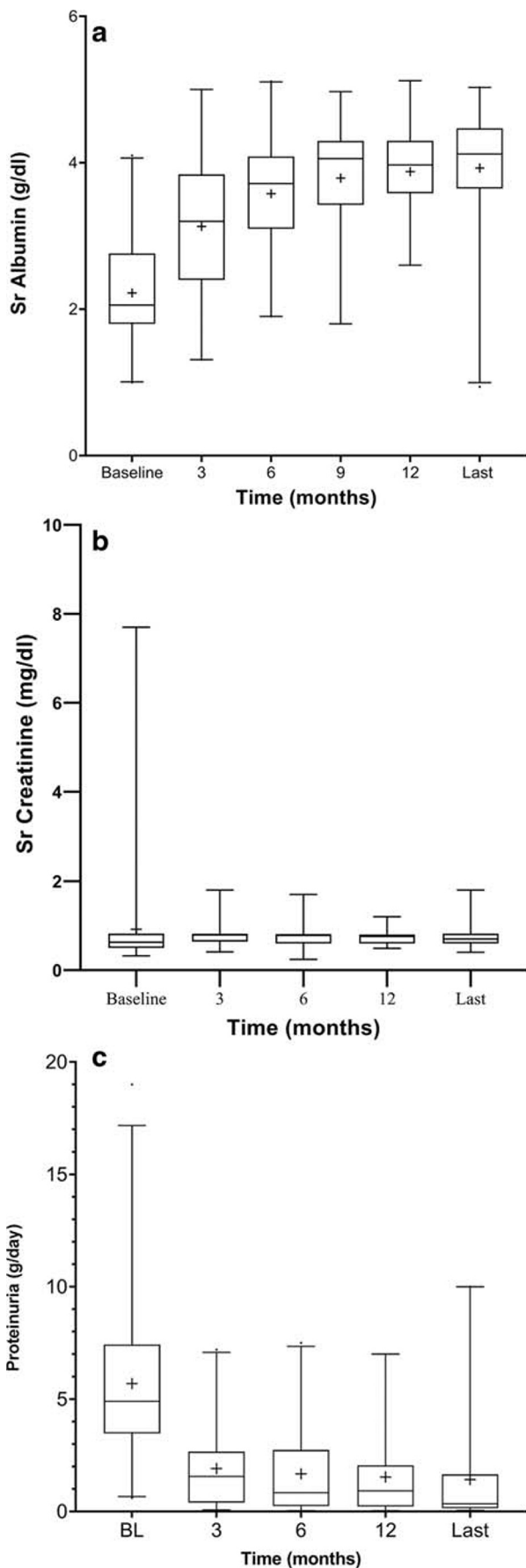
Resistant disease

Ten episodes of resistance in eight patients received rescue therapy. At 6 months, 14 patients were resistant as per definition, while seven of them remitted during extended follow-up without the addition of a second-line agent. Of the remaining seven, four received second-line agents. Two had a disease course complicated by two distinct episodes of resistance: patient 8 responded to cCYC/GC after being declared refractory to Tac and steroids at 12-month follow-up; she later relapsed and was found refractory to a second course of cCYC/GC, which was successfully treated with rituximab. Patient 43 showed initial resistance to

Table 1 Baseline characteristics of patients on various first-line agents

Parameters	cCYC/GC ($n = 25$)	Tac/GC ($n = 10$)	Rituximab ($n = 7$)	Steroid ($n = 2$)	MMF ($n = 1$)	AZA ($n = 2$)	None ($n = 1$)
Age (in years)	16.44	14.70	17.14	15.5	13	12.5	19
Gender (M:F)	1.1:1	0.66:1	0.75:1	2:0	1:0	1:1	1:0
Mean proteinuria (g per TV)	5.67	5.99	6.01	5.13	3.8	2.08	10.9
Mean serum Albumin (g/dL)	2.08	2.42	2.51	2.2	2.1	3.02	3.09
Mean serum creatinine (mg/dL)	1.06	0.82	0.67	0.9	0.8	0.57	1.02
Mean follow-up period in months	36	62	14	21	72	9	12
Median anti PLA2R levels (RU/mL)	96.15	390.90	80.24	0.39	119.5	0.60	0.60
PLA2R-related PMN	18 (72%)	7 (70%)	5 (71.4%)	1	1	1	0

cCYC/GC cyclical cyclophosphamide and glucocorticoids, Tac/GC tacrolimus/glucocorticoids, MMF mycophenolate mofetil, AZA azathioprine, PLA2R M-type phospholipase A2 receptor, PMN primary membranous nephropathy



◀ **Fig. 2** a Mean serum albumin trend during treatment and follow-up. b Mean serum creatinine trend during treatment and follow-up. c Mean 24-h proteinuria during treatment and follow-up

mycophenolate mofetil but was successfully managed with cyclical CYC/GC after which disease relapsed and was found refractory to Tac/GC and later responded to rituximab.

Three patients (patient 1, 2 and 8, Supplemental Table 2) demonstrated unique resistant profiles with Tac/GC as the first-line agent. After the first 6 months (glucocorticoids tapered to stop), these patients showed no response (relapse) while maintained at the same dose and adequate trough levels at 12 months, and were declared resistant. One was treated with rituximab and achieved remission (Patient 1) (Supplemental Table 2), while the other two were resistant to cyclical CYC/GC as well. Patient 8 (Supplemental Table 2) was offered rituximab as a third-line agent to which she responded, while patient 2 (Supplemental Table 2) refused any third-line agent due to financial constraints. Another patient (patient 11) (Supplemental Table 2) relapsed after successful treatment with cyclical CYC/GC and was declared resistant to azathioprine before being successfully treated with rituximab. The median proteinuria, serum albumin and creatinine prior to next immunosuppressive therapy were 4.5 g/day (4.05–6.07), 2.7 g/dL (2.03–3.20) and 0.8 mg/dL respectively. The second-line agent was rituximab, cCYC/GC and azathioprine in six, three and one patient, respectively. As a second-line agent, rituximab was successful in four patients previously treated with Tac, azathioprine or cyclical CYC/GC and as a third-line agent in two patients who failed cyclical CYC/GC as

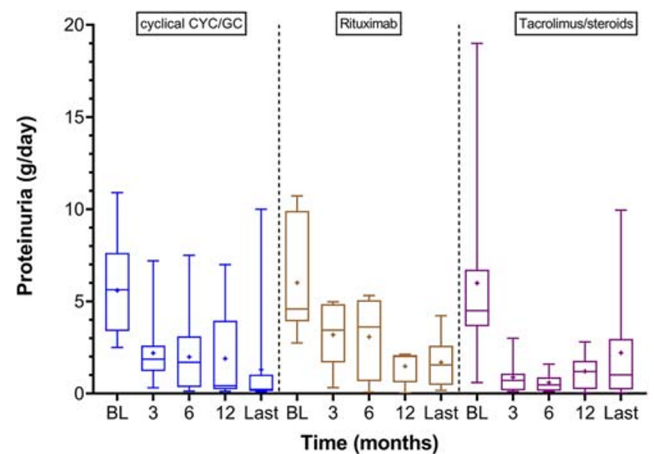


Fig. 3 Comparison of clinical response measured by 24-h proteinuria among various immunosuppressive regimens used. Foot notes: Values expressed as mean ± SD; CYC/GC, cyclical cyclophosphamide/glucocorticoids

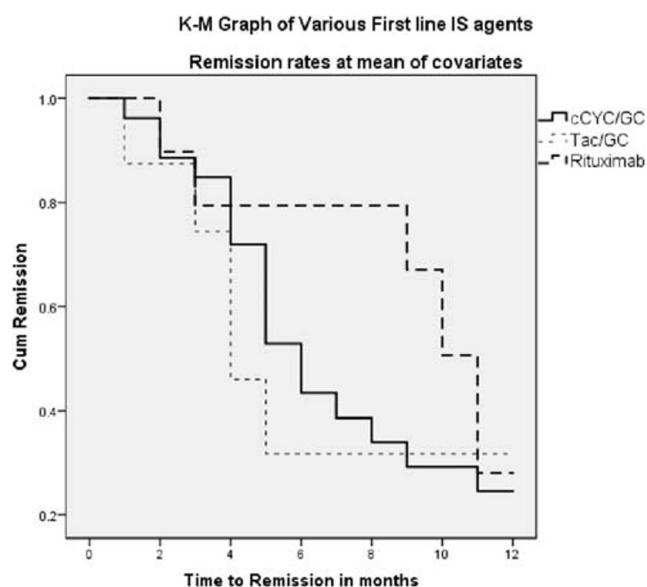


Fig. 4 Response rate of various IS agents- Kaplan- meier graph (using multivariate cox regression analysis)

second-line therapy. At the last follow-up, except for one (patient 2) (Supplemental Table 2), all ten episodes of resistant disease responded meaningfully, with median proteinuria, serum albumin and serum creatinine of 0.4 g/day (0.11–1.53), 4.2 g/dL (4.0–4.4) and 0.8 (0.7–0.84) mg/dL. Of the nine patients with PLA2R-related resistant disease, two responded serologically with a

negative titre, despite persistence of nephrotic state, and later responded clinically to rituximab (Supplemental Table 2).

Rituximab in PMN

A total of 17 patients received rituximab therapy. The mean age of the patients was 16.3 ± 2.3 (range 12–20). The indications for rituximab as therapy were the following: treatment naïve (first-line agent), relapsing disease and resistant disease in seven, four and six patients, respectively. The mean proteinuria, serum albumin and serum creatinine prior to starting rituximab therapy were 4.6 g/day (3.9–6.1), 2.6 g/dL (2–3.1) and 0.8 mg/dL (0.67–0.80), respectively. Ten, two and five patients received 1 g rituximab (days 0 and 15), 375 mg/m² (\times 4 weekly doses) and CD19-targeted rituximab therapy, respectively. One patient was lost to follow-up (treatment naïve) post rituximab infusion. After a median period of 15 (IQR 12–36) months, 12 patients achieved remission (5 CR and 7 PR). The overall response rate was 75%. Among patients treated for therapy naïve and relapsing disease, a 60% response rate was noted (2 CR and 4 PR) at the end of 1 year. Quite impressively, all resistant PMN cases responded favourably (3 CR and 3 PR) to rituximab treatment.

PLA2R antibody and clinical response

In patients with PLA2R-related PMN, the median PLA2R at 6 and 12 months was 21.34 (1.0, 72.55) and 3.25 (0.6, 44.87)

Table 2 Follow-up course of patients on various first-line agents

Parameter	Total (n = 48)	cCYC/GC (n = 25)	Tac/GC (n = 10)	Rituximab (n = 7)
At 6 months				
Proteinuria (g/TV)	0.83 (0.24, 2.73)	1.45 (0.33, 2.96)	0.47 (0.15, 0.87)	3.61 (0.67, 5.06)
Serum albumin (g/dL)	3.71 (3.11, 4.12)	3.59 (3.05, 3.99)	4.00 (3.76, 4.24)	3.04 (2.10, 4.03)
Serum creatinine (mg/dL)	0.8 (0.6–0.8)	0.8 (0.6–0.8)	0.6 (0.5–0.6)	0.8 (0.6–0.9)
At 12 months				
Proteinuria (g/TV)	0.91 (0.23–2.02)	0.37 (0.24–3.05)	1.20 (0.25, 1.78)	2.02 (0.61, 2.08)
Serum albumin (g/dL)	3.98 (3.60–4.27)	3.85 (3.61–4.52)	3.85 (2.91, 4.27)	3.94 (3.64, 4.13)
Serum creatinine (mg/dL)	0.7 (0.6–0.8)	0.7 (0.6–0.8)	0.6 (0.5–0.6)	0.8 (0.6–0.8)
At last follow-up				
Proteinuria (g/TV)	0.31 (0.14–1.64)	0.20 (0.12–1.15)	1.02 (0.23–2.95)	1.55 (0.47, 2.58)
Serum albumin (g/dL)	4.12 (3.64–4.47)	4.20 (3.62–4.68)	4.07 (3.62, 4.35)	3.79 (1.79, 4.12)
Serum creatinine (mg/dL)	0.7 (0.6–0.8)	0.8 (0.6–0.9)	0.7 (0.5–0.8)	0.7 (0.5–0.8)
Remission at 6 months	28/45 ^a (62.2%)	13/25 (52%)	8/8 (100%)	2/6 (33.3%)
Remission at 12 months	31/44 ^b (70.5%)	16/24 (66.7%)	5/8 (62.5%)	5/6 (83.3%)
Remission at last follow-up	34/44 (77.3%)	18/24 (75%)	6/8 (75%)	4/6 (66.7%)

^a Three patients lost to follow-up at 6 months (two in Tac/GC and one in rituximab group)

^b One additional patient lost to follow-up at the 12th month (in cCYC/GC group)

All variables median (interquartile range), cCYC/GC cyclical cyclophosphamide and glucocorticoids, Tac tacrolimus

Table 3 Cox regression multivariate analysis comparing baseline parameters with response time among patients given cCYC/GC, rituximab and tacrolimus/GC therapy as first-line agent ($n = 38$)

S no.	Group	HR	SE	P value (95% CI)
1	Age < 10 years	11.46	0.957	0.011 (1.756–74.786)
2	Male gender	0.858	0.484	0.752 (0.332–2.218)
3	No haematuria	3.654	0.796	0.103 (0.768–17.39)
4	Serum albumin > 3 g/dL	3.094	0.546	0.038 (1.062–9.017)
5	Serum anti PLA2R < 100 RU/ml	1.666	0.591	0.387 (0.524–5.3)
6	Serum creatinine < 1.0 mg/dL	1.131	0.350	0.726 (0.569–2.247)
7	Proteinuria < 8 g/TV	0.751	0.549	0.602 (0.256–2.203)
8	PLA2R related	0.930	0.599	0.904 (0.288–3.006)
9	Rituximab	0.611	0.562	0.381 (0.203–1.837)
10	Tac/GC	0.919	0.581	0.885 (0.294–2.871)

Tac tacrolimus, GC glucocorticoids, PLA2R M-type phospholipase A2 receptor

RU/ml, respectively (Fig. 5). There was an association of anti-PLA2R to the clinical outcome ($p < 0.05$). Of the patients who completed 12 months of follow-up ($n = 44$), only 4 patients had a clinico-serological dissociation. Two of these were on Tac/steroid therapy; they had remission in proteinuria with persisting antibodies at 1 year; both had relapse of nephrotic syndrome on stopping Tac. One patient had persistent antibodies at 12 months after successful completion of cCYC/GC course, he suffered relapse after 5 months. Conversely, only one patient who was given cCYC/GC as first-line therapy had persisting nephrotic range proteinuria and hypoalbuminemia despite having achieved serological remission (disappearance of autoantibodies); he was considered as having refractory

disease and was given rituximab with which clinical remission was achieved.

Adverse events

Three patients died within the first month of starting immunosuppression (all three were given cyclical CYC/GC) due to infectious complications, and were managed at a hospital elsewhere. Of the remaining; a total of 15 patients had infectious complications in the form of upper/lower respiratory tract infections—none required hospitalization. Four patients treated with cCYC/GC had leucopenia, which responded to lowering of the dosage. Rituximab was well tolerated, with upper respiratory tract infection in 3 patients and minor infusion reactions in 4 patients.

Discussion

In the present study, we report PLA2R-related MN in three-fourths of children with PMN. Also, two-thirds of the patients responded to conventional immunosuppression. Rituximab is useful in the management of treatment naïve and resistant PMN.

PMN is a rare cause of nephrotic syndrome in children [2]. Disparate to adults, secondary causes like systemic lupus erythematosus and chronic hepatitis B infection are common causes of MN in children [3, 4]. In addition to various morphological parameters, with the advent of detection techniques for PLA2R in glomeruli and autoantibodies in serum, the primary and secondary forms of MN may be accurately differentiated. In adults, 75–82% of PMN cases are PLA2R related [2, 19]. We report 70% of the PMN cases in children and adolescents to be PLA2R related, which is slightly lower than the adult population. The finding is significant as this current report is the most extensive series of paediatric (child and adolescent) PMN cases reported to date. Literature on PLA2R-related PMN in children/adolescents is limited to case

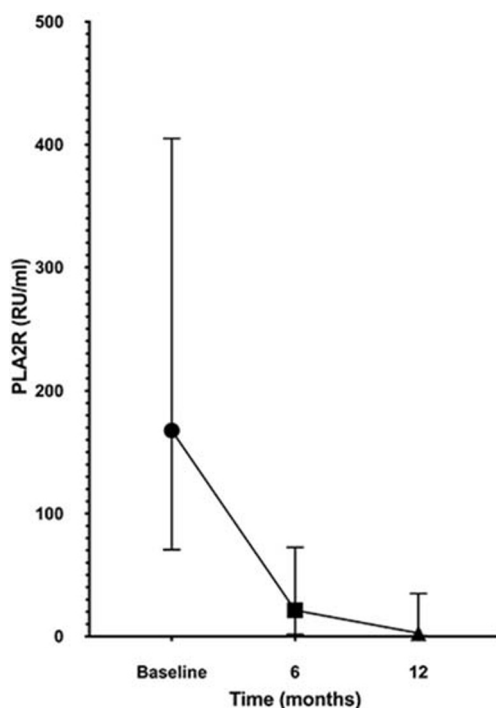


Fig. 5 Serum PLA2R antibody titres at baseline and after 6 and 12 months of therapy. Foot notes: values expressed as mean \pm SD; PLA2R, phospholipase 2 receptor antibody

series with diminished sample size (Supplemental Table 3). The existing literature proposes paediatric MN to be less commonly related to PLA2R [9, 10]: the prevalence of anti-PLA2R ranges from 6 to 83% [14, 20, 21] depending on the age at presentation and ethnicity. Studies including adolescents, in contrast to children less than 10 years old, have consistently shown similarity with adults in this context. Since this study consists mainly of adolescents, it reproduces similar findings and thus explains the high PLA2R positivity.

In children, the intensity of glomerular staining and serum autoantibody titres was also reported to be lower than that of adults due to various plausible reasons [10, 12, 21]. Lower proteinuria, preserved glomerular filtration rate, early clinical detection, pre-biopsy steroid therapy and early histological features on biopsy are among the commonly cited reasons for lower serum anti-PLA2R antibody titres. A small minority of the PMN cases in children may be steroid sensitive and are unlikely to be biopsied, lowering the sensitivity of autoantibody testing. We report high baseline antibody titre (more than 100 RU/mL) in up to 20 (42%) patients.

Up to 16% of PLA2R-unrelated PMN in adults may be THSD7A related [22]; however, none of the paediatric PMN patients has shown detectable serum THSD7A autoantibodies or enhanced glomerular staining in the existing literature or the current study [10, 12]. In stark distinction to adults, the French group demonstrated autoantibodies to BSA in children with PMN [6]. However, there are no reports of BSA-related MN from other ethnicities/geographical regions. In the present report, there was an association of PLA2R antibody with clinical remission. The above finding may be explained by the regional variation and ethnicity of the cohort examined. A summary of PMN studies in children (post-PLA2R era) is described in Supplemental Table 3.

Management of adult PMN cases is relatively standardized with cCYC/GC and CNIs with or without glucocorticoids and rituximab as the first-line agents. A clinical response rate of around 60% has been demonstrated by 12 months with the use of cCYC/GC, CNI/GC and rituximab. Literature is scarce regarding the clinical course of paediatric PMN patients treated with or without immunosuppressive agents. In a study by Lee et al. [4], a clinical remission rate of 68% was noted in 19 paediatric PMN cases treated with immunosuppressive agents. Valentini et al. [23] in 2009 demonstrated a clinical remission in 75% of treated children. In a recent uncontrolled study of 12 paediatric PMN cases from Germany, 7 (75%) responded to immunosuppressive therapy by 24 months, five achieved CR and 2 achieved only PR [12]. The paediatric literature is devoid of controlled trials for MN; henceforth, it remains a challenge to choose one immunosuppressive agent over another preferentially. Children diagnosed with MN frequently undergo biopsy after pre-emptive steroid therapy and being declared steroid resistant. The strategy, as mentioned above, also complicates the adoption of the “Kidney

Disease: Improving Global Outcomes” (KDIGO) recommended algorithm for adults [18] and obviates the need for assessing the efficacy of steroids as sole immunosuppressive agent. A recent report highlighted the unpopularity of KDIGO recommendations among paediatricians [24]. Alkylating agents are the backbone of adult PMN management in limited-resource settings, but fear of gonadotoxicity generates scepticism about their utilization in children. Valentini et al. [23] in an uncontrolled study demonstrated a 75% response to a 12-week course of oral cyclophosphamide (2 mg/kg/day) with alternate-day steroids. Among seven steroid-resistant cases, 86% achieved CR. Calcineurin inhibitor use in paediatric MN is limited to only case series, where it is difficult to ascertain their efficacy. Lee et al. [4] reported cyclosporine use in three steroid-resistant children with 100% response within 6 months, followed by relapse on drug withdrawal, and Chen et al. [25] reported CNI use in 5 patients (cyclosporine in 3 and Tac in 2) with similar results. There has been a single series of 4 children treated with mycophenolate mofetil (1200 mg/m² per day), and the authors report PR in proteinuria at 6 months with steroids and ACE inhibitors [26]. In the present study, 62–83% of the patients achieved remission at the end of 12 months of starting various therapies; the response was similar in patients receiving cyclical CYC/GC, rituximab and tacrolimus and steroids. Also, the response to second-line therapy was impressive, with most patients responding favourably to subsequent immunosuppressive therapy. In the current report, we may conclude that rituximab, cCYC/GC or CNI/GC (Tac) may be chosen as the first-line agent in children, factoring in financial availability.

Even before the discovery of the pathogenic PLA2R autoantibody, the efficacy of rituximab (B cell-targeted therapy) in PMN was established (since 2002) [27]. Amidst unacceptable and unavoidable long-term adverse effects of existing PMN therapies, rituximab is a beacon of hope for paediatric PMN patients, but surprisingly, medical literature is devoid of any controlled study so far. In a Chinese report, a 10-year-old boy with steroid-resistant nephrotic syndrome diagnosed with PMN on kidney biopsy and treated with four weekly doses of 375 mg/m² responded and attained CR in just 2 months [28]. Malatesta-Muncher et al. [29] reported the successful use of 2 doses of 375 mg/m² rituximab 2 weeks apart in two adolescent MN cases with subsequent remission of proteinuria noted in 3 months.

The present report is the largest to date on the utility of rituximab in children with PMN. Three-fourths of the patients treated with rituximab therapy achieved remission, and at the end of 6 months of therapy all but two achieved serological remission. At the last follow-up, even the patients with antibody positivity at 6 months responded with negative antibody titres. The response rate in patients with resistant disease was numerically better than in treatment naïve/relapsing disease. The higher response rate in cases with the resistant disease is

probably due to the cumulative effect of multiple immunosuppressive therapies that these patients received. Three different rituximab schedules used in the patients are an adaptation of the previously published studies [30–32]. Patients with PMN have delayed remission, and an extended follow-up may accurately characterize the clinical response to CD20 depleting agents.

The response of the PLA2R antibody to immunosuppressive agents is the same as observed in adults [15]. All but one patient had a clinico-serological association; lack of an extended follow-up may explain the discrepancy. Two patients had undetectable antibody before immunosuppressive therapy, and the antibodies became apparent at the 6-month testing. Such cases mentioned above may be due to the “kidney as a sink” phenomenon, which we described earlier [33]. On the observations of the current report, we recommend PLA2R antibody testing in all children/adolescents with PMN, with repeat testing after therapy completion or before starting a second-line agent.

The present study is the most extensive study of PMN in children/adolescents with an acceptable follow-up and anti-PLA2R monitoring. However, treatment variability, short duration of follow-up in patients receiving rituximab as the first-line agent and no assessment of long-term complications limit the findings of the study. To conclude, PMN in children is PLA2R related in 73% of cases; over two-thirds of the patients respond to immunosuppressive therapy, and rituximab is useful both as a first-line and rescue therapy.

Authors' contributions RR conceptualized the study, collected the data, analyzed results and helped in writing manuscript. SN helped in data collection and manuscript writing. NA, RB, KL, MR and HSK helped in data collection. VK helped in statistics and result analysis. AK and RN performed serum PLA2R and tissue level testing respectively.

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Data availability All relevant data will be available from the corresponding author and will be reproduced on demand.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study was approved by the Institutional Ethic committee (PGI/IEC/2014/241).

Consent of participation Written informed consent for participation was taken from all the patients and parents in case of age < 18 years.

Consent for publication All authors have approved the manuscript for publication.

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