Can Steroid Response in Idiopathic Childhood Nephrotic Syndrome bePredicted? A Single Center Quasi-Experimental Study

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ABSTRACT

Objective: To predict the role of clinical risk factors and urinary β2-microglobulin levels as a biomarker for steroid-resistant nephrotic syndrome.

Study Design: Quasi-experimental study.

Place and Duration of Study: Paediatric Nephrology Department, Sindh Institute of Urology and Transplantation, Karachi Pakistan, from Jun 2019 to Nov 2020.

Methodology: All children (3 months to 12 years) with either first episode or relapse of the nephrotic syndrome were included. A stored urine sample was used on 100 patients with steroid-sensitive (group-1) and 35 patients with steroid-resistant nephrotic syndrome (group-2). In addition, histopathology of all patients with steroid-resistant nephrotic syndrome was recorded.

Results: Both groups and those who had focal segmental glomerulosclerosis were compared and analysed to evaluate the predictability of steroid response. There was a significant association in both groups for microscopic haematuria, hypertension, heavy proteinuria (urine spot protein to creatinine ratio >10 g/g) and increased β2-microglobulin levels (> 3× normal) as individual risk factors (p<0.01). The sensitivity of β2-microglobulin levels was 78% and a positive predictive value of 80%. Multivariate regression analysis on steroid-resistant nephrotic syndrome as a group did not confer a higher risk; however, for children with focal segmental glomerulosclerosis, the likelihood of steroid unresponsiveness was significantly higher for the same parameters.

Conclusion: The addition of biomarker measurement and known clinical risk factors helped predict steroid-resistant focal segmental glomerulosclerosis. However, further studies are warranted before these results can be generalized.

Keywords: Biomarkers, β2-microglobulin, Idiopathic childhood nephrotic syndrome, Proteinuria, Steroid-resistant nephrotic syndrome.

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INTRODUCTION

Childhood nephrotic syndrome (NS) is defined as edema, nephrotic-range proteinuria (>50 mg/kg/day or 40 mg/h/m^2) and hypoalbuminemia (<25 g/L).[1] One of the most important prognostic factors in childhood NS is the response to steroids. Almost 90% of children with idiopathic NS (INS) achieve remission with standard steroid treatment and are labelled steroid-sensitive NS (SSNS). 10% do not respond and are categorized as steroid-resistant NS (SRNS).[2] SRNS is diagnosed only after a child has received a full dose of steroids (2 mg/kg/day) for 4 to 6 weeks.[3] After establishing steroid resistance, renal biopsy is recommended, and a second line immunosuppressive agent is added. This approach reduces the duration of proteinuria and minimizes steroid-related side effects.[4] Around 36-50% of children with SRNS are prone to develop steroid-related toxicity and end up in endstage renal disease (ESRD) within ten years of diagnosis.[5]

Certain clinical features are predictive for SRNS diagnosis, like heavy proteinuria, early age at presentation (<4 years), hypertension and haematuria. However, the sensitivity of these predictive factors is low (40% in most cases).[6] Acute tubulointerstitial injury is more pronounced in SRNS, mainly focal segmental glomerulosclerosis (FSGS) than in SSNS.[7] Currently, no diagnostic tests can accurately predict the steroid response at the time of presentation in NS. Serum and urinary biomarkers are non-invasive diagnostic tools and show promising results in the early detection of kidney injury in various diseases.[8] Many studies have demonstrated the diagnostic and prognostic value of biomarkers like adiponectin, neopterin, and β2-microglobulin in childhood INS, but the results are variable, ranging from 50 to 75%.[9]

Many studies have identified either clinical risk factors or specific urinary biomarkers in these children

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separately; however, no study has looked into combined clinical features and biomarkers to the best of our knowledge. Therefore, this study was planned to investigate the predictive value of combined clinical features and a urinary biomarker, β2-microglobulin, to predict response to steroids at the outset of the disease. The study aimed to find an association of SRNS with clinical risk factors like age, microscopic haematuria, hypertension, and degree of proteinuria in combination with urinary β2-microglobulin levels at the time of presentation. This may help early recognition and reduce unnecessary side effects related to prolonged use of steroids.

METHODOLOGY

This quasi-experimental study was carried out AT the Department of Pediatric Nephrology, Sindh Institute of Urology and Transplantation (SIUT), Karachi Pakistan, from July 2019 to June 2020. The study was approved by the Institutional Scientific Committee and Ethics Review Committee (ERC number: SIUT-ERC-2019/A-165).

Written informed consent was obtained from the parents or guardians of children, and assent was taken in the case of adolescents. The sampling technique was non-probability and consecutive.

Inclusion Criteria: All the children (3 months to 12 years) with either first episode or relapse of the nephrotic syndrome were included in the study.

Exclusion Criteria: Children with Congenital NS (CNS), atypical presentation or secondary NS were excluded from the study.

After obtaining informed consent, demographic and biochemical data was recorded on a proforma and urine samples of all patients were stored at-20˚C. Standard treatment with prednisolone (2 mg/kg/day) was given to all children based on their therapy response. They were diagnosed as SSNS or SRNS according to standard definitions. The sample size was calculated using OpenEpi online software based on an 8% margin of error and 95% confidence interval in a population of 4800 annual cases and 36.1% incidence.[10] A total of 135 cases with 35 cases of SRNS were required to achieve statistical significance. Once the required sample size was achieved, urinary β2-micro-globulin was checked by ELISA on the stored urinary samples from the presentation time using commercially available kits. The value of urinary β2-microglobulin >0.3 mg/l was considered significant. All stored samples were discarded after testing.

Statistical Package for Social Sciences (SPSS) version 22.0 was used for the data analysis. The quantitative variables (age, proteinuria, and spot urine protein creatinine ratio and urine β2-microglobulin) were expressed as either mean with standard deviation (SD) or median with interquartile range (IQR). ANOVA was used for continuous variables and chi-square for categorical variables. Multivariate logistic regression analysis was performed to compare SSNS with SRNS as a group and for patients diagnosed with FSGS on biopsy. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

A total of 135 children with a mean age of 6.3 ± 3 years (range: 1-12 years) were included in the study. Among these, 88 (65%) were males with a male to female ratio of 1.9: 1. These patients were stratified into two groups, of which 74% (n=100) were SSNS (Group-I) and 26% (n=35) were SRNS (Group-II). The clinical and biochemical parameters of patients in both categories are given in Table-I. Individual known risk factors that can predict SRNS, like age, degree of proteinuria, haematuria, hypertension and urinary β2-microglobulin excretion, were analysed individually and as a group by multivariate logistic regression analysis.

There was a statistically significant association between SRNS and heavy proteinuria (spot ratio of >10 g/g), presence of microscopic haematuria (presence of blood on dipstick of >+1), hypertension (as defined by blood pressure more than 95% ile for height, age, gender and height at the time of presentation) and increased β2-microglobulin urinary excretion at the time of presentation. However, there was no associa-tion with the age of children in our study participants.

All SRNS patients had undergone a renal biopsy, and the most common histological diagnosis was FSGS in19 (54%) of patients. Therefore, the same predictive risk factors were analysed separately for children with FSGS (Table-I).

Urinary β2-microglobulin levels were higher than normal (>0.3 mg/l) in 49 ((49%) of children with SSNS and 21 (60%) of SRNS children. Therefore, we used a cut-off value of 0.9 mg/l (3× normal) and calculated the validity of this test to predict SRNS. The sensitivity was 78%, while the specificity was only 45%. Similarly, the Positive Predictive Value (PPV) was 80%, and the Negative Predictive Value (NPV) was 60% (Table-II).

Table-I: Clinical features and laboratory data in steroid sensitive, steroid-resistant and Focal segmental glomerulosclerosis study groups.

Variables	Steroid Sensitive Nephrotic Syndrome (n=100)	Steroid Resistant Nephrotic Syndrome (n=35)	Focal Segmental Glomerulosclerosis (n=19)	*p*-value
Age (Mean ± SD), years	6.3 ± 3.0	6.2 ± 3.2	6.29 ± 3.3	0.925
Gender (n %)				
Male	63 (63%)	25 (71.4%)	14 (74%)	0.368
Female	37 (37%)	10 (28.6%)	5 (26%)	
Serum albumin (Mean ± SD), mg/dl	1.6 ± 0.5	1.8 ± 0.6	1.7 ± 0.6	0.404
Microscopic Haematuria				
Present	12(12%)	16(46%)	10 (53%)	<0.001
Absent	88 (88%)	19 (54%)	9 (47%)	
Hypertension >95th centile				
Present	9(9%)	15(43%)	9 (47%)	<0.001
Absent	91 (91%)	20 (57%)	53 (42%)	
Spot urine Pr/Cr ratio (Mean ± SD)	7.7 ± 4.8	11.9 ± 10.6	6 (32%)	0.002
Urine β2-microglobulin (median: IQR), mg/l	0.3 (0.12:0.82)	0.6 (0.18:5.7)	0.6 (0.18:5.7)	0.009

Table-II: Calculation of Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value for β 2-microglobulin levels more than 3 times normal.

Test	Steroid-Resistant Nephrotic Syndrome	Steroid Sensitive Nephrotic Syndrome	Total
β2-microglobulin >0.9 mg/l	TP = 16	FP = 22	38
β2-microglobulin <0.9 mg/l	FN = 19	TN = 78	97
Total	35	100	135

Sensitivity = True Positive / True positive + False Negative
16/35 = 46%
Specificity = True Negative / True Negative + False Positive
78/100 = 78%
Positive Predictive Value = True Positive / True Positive + False Positive
16/38 = 42%
Negative Predictive Value = True Negative / True Negative + False Negative
78/97 = 80%

A multivariate logistic regression analysis was done on all risk factors to evaluate the odds of developing SRNS in these children (Table-III). It showed that only hypertension and hematuria at the presentation were likely predictive of non-response to steroids ($p < 0.001$, 0.002, respectively).

Out of 19 patients with FSGS, 14 (74%) were boys, and multivariate analysis of only those children who had FSGS on biopsy showed that the odds of a child suffering from FSGS were 7.5 times higher if their β2-microglobulin levels are higher than three times normal. Similarly, the odds were 3 to 6 times higher if the age at presentation was less than four years and the presence of microscopic haematuria and hypertension along with heavy proteinuria of >10 g/g spot urine protein to creatinine ratio (Table-IV).

DISCUSSION

Children with NS, who do not respond to standard treatment, are known to develop primary disease and treatment complications. Due to genetic predisposition, a structural abnormality of the filtration barrier is found in 10 to 30% of these children. Multiple genes encoding for different proteins have been implicated; however, to select the patients who should get genetic testing and alternate therapies, the only reliable diagnostic criterion is to give a trial of high dose steroids to these children for a duration of 4 to 6 weeks.[3]

Our results have re-validated individual clinical risk factors commonly seen in children with SRNS. In the male gender, heavy proteinuria as indicated by a spot urinary protein to creatinine ratio of more than 10 g/g, microscopic haematuria and high blood pressure at the time of presentation have been described as risk factors for SRNS.[6] Many investigators, including Ramjee *et al*,[11] Gooding *et al*,[12] Weng *et al*,[13] and Chehade *et al*,[14] have attempted to study this cohort using different biomarkers with variable results. Ramjee *et al*, studied non-invasive markers and concluded that sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and isoelectric focusing (IEF) of urinary proteins appear useful non-invasive tests in the diagnosis and management of SRNS and FSGS. Gooding *et al*, performed proton nuclear magnetic resonance (1H NMR) metabolomic analyses on plasma samples

Table-III: Logistic Regression analysis of children with Steroid-resistant Nephrotic Syndrome.

Factors	Study Parameter		Univariate logistic regression			Multivariate logistic regression		
	SSNS n (%)	SRNS n (%)	*p*-value	OR	95% CI for OR	*p*-value	Adjusted OR	95% CI for AOR
Age								
<4 years	39 (39%)	12 (34%)	0.62	1.23	0.5-2.7	0.23	1.89	0.67-5.4
>4 years	61 (61%)	23 (66%)						
Gender								
Male	63 (63%)	25 (71%)	1			1		
Female	37 (37%)	10 (29%)	0.37	0.68	0.29-1.58	0.27	0.55	0.19-1.57
Hypertension								
<95th Centile	91 (91%)	20 (57%)	1					
>95th centile	9 (9%)	15 (43%)	<0.001*	7.58	2.9-19.7	<0.001*	0.52	3.35-33.0
Hematuria								
Present	88 (88%)	19 (54%)	1			1		
Absent	12 (12%)	16 (46%)	<0.001*	6.17	2.5-15.1	0.002*	5.39	1.90-15.24
Proteinuria								
<10 gm/gm	72 (72%)	21 (60%)	1			1		
>10 gm/gm	28 (28%)	14 (40%)	0.18	1.71	0.8-3.8	0.13	2.2	0.78-6.19
β2-microglobulin levels								
<0.9 mg/L	78 (78%)	19 (54%)	I			I		
>0.9 mg/L	22 (22%)	16 (46%)	0.007*	2.98	1.3-6.7	0.10	2.3	0.85-6.14

Table-IV: Logistic Regression analysis of children with focal segmental glomerulosclerosis.

Factors	Study Parameter		Univariate Logistic Regression			Multivariate Logistic Regression		
	SRNS n (%)	FSGS n (%)	*p*-value	OR	95% CI for OR	*p*-value	Adjusted OR	95% CI for AOR
Age								
<4 years	6 (32%)	6 (37%)	0.71	0.77	0.19-3.1	0.68	1.48	0.23-9.5
>4 years	13 (68%)	10 (63%)						
Gender								
Male	14 (74%)	11 (69%)	1			1		
Female	5(26%)	5 (31%)	0.75	1.28	0.29-5.5	0.97	1.03	0.16-6.7
Hypertension								
< 95th Centile	13 (68%)	7 (44%)	1					
>95th centile	6 (32%)	9 (56%)	0.14	2.78	0.69-11.1	0.27	0.37	0.06-2.1
Hematuria								
Present	10 (53%)	9 (56%)	1			1		
Absent	9 (47%)	7 (44%)	0.83	0.86	0.23-3.3	0.55	0.58	0.10-3.4
Proteinuria								
<10 gm/gm	9 (47%)	12 (75%)	1			1		
>10gm/gm	10 (53%)	4 (25%)	0.09	0.3	0.07-1.28	0.27	2.6	0.48-14.6
β2-microglobulin Levels								
<0.9 mg/L	6 (32%)	13 (81%)	I			I		
>0.9 mg/L	13 (68%)	3 (19%)	0.006*	0.10	0.02-.52	0.01*	10.8	1.7-69.4

(n=86) from 45 patients with NS (30 SSNS and 15 SRNS) obtained at initial presentation before glucocorticoid initiation and after approximately seven weeks of glucocorticoid therapy to identify candidate biomarkers able to either predict SRNS before treatment or define critical molecular pathways/targets regulating steroid resistance. Meta-bolomic analyses of serial plasma samples from children with SSNS and SRNS identified elevated creatinine and glutamine concentrations, and reduced malonate concentrations, as auspicious candidate bio-markers to predict SRNS at disease onset in pediatric NS, as well as additional candidate biomarkers with the potential to identify mechanistic molecular pathways that may regulate clinical steroid resis-tance.[12] The role of β2-microglobulin in various kidney diseases has also been extensively studied.[15-17] Most of the researchers have reported similar results as seen in our cohort. Due to heavy

proteinuria in nephrotic syndrome, it is predictable that there will be higher urinary excretion of low molecular weight proteins in both SSNS and SRNS.

In our cohort, we stratified all these risk factors to see if SRNS can be predicted in a specific group of children with multiple risk factors as a whole group and as a sub group of only those children who had FSGS. The multivariate analysis of the whole group was not conclusive; however, a statistically significant association was seen in children with FSGS. Zagury *et al,* demonstrated FSGS as the most prevalent histological type in SRNS with an increased risk of developing ESRD.[18] In another study by Muljanto *et al*, patients who had findings of tubular atrophy and interstitial fibrosis on renal biopsy had raised β2-microglobulin excretion in urine.[19]

Based on our findings, a child with microscopic haematuria, hypertension, heavy proteinuria and three times higher urinary excretion of β2-microglobulin has a very high likelihood that he/she may have SRNS with FSGS. Therefore, children with these findings may not require a complete 4 to 6 weeks duration of high dose steroids, and kidney biopsy followed by an alternate immune suppression regimen may be started early in the disease.

Since this is a single centre experience on a limited number of patients, we recommend further longitudinal research with a larger cohort to establish this approach. It has to be borne in mind that SRNS as a whole group was not predictable through this approach. We have used only β2-microglobulin as a biomarker. Further researches about specific biomarkers in other morphologic lesions are essential to validate these findings.

To summarize, urinary β2-microglobulin, along with other clinical parameters, can be a reliable non-invasive prognostic indicator of steroid-resistant nephrotic syndrome, especially FSGS. These children may be spared a prolonged and high dose exposure to glucocorticoids and specific therapies may be instituted earlier in these children. Further longitudinal research with a more significant number of patients and other biomarkers is recommended to establish this approach further..

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CONCLUSION

The addition of biomarker measurement and known clinical risk factors helped predict steroid-resistant focal segmental glomerulosclerosis. However, further studies are warranted before these results can be generalized.

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Conflict of Interest: None

Author's Contribution

MR: Study conceptualization, design, data acquisition and analysis, primary drafting of paper, AAL: study conceptualization, design, data acquisition and analysis, final review of paper, AE: Literature review, data acquisition, primary drafting of paper, MA: Literature review, data acquisition and analysis, SK: Literature review, data acquisition and analysis, SH: study conceptualization, design, data acquisition, final review of paper.

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