Original Article

Steroid Responsiveness and Urinary Monocyte Chemotactic Protein-1 in Children with Nephrotic Syndrome.

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Abstract

Introduction: Idiopathic nephrotic syndrome is a clinical syndrome marked by a significant loss of urine protein, resulting in hypoproteinemia and edema.The recruitment of monocytes/macrophages into the renal tubulointerstitium is aided by the moncyte chemotactic protein-1.
Aim of the study: The aim of our study was planned to measure the level of monocyte chemotactic protein 1 in the urine of children with idiopathic nephrotic syndrome during disease activity and remission as well as in steroid sensitive and steroid resistant cases to identify a possible predictive biomarker of disease activity and/or steroid responsiveness.
Methods: This prospective study comprised 50 patients with nephrotic syndrome who were followed up on at Pediatric Nephrology Clinic and Department, Benha University, and were divided into two groups: group A (cases in remission) and group B (cases in activity). Also, 20 age and sex matched healthy children have been included as a control group.
Results: We found a significant increase in urinary moncyte chemotactic protein-1(uMCP-1) in idiopathic nephrotic syndrome patients as compared to control group ($p < 0.001$). Also, the greatest levels of uMCP-1 were found in group B, followed by group A, and then the control group ($p<0.001$). The steroid resistant nephrotic syndrome patients had significantly higher uMCP-1 levels than the steroid sensitive nephrotic syndrome patients ($p<0.001$).
Conclusion: Urinary MCP-1 can be considered a useful biomarker for identification of disease activity in children with INS, as well as a potential predictive biomarker of steroid responsiveness among these patients.

Keywords: Nephrotic Syndrome; Relapse; Remission; Steroid Sensitive; Steroid Resistant; Urinary monocyte chemotactic protein-1.

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Introduction

Nephrotic syndrome is defined as hypoalbuminemia, edema, and proteinuria (24 hour urine protein > 40 mg/m^2/hr or urinary protein to creatinine ratio > 2000 mg/gm or protein > 300 mg/dL or 3+ on urine dipstick [1]. Nephrotic syndrome is a frequent pediatric kidney disorder that affects 2–16 per 100,000 children each year. It can occur at any age during childhood and adolescence [2].

Steroid-sensitive nephrotic syndrome (SSNS) is described as remission within the initial four weeks of corticosteroid therapy [3]. The failure to achieve complete remission after eight weeks of corticosteroid therapy, namely a urinary protein to creatinine ratio <200 mg/gm creatinine or <+1 of protein on a urine dipstick for three consecutive days is described as steroid-resistance nephrotic syndrome (SRNS) [3].

Although the pathogenesis and pathophysiology of idiopathic nephrotic syndrome (INS) are unknown, it has been suggested that the immune system plays an important role. A link between several cytokines and chemokines and INS has been reported [4]. Histological reports suggest that macrophages play an important role in the pathogenesis of focal segmental glomerulosclerosis (FSGS), a form of steroid-resistant nephrotic syndrome [5].

Monocyte chemotactic protein-1 (MCP-1) belongs to the CC-chemokine family, is encoded on chromosome 17, and is composed of 76 amino acids. It is produced by mesangial, tubular, and epithelial cells in the kidney, as well as smooth muscle cells. It is mainly expressed by monocytes, activated macrophages, T cells, and natural killer cells [6]. MCP-1 causes monocyte migration and retention, as well as fibroblast transformation, in the glomeruli, and hence plays a significant role in glomerular inflammation. The pathophysiology of renal glomerular and tubular injury was linked to urinary MCP-1 [7].

The aim of the study: was planned to measure the level of monocyte chemotactic protein 1 in the urine of children with idiopathic nephrotic syndrome during disease activity and remission as well as in steroid sensitive and steroid resistant cases to identify a possible predictive biomarker of disease activity and/or steroid responsiveness.

METHODS

This prospective study was carried out on 50 children attending nephrology unit and clinic of the pediatric department at our University Hospital from January 2021 to July 2021. The patients were classified into 2 groups:

Group A: This group included 25 nephrotic patients in remission (marked reduction in proteinuria to less than 4 mg/m^2/hr or urine albumin dipstick of 0 to trace for three consecutive days in association with relief of edema) [3].

Group B: This group included 25 nephrotic patients in activity (proteinuria more than 40 mg/m^2/hr or urine albumin dipstick ++ or more on three consecutive days, often with recurrence of edema) [8].

Control group: This group included 20 age and sex matched healthy children

attending general pediatric clinic at our University Hospital.

Inclusion criteria: Patients with idiopathic nephrotic syndrome, ranging in age from 2 to 18 years, with preserved renal function. Exclusion criteria: Patients with secondary nephrotic syndrome, patients with any manifestations of systemic disease, patients < 2 years or > 18 years, INS patients at stages 2–5 of chronic kidney disease, and the presence of acute infections and allergies at the time of urine collection.

All children will be subjected to the following: Full history taking (including symptoms of nephrotic syndrome, duration of the disease, response to steroid therapy, and frequency of relapses). Clinical examination including: a) General examination including: vital signs, pulse, blood pressure, its centile, and anthropometric measurements. b) Local examination including abdominal examination, chest examination, heart examination, and neurological examination. Investigations: Routine lab investigations including complete blood count (CBC), C-reactive protein (CRP), serum creatinine, blood urea, serum albumin, serum total cholesterol, complete urine analysis, and urine protein/creatinine ratio. And the Specific tests urinary monocyte chemotactic protein-1 (uMCP-1) was done by ELISA technique.

Urine Sampling: Random mid-stream urine samples were taken from patients and controls in clean containers at about 10 am, and divided into two portions: the first portion for complete urine analysis, urinary protein/creatinine ratio, the second portion was collected in sterile tube and centrifuged at 1000 RPM for 20 minutes. The supernatant was then collected, aliquoted and immediately frozen at -20°C till the time of assessment of MCP-1 using a commercially available ELISA kit supplied by Elabscience, Inc, USA, catalog number: E-EL-H6005, sensitivity: 37.5pg/mL, and detection range: 62.5-4000 pg/mL.

Statistical analysis

Statistical program for social sciences (IBM-SPSS), version 24 (May 2016) was used for data entry, processing, and statistical analysis; IBM-Chicago, USA will be utilized for statistical data analysis. Kruskal-Wallis, Mann–Whitney U test, Chi square, F value of ANOVA, and Spearman's correlation were utilized as tests of significance. Data were presented and appropriate analysis was performed according to the kind of data (parametric and nonparametric) obtained for each variable. P-values less than 0.05 (5%) was considered to be statistically significant. Statistical significance was defined as a P-value of less than 0.05 (5%).

RESULTS

The study included 50 nephrotic patients, with a 54 percent female predominance. Our study's patient group has an average age of 8.4 years ± 2.5 years. Comparative data between patients and controls are illustrated in **Table 1, 2**. Group A included 9 steroid sensitive, 7 steroid resistant, 5 steroid dependent, and 4 frequent relapse

nephrotic syndrome, while group B included 7 steroid sensitive, 11 steroid resistant, 1 steroid dependent, and 6 frequent relapse nephrotic syndrome. 36% of patients in group A were treated with steroids alone compared to 28% in group B. The other patients used multiple steroid sparing drugs as cyclophosphamide, cyclosporine, and mycophenolate mofetil.

There was a highly statistically significant difference in uMCP-l levels between the patients and control group ($p < 0.001$). Also, the greatest levels of uMCP-1 were identified in group B (patients in activity), followed by group A (patients in remission), and finally the control group. There was a statistically significant difference between the three groups ($p < 0.001$) **Table 3, 4** and **Figure 1, 2**.

There was statistically significant difference in uMCP-l levels between SRNS in group A and SSNS in group B **Table 5**. As regard uMCP-1, there were statistically significant difference between SRNS and SSNS in group A **Table 6**, and highly statistically significant difference between SRNS and SSNS in group B **Table 7**. It was shown that uMCP-1 can be used to discriminate between SRNS and SSNS groups in all studied patients at a cut off level of > 68.7 pg/ml, with 88.9% sensitivity, 93.8% specificity, 93.5% PPV, and 89.4% NPV (AUC = 0.96 & $p < 0.001$).

Our study revealed that uMCP-1 level in group B (patients in activity) and group A (patients in remission) were highly positively correlated with urinary protein/creatinine ratio and serum total cholesterol, and highly negatively correlated with serum albumin **Table 8**.

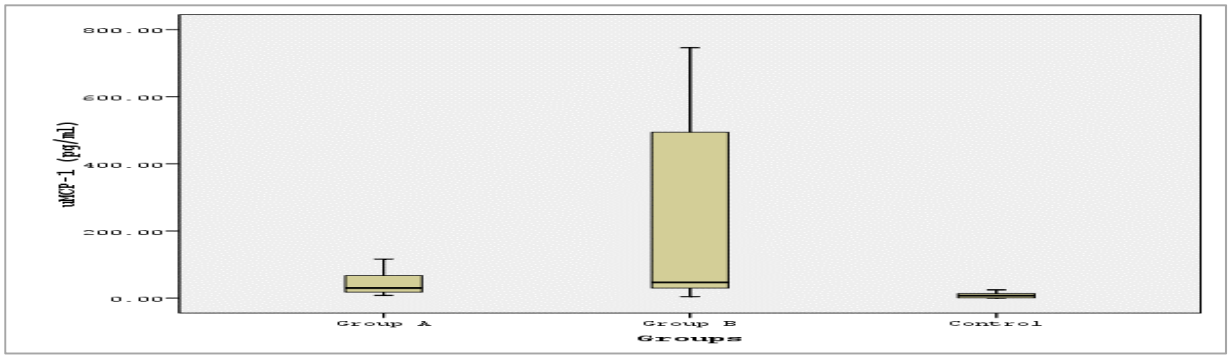

Figure 1: Comparison between the studied groups as regard uMCP-1.

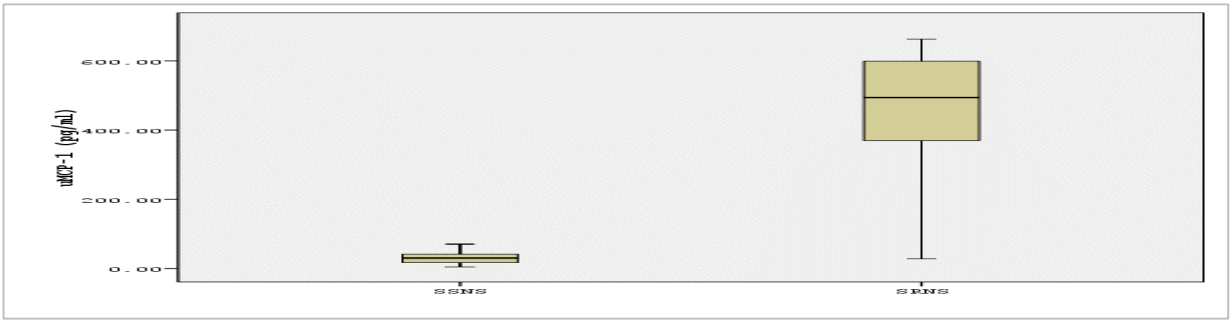

Figure 2: Comparison between SRNS and SSNS as regard uMCP-1.

Table 1: Demographic and Laboratory data of studied groups

Groups / Parameters		Group A (n = 25)		Group B (n = 25)		Control Group (n = 20)		Stat. test	P value
Age (years)	**Mean ± SD**	8.6 ± 2.2		8.3 ± 2.9		9.0 ± 2.4		F = 0.33	0.720 NS
Sex	**Male**	11	44%	12	48%	8	40%	X^2 = 0.28	0.865 NS
	Female	14	56%	13	52%	12	60%		
Duration of the disease (years)	**Median**	2		2		----		MW = 273.5	0.443 NS
	IQR	1.75 - 4.5		1 - 4.25		----			
Blood Pressure	**Normal**	25	100%	22	88%	20	100%	X^2 = 5.6	0.06 NS
	High	0	0%	3	12%	0	0%		
Edema	**Absent**	25	100%	6	24%	20	100%	X^2 = 46.9	**< 0.001 HS**
	Present	0	0%	19	76%	0	0%		
Proteinuria	**Negative**	15	60%	0	0%	20	100%	X^2 = 86	**< 0.001 HS**
	Trace	10	40%	0	0%	0	0%		
	++	0	0%	4	16%	0	0%		
	+++	0	0%	21	84%	0	0%		
S. creatinine (mg/dl)	**Mean ± SD**	0.6±0.2		0.5 ± 0.2		0.5 ± 0.2		F=0.93	0.399 NS
Blood urea (mg/dl)	**Mean ± SD**	25.6 ± 8.0		24.2 ± 6.7		28.3 ± 9.6		F=1.37	0.260 NS
CRP (mg/L)	**Median**	3.0		2.7		2.7		KW = 0.64	0.527 NS
	IQR	1.4		1.0		1.4			

F value of ANOVA and X^2: Chi-square tests were used

Table 2: Comparisons between studied groups as regard protein/creatinine ratio, albumin and cholesterol

Groups / Parameters		Group A (n = 25)	Group B (n = 25)	Control Group (n = 20)	Stat. test	P value
Protein/Creatinine ratio (mg/g Cr)	Mean ± SD	106.3 ± 40.6	6899.8±4288.8	44.2±10.6	F=52.7	**< 0.001 HS**
Serum albumin(g/dl)	Mean ± SD	3.0±0.3	2.3±0.4	4.1±0.5	F=104.2	**< 0.001 HS**
Serum total cholesterol(mg/dl)	Mean ± SD	160.8±21.3	241.9±65.1	95.5±16.7	F=68.9	**< 0.001 HS**

F value of ANOVA test was used

Table 3: uMCP-1 levels in studied groups

		Group A (n = 25)	Group B (n = 25)	Control Group (n = 20)	KW	P-value
uMCP-1 (pg/ml)	**Median**	30.1	46.9	7.2	31.9	**< 0.001 HS**
	IQR	16.2 - 74.8	29.01 - 501.7	0.57 - 13.9		

Kruskal Wallis (KW) test was used

Table 4: uMCP-1 levels in steroid resistant, and in steroid sensitive nephrotic syndrome patients

		SRNS (n = 18) (11 in relapse, 7 in remission)	SSNS (n = 16) (7 in relapse, 9 in remission)	Stat. test	P-value
uMCP-1 (pg/ml)	**Median**	300	30.1	MW = 10	< 0.001 HS
	IQR	104.4 - 528.1	15.3 - 46.9		

Mann Whitney U test was used

Table 5: Comparison between SRNS in group A and SSNS in group B as regard uMCP-1

		SRNS Group A (n = 7)	SSNS Group B (n = 7)	Stat. test	P-value
uMCP-1 (pg/ml)	Mean	120.4	31.7	T = 3.4	**0.005 S**
	±SD	64.6	22.2		

Independent sample T test was used

Table 6: Comparison between SRNS and SSNS as regard uMCP-1 in group A

		Group A		Stat. test	P-value
		SRNS (n = 7)	SSNS (n = 9)		
uMCP-1 (pg/ml)	Mean	120.4	34.7	T = 3.7	**0.002 S**
	±SD	64.6	20.5		

Independent sample T test was used

Table 7: Comparison between SRNS and SSNS as regard uMCP-1 in group B

		Group B		Stat. test	P-value
		SRNS (n = 11)	SSNS (n = 7)		
uMCP-1 (pg/ml)	Mean	450.3	31.7	T = 5.7	< 0.001 HS
	±SD	190.3	22.2		

Independent sample T test was used

Table 8: Correlation between uMCP-1 and other studied data in all studied patients.

Variables	All studied patients	
	r	p-value
uMCP-1 versus serum albumin	-0.42	**< 0.001 HS**
uMCP-1 versus serum total cholesterol	0.31	0.008 S
uMCP-1 versus urinary protein/creatinine ratio	0.40	0.001 S

DISCUSSION

We found that urine MCP-1 was higher in children with INS, implying that immune cells are involved in the development of NS. MCP-1 levels in the urine were substantially higher during relapse than during remission. MCP-1 causes monocyte migration and retention, as well as fibroblast transformation, in the glomeruli, and hence plays a significant role in glomerular inflammation. The pathophysiology of renal glomerular and tubular injury was linked to urinary MCP-1 [7]. Similalry, many studies showed that uMCP-1 was substantially greater in children with minimal change disease (MCD) in relapse than in group MCD after proteinuria regression and controls [9, 10, 11]. Furthermore, our study is in agreement with other studies that found MCP-1 levels in the urine were substantially greater in INS children at onset than in remission and when compared to the control group [12, 13, 14].

We found significant negative correlation between uMCP, and serum

albumin, and also significant positive correlations between uMCP and serum cholesterol, and UPCR, which indicate that uMCP may be considered a sensitive biomarker of disease activity. Moreover, many studies observed that patients with active idiopathic nephrotic syndrome had considerably higher urine MCP-1 excretion than patients in remission or controls [15, 16].

Our findings that urine MCP-1 was considerably higher in steroid resistant nephrotic syndrome (SRNS) patients than in steroid sensitive nephrotic syndrome (SSNS) patients imply that urinary MCP-1 could be a predictive biomarker for steroid resistance in INS. According to Matsumoto et al., [4], urine MCP-1 levels were considerably higher in SRNS patients compared to SSNS patients. Increased MCP-1 excretion in the urine of children with SRNS is a predictive biomarker of steroid resistance in INS. Also, our study was consistent with the study done by Besbas et al., [17] that found urinary concentrations of MCP-1 were significantly higher in SRNS compared to SSNS and healthy individuals.

In another study, Khatibi et al., [18] showed a higher level of uMCP-1 in SRNS patients compared to SSNS patients, indicating that uMCP-1 can predict glucocorticoid resistance. In addition, our findings were consistent with those of Agrawal et al., [19] who found that uMCP-1 may distinguish children with SRNS from those with SSNS at the time of initial disease presentation. Moreover, Angela [16] noted that patients with SRNS had higher concentrations of MCP-1 in the urine compared to SSNS patients ($p < 0.001$). On the other hand, Souto et al., [20] did not find any statistically significant differences between steroid-sensitive and steroid-resistant patients or between patients in relapse and remission as regard uMCP-1.

In our study number of patients was (50) compared to (32) in Souto et al's study and number of controls in this study was (20) compared to (12) in Souto et al's study, and Souto et al's study was done in Brazil while this study was done in Egypt.

CONCLUSION

Urinary MCP-1 can be considered a useful biomarker for identification of disease activity in children with INS, as well as a potential predictive biomarker of steroid responsiveness among these patients, because its level was markedly increased among patients with active disease and among steroid resistant patients. Further studies are needed to determine its role in the pathogenesis of nephrotic syndrome.

ABBREVIATIONS

ANOVA	A one-way analysis of variance
CBC	Complete blood count
CCL 2	C-C chemokine ligand 2
CRP	C-reactive protein
ELISA	Enzyme linked immunosorbent assay
FSGS	Focal segmental glomerulosclerosis
INS	Idiopathic nephrotic syndrome
MCD	Minimal change disease
MCNS	Minimal change nephrotic syndrome
MCP-1	Monocyte chemotactic protein-1
NS	Nephrotic syndrome
SPSS	Statistical Program for Social Science
SRNS	Steroid resistant nephrotic syndrome
SSNS	Steroid sensitive nephrotic syndrome
uMCP-1	Urinary monocyte chemotactic protein-1

REFERENCES

1. Cattran DC, Feehally J, Cook HT, Liu ZH, Fervenza FC, Mezzano SA, Wetzels JF. Kidney disease: improving global outcomes (KDIGO) glomerulonephritis work group. KDIGO clinical practice guideline for glomerulonephritis. Kidney Internat Supp. 2012; 2(2): 139-274.
2. Esezobor CI, Solarin AU, Gbadegesin R. Changing epidemiology of nephrotic syndrome in Nigerian children: A cross-sectional study. Plos one. 2020; 15(9): e0239300.
3. Lee JM, Kronbichler A, Shin JI, Oh J. Review on long term non renal complications of childhood nephrotic syndrome. Acta Paediatr. 2020; 109(3): 460-470.
4. Matsumoto Y, Ikezumi Y, Kondo T, Nakajima Y, Yamamoto Y, Morooka M, Yoshikawa T. Urinary monocyte chemotactic protein 1 as a predictive marker of steroid responsiveness in children with idiopathic nephrotic syndrome. Fujita Med J. 2018; 4(1):17-22.
5. Eom M, Hudkins KL, Alpers CE. Foam cells and the pathogenesis of kidney disease. Curr Opin Nephrol Hypert. 2015; 24(3): 245-258.
6. Aragón CC, Tafúr RA, Suárez-Avellaneda A, Martínez MT, de Las Salas A, Tobón GJ. Urinary biomarkers in lupus nephritis. J Transl Autoimm. 2020; 3:100042.
7. Mao S, Wu L. Association between MCP-1 2518 A> G gene olymorphism and chronic kidney disease. Internat Urol Nephrol. 2018; 50(12): 2245-2253.
8. Constantinescu AR, Marunţelu S, Velis E, Bizerea-Moga TO, Almaguer MC. Strategies to minimize growth retardation in children with steroid-sensitive nephrotic syndrome. J Clin Exp Nephrol. 2019; 4(2): 06-13.
9. Wasilewska A, Zoch-Zwierz W, Taranta-Janusz K, Kołodziejczyk Z. Urinary monocyte chemoattractant protein-1 excretion in children with glomerular proteinuria. Scand J Urol Nephrol. 2011; 45(1): 52-59.
10. Filha RDS, Pinheiro SVB, Macedo e Cordeiro T, Feracin V, Vieira ÉLM, Miranda AS, Simões e Silva AC. Evidence for a role of angiotensin converting enzyme 2 in proteinuria of idiopathic nephrotic syndrome. Biosci Rep. 2019; 39(1): BSR20181361.
11. Cho MH, Lee HS, Choe BH, Kwon SH, Chung KY, Koo JH, Ko CW. Interleukin-8 and tumor necrosis factor-alpha are increased in minimal change disease but do not alter albumin permeability. Am J Nephrol. 2003; 23(4): 260-266.
12. Woroniecki RP, Shatat IF, Supe K, Du Z, Kaskel FJ. Urinary cytokines and steroid responsiveness in idiopathic nephrotic syndrome of childhood. Am J Nephrol. 2008; 28(1): 83-90.
13. Vianna HR, Soares CMBM, Silveira KD, Elmiro GS, Mendes PM, de Sousa Tavares M, e Silva ACS. Cytokines in chronic kidney disease: potential link of MCP-1 and dyslipidemia in glomerular diseases. Pediatr Nephrol. 2013; 28(3): 463-469.
14. Simoes e Silva AC, Pereira AB, Teixeira MM, Teixeira AL. Chemokines as potential markers in pediatric renal diseases. Dis Markers. 2014; 3(2): 1-10.
15. Mariani LH, Eddy S, Martini S, Eichinger F, Godfrey B, Nair V, Kretzler M. Redefining Nephrotic Syndrome in Molecular Terms: Outcome-associated molecular clusters and patient stratification with noninvasive surrogate biomarkers. 2018; bioRxiv, 427880.
16. Angela C. The role of cell signaling molecules in the pathogenesis of glomerulonephritis in children. Mold Med J. 2021; 64(2): 37-41.

17. Besbas N, Kalyoncu M, Cil O, Ozgul RK, Bakkaloglu A, Ozaltin F. MCP1 2518 A/G polymorphism affects progression of childhood focal segmental glomerulosclerosis. Ren fail. 2015; 37(9): 1435-1439.

18. Khatibi SMH, Ardalan M, Abediazar S, Vahed SZ. The impact of steroids on the injured podocytes in nephrotic syndrome. J St Bioch Molec Biol. 2020; 196: 105490.

19. Agrawal S, Brier ME, Kerlin BA, Smoyer WE, Mahan J, Patel H, Greenbaum LA. Plasma Cytokine Profiling to Predict Steroid Resistance in Pediatric Nephrotic Syndrome. Kid Internat Rep. 2021; 6(3): 785-795.

20. Souto MF, Teixeira AL, Russo RC, Penido MGM, Silveira KD, Teixeira MM, Silva ACSE.. Immune mediators in idiopathic nephrotic syndrome: evidence for a relation between interleukin 8 and proteinuria. Pediatr Res. 2008; 64(6): 637-642.

AUTHORS' CONTRIBUTIONS

Conception and design of study: All authors.
Acquisition of data: 2nd author.
Analysis and/or interpretation of data: 1st & last author.
Drafting the manuscript: All authors.
Revising the manuscript critically for important intellectual content: 1st & last author.
Approval of the version of the manuscript to be published: All authors.

STATEMENTS

Ethics approval and consent to participate:
This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of Benha University hospital and informed written consent was obtained in every case from their legal guardians.

Consent for publication:
The contents and material of the manuscript have not been previously reported at any length or being considered for publishing elsewhere.

Availability of data and material:
The data and material are factual and genuine.

Conflict of interest:
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