

ORIGINAL ARTICLE

Iran J Allergy Asthma Immunol
December 2020; 19(6):632-639.
Doi: 10.18502/ijaai.v19i6.4932

**Predictive Value of Serum Interleukins in Children
with Idiopathic Nephrotic Syndrome**

Azar Nickavar¹, Ehsan Valavi², Baranak Safaeian³, Parisa Amoori², and Mostafa Moosavian⁴

¹ *Department of Pediatric Nephrology, Iran University Medical Sciences, Tehran, Iran*

² *Chronic Renal Failure Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

³ *Neonatal and Children's Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran*

⁴ *Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Received: 26 June 2020; Received in revised form: 1 September 2020; Accepted: 5 September 2020

ABSTRACT

Pro-inflammatory cytokines have been suggested in the pathogenesis of idiopathic nephrotic syndrome (INS), with conflicting results. This study was performed to identify alteration of different serum interleukins (ILs) in children with INS, and their predictive value in response to steroid treatment.

Three groups of children (27; steroid-sensitive INS, 21; steroid-resistant INS, and 19 healthy controls) with normal serum C3, negative serologic tests of hepatitis B virus (HBV), hepatitis C virus (HCV), human immune deficiency virus (HIV), and parasitic infections were included in this study. Serum concentrations of IL-1 β , IL-2, IL-6, IL-8, IL-13, and IL-18 were measured, using quantitative colorimetric sandwich ELISA kits. Children with secondary nephrotic syndrome, inflammations, systemic disorders, and chronic kidney disease were excluded.

The serum concentration of all ILs; except IL-13 and IL-18; was significantly higher in children with INS, compared with the healthy controls. Serum IL-2 had the highest sensitivity of (95.24%) in patients with INS. All of the serum ILs had acceptable accuracy in children with INS, compared with the control group. The serum concentration of IL-1 β , IL-6, and IL-8 was significantly higher in children with steroid-sensitive nephrotic syndrome (SSNS), compared with steroid-resistant nephrotic syndrome (SRNS). All of these ILs had acceptable accuracy for the prediction of steroid response in patients with INS.

Our findings suggested the pathogenic role of pro-inflammatory cytokines in children with INS, of which IL-1 β , IL-6, and IL-8 were accurate biomarkers for the prediction of steroid response in these patients.

Keywords: Child; Cytokines; Idiopathic nephrotic syndrome; Steroids

Corresponding Author: Ehsan Valavi, MD;
Chronic Renal Failure Research Center, Ahvaz Jundishapur
University of Medical Sciences, Ahvaz, Iran. Tel: (+0 613) 444 352,
E-mail: dr_ehsan_valavi@yahoo.com

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is one of the most common glomerular disorders in children,

Serum Interleukins in Idiopathic Nephrotic Syndrome

characterized by heavy proteinuria, hypoalbuminemia, and edema. The primary pathogenic feature of nephrotic syndrome (NS) is the loss of glomerular filtration selectivity, which occurs secondary to glomerular structural damage of podocyte, basement membrane, and endothelial surface area.^{1,2} Medical response to corticosteroid treatment has a great prognostic value in these patients.¹

The pathogenesis of INS has not been completely understood. It has been suggested a primary immune-mediated disorder, characterized by an imbalance between Th1 and Th2 cells along with an increased Th2 cytokine level.³ Increased cytokine production by abnormal T cells is the leading cause of glomerular damage and eventual proteinuria in these patients.^{4,5} In addition, massive proteinuria might induce a pro-inflammatory response with cytokine induction and inflammatory cell infiltration.² Favorable clinical response to immunomodulating drugs such as steroids and cyclosporine A supports the involvement of immune cytokines in the pathogenesis of INS.⁶

Since the mid-1990s, alteration of several cytokines has been suggested as the primary immunopathogenic mechanism of INS.⁴ However, conflicting results have been reported about the predominant type 1 or type 2 cytokine pattern in these patients.⁷ According to the controversial cytokine expression, this study was performed to identify alteration of different interleukins (ILs) concentration in children with primary INS, and their predictive value in response to steroid treatment.

MATERIALS AND METHODS

Patient Selection

A total of 48 patients with INS and 19 age-matched healthy controls were enrolled in a prospective multicentric case-control study during 2018–2019. The study was approved by the local medical ethics committee (ethical code; IRAJUMS.REC.1398.537, License code; CRD-9801), and informed consent obtained from children's legal guardians.

NS was defined as urine protein excretion >50 mg/kg/24 h or Pro/Cr ≥ 2 in the first-morning urine sample, associated with hypoalbuminemia (serum albumin < 2.5 g/dL) and edema.

Children with the first episode or relapse of NS with normal serum C3 level, and negative serologic tests of hepatitis B virus (HBV), hepatitis C virus (HCV), human immune deficiency virus (HIV), and parasitic infections were included in this study. Patients with

infectious or inflammatory disorders, congenital abnormalities of the kidney and urinary tract (CAKUT), family history of NS, chronic kidney disease, and immunosuppression during the last 2 weeks were excluded.

Steroid-resistant nephrotic syndrome (SRNS) was defined as ≥ 2+ of urine dipstick protein after 8 weeks of oral steroid treatment. Steroid-sensitive nephrotic syndrome (SSNS) was defined as 0-trace urine albumin excretion in 3 consecutive days, with the resolution of edema and serum albumin > 2.5 g/dL after steroid treatment.¹

Five mL peripheral blood was collected at the time of active disorder (initial episode, relapse) without immunosuppressive treatment during the past 2 weeks. The serum was isolated and kept at -80°C.

Cytokine Assay

Concentrations of IL-1β, IL-2, IL-6, IL-8, IL-13, and IL-18 were measured, using quantitative colorimetric sandwich ELISA kits (R&D China Co. Ltd., Shanghai), and expressed as pg/mL.

Statistical Analysis

Analysis of data was performed by SPSS 24.0 for Windows and MedCalc version 15.4.

Demographic and laboratory values with normal distribution are expressed as mean ± SD. Serum ILs with non-normal distribution are presented as median (Q1, Q3). Normality was determined by Kolmogorov Smirnov statistical test. Categorical variables were compared using the chi-square test. In addition, the comparison of continuous variables with normal or non-normal distribution was performed by Student's t-test and Mann-Whitney U test. A receiver operating curve (ROC) analysis was used to identify the sensitivity and specificity of different ILs in children with INS, and those ILs for prediction of steroid response in these patients. The highest values of sensitivity and specificity were used to determine the optimal IL cut-off value of each serum IL. *p* value < 0.05 was considered as significant.

RESULTS

Demographics

The serum concentration of different ILs was measured in 48 patients (case) with INS and compared with 19 healthy children (control). The mean age of all

patients with INS (53.89±32.76 months) and controls (58.10±29.93 months) had no significant difference ($p=0.947$). The majority of patients were males (M/F=1.82), and females outnumbered males in the control group (F/M=2.8). About 27 (56.25%) children with INS were resistant to corticosteroids, and 21 (43.75%) responded to steroid treatment. The mean age had no significant difference between the two groups of INS ($p=0.284$). Males outnumbered females in both groups, with no significant difference ($p=0.732$). Patients with SRNS had a significantly higher incidence of hypertension ($p=0.003$), increased ESR ($p=0.034$), and serum creatinine ($p<0.001$), compared with SSNS. The demographic and clinical characteristics of both groups of patients with INS are shown in Table 1.

Cytokines Measurement

Median serum levels of IL-1 β , IL-2, IL-6, and IL-8 were significantly higher in children with INS,

compared with the normal control group. In contrast, mean serum levels of IL-13 and IL-18 were significantly higher in the control group, compared with INS (Table 2, Figure 1). The best cutoff values with the highest sensitivity and specificity of each IL are shown in Table 3, Figure 2. Accordingly, IL-2 had the highest sensitivity, followed by IL-1 β , IL-8, and IL-6. In addition, serum IL-8 concentration with equally IL-13 and IL-18 had the highest specificity. Based on the area under the curve (AUC), all serum ILs had acceptable accuracy in children with INS. Serum concentrations of IL-1 β , IL-6, and IL-8 were significantly lower in children with SRNS, compared with SSNS (Table 4, Figure 3). Serum IL-8 and IL-2 had the highest sensitivity and specificity for the prediction of steroid response, respectively. In addition, all of these ILs were accurate biomarkers for the suggestion of steroid response in INS (Table 5, Figure 4).

Table 1. Demographic and clinical characteristics of patients with INS

Variables (mean±SD)	SSNS (n=21)	SRNS (n=27)	<i>p</i>
Age (month)	56.55 ± 31.47	50.47 ± 34.83	0.284
Gender (M/F) (N;%)	18 (66.7%)/9 (33.3%)	13 (61.9%)/8 (38.1%)	0.732
Hematuria (N;%)	7 (25.9%)	6 (28.6%)	0.838
Hypertension (N;%)	0 (0%)	6 (28.6%)	0.003
ESR (mm/h)	64.07 ± 31.82	68.76 ± 28.90	0.034
Sodium (meq/L)	139.44 ± 4.29	136.76 ± 4.07	0.800
Potassium (meq/L)	4.16 ± 0.58	4.24 ± 0.79	0.800
Calcium (mg/dL)	8.13 ± 0.72	8.06 ± 0.62	0.578
BUN (mg/dL)	17.33 ± 9.24	27.00 ± 10.92	0.201
Creatinine (mg/dL)	0.52 ± 0.33	0.67 ± 0.29	<0.001
Albumin (g/dL)	1.92 ± 0.49	2.19 ± 0.71	0.576
Cholesterol (mg/dL)	370.31 ± 89.00	367.17 ± 117.09	0.313
Triglycerides(mg/dL)	307.09 ± 102.56	378.00 ± 144.01	0.310

Idiopathic nephrotic syndrome (INS); Steroid-sensitive nephrotic syndrome (SSNS); Steroid-resistant nephrotic syndrome (SRNS)

Table 2. Comparison of serum interleukins in nephrotic syndrome and control group using Nonparametric Mann-Whitney U test

IL (pg/mL)	Case (n=48)	Control (n=19)	<i>p</i>
	Median (Q1,Q3)	Median (Q1,Q3)	
IL-1 β	888(697.65,1137.50)	470 (470,470)	0.001
IL-2	165.64 (111.47,237.62)	24.30 (24.30,220.88)	0.009
IL-6	37.50 (14.48,47.60)	11.78 (6.40,26.67)	< 0.001
IL-8	53.10 (42.10,97.70)	10.41 (8.22,21.34)	< 0.001
IL-13	7 (1.20,24.20)	53.40(23.80,1059.40)	< 0.001
IL-18	5(4.1,7.00)	8.90 (5.90,44.00)	< 0.001

Serum Interleukins in Idiopathic Nephrotic Syndrome

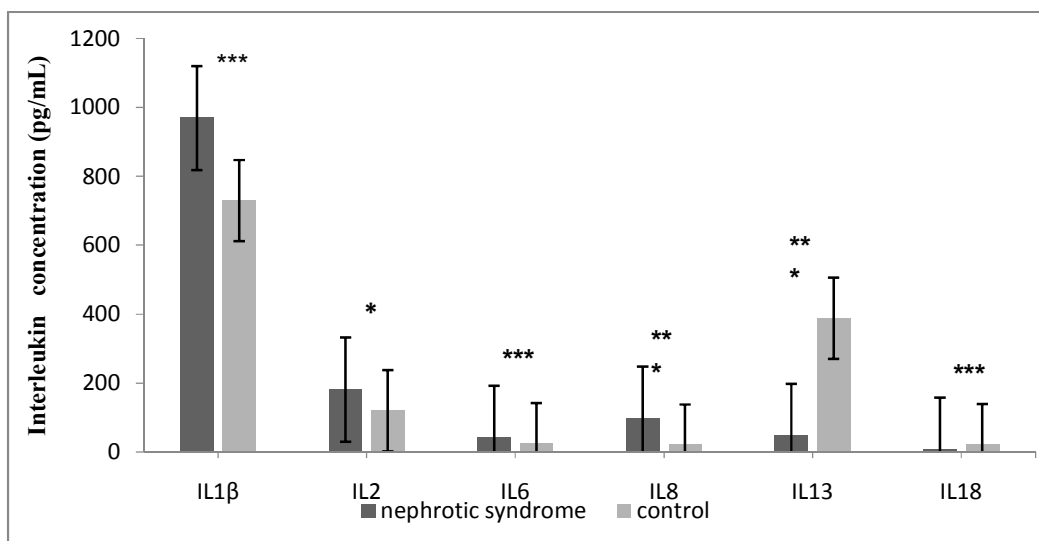


Figure 1. Comparison of serum interleukins in nephrotic syndrome and control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Table 3. Sensitivity, specificity, and accuracy of serum interleukins in patients with idiopathic nephrotic syndrome (INS)

IL (pg/mL)	Cut Point	Sensitivity	Specificity	AUC	95% (CI)	SE	<i>p</i>
IL-1β	>470	88.64	78.95	0.755	0.630 - 0.854	0.077	<0.001
IL-2	>24.30	95.24	68.42	0.708	0.578 - 0.817	0.091	<0.023
IL-6	>11.93	86.05	57.89	0.714	0.585 - 0.821	0.080	0.008
IL-8	>30.87	87.23	94.74	0.890	0.789- 0.954	0.051	<0.001
IL-13	<13.8	70.21	89.47	0.834	0.722 -0.914	0.057	<0.001
IL-18	<5.6	68.09	89.47	0.832	0.720 -0. 913	0.053	<0.001

AUC: area under the curve, CI: confidence interval, SE: standard error

Table 4. Comparison of serum interleukins in 2 groups of patients with idiopathic nephrotic syndrome (INS); using Nonparametric Mann–Whitney U test

IL (pg/mL)	SSNS (n=48)	SRNS (n=19)	<i>p</i>
	Median (Q1,Q3)	Median (Q1,Q3)	
IL-1β	1074.05(865.12,1259. 07)	684.20 (467.40,930.55)	< 0.001
IL-2	177.45 (118.55,246.78)	154.60 (72.47,226.17)	0.314
IL-6	40.80 (28.20,53.30)	21.45 (13.07,40.05)	0.042
IL-8	68.60 (46.82,138.15)	50.00(34.56,66.28)	0.034
IL-13	7.50 (2.67,22.47)	5.50(0,26.45)	0.520
IL-18	5.00(4.22,7.30)	4.90 (4.05,7.20)	0.769

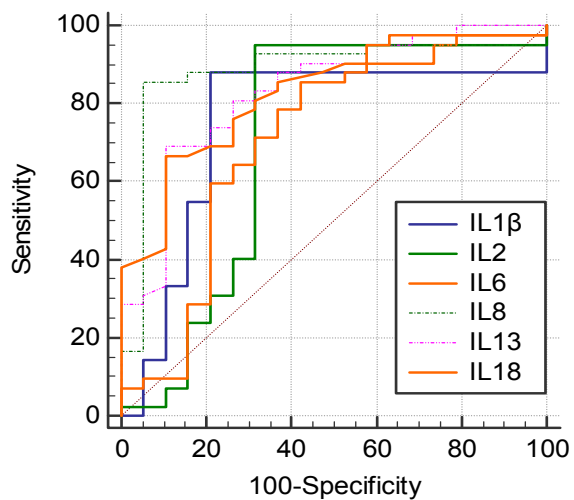


Figure 2. Receiver operating characteristic (ROC) analysis demonstrating an overall accuracy of different interleukins in patients with idiopathic nephrotic syndrome (INS)

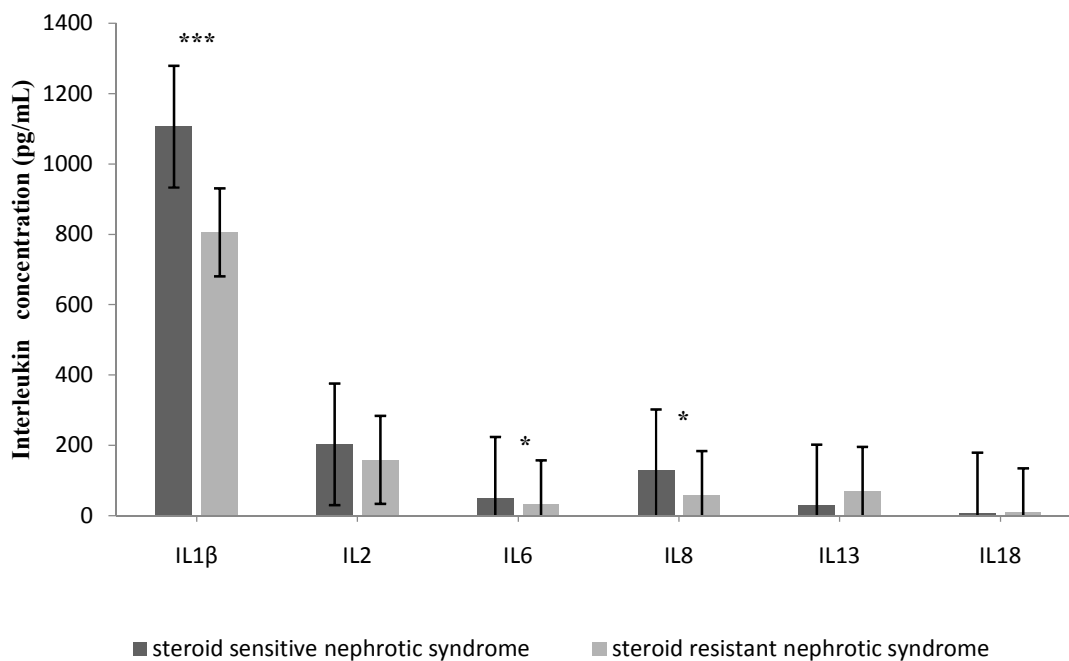


Figure 3. Comparison of serum interleukins in 2 groups of patients with idiopathic nephrotic syndrome (INS) (* $p < 0.05$, * $p < 0.001$)**

Serum Interleukins in Idiopathic Nephrotic Syndrome

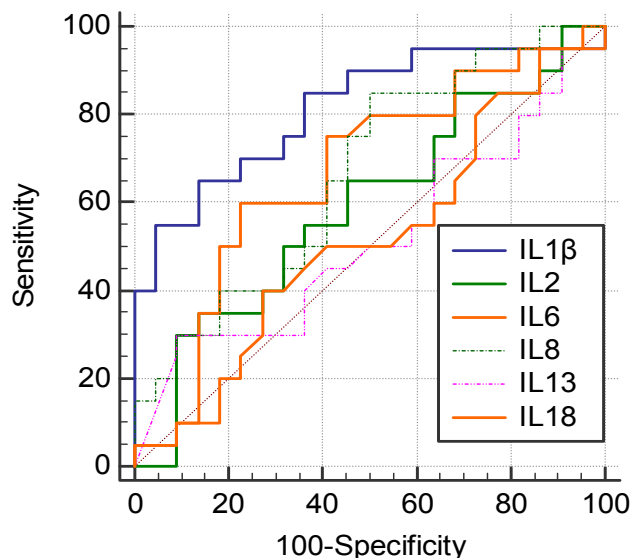


Figure 4. Receiver operating characteristic (ROC) analysis demonstrating an overall accuracy of different interleukins in 2 groups of patients with idiopathic nephrotic syndrome (INS)

Table 5. Sensitivity, specificity, and accuracy of serum interleukins in 2 groups of patients with idiopathic nephrotic syndrome (INS)

IL (pg/mL)	Cut Point	Sensitivity	Specificity	AUC	95% (CI)	SE	<i>p</i>
IL-1 β	<751.4	65	78.50	0.819	0.674 - 0.918	0.068	<0.001
IL-2	<110.80	35	86.36	0.591	0.429 - 0.740	.090	0.315
IL-6	<25.40	60	78.30	0.682	0.522- 0.819	0.085	0.032
IL-8	<71.7	85.7	50	0.681	0.529- 0.810	0.078	0.021
IL-13	<0.4	33.30	92.31	0.555	0.403 -0.700	0.089	0.537
IL-18	<4.5	42.86	73.08	0.523	0.372 -0. 671	0.088	0.795

AUC: area under the curve, CI: confidence interval, SE: standard error

DISCUSSION

The pathogenesis of INS has not been completely identified. However, activation and imbalance of both Th1 and Th2 cells along with the expression of different cytokines have been postulated for increased glomerular permeability in these patients.⁶ Th1 cells are involved in cellular immune reaction and production of inflammatory IL-2 cytokine, while Th2 cells are responsible for IL-4 and IL-13 cytokine release.⁷ This study was performed to identify the alteration of serum ILs and their predictive value of steroid response in children with INS.

The serum IL-8 was significantly higher in our

children with INS compared with the control group, with acceptable sensitivity, specificity, and accuracy. IL-8 is a chemotactic chemokine, secreted by monocytes, lymphocytes, macrophage, and non-immune cells including podocytes, endothelial, mesangial, and proximal or distal renal tubular cells. It is the mediator of inflammatory reactions by attracting and activating neutrophils and lymphocytes during an inflammatory process.^{2,5} IL-8 induces proteinuria by alteration of glomerular basement membrane sulfide metabolism, through increasing catabolism and decreasing negative charge of glomerular heparan sulfate.

Similar to our study, a significant increase of

plasma IL-8 concentration has been reported during the active phase of minimal change disease (MCD), compared with the remission phase and healthy individuals, which could predict steroid sensitivity in these patients.^{2,8} Serum IL-8 was significantly higher in children with SSNS in our study, with high sensitivity and acceptable accuracy for the prediction of steroid response in these patients. Similar to our results, serum IL-8 was a relatively accurate predictor of steroid sensitivity in the Xie et al study, which decreased during the remission phase in these patients.⁸

IL-18 is a member of the IL-1 cytokine family, which stimulates both type-1 and type-2 immune reactions. Correlation of serum IL-18 with IL-4 and IL-13 is an indicator of predominant type-2 cytokine pattern in children with active SSNS.³

Increased serum and urine IL-18 concentration has been reported during the active phase of both SSNS and SRNS, in accordance with the disease activity.^{3,9} IL-18 was a specific and accurate biomarker in children with INS in our study, which was significantly decreased during the active phase of NS, compared with the healthy control group.

Jiang et al reported increased serum IL-18 in both SSNS and SRNS, with no significant change after treatment of SRNS, but decreased in patients with SSNS.⁹ Serum IL-18 had no significant difference between the 2 groups of patients with NS in our study.

IL-2 is an immunoregulatory cytokine, which is produced by type-1 cellular immunity. It has an important role in both cellular and humoral immune responses. The pathogenic role of regulatory T cells in INS has been supported by a protective effect of direct T regulatory infusion or stimulation by IL-2.¹⁰

Serum IL-2 increased in the active phase of NS in our study, with high sensitivity and acceptable accuracy. However, it was not a significant predictor of steroid response in our patient population. In addition, serum IL-2 mRNA was higher in the acute phase of INS, compared with the remission phase as stated in Shimoyama et al study, which suggested upregulation of serum IL-2 in the pathogenesis of INS.⁶

IL-13 has been suggested as an important cytokine in the pathogenesis of MCD. Overexpression of IL-13 leads to decreased glomerular nephrin, podocin, and dystroglycan contents, in addition to increased glomerular podocyte B7-1 (CD-80) expression with eventual glomerular injury and minimal change nephropathy.¹¹

IL-13 was significantly higher during the active phase of SRNS and SSNS in the previous studies, with a positive correlation with urine protein excretion, and decreased during the remission phase.^{11,12} Concentration of IL-13 was higher in patients with frequent relapse than the first episode of NS in the Mishra et al study.¹² However, serum IL-13 was lower in our patients with NS, compared with the healthy control group, with high specificity and acceptable accuracy. In addition, it was not a significant predictor of steroid response in our study.

Dysregulation of both IL-1 and IL-6 has been suggested in the pathogenesis of proteinuria in MCD.⁵ The highest concentration of IL-6 and IL-1 β has been reported during the first presentation and relapse of MCD and non-MCD, compared with the remission and healthy control in some reports.¹³ We also showed a significantly higher level of serum IL-1 β and IL-6 in our patients, with high sensitivity, and acceptable accuracy. In addition, serum IL-1 β and IL-6 were significantly higher in SSNS, with acceptable accuracy for the prediction of steroid response in these patients.

Despite these implications, some studies showed no documented change or decrease of serum ILs in patients with INS.¹⁴ Similarly, serum IL-13 and IL-18 decreased significantly during the active phase of INS in our study, which suggested further multicentric studies of serum and urine cytokine alteration in these patients, to identify the potential value of these biomarkers for diagnosis and prediction of response to steroid or other immunosuppressive drugs, and provide the best specific treatment with the least medical complication in these patients.

In conclusion, we found a significant alteration of proinflammatory cytokines in children with INS, which suggested predominant pathogenic T cell dysfunction in these patients. In addition, serum IL-1 β , IL-6, and IL-8 were significant and accurate predictive biomarkers of steroid response in patients with INS.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

This article was performed by the funding support from the Ahvaz Jundishapur University of Medical Sciences.

Serum Interleukins in Idiopathic Nephrotic Syndrome

REFERENCES

1. Nickavar A, Safaeian B, Sadeghi-Bojd S, Lahouti Harah dashti A. Urine Neutrophil Gelatinase Associated Lipocalin to Creatinine Ratio: A Novel Index for Steroid Response in Idiopathic Nephrotic Syndrome. *Indian J Pediatr.* 2016;83(1):18-21.
2. Alsharidah AS, Alzogaibi MA, Bayoumy NM, Alghonaim M. Neutrophil chemokines levels in different stages of nephritic syndrome. *Saudi J Kidney Dis Transpl.* 2017;28(6):1256-63.
3. Shalaby SA, Al-Edressi HM, El-Tarhouny SA, Fath El-Bab M, Zolaly MA. Type1/type2cytokineserumlevels and role of interleukin-18 in children with steroid-sensitive nephritic syndrome. *Arab J Nephrol Transplant.* 2013;6(2):83-8.
4. Tain YL, Chen TY, Yang KD. Implications of serum TNF-beta and IL-13 in the treatment response of childhood nephrotic syndrome. *Cytokine.* 2003;21(3):155-9.
5. Al-Eisa AA, Al Rushood M, Al-Attayah RJ. Urinary excretion of IL-1 β , IL-6 and IL-8cytokines during relapse and remission of idiopathic nephritic syndrome. *J Inflamm Res.* 2017;23;10:1-5.
6. Shimoyama H, Nakajima M, Naka H, Maruhashi Y, Akazawa H, Ueda T, et al. Up-regulation of interleukin-2mRNA in children with idiopathic nephrotic syndrome. *Pediatr Nephrol.* 2004;19(10):1115-21.
7. Youssef DM, Elbehidy RM, El-Shal AS, Sherief LM. Thelper1 and Thelper2cytokines in atopic children with steroid-sensitive nephrotic syndrome. *Iran J Kidney Dis.* 2015;9(4):298-305.
8. Xie H, Fang M, Lin H, Li P, Chen J, Sun Y et al. Intermittent high-volume hemofiltration promotes remission in steroid-resistant idiopathic nephritic syndrome. *Ren Fail.* 2015;37(6):966-73.
9. Jiang HK, Luo G, Jiang H. Interleukin-18 expression in peripheral blood mononuclear cells in children with steroid-resistant nephrotic syndrome. *Zhongguo Dang Dai Er Ke Za Zhi.* 2009;11(5):337-40.
10. Bertelli R, Bonanni A, Di Donato A, Cioni M, Ravani P, Ghiggeri GM. Regulatory Tcells and minimal change nephropathy: in the midst of a complex network. *Clin Exp Immunol.* 2016;183(2):166-74.
11. Ha TS, Nam JA, Seong SB, Saleem MA, Park SJ, Shin JI. Montelukast improves the changes of cytoskeletal and adaptor proteins of human podocytes by interleukin-13. *Inflamm Res.* 2017;66(9):793-802.
12. Mishra OP, Teli AS, Singh U, Abhinay A, Prasad R. Serum immunoglobulin E and interleukin-13levels in children with idiopathic nephrotic syndrome. *J Trop Pediatr.* 2014;60(6):467-71.
13. Wang L, Li Q, Wang L, Li C et al. The role of Th17/IL-17 in the pathogenesis of primary nephrotic syndrome in children. *Kidney Blood Press Res.* 2013;37(4-5):332-45.
14. Pereira Wde F, Brito-Melo GE, Guimarães FT, Carvalho TG, Mateo EC, Simões e Silva AC. The role of the immune system in idiopathic nephritic syndrome: a review of clinical and experimental studies. *Inflamm Res.* 2014;63(1):1-12.