Original Research

Serum Cytokines and Histopathological Pattern of Idiopathic Nephrotic Syndrome in Saudi Children

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Abstract

Objective: Nephrotic syndrome (NS) defined by massive continued losses of urinary proteins, resulting in hypoalbuminemia and edema. These are associated with complications such as increased susceptibility to infections, thromboembolism, altered lipid and carbohydrate metabolism and losses in binding proteins in the urine. Aim of the study: To evaluate the cytokines levels in different stages of idiopathic nephrotic syndrome (INS) and to determine the histopathological pattern of INS.

Methods: Fifty two children with INS were divided into; steroid-sensitive group I (SS) (18/52) and steroid-resistant group II (SR) (34/52). Both groups subdivided into SS in relapse IA (8/18), SS in remission IB (10/18), and SR in relapse group IIA (25/34) and in remission group IIB (9/34). Twenty age-matched controls compared with data. The following parameters had assessed; serum levels of IFN-γ, IL-2, IL-4, IL-13 and IL-18 by using quantitative colorimetric ELISA test. Renal biopsy specimens were histopathologicaly studied.

Results: Serum IFN-γ levels were significantly lower in the relapse phase of SS compared with the remission phase and controls. On the contrary, serum IL-4 and IL-13 levels were significantly higher in the relapse phase of SS and SR compared with the remission phases and controls. IL-18 levels were significantly higher in the relapse phase of SS and SR subgroups compared with the remission phases and the controls respectively. IL-4 and IL-13 significantly correlated with IL-18. All 34 SR nephrotic patients were submitted to renal biopsies, which showed focal segmental glomerulosclerosis (FSGS) (59%) the most common diagnosed entities of INS.

Conclusions: Type-2 cytokines predominate in relapse phase of SS and SR patients and one could predict a good response to steroid therapy. IL-18 expression significantly correlated with this type-2 immune response. The primary glomerular diseases in Saudi children and FSGS are the most common diagnosed entities.

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in childhood and characterized by heavy proteinuria, edema and hypoalbuminemia [1]. Histological variants of childhood idiopathic nephrotic syndrome may be due to a variety of glomerulopathies, including minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous glomerulopathy (MG), mesangioproliferative glomerulonephritis (MesPGN), and diffuse mesengial proliferation (DMP) [2].

The International Study of Kidney Diseases in Children (ISKDC) demonstrated in the 1970s that, the most

common histological lesion in INS patients was MCD [3-4]. The early studies showed that, MCD in 70 -90**%** of cases, while FSGS found in only 5-7**%** of biopsies was a rare cause of INS in children [5-7]. Recent reports from many parts of the world, particularly the USA, suggested that the incidence of FSGS is on the rise in both adults and children [8].

The pathogenesis of INS still not yet fully understood. The role for the immune mechanism proposed by many studies [2, 9-11] supported by a favorable response to anti-inflammatory drugs [2], a relation between relapses and viral infections or allergic reactions [10], the recurrence of the disease in transplanted patients [11] and an association with immunologic disorders [2].

Recently, it has been proposed that alterations in the cytokine profile of INS patients might contribute to proteinuria and glomerular damage [12]. Idiopathic nephrotic syndrome (INS) was proposed to be a disorder of T-cell dysfunction [13]**.** Since then, many investigators had tried to identify the mechanism by which T cells might increase glomerular permeability [14, 15]. It was suggested that this may be due to a factor (cytokine), which was released by activated T cells [14]. According to the cytokines which prevail, the immune response was functionally subdivided into type-1 and type-2. Type-1 response, which normally prevails, dominated by interferon-gamma (IFN-γ) and interleukin (IL)-2 and mainly had anti-infectious function, whereas type-2 response dominated by IL-4 and IL-13 and favored atopic expression [14].

IL-18 is a unique cytokine that stimulates both type-1 and type-2 immune responses depending on the cytokine milieu [16]. During the last two decades several studies have been carried out to characterize the cytokine pattern in steroid sensitive nephrotic syndrome (SSNS) with sometimes conflicting results [17-20]. Moreover, very little was known about the involvement of IL-18. Adults with SSNS, IL-18 levels were elevated in the active stage of the disease and that there was a positive correlation between IL-18 production and disease activity [21].

Aim of the study is to evaluate the immune mediators (cytokines) in different stages of INS by measuring the serum levels of IL-2, IFN-γ, IL-4, IL-13 and IL-18 concentrations, define the potent involvement of IL-18 and determine the histopathological pattern of INS in Saudi children.

PATIENTS AND METHODS

Study design

The present cross-sectional study used a convenience sample of children and adolescents with INS, followed-up at the Pediatric Nephrology Departments of Al-Nour Specialty Tertiary Referral Hospital, Makah and Al-Jedaany Hospitals, Jeddah, KSA and seen between February 2007 and May 2011. Diagnostic criteria for INS were based on the International Study of Kidney Disease in Children [22]. Pediatric Nephrology Departments has followed-up children with nephrotic syndrome, according to a protocol that includes definition of disease etiology, assessment of clinical course and laboratory alterations, institution of treatment protocols and indication of renal biopsy based on clinical (corticosteroid unresponsiveness) and laboratory findings.

Patients with nephrotic syndrome: Inclusion criteria included 52 children and adolescents with well-established INS with still preserved renal function, followed-up, whose parents gave their consent to participate in the study protocol. Mean age at the investigation was 9.3 ± 4.3 years (range, 2 to 18 years), and the boy-girl ratio was 3:1. Patients with congenital or secondary forms of nephrotic syndrome and INS patients at stages 2–5 of chronic kidney disease automatically excluded from the study. None of the patients had been on cytotoxic immunosuppressive therapy within the 6 months preceding the study and none assessed at the first attack of NS.

Controls: The control group consisted of sex and age-matched 20 healthy subjects (8 girls, 12 boys) with a mean age of 11.0±1.8 years from our Pediatric Primary Care Center. Healthy status either determined through the subjects' medical history and a parental report or self-report to rule out the presence of chronic or acute diseases. Blood samples in control group drawn simultaneously to other routine blood exams.

Control tissues from nephrectomy specimens of kidney removed because of trauma was included in this study.

Our study approved by the clinical research committee of the hospitals and performed according to ethical procedures. All parents of the patients and the controls informed about the study and their written consent obtained.

Study protocol

Our fifty-two INS patients will allocated according to their corticosteroid treatment responsiveness (fulfilled the ISKDC criteria for diagnosis of NS) [3,4] into two groups; complete remission obtained after an 8-wk course of corticosteroids (Steroid-sensitive group I [SSNS] [18/52]). No or partial remission occurred with corticosteroid treatments or other medications used as an attempt to achieve disease control (Steroid-resistant [SRNS] group II) (34/52) [23]. We also subdivided SS patients in two subgroups, according to disease activity (8/18 SSNS in relapse IA and 10/18 SSNS in remission

IB) and SR patients in two subgroups, according to disease activity (25/34 SRNS in relapse IIA and 9/34 SRNS in remission IIB). In accordance to standard recommendations, our INS patients were considered in remission if their proteinuria levels were below or equal to 100 mg/m2/24 h, and in relapse if their proteinuria levels were above 100 mg/m2/24 h [2, 23].

Patients considered having MCD if they had nephrotic proteinuria, no hematuria, no hypocomplementemia, and normal blood pressure and renal function [23].

Thirty-Four patients considered having SRNS and patients underwent a percutaneous renal biopsy for suspected significant renal lesions. Renal specimens were examined by light microscopy (LM), electron microscopy (EM) and immunohistochemistry (IHC). Histopathological findings interpreted by the same histopathologist and the histopathological diagnosis were made as per standard case definitions [3, 24]. Adequacy of biopsy was defined as the presence of at least 5 glomeruli in the specimen on light microscopy [25].

Serum cytokine assay

Blood samples collected and stored at -80 $^{\circ}$C for future serum cytokine assessment of Th1 cells cytokines (IL-2, IFN-γ) and Th2 cells cytokines (IL-4, IL-13 and IL-18) and using quantitative colorimetric sandwich ELISA kits (R&D Systems, UK) following the manufacturer's instructions as described elsewhere [26]. Each cytokine sample run in duplicate way and the mean cytokine concentration was calculated. Assay sensitivities were 7.2 pg/ml, 13.8 pg/ml, 6.6 pg/ml, 14.3 pg/ml and 12.5 pg/ml for IL-2, IFN- γ, IL-4, IL-13 and IL-18, respectively.

Histopathological evaluation

Light microscopic study: Tissue specimens fixed in 10% neutral buffered formalin embedded in paraffin and cut in sequential 4–5 μm thickness. These sections were stained with hematoxylin and eosin stain (H & E) [27] and periodic acid Schiff's stain [28] which were useful for optimal evaluation of the morphologic details.

Transmission Electron Microscopic Study [29]**:** Biopsy specimens were fixed in a mixture of 2,5% gluteraldhyde in (0,2M) cacodylate buffer (pH.7,4) for 24 hours, washed in two changes cacodylate buffer, post fixed for 2 hours in osmium tetroxide, and specimens were dehydrated in ascending grade of ethyl alcohol and embedded in Epon 812. The semithin section (1μm) cut by ultramicrotome, stained with toluidine blue, and examined by light microscope to show the tissue and for good selection and localization of the needed part examined in thin section. The ultrathin section (100nm) prepared and stained with uranyl acetate and leads citrate and examined by JEOL model XC100 transmission electron microscope.

Immunohistochemical Study [30]**:** Renal sections of 5μm thick, Bouin- or formalin-fixed were deparaffinized through xylene, alcohol and distilled water. Endogenous peroxidase blocked by 3% H_2O_2 for 15 min and then the sections were treated in a microwave oven in a solution of 0.1 mM citrate buffer pH 6.0 for 10 min or EDTA buffer 1 mM pH 8.0 for synaptopodin detection. After blocking, the sections were incubated overnight at 4°C with the specific primary antibody {mouse anti-human synaptopodin clone G1D4 (Progen, Heidelberg, Germany)}. The sections were incubated with the correspondent biotinylated secondary antibodies for 30 min at 22°C. After three rinses in Tris saline buffer, they were incubated with streptavidin-peroxidase (Dako) 1/1000 for 30 min. Color was developed with substrate (Dako) and then counterstained with haematoxylin, dehydrated, and mounted with Canadian balsam (Polysciences, Inc.). The specificity checked by omission of primary antibodies and use of non-immune sera. Synaptopodin stands out as a distinct brown stain against the light background of the remaining glomerulus.

Statistical analysis

Data were entered on an IBM compatible PC and Statistical analysis performed using Statistical Package for the Social Sciences for Windows (SPSS version 17, Chicago, IL, USA). Descriptive statistics were done in the form of median and ranges. The non-parametric Wilcoxon signed-rank test used to compare differences between study groups with paired data. For non-paired data, statistical significance analyzed by the Mann–Whitney U test. Spearman's coefficient of correlation (r) and Regression analysis model used to determine the correlations. $P < 0.05$ considered statistically significant.

RESULTS

Fifty-two patients were included in this study. All these patients presented with INS to the pediatric nephrology clinics of Al-Nour Specialty Tertiary Referral Hospital, Makah and Al-Jedaany hospitals, Jeddah, KSA. Mean age at the investigation was 9.3± 4.3 years (range, 2 to 18 years), and the boy-girl ratio was 3:1 (39 boys and 13 girls). The mean values of weight, height, body mass index, systolic and diastolic pressures, and renal function parameters were within normal range**.** Fifty-two INS patients were divided according to steroid-responsiveness (18/52 were SS and 34/52 were SR). The clinical and laboratorial features of each group at the time of blood and urine sampling; no differences were detected in age, sex distribution, weight, height, body mass index, nitrogen waste levels (urea and

creatinine), uric acid, albumin, triglycerides, total cholesterol and GFR among INS groups and controls.

Table 1 summarized results regarding serum IL-2, IFN-γ, IL-4, IL-13 and IL-18 levels (Th1 and Th2 cytokines) in steroid sensitive nephrotic patients according to corticosteroid responsiveness: No difference in IL-2 levels found between SS nephrotic patients of relapse, remission phase and the controls ($p > 0.05$). Serum IFN-γ levels were significantly lower in the relapse phase of SSNS compared with the remission phase ($p = 0.03$) and the controls ($p = 0.07$) and no significant difference was identified between the controls and the remission phase ($p > 0.05$). On the contrary, serum IL-4 and IL-13 levels were significantly higher in the relapse phase of SSNS compared with the remission phase and the controls ($p < 0.001$). IL-18 levels were significantly higher in the relapse phase of SSNS compared with the remission phase ($p = 0.03$) and the controls ($p < 0.001$).

Table 2 summarized results regarding serum IL-2, IFN-γ, IL-4, IL-13, and IL-18 levels (Th1 and Th2 cytokines) in steroid resistant nephrotic patients according to corticosteroid responsiveness: No difference in serum IL-2 and IFN-γ levels found between SR nephrotic patients in relapse, remission phases and the controls ($p > 0.05$). On the contrary, serum IL-4 levels were significantly higher in the relapse phase of SRNS compared with the remission phase ($p = 0.03$) and the controls ($p = 0.06$. Similarly, serum IL-13 levels were significantly higher in relapse phase of SRNS compared with the remission phase ($p < 0.001$). IL-18 levels were significantly higher in the relapse phase of SRNS compared with the remission phase ($p = 0.05$) and the controls ($p < 0.001$).

Serum IL-18 and IL-4 levels were significantly correlated in nephrotic patients in the remission phase of SS and SR groups ($r = 0.82$ and $p<0.001$, $r = 0.61$ and $p < 0.01$ respectively). In patients in active relapse stage of SS and SR, serum IL-18 and IL-4 levels, were also significantly correlated ($r = 0.67$ and $p = 0.01$, $r = 0.64$ and $p<0.01$ respectively) **(Table 3).** Serum IL-18 and IL-13 levels were significantly correlated in nephrotic children in the remission phase ($r = 0.81$ and $p < 0.001$, $r = 0.74$ and $p < 0.01$ respectively). In patients with active relapse stage of SS and SRNS, serum IL-18 and IL-13 levels were also significantly correlated ($r = 0.83$ and $p < 0.001$, $r = 0.71$ and $p < 0.01$ respectively) **(Table 3).**

Correlation of serum IL-4, IL-13 and IL-18 levels in children with SS and SRNS by Regression analysis: We found that in all stages of SSNS and SRNS, IL-4 and IL-13 were significantly correlated with IL-18 ($r = 0.80$ and $r_2 = 0.64$) and IL-13 had a better predictor of the value of IL-18 than IL-4 **(Table 3)**.

Table 1. Th1 and Th2 serum cytokine measurements in steroid sensitive nephrotic patients and in healthy control.

Serum cytokine levels		SSNS Group I (n=18)		Control (n=20)
		Relapse Group IA (n=8)	Remission Group IB (n=10)	
IL-2 (pg/ml)	*Median*	8.2[1]	8.7[1]	7.8
	Range	7.3-13.4	7.5-12.7	6.5-11.8
IFN-γ (pg/ml)	*Median*	16.9[2]	25.4[1]	22.10
	Range	14.5-28.28	17.3-36.4	14.5-33.21
IL-4(pg/ml)	*Median*	66[3]	25.43[4]	14.2
	Range	18.7-184	11.6-52.1	7.03-35.23
IL-13(pg/ml)	*Median*	77.56[5]	31.12[6]	16,1
	Range	37.1-190	13.5-91.2	13.6-20.1
IL-18 (pg/ml)	*Median*	1511[7]	933[8]	123
	Range	554.1-2692	311-1435	41-247

[1] $p > 0.05$.

[2] $p = 0.03$, $p = 0.07$ for SS in relapse versus remission, and controls, respectively.

[3] $p < 0.001$, $p < 0.001$ for SS in relapse versus remission, and controls, respectively.

[4] $p < 0.001$ for SS in remission versus controls.

[5] $p < 0.001$, $p < 0.001$ for SS in relapse versus remission, and controls, respectively.

[6] $p < 0.001$ for SS in remission versus controls.

[7] $p = 0.03$, $p < 0.001$ for SS in relapse versus remission, and controls, respectively.

[8] $p < 0.001$ for SS in remission versus controls.

Table 2. Th1 and Th2 serum cytokine measurements in steroid resistant nephrotic patients and in healthy control.

Serum cytokine levels		SRNS Group II (n=34)		Control (n=20)
		Relapse Group IIA (n=25)	Remission Group IIB (n=9)	
IL-2 (pg/ml)	*Median*	8.3[1]	8.5[1]	7.8
	Range	7.2-12.9	7.9-12.2	6.5-11.8
IFN-γ (pg/ml)	*Median*	20.8[1]	23.75[1]	22.10
	Range	13.7-33.02	14.81-35.35	14.5-33.21
IL-4(pg/ml)	*Median*	57[2]	33.30[2]	14.2
	Range	17.3-176	13.11-74.7	7.03-35.23
IL-13(pg/ml)	*Median*	73.72[3]	46.4[4]	16,1
	Range	34.8-176	15.4-141	13.6-20.1
IL-18 (pg/ml)	*Median*	1323[5]	1187[5]	123
	Range	487.9-2481	398-2501	41-247

[1] $p > 0.05$.

[2] $p = 0.03$, $p = 0.06$ for SR in relapse versus remission and controls, respectively.

[3] $p = <0.01$ for SR in relapse versus controls.

[4] $p = 0.04$ for SR in remission versus controls.

[5] $p < 0.001$, $p < 0.001$ for SR in relapse versus remission and controls, respectively.

[5] $p= 0.05$ for SR in remission versus control

Table 3. Correlation of IL-18, IL-4 and IL-13 in idiopathic nephrotic patients.

IL-18		SSNS Group I (n=18)		SRNS Group II (n=34)	
Serum cytokine levels		Relapse Group IA (n=8)	Remission Group IB (n=10)	Relapse Group IIA (n=25)	Remission Group IIB (n=9)
IL-4 (pg/ml)	r	0.651	0.82	0.64	0.61
	p	0.01	<0.001	0.01	<0.01
IL-13 (pg/ml)	r	0.83	0.81	0.71	0.74
	p	<0.001	<0.001	<0.01	<0.01

Histopathological findings:

Thirty four SRNS patients were submitted to renal biopsies as expected, which showed 20 patients (59%) had focal segmental glomerulosclerosis (FSG), seven patients (20.5%) had mesangioproliferative glomerulonephritis (MesPGN), 3 patients (8.8%) had membranoproliferative glomerulonephritis (MPGN), 3 patients (8.8%) had membranous glomerulopathy (MG) and one patient (2.9%) had evidenced minimal change disease (MCD).

In histopathologic examination of sections; FSGS with INS showed sclerosis in some segments of some glomeruli (**Fig. 1-A)**. MesPGN showed mild segmental mesangial hypercellularity and the glomerular capillary walls with regular thickness (**Fig. 1-B)**. MPGN showed diffuse mesangial hypercellularity, thickened capillary wall and increased lobular appearance (**Fig. 1-C)**. MG showed increased thickness of capillary loop (**Fig. 1-D)**. In all figures, mesangium, glomerular capillary walls, Bowman membranes and brush borders and basement membrane in tubules are positive with PAS.

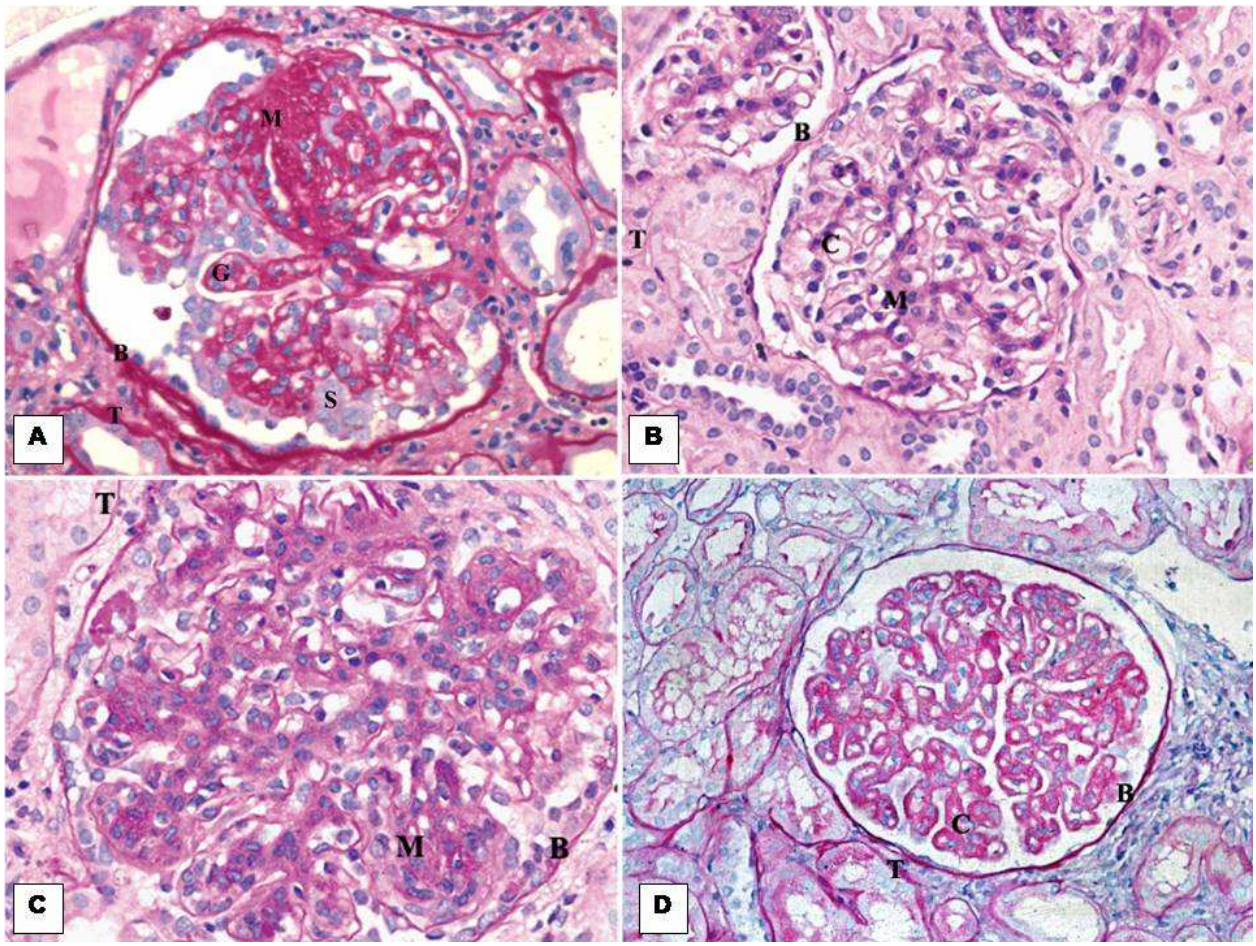

Figure 1. Histologic sections in INS: **A.** FSGS showing sclerosis in some segments of some glomeruli (S). **B.** MesPGN showing mild segmental mesangial hypercellularity (M) and the glomerular capillary walls (C) with regular thickness. **C.** MPGN showing diffuse mesangial hypercellularity, thickened capillary wall and increased lobular appearance (M). **D.** MG showing increased thickness of capillary loop(C). In all figures, mesangium, glomerular capillary walls, Bowman membranes (B) and brush borders and basement membrane in tubules (T) are positive with PAS (PAS, x400).

Transmission electron microscopic findings: FSGS with INS showed thickened basement membrane with tortuous course, diffuse foot process effacement, and endothelial cell nuclei bulged into capillary lumen **(Fig. 2-A)**. MesPGN with INS showed granular electrone dense immune deposits in the mesangial regions **(Fig. 2-B)**. MPGN with INS showed subendothelial electron-dense deposits in the glomerular basement membrane and effacement of foot process (**Fig. 2-C**). MG with INS showed subepithelial electron-dense deposits in the glomerular basement membrane and effacement of foot process **(Fig.2-D)**.

Immunohistochemical findings:

Immunostaining sections for synaptopodin in glomerular podocytes, Control and MCD showed strong brown staining **(Fig. 3-A)**. MPGN with INS showed moderate brown staining **(Fig. 3-B)**. FSGS with INS showed weak brown staining **(Fig. 3-C)**.

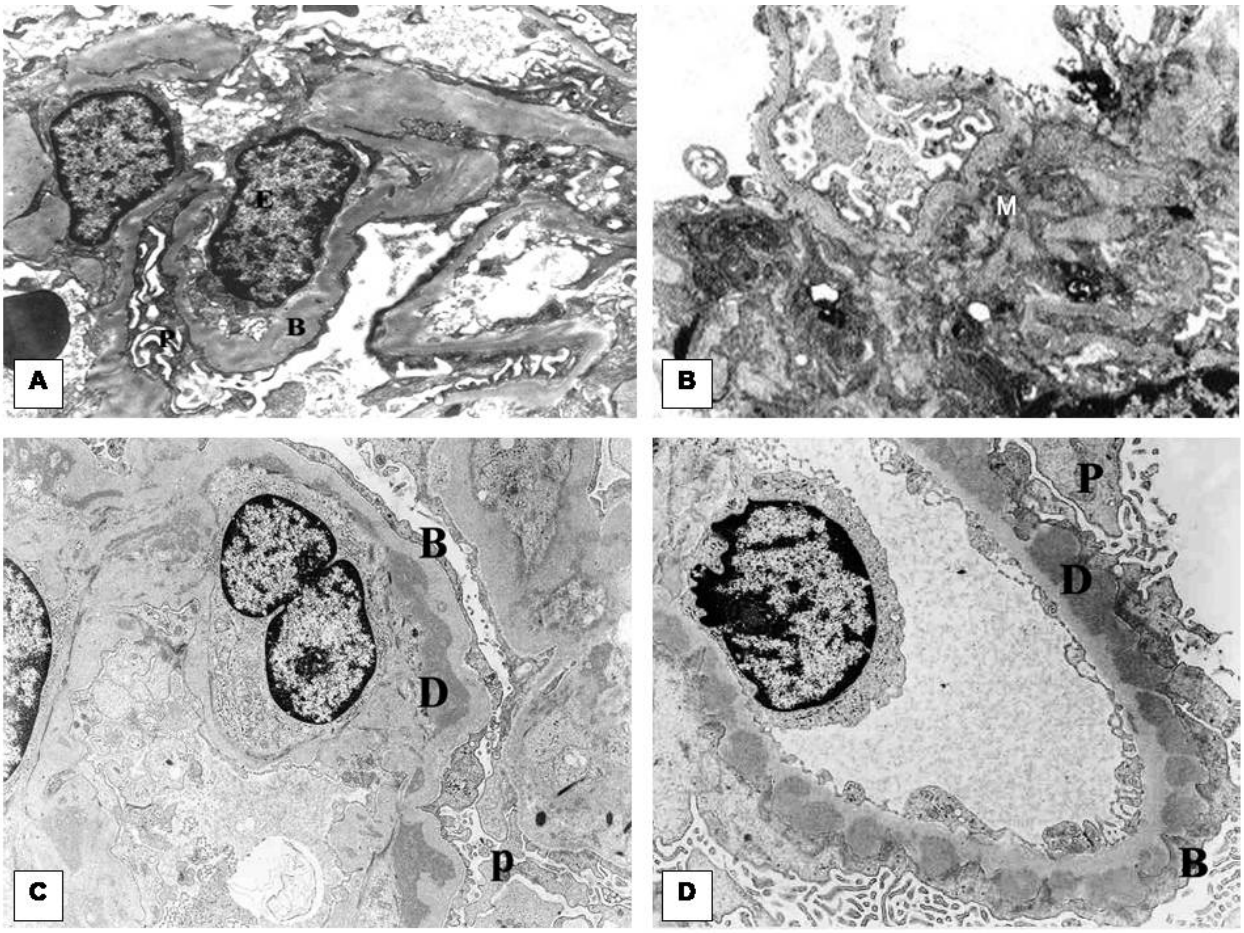

Figure 2. Transmission electron micrographs: **A**. FSGS with INS showing thickened basement membrane with tortuous course (B), diffuse foot process effacement (P), and endothelial cell nuclei bulged into capillary lumen (E) (x4500). **B.** MesPGN with INS showing granular electrone dense immune deposits in the mesangial regions (M)(x4500). **C.** MPGN with INS showing subendothelial electron-dense deposits (D) in the glomerular basement membrane (B) and effacement of foot process(x4500). **D.** MG with INS showing subepithelial electron-dense deposits (D) in the glomerular basement membrane (B) and effacement of foot process (P) (x6000).

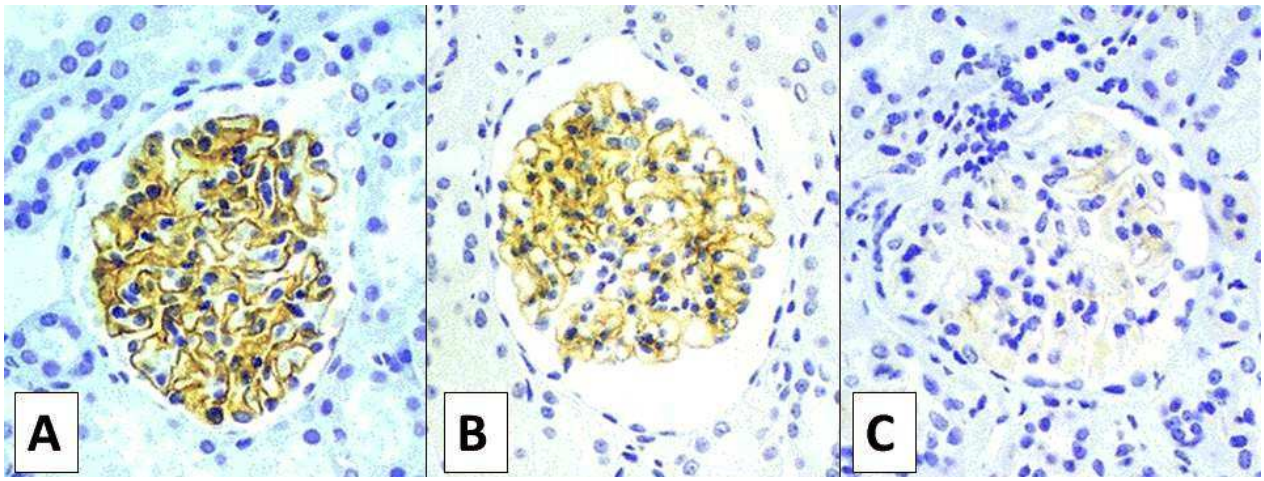

Figure 3. Immunostaining sections for synaptopodin in glomerular podocytes: **A.** Control and MCD showing strong brown staining. **B.** MPGN with INS showing moderate brown staining. **C.** FSGS with INS showing weak brown staining (x400).

DISCUSSION:

The pathogenesis of INS is still controversial. The immune system is thought to play a pivotal role, and there is a lot of evidence that supports this theory [2, 9-11]. Lymphocytic dysfunction, increased cytokines production and release of free radicals had been suggested to be pathogenic mechanism for renal injury in childhood INS [31].

Our study specifically focused on the evaluation of immune mediators (cytokines) in different stages of INS by measuring the serum levels of IL-2, IFN-γ, IL-4, IL-13 and IL-18 concentrations, elucidate the role of IL-18 and determine the histopathological pattern of INS in Saudi children. Since previous reports described alterations of these immune mediators in diverse renal diseases, including glomerulopathies [12, 32, 33]. Our nephrotic patients studied in sequential stages of activity and remission, in order to have a paired data, and compared the results with those obtained by healthy age matched controls. The main findings of our study were that nephrotic patients in all disease stages had an imbalance between type1/type-2 cytokine synthesis and secretory pattern with prevalence of type-2 cytokines with significantly increased IL-18 levels. Moreover, IL18 up-regulation in nephrotic patients correlated with this type-2 immune response.

The immune response strongly influenced by the pattern of cytokines produced by T cells. IL-2, a powerful immunoregulatory cytokine and produced by type-1 cells and plays a central role in cellular and humoral immune responses. In agreement with our findings, previous studies reported that, no significant difference of IL-2 serum levels in children with SSNS and SRNS [34]. This is consistent also with other results, who found no significant difference in IL-2 mRNA expression in patients with INS [19]. In contrast, by using a three-color flow cytometric assay, found high intracellular expression of IL-2 [35]. The interpretation of the above data is difficult but increased cytokine intracellular production does not necessary imply that the cytokine secreted.

Our study demonstrated a down-regulation of type-1 immune response, by decreased production of IFN-γ in SSNS, during relapse phase compared with remission phase and no significant difference during remission phase compared with healthy control. Our findings are in agreement with previous study who found a decreased production of IFN-γ by stimulated peripheral blood mononuclear cells in patients with relapse phase of SSNS [36]. Two studies assessed the percentage of IFN-γ producing NK cells in patients with SSNS, did not find significant difference between patients and controls [37, 38]. These discrepancies in results may probably be due to the heterogeneity of patients studied (different patient groups in each disease stage in contrast with our paired data).Our study indicated that no difference in serum IFN-γ levels was found between SR nephrotic patients in relapse, remission phases and controls. Indeed, the small number of SS nephrotic patients as well as the use of other medications in SR group such as angiotensin converting enzyme inhibitors (ACEi) and prednisone could interfere with the results. The use of these medications might have reduced serum levels of IL-2 and IFN-γ in our SR patients, thus attenuating the differences between INS groups in our study. Another important aspect was the collection of samples from patients at different time-points during disease evolution. This fact could also interfere with the measurements.

The shift to type-2 immune response in our patients with SSNS and SRNS demonstrated by the increase of the serum levels of IL-4 of relapse phase compared with the remission phase and controls. Our findings are in agreement with studies had reported an increased synthesis of IL-4 by stimulated peripheral mononuclear cells in patients with relapse phase of INS [39, 40]. In contrast, previous data reported inability to demonstrate any increase in the percentage of IL-4 producing T cells [12, 37]. Studies of the type-1/type-2 cytokine patterns in the sera of patients with INS have generally been variable and inconsistent. This conflicting result may be due to the differences in immunologic techniques, which used to assess cytokine synthesis [12].

The elevated IL-13 serum levels in all our patients with SSNS and SRNS during the relapse phase compared with the remission phase and healthy controls also indicated that the balance in these patients were pointed in favor of type-2 cytokine pattern. These results were in agreement with earlier published works [19, 37].

In our study, there was an increased level of IL-18 in all disease stages of SSNS and SRNS, particularly in the active relapse phase of SSNS and SRNS compared with the controls. This elevation was of great interest considering that IL-18 is a unique cytokine that stimulates both type-1 and type-2 immune responses depending on its cytokine milieu [38]. IL-18 identified mainly as an IFN-γ inducing factor, which plays a critical role in the host defenses [41]. IL-18 reported to induce IL-13 and/or IL-4 production by NK cells, mast cells, and basophils [42]. The potent roles of IL-18 in various pathological conditions are currently of great interest. Until now, its implication examined in various diseases such as insulin dependent diabetes mellitus, rheumatoid arthritis, Crohn's disease, and atopy. It is

well recognized that IL-18 has at least two effects; as a pro-inflammatory cytokine and as a strong inducer of the atopic immune response. Regarding SSNS, in adults with SSNS, IL-18 levels were elevated in the active stage of the disease and that there was a positive correlation between IL-18 production and disease activity [21]. Furthermore, our results showed that in SSNS and SRNS, IL-18 significantly correlated with type-2 cytokines IL-4 and IL-13. These results were in agreement with published works who concluded that, increased serum and urine IL-18 levels observed during relapse of INS [43]. These findings indicate the association between the active phase of INS and the levels of IL-18 and could suggest the role of this cytokine in the INS development. However, we suggested that an increased serum IL-18 in SR nephrotic patients could be another pathogenic factor for the progression of renal disease. Further studies are obviously necessary to address this issue.

The optimum approach to laboratory investigation of renal biopsy in developed countries included light microscopy, immunohistochemistry and EM examination [44]. On the other hand, renal biopsy diagnosis was often rendered solely on LM examination in developing countries. LM provided a morphological pattern not a specific diagnosis for glomerular disease [44].

An accurate diagnosis of glomerulonephritis causing NS required integration of clinical data, serological and detailed investigations of renal biopsy by L/M, EM and immunohistochemistry studies and a correlation of all the above findings [45].

The visceral glomerular epithelial cell or podocyte was a terminally differentiated cell in the glomerulus that played an important role in glomerular ultrafiltration [46]. Podocytes had three structurally and functionally different segments: the cell body, major processes, and foot processes [47]. The foot processes and slit diaphragms of the podocyte form a barrier, which allowed only certain molecules to filter into the urine, depending on their size, charge, and shape. Synaptopodin was a linear, proline-rich protein, intimately associated with the actin microfilament, and it was expressed exclusively in the foot process of podocytes in the kidney and in the dendritic spines of the telencephalic synapses [48]. Synaptopodin was believed to modulate the actin-based shape and motility of the podocyte foot processes [49]. This protein was believed to play a crucial role in the normal blood-filtering mechanism of the kidney [49]. Each type of glomerulonephritis can be differentiated by EM and immunohistochemistry examination.

All forms of nephrotic syndrome are characterized by abnormalities in the podocyte and injury of this cell type typically leads to marked proteinuria [50]. Glomerulopathies therefore were considered as podocyte injury diseases (podocytopathies) [51].

As expected, all thirty-four SR nephrotic patients were submitted to renal biopsies, which showed 20 patients (59%) had FSGS , seven patients (20.5%) had MesPGN, 3 patients (8.8%) had MPGN, 3 patients (8.8%) had MG, and only one patient (2.9%) had histologic pattern evidenced MCD. The present study confirmed that the primary glomerular diseases in children in the Saudi Arabia are more frequent than secondary glomerulopathy. Furthermore, the study illustrated that FSGS are the most common diagnosed entities occurring with steroid resistant nephrotic patients (59%).

In our studies, most common pattern was FSGS (59%), observed by LM and confirmed by EM which showed a thick glomerular basement membrane with a tortuous course and complete foot process effacement and immunohistochemical examination revealed weak brown staining for synaptopodin in glomerular podocytes.

A dramatic increase in the incidence of FSGS during recent years (47%) [6]. Also from western region of Saudi Arabia and India observed FSGS incidence was (59%) of all SRNS cases [52, 53].

A decline in the incidence of minimal change disease and an increased incidence of focal segmental glomerulosclerosis had observed in recent years in the western area of the Kingdom of Saudi Arabia [54].

Mesangioproliferative pattern (MesPGN) found in (20.5%) of our SRNS cases by LM and confirmed by EM which showed granular immune deposits in the mesangial regions. In agreement with our findings, an increasing rates of MesPG have been reported from Pakistan (12%), Saudi Arabia (24%) [54] and Jamaica (31.2%) [55] of cases.

Membranoproliferative glomerulonephritis (MPGN) observed in 8.8% of our SRNS by LM and confirmed by EM which showed electron-dense deposits in the endothelial aspect (internal) of the glomerular basement membrane with foot process effacement and immunohistochemical examination showed moderate brown staining for synaptopodin in glomerular podocytes.

In our studies, MPGN reported a similar frequency to studies from the USA [6] and Iran [55]. High rates of MPGN markedly found in studies from New Zealand (23%) [56] but in Pakistan, MPGN reported in 14% of cases [8]. Previous works explained the reasons for disparities in the prevalence of MPGN were due to environmental factors such as chronic infection and malnutrition [57].

Membranous glomerulopathy (MG) observed 8.8% of our SRNS patients by LM and confirmed by EM that showed an electron-dense deposits the epithelial aspect (external) of the glomerular basement membrane with foot process effacement. A low rate of MG reported throughout the world ranging from 0.4% to 5.7% [8, 58].

Serum cytokine assay cannot distinguish between each type of glomerulonephritis and cannot used alone in diagnosis glomerulonephritis. An accurate diagnosis of glomerulonephritis causing NS required association of serum cytokine assay and detailed investigations of renal biopsy by LM, EM and immunohistochemical studies and a correlation of all the above findings.

CONCLUSIONS

Type-2 cytokine synthesis and production patterns predominate in children with relapse phase of SS and SR patients and one could predict a good response to steroid therapy. IL-18 expression significantly correlated with this type-2 immune response. In addition, the cytokine levels in SR patients had a trend to be higher than in SS patients. The present study confirms that the primary glomerular diseases in children in the Saudi Arabia are more frequent than secondary glomerulopathy. Furthermore, the study illustrates that FSGS are the most common diagnosed entities occurring with steroid resistant nephrotic patients (59%).

DISCLOSURE OF BENEFIT

None

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