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Original Research Article

Urinary Interleukin 18 in Children with Nephrotic Syndrome and Its Role in Steroid Responsiveness

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Abstract:

Introduction: Nephrotic syndrome includes the clinical manifestation of glomerular diseases associated with heavy proteinuria i.e., nephrotic range (40 mg/m²/hr or >1000 mg/m²/day; spot Up/Uc>2 mg/mg; 3-4+ by urine dipstick); hypoalbuminemia (albumin <3.0 g/dL); and edema. In children, the most common cause of nephrotic syndrome is idiopathic or primary nephrotic syndrome (INS), also called nephrosis. In the kidney, the predominant source of IL-18 is the tubular epithelial cells. In the recent years, the biological and pathological roles of IL-18 have been studied in many diseases. In clinical practice, IL-18 is expected to be useful in the diagnosis of diseases and the estimation of disease severity and prognosis.

Methods: This case control study was conducted in 60 children with nephrotic syndrome, 30 children each in steroid sensitive group and steroid resistant group, compared with 30 controls who were admitted for illnesses other than nephrotic syndrome. Interleukin 18, a urinary biomarker for predicting steroid resistance was compared between the steroid sensitive and steroid resistant group and controls.

Results: Urine interleukin 18 levels were found to be significantly higher in the steroid resistant group than the steroid sensitive group which in turn is higher than the control group. A positive correlation was established between the urinary IL 18 levels and the degree of proteinuria both in the steroid sensitive and steroid resistant groups. Other parameters such as serum creatinine, serum cholesterol, urine spot PCR were found to be higher in children with steroid resistant nephrotic syndrome

Conclusion: This study evaluated the prediction of steroid resistance in children with nephrotic syndrome using urinary interleukin 18 which is one of the biomarkers studied in proteome profiling of urine. Children with steroid resistant nephrotic syndrome showed higher levels of urinary IL 18 which will enable us to predict subsequent clinical course and early initiation of steroid sparing drugs as well as to search for molecular pathways and targets associated with steroid resistance. Other biochemical parameters like serum cholesterol, serum creatinine, urine spot PCR and 24 hour urine protein were also found to be significantly higher in the steroid resistant group, thus enabling us in predicting steroid resistance.

Keywords: Nephrotic Syndrome, Urinary IL-18.

Introduction

Nephrotic syndrome includes the clinical manifestation of glomerular diseases associated with heavy proteinuria i.e., nephrotic range (40 mg/m²/hr or >1000 mg/m²/day; spot Up/Uc>2 mg/mg; 3-4+ by urine dipstick); hypoalbuminemia (albumin <3.0 g/dL); and edema.

In children, the most common cause of nephrotic syndrome is idiopathic or primary nephrotic syndrome (INS), also called nephrosis.[1] Idiopathic nephrotic syndrome is defined by the combination of a nephrotic syndrome (proteinuria, hypoalbuminemia, hyperlipidemia, and edema) and non-specific histological abnormalities of the kidney including minimal change nephropathy, focal and segmental glomerular sclerosis (FSGS), and diffuse mesangial proliferation. INS accounts for only 25% of adult cases; it is by far the most common cause of nephrotic syndrome in children. Almost all nephrotic children between 1 and 6 years of age in Western countries suffer from INS.[2] The annual incidence of INS in children in the USA and in Europe has been estimated to 1–3 per 100,000 children below age of 16, with a cumulative prevalence of 16 per 100,000 children. In India the annual incidence ranges from 1.2 to 16.9 per 100,000 children.[3]

Glomeruli show a fusion of epithelial cell foot processes on electron microscopy and no

significant deposits of immunoglobulins or complement factors on immunofluorescence. It is considered that minimal change disease, diffuse mesangial proliferation and FSGS are separate diseases because of differences in response to corticosteroids and subsequent clinical course.

Indeed, these various pathologic features carry prognostic significance.[4] Patients with FSGS and diffuse mesangial proliferation have more frequent haematuria and are often resistant to corticosteroid treatment. They progress more often to renal failure and may have a recurrence of the nephrotic syndrome soon after renal transplantation. However, in the early stages, FSGS and minimal change disease are indistinguishable. The International Study of Kidney Disease in Children found minimal change disease in 76.6% of children with primary nephrotic syndrome.[5]

A significant number of patients with FSGS respond to corticosteroids, whereas some steroid resistant patients have no sclerotic changes on biopsy specimens containing adequate renal tissue. Therefore, it is believed that, although histological variants of the idiopathic nephrotic syndrome carry prognostic significance, they cannot be considered as separate entities.[6]

The term minimal change disease has become synonymous with steroid sensitive INS although renal biopsy is usually not performed in patients who are responsive to steroid therapy. Indeed, in many centres, renal biopsy is recommended only for those patients who fail to respond to steroids. Consequently, renal biopsy findings in recent published series are not representative of the true incidence of various histopathological categories seen in INS. It is therefore more appropriate to classify the patients according to their response to steroid therapy. Response to corticosteroid therapy carries a greater prognostic weight than the histological features seen on initial renal biopsy. Thus, two types of INS can be defined: steroid-responsive nephrotic syndrome, in which the proteinuria rapidly resolves and steroid-resistant nephrotic syndrome, in which steroids fails to induce remission.

A major part of the immunopathogenesis of nephrotic syndrome is T cell mediated immune response where there is imbalance between T helper type 1/ type 2 cytokines, which plays a key role with shift towards type 2 mechanism.[6] T helper type 2 cytokines such as IL 4, IL5, IL13 and IL 18 are increased during the active phase of the disease and were used in several studies as biomarkers to predict steroid resistance. IL 18 is a pro inflammatory cytokine belonging to IL 1 cytokine super family. It plays an integral role in causing systemic and local inflammatory response leading to glomerular and tubular injury and development of renal dysfunction in nephrotic syndrome. IL-18 is produced by macrophages, dendritic cells, epithelial cells, keratinocytes, chondrocytes, osteoblasts, synovial fibroblasts and adrenal cortex cells, and plays an important role in inflammatory pathology. In the kidney, the predominant source of IL-18 is the tubular epithelial cells. IL-18 gene expression could be enhanced by stimulation with microbe products such as LPS and cytokines such as IFN-α/β/γ and TNF-α. Nitric oxide causes suppression of the secretion of IL-1β and IL-18 by inhibiting caspase-1. An inhibitor of mammalian target of rapamycin (mTOR), has been widely used as an autophagy inducer.

The induction of autophagy by rapamycin can suppress the production and secretion of IL-1β and IL-18 and limit excessive inflammation. In the recent years, the biological and pathological roles of IL-18 have been studied in many diseases. Inflammation underlies the pathogenesis of many acute or chronic kidney diseases, and IL-18 plays an important role. Inflammation underlies the pathogenesis of many renal diseases, including Nephrotic syndrome, acute kidney injury (AKI) and chronic kidney disease (CKD), and the role of IL-18 in inflammation has been reported in many experimental animal models.[7] In clinical practice, IL-18 is expected to be useful in the diagnosis of diseases and the estimation of disease severity and prognosis. Thus, in our study, we aim to determine the role of interleukin 18 in steroid responsiveness in children with nephrotic syndrome.

Methodology

Sample Selection: The current case control study included pediatric patients of age between 2 and 12 years, diagnosed with nephrotic syndrome in Institute of Child Health and Research centre, GRH, Madurai (SSNS/ SRNS) according to the diagnostic criteria of International Study of Kidney Disease in Children. An equivalent group of Non nephrotic healthy children admitted for other illnesses were taken as control. The patients with congenital nephrotic syndrome and patients on immunomodulator therapy were excluded from the study. All the patients were enrolled into the study after getting informed written consent from their parents. The Study was approved by Institutional Ethical Committee in GRH, Madurai.

Data Collection: 30 children each group (steroid sensitive nephrotic syndrome, steroid resistant nephrotic syndrome) and 30 controls were enrolled into the study. Age distribution, gender distribution and urinary interleukin 18 were studied. Other parameters such as serum urea, creatinine, cholesterol, serum albumin and 24 hour urinary protein were also compared among the groups. The steroid sensitive and steroid resistant groups were

also studied for correlation between their urinary interleukin 18 levels and the degree of proteinuria.

Laboratory Analysis: About 10 ml of the 24-hour urine were collected from each participant at the time of admission. All samples collected were centrifuged at 1000 rpm for 10 min at 4 degree C and the supernatant were separated and stored at minus 80 degree C in the Medical Research Unit (MRU) of GRH, Madurai. The concentration of interleukin 18 in the urine was measured by Enzyme Linked Immunosorbent Assay (ELISA) method using commercially available antibody coated ELISA kit. A monoclonal antibody specific for human IL-18 has been coated into the wells of the microtiter strips provided. Samples including standards of known human IL-18 content, control specimens and unknowns are pipetted into these wells and IL-18 present in a sample is bound to the immobilized antibody-coated wells. The wells are washed and biotinylated anti-human IL-18 antibody is added.

During the first incubation, the human IL-18 antigen binds to the immobilized antibody on one site and to the solution phase biotinylated antibody on a second site. After removal of excess second antibody, streptavidin-peroxidase enzyme is pipetted to the wells. This binds to the biotinylated antibody to complete the four member sandwich. After a second incubation and washing to remove the entire unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color which is measured at 450 nm. The intensity of this color is directly proportional to the concentration of human IL-18 present in the original specimen.

Statistical Analysis: Data were analysed by Statistical package for the Social Sciences (SPSS) version 15.0 for windows. Data are presented as mean ± SD. Nominal variables are given as numbers with appropriate percentage whereas continuous variables are presented as means with standard deviation if normally distributed. Normality was checked by Shapiro- Wilk test. Chi-square test was used to test associations between categorical variables. For pairwise comparisons of continuous variables, the student's t-test was used. For multi-group comparison one-way analysis of variance (ANOVA) was used. Pearson correlation was used to estimate the strength of linear relationship between two continuous variables. P value of < 0.05 was considered as statistically significant.

Results

The demographic and characteristics of the studied participants are shown in Table 1.

In our study steroid sensitive nephrotic syndrome was more common in females whereas in males steroid resistance was predominant. But this was not statistically significant.

Table 1: Baseline characteristics of the studied children

Variables	Steroid sensitive (n=30)	Steroid resistant (n=30)	Control group (n=30)	P value
Age (years)				
Mean (SD)	5.65(2.662)	5.73(3.129)	7.03(3.232)	0.144
Sex				
Male (*n*, %)	11(36.7%)	18(60%)	15(50%)	0.193
Female (*n*, %)	19(63.3%)	12(40%)	15(50%)	

The mean serum urea value in the steroid sensitive group was 17±3.3 mg/dl, which is significantly lower than that in the steroid resistant group 32.6±15 mg/dl (p value <0.001).

Mean serum urea level in the steroid sensitive group was higher than the controls however this was not statistically significant (p value 0.314).

The mean serum creatinine level was higher among the steroid resistant group 0.84±0.29 mg/dl, than the steroid sensitive group 0.5±0.17 mg/dl and the difference was found to be significant. The cholesterol level among the steroid resistant group 601±84 mg/dl was higher than that in the steroid sensitive group 376±108 mg/dl, which in turn is higher than the control group 163±19 mg/dl.

The serum albumin level among steroid resistant group 1.017±0.5 g/dl was lower than the steroid sensitive group 2.28±0.4 g/dl.

Controls had higher serum albumin levels 3.5±0.5 g/dl, than the steroid sensitive group. This difference was statistically significant.

Table 2: Comparison of laboratory data between Steroid Sensitive, Steroid resistant and the control groups

Variable	Steroid Sensitive (*n*=30)	Steroid Resistant (*n*=30)	Control group (n=30)	*P* value
Urea (mg/dL)				<0.001
Mean (SD)	17.13 (3.371)	32.63(15.804)	16.07(4.66)	
Creatinine (mg/dL)				<0.001

Mean (SD)	0.513(0.172)	0.843(0.297)	0.55(0.125)	
Albumin (mg/dL)				<0.001
Mean (SD)	2.28(0.424)	1.017(0.531)	3.56(0.498)	
Cholesterol (mg/dL)				<0.001
Mean (SD)	376.7(108.974)	601.2(84.068)	167(19.47)	

Table 3: Comparison of IL-18, 24 hour urinary protein and Urine spot PCR between Steroid sensitive and resistant group

Variable	Steroid Sensitive	Steroid Resistant	P value
IL-18 (ng/L)			
Mean (SD)	688.81(157.459)	3253.23(862.464)	<0.001
24 Hour Urine Protein (mg/m²/hr)			<0.001
Mean (SD)	69.82(13.716)	106.5(22.09)	
Urine Spot PCR			<0.001
Mean (SD)	13.263(6.89)	20.617(6.454)	

The 24 hour urinary protein value in the steroid resistant nephrotic syndrome group was 106.5±22.09 mg/m²/hr , which was significantly higher thanthat in the steroid sensitive group which was 69.8±13.7 mg/m²/hr (p value< 0.001). Urinary interleukin 18 levels were found to be higher in steroid resistant group than steroid sensitive group which in turn is higher than the controls and the difference is significant p value <0.001

Table 4: Correlation between Interleukin-18 level and 24 hour urinary protein of the steroid sensitive and steroid resistant group

Variable	24 hour urinary protein of the Steroid sensitive group	24 hour urinary protein of the Steroid sensitive group
Interleukin-18	r = 0.889	r = 0.179
	p <0.000*	p =0.343
r : Pearson correlation, *p <0.05 significant		

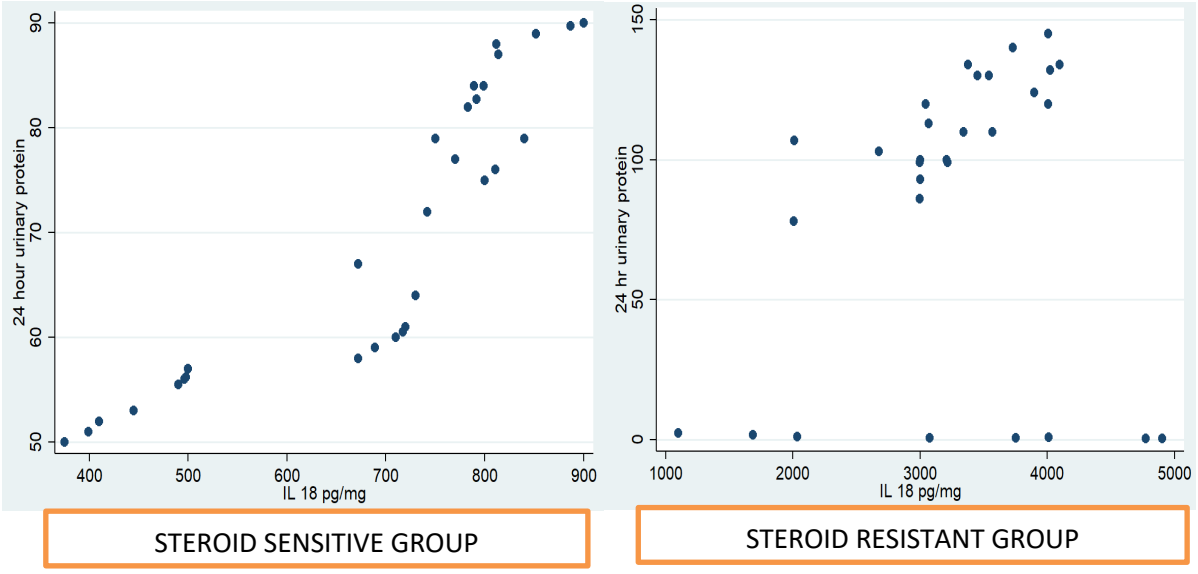

Figure 1: Scatter plot showing Correlation between interleukin-18 level and steroid sensitive and resistant group

A positive correlation between urinary IL 18 and the degree of proteinuria in the steroid sensitive and resistant group respectively and it was found to be statistically significant in the former (Table 4 and Figure 1)

Discussion

In our study urinary interleukin 18 levels were higher in children with steroid resistant nephrotic syndrome than those who are steroid sensitive. A study done by Zhongguo Dang dai et al[8] in Chinese population also showed similar results with IL 18 levels being higher in children with steroid resistance.

Our study also showed a positive correlation between the degree of proteinuria and the urinary interleukin 18 levels, both in steroid resistant and steroid sensitive nephrotic syndrome children. These findings were consistent with the results of

another study conducted in Poland by Katarzyna Killis Pstrusinska et al [9] where similar correlation was found. Printza et al [10] conducted a study in children with steroid sensitive nephrotic syndrome and found a positive correlation between IL 18 levels and the disease activity, a finding similar to our study. According to a study conducted by Youssef DM et al[11], demographic data were compared between the steroid resistant and steroid sensitive children and found that male gender and young age at onset were important predictors of steroid resistance.

In our study steroid resistant nephrotic syndrome was more common among males which were similar to the previous study but prediction of steroid resistance could not be established with respect to age factor. In our study the mean age at presentation was not significantly different between the steroid resistant and steroid sensitive groups. This was much similar to a study by Zaorska et al[12] where no difference was observed between the steroid resistant and steroid sensitive groups with respect to demographic data.

Our study showed higher levels of serum creatinine among the steroid resistant group when compared to the steroid sensitive group. Gooding JR et al[13] also demonstrated a very good association of higher serum creatinine levels with steroid resistance. Anitha Palaniyandi et al[14] conducted a study in Chennai where they compared several clinical and laboratory profile among steroid sensitive and steroid resistant nephrotic children group and found that parameters such as serum creatinine and urine spot PCR were higher in the steroid resistant group.

Serum albumin level was found to be lower in the steroid resistant group. Similar findings were also observed in our study. Taiwo Augustina et al[15] showed higher levels of serum cholesterol to be associated with steroid resistance in children with nephrotic syndrome.

Our study also showed significantly higher levels of serum cholesterol among the steroid resistant group when compared to the steroid sensitive group. Zhou J et al[16] conducted a study where they found that the levels of inflammatory factors such as IL-18 and urinary protein in children with nephrotic syndrome before treatment were significantly higher than those in children in the healthy control group, and significantly lower after treatment than those before treatment which is supporting the findings of our study.

Conclusion

This study evaluated the prediction of steroid resistance in children with nephrotic syndrome using urinary interleukin 18 which is one of the biomarkers studied in proteome profiling of urine. Children with steroid resistant nephrotic syndrome showed higher levels of urinary IL 18 which will enable us to predict subsequent clinical course and early initiation of steroid sparing drugs as well as to search for molecular pathways and targets associated with steroid resistance. Other biochemical parameters like serum cholesterol, serum creatinine, urine spot PCR and 24-hour urine protein were also found to be significantly higher in the steroid resistant group, thus enabling us in predicting steroid resistance.

Limitations: There are a number of limitations to our study. Our study population was children attending our tertiary care centre which is a unicentric study including one geographic area, hence the results could not be generalized to a wider population. ELISA test was used to quantify urinary IL 18 which would require larger volume of sample when compared to the immunoblot assay where smaller quantities are sufficient.

Immunoblot has higher specificity than ELISA in detecting biomarkers such as IL 18 which could not be afforded in our study. The predictive power of our study would have increased with the use of muti- centric study and better specific tests.

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