Serum interleukins and urinary microglobulin in children with idiopathic nephrotic syndrome

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المستويات المصلية للإنترلوكين 1 – بيتا، والإنترلوكين — 6، والعامل المُنَخِّر للورم، ومستوى البيتا — 2 — مكروغلوبولين في البول، في الأطفال المصابين بمتلازمة كُلائية مجهولة السبب

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الخلاصة: درس الباحثون 60 طفلاً مصاباً بالمتلازمة الكُلائية المجهولة السبب، مع عشرين آخرين يماثلونهم من حيث العمر والجنس على أنهم شواهد. وقد قسم الأطفال المصابون بالمتلازمة الكُلائية المجهولة السبب إلى ثلاث مجموعات، كلٌّ منها تضم 20 طفلاً، في الأولى من شوهد فيهم لأول مرة، وفي الثانية من هم في فترة هدأة، وفي الثالثة من هم في فترة نكس. وأجرى الباحثون دراسة متكاملة لعناصر الدم والبول، مع تحليل لمستويات الإنترلوكين1 – بيتا، والإنترلوكين — 6، والعامل المُنَخِّر للورم، وعيار كميات بيتا — 2 — مكروغلوبولين المفرغ في البول. وقد وجد الباحثون أن مستوى الإنترولوكين 1 – بيتا والإنترولوكين — 6، مرتفع لدى المجموعات المدروسة، ويبلغ أقصاه لدى الذين شوهدوا لأول مرة والذين نكسوا. أما مستوى العامل المُنَخِّر للورم، والمفرغ من البيتا — 2 مكروغلوبولين في البول فقد كان أكثر ارتفاعاً لدى من شوهدوا لأول مرة والناكسين بشكل واضح. وبناءً على ذلك فإن تراكيز كل من الإنترلوكين 1 – بيتا والإنترلوكين — 6 والعامل المُنَخِّر للورم، يمكنها أن تحدّد تحديداً إيجابياً (بنسبة مئة بالمئة) مجموعات الذين شوهدوا لأول مرة ومجموعات النكس، فيما تحدّد هذه التراكيز إضافةً إلى المفرغ من البيتا — 2 مكروغلوبولين في البول تحديداً سلبياً المجموعة الشاهدة بنسبة مئة بالمئة.

ABSTRACT We studied 60 children affected with idiopathic nephrotic syndrome (INS) plus 20 age and sex matched controls. The children with INS were divided into 3 groups of 20: first presentation, remission and relapse. A complete blood picture and complete urinalysis were done. Serum interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF) and quantitative urinary β-2-microglobulin (β-2-m) excretion were estimated. IL-1β and IL-6 were significantly higher in the study groups, the first presentation and relapse groups having the highest concentrations. Serum TNF concentration and urinary β-2-m excretion were significantly higher in the first presentation and relapse groups. Serum IL-1β, IL-6 and TNF concentrations were able to select positively (100%) the first presentation and relapse groups, while these plus urinary β-2-m excretion selected negatively (100%) the control group.

Interleukines sériques et β microglobuline urinaire chez des enfants présentant un syndrome néphrotique idiopathique

RÉSUMÉ Nous avons étudié 60 enfants présentant un syndrome néphrotique idiopathique plus 20 témoins appariés selon l'âge et le sexe. Les enfants présentant un syndrome néphrotique idiopathique ont été répartis en trois groupes de 20 : première manifestation, rémission et récidive. Un hémogramme et un examen des urines complets ont été réalisés. On a estimé l'interleukine 1β, l'interleukine 6, la cachectine (TNF) et l'excrétion quantitative de β2-microglobuline urinaire. Les taux sériques d'interleukine 1β et d'interleukine 6 étaient significativement plus élevés dans les groupes de l'étude, les groupes de première manifestation et de récidive ayant les concentrations les plus fortes. La concentration sérique de TNF et l'excrétion de β2-microglobuline urinaire étaient significativement plus élevées dans les groupes de première manifestation et de récidive. Les concentrations sériques d'interleukine 1β, d'interleukine 6 et de TNF permettaient de sélectionner positivement (100 %) les groupes de première manifestation et de récidive alors que ces concentrations plus l'excrétion urinaire de β2-microglobuline urinaire sélectionnaient négativement (100 %) le groupe témoin.

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Introduction

Nephrotic syndrome is neither a single disease nor even a heterogeneous group of related diseases. Rather, it is a clinical state characterized by heavy proteinuria and hypoalbuminaemia, often associated with oedema, hypercholesterolaemia and generalized hyperlipidaemia [*1*].

Idiopathic nephrotic syndrome (INS) accounts for 90% of nephrosis in childhood; minimal change nephrotic syndrome is found in approximately 85% of INS, mesangial proliferative glomerulonephritis in 5% and focal glomerular sclerosis in 10%. In the remaining 10% of children with nephrotic syndrome, it is largely mediated by some form of glomerulonephritis, membranous and proliferative being the most common [*2*].

Idiopathic nephrotic syndrome in childhood occurs at an annual incidence of 2 cases/100 000 population under the age of 18 years [*3*]; minimal change nephrotic syndrome is most common in children 2–4 years old, but may occur at any age [*4*].

The basic pathogenic abnormality in nephrosis is proteinuria, which results from an increase in glomerular capillary wall permeability. The mechanism of the increase in permeability is unknown but may be related, at least in part, to loss of negatively charged glycoproteins within the capillary wall [*2*]. The glomerular lesion, especially in minimal change nephrotic syndrome, may be mediated by circulating permeability factors. They are thought to be T-lymphocyte derived cytokines, which cause podocyte swelling, alterations in charge density, and foot process effacement with consequent increase in vascular permeability [*5*].

Interleukin (IL)-1 and tumour necrosis factor (TNF) are structurally unrelated cytokines, yet their spectra of biological effects are so similar that these 2 cytokines are almost interchangeable [*6*]. It has been suggested that IL-1 has a significant role in the immunopathogenesis of proteinuria [*7*] and that TNF-α plays a pathogenic role in the induction and/or maintenance of glomerular barrier dysfunction [*8*]. Interleukin-6 is involved in inflammatory responses and immune reactions in the host. It is produced by a variety of cells, including monocytes and mesangial cells in the kidney [*9*]. It is not possible at present to distinguish whether IL-6 contributes to renal dysfunction or whether it reflects renal damage [*10*].

β-2-microglobulin (β-2-m) is a low molecular weight protein found on the surface of all nucleated cells which synthesize it. About 95% of free β-2-m is filtered by the normal glomerulus and a normal kidney is able to reabsorb 99.9% through the proximal tubules. Malfunction of the proximal tubules with a normal glomerular filtration rate is accompanied by decreased tubular reabsorption and increased urinary excretion of β-2-m [*11*].

The aim of this study was the evaluation of serum concentrations of IL-1β, IL-6, TNF and urinary β-2-m in children suffering from idiopathic nephrotic syndrome in various situations: before treatment, during remission and on relapse before reinstitution of therapy. An additional aim was the comparison of these levels to those in a matched control group.

Methods

This study was carried out at Alexandria University Children's Hospital from January 1998 to December 1999 (2 years).

Eighty children were included in the study, 60 had idiopathic nephrotic syndrome (INS) and 20 were healthy children who were randomly selected from those

attending the growth and development follow-up clinic who were included as a control group. The sick children were recruited in the paediatric nephrology clinic and selected sequentially until the required number was reached. They were classified into 3 groups (20 children in each): first presentation, diagnosed as nephrotic for the first time (these were selected first owing to their small number); remission, selected as being in remission for ≥ 3 months; and relapse, selected as having had their first relapse recently but before reinstitution of therapy (after the first group was complete, we selected a child in remission and a child with relapse sequentially until the required number was reached). Informed consent was obtained from all parents and the protocol of the study was approved by the Alexandria University ethics committee. All patients with a history of recent (within the previous 6 months) infective and/or inflammatory conditions and all patients with abnormal urinary sediments (abnormal casts or crystalluria) were excluded from the study.

A detailed history of the present condition of all the participants was obtained and a thorough clinical examination was performed.

A morning blood sample of 8 mL was collected; 2 mL of the sample were added to EDTA tubes for a complete blood picture. The remaining 6 mL were collected in test tubes for clinical tests. Sera were separated and collected in Eppendorf tubes. These were kept frozen (–70 °C) till the time of the specific tests. All investigations were carried out in the laboratories of the university hospital.

The specific tests performed were: complete blood picture, blood urea, serum creatinine, serum protein and serum cholesterol. Specific immunologic tests included: serum IL-1β by enzyme immunoassay using Medgenix kits (Medgenix Diagnostics, Fleurus, Belgium) [intra-assay coefficient of variation (CV) 2.8%, interassay CV 4.5%]; serum IL-6 by enzyme immunoassay using Medgenix kits (intra-assay CV 5.1%, interassay CV 4.6%); TNF-α by enzyme immunoassay using Medgenix kits (intra-assay CV 4.8%, interassay CV 8.7%). all tests were carried out according to the manufacturer's instructions.

An early morning sample of urine was collected, examined and centrifuged. The clear supernatant was preserved for the definitive test. Urine output was estimated by collecting all urine voided from 08.00 till 08.00 the next day. For the control group, mothers were asked to collect the urine and deliver it to the clinic for analysis. β-2-m was estimated in urine quantitatively using an enzyme-linked immunosorbent assay technique using Eurogenetics kits coated microtitre strips (Eurogenetics, Tessenderlo, Belgium) (intra-assay CV 6.4%, inter-assay CV 8.0%).

Data were collected and tabulated using *SPSS*, version 6, for statistical analysis. Descriptive measures included: count, percentage, minimum, maximum, arithmetic mean and standard deviation. Statistical tests included chi-squared for testing association, 1-way analysis of variance (*F*-test) for comparing means of more than 2 groups, Tukey β difference test for pair-wise comparison and Kruskal–Wallis analysis of variance (χ^2) for non-parametric or non-normally distributed variables. Pearson correlation was utilized to study association among quantitative variables. The selected level of significance was $P \leq 0.05$.

Results

There were no statistically significant differences between the 4 groups for mean age,

sex distribution and height percentile from 50th centile for age and sex. The weight percentiles were significantly higher in the first presentation and relapse groups than in the control and remission groups from 50th centile for age and sex. Systolic and diastolic blood pressures were significantly higher in the 3 nephrotic groups compared to the control group (Table 1).

Mean serum protein concentration was significantly lower in the 3 study groups, and in the relapse group it was significantly lower than in the other INS groups (Table 2). The serum albumin concentration was significantly lower in the 3 study groups compared to the control group, and also in the first presentation and relapse groups compared to the remission group. Mean serum cholesterol was significantly higher in the 3 INS groups than in the control group. Mean serum creatinine was significantly higher in the first presentation and remission groups compared to the control group, but mean blood urea did not show any statistically significant difference across the 4 groups. The mean leukocyte count was

Table 1 **Personal and clinical characteristics of 3 groups of children with nephrosis and a control group**

Characteristic	Group				*F*
	First presentation	Remission	Relapse	Control	
Age (years)					
Range	2.1–11.5	2.3–14.3	2.4–12.6	2.3–10.2	
Mean (SD)	5.7 (3.0)	6.7 (3.4)	5.7 (3.8)	4.0 (2.2)	2.4543
Sex					
Males [No. (%)]	11 (55)	11 (55)	14 (70)	8 (40)	$\chi^2 = 3.636$[a]
Females [No. (%)]	9 (45)	9 (45)	6 (30)	12 (60)	
Weight percentile[b]					
Range	116.4–135.1	76.5–101.7	81.2–194.5	79.3–101.8	
Mean (SD)	126.5 (18.1)[c,d]	94.0 (6.3)	125.7 (31.8)[c,d]	92.6 (6.1)	20.255[e]
Height percentile[a]					
Range	84–144.8	86.8–160.0	84–149.7	84–138.3	
Mean (SD)	111.8 (19.0)	118.3 (20.6)	111.1 (23. 9)	101.2 (14.7)	2.5238
Systolic blood pressure (mmHg)					
Range	100–150	100–140	80–140	90–110	
Mean (SD)	105.5 (11.8)[d]	103.1 (12.2)[d]	121 (16.8)[d]	92.5 (5.5)	18.425[e]
Diastolic blood pressure (mmHg)					
Range	60–80	60–85	50–100	60–70	
Mean (SD)	65.5 (7.2)[d]	64.2 (6.8)[d]	73.75 (12.9)[d]	60.5 (2.2)	9.323[e]

[a] *Kruskal–Wallis test.*
[b] *Presented as % from 50th centile for age and sex.*
[c] *Significantly different from remission group.*
[d] *Significantly different from control group.*
[e] *Significant at* $P < 0.05$.
SD = standard deviation.

Table 2 **Laboratory results from blood and urine tests for 3 groups of children with nephrosis and a control group**

Test	Group				*F*
	First presentation	Remission	Relapse	Control	
Serum protein (g/dL)					
Range	3.3–7.4	5.4–8.2	2.8–7.2	7.0–8.2	
Mean (SD)	6.0 (0.9)[a]	6.1 (1.2)[a]	4.5 (1.3)[a,b,c]	7.8 (0.4)	36.092[d]
Serum albumin (g/dL)					
Range	1.2–2.0	3.1–4.2	1.4–2.0	3.0–4.5	
Mean (SD)	1.7 (0.2)[b,a]	3.7 (0.4)[a]	1.8 (0.2)[b,a]	3.9 (0.3)	318.000[d]
Serum cholesterol (mg/dL)					
Range	120–640	140–540	216–561	145–200	
Mean (SD)	401.9 (137.8)[a]	350.0 (129.0)[a]	402.9 (105.6)[a]	168.0 (15.3)	20.051[d]
Blood urea (mg/dL)					
Range	17–51	18–51	10–56	20–35	
Mean (SD)	34.2 (10.3)	29.9 (8.6)	32.5 (14.1)	25.9 (4.3)	2.603
Serum creatinine (mg/dL)					
Range	0.2–1.2	0.5–1.1	0.5–1.2	0.3–0.9	
Mean (SD)	0.9 (0.2)[a]	0.8 (0.2)	0.8 (0.3)[a]	0.6 (0.2)	5.669[d]
Leukocytes (× 1000/mm^3)					
Range	5.7–12.3	6.9–16.2	5.4–20.7	6.2–9.4	
Mean (SD)	8.9 (1.6)	10.0 (2.6)[a]	11.4 (3.9)[a,c]	7.8 (1.2)	7.589[d]
Haemoglobin (g/dL)					
Range	8.0–13.7	9.6–14.0	7.9–15.6	8.3–13.8	
Mean (SD)	10.4 (1.6)	11.8 (1.3)	12.2 (2.2)[c]	11.0 (1.6)	4.359[d]
Platelets (× 1000/mm^3)					
Range	180–693	211–630	280–544	238–430	
Mean (SD)	349.8 (103.2)	369.7 (105.1)	420.7 (71.4)[a]	314.8 (66.3)	5.019[d]
Leukocytes in urine/HPF					
Range	1–100	1–9	2–55	1–2	
Mean (SD)	17.4 (22.1)[a]	3.45 (2.0)[c]	12.3 (13.1)[a]	1.36 (0.5)	6.856[d]
Urinary volume (mL/kg per day)					
Range	9.1–125.0	24.0–150.0	25.0–38.5	50.0–68.2	
Mean (SD)	35.8 (27.1)[a,b]	60.5 (35.8)	31.0 (3.3)[a,b]	60.4 (4.4)	9.625[d]

[a]*Significantly different from control group.*
[b]*Significantly different from remission group.*
[c]*Significantly different from first presentation group.*
[d]*Significant at* P *< 0.05.*
SD = standard deviation.
HPF = high power field.

significantly higher in the remission and relapse groups compared to the controls, and in the relapse group it was significantly higher than in the first presentation group. Mean platelet count was significantly higher in the relapse group compared to controls and mean haemoglobin concentration was significantly higher in the relapse group compared to the first presentation group.

Mean urinary leukocyte count was significantly higher in the first presentation and relapse groups compared to the control group, while in the first presentation group the count was significantly higher than in the remission group. Mean 24-hour urinary output was significantly lower in the first presentation and relapse groups compared to the other 2 groups.

Mean serum IL-1β concentration was significantly higher in the first presentation and relapse groups compared to the remission and control groups, while in the remission group the level was significantly higher than in the controls (Table 3). Mean serum IL-6 concentration was significantly higher in the 3 groups with nephrosis compared to the controls, and in the first presentation and relapse groups the level was significantly higher than the remission group. Mean serum TNF concentration in the first presentation and relapse groups was significantly higher than that of the remission and control groups, and in the first presentation group it was significantly higher than in the relapse group. Mean urinary β-2-m concentration in the first presentation and relapse groups was

Table 3 **Serum concentrations of interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF) and urinary β-2-microglobulin (β-2-m) in children with nephrosis and a control group**

Variable	Group				Kruskal–Wallis test, χ^2
	First presentation	Remission	Relapse	Control	
IL-1β (pg/mL)					
Mean (SD)	1208.2 (1977.5)	73.4 (91.6)	657.3 (790.5)	6.0 (2.7)	59.74[a]
Negative [No. (%)]	0 (–)	8 (40)	0 (–)	20 (100)	
Positive [No. (%)]	20 (100)	12 (60)	20 (100)	0 (–)	58.90[a]
IL-6 (pg/mL)					
Mean (SD)	448.5 (268.3)	32.0 (40.6)	171.3 (182.1)	4.1 (2.2)	
Negative [No. (%)]	0 (–)	7 (35)	0 (–)	20 (100)	65.682[a]
Positive [No. (%)]	20 (100)	13 (65)	20 (100)	0 (–)	59.65[a]
TNF (pg/mL)					
Mean (SD)	56.6 (9.3)	29.3 (58.8)	43.9 (24.6)	5.6 (2.8)	51.424[a]
Negative [No. (%)]	0 (–)	12 (60)	0 (–)	20 (100)	60.00[a]
Positive [No. (%)]	20 (100)	8 (40)	20 (100)	0 (–)	
Urinary β-2-m (μg/mL)					
Mean (SD)	6.0 (5.2)	2.5 (4.9)	4.9 (7.6)	1.4 (0.9)	21.86[a]
Negative [No. (%)]	13 (65)	17 (85)	9 (45)	20 (100)	17.76[a]
Positive [No. (%)]	7 (35)	3 (15)	11 (55)	0 (–)	

[a] *Significant at* P < 0.05.
Negative = ≤ normal.
Positive = > normal.
SD = standard deviation.

significantly higher than in the remission and control groups.

Serum concentrations of IL-1β, IL-6 and TNF were able to select positively the first presentation and relapse groups (100%), while these 3 cytokines along with urinary β-2-m excretion could select negatively the controls (100%).

Discussion

Increased level of IL-1β encountered in a study is indicative of its significant role in the immunopathogenesis of proteinuria [*7*]. The effect of increased IL-1β has been explained on the basis of its local and systemic effects. The main vascular effects of IL-1β are increased platelet adherence, increased capillary permeability, increased prostaglandin synthesis and hypertension [*12*]. Moreover, IL-1β with TNF may stimulate increased synthesis of eicosanoids [*2*]. These could pass through the endothelial cells, affecting the negatively charged podocytes, which usually prevent the passage of albumin, leading to their neutralization and hence excessive albuminuria.

Although the mean blood pressure measurements among the INS groups in our study were significantly higher than that of the control group, they were within the normal range for age and sex in the majority of children [*2*]. It has been postulated that increased mesangial cellularity [*13*] could compromise renal afferent and/or efferent circulation in the glomeruli. Abrass reported that 19% of children remain hypertensive after induction of remission in nephrotic syndrome [*14*]

An important effect of increased IL-1β combined with IL-6 and TNF is the increased leukocyte count from 2 sources: bone marrow and leucocytes attached loosely to endothelial cells [*15*]. This high leukocyte count could possibly indicate that the infiltrating monocytes-macrophages are the major source of inflammatory cytokines, especially IL-1β, IL-6 and TNF, rather than the resident glomerular cells. IL-1β stimulates hepatic protein synthesis due to hypoalbuminaemia and inhibits lipoprotein lipase [*12*], which will lead to hypercholesterolaemia. In this study, the main cause of hypercholesterolaemia in the remission group was inhibition of lipoprotein lipase since the serum protein was within the normal range for age and sex [*2*]. Although IL-1β increased hepatic protein synthesis, its vascular leak effects surpass it, with a net hypoteinaemia.

The expression of TNF is amplified by IL-1β and is suppressed by immunosuppressive drugs such as corticosteroids [*16*]. In renal disease, TNF expression has been found in both resident cells and infiltrating monocytes-macrophages [*17*]. It acts in a paracrine way to recruit monocytes and macrophages to the glomerular region, besides acting with other mediators to increase vascular permeability [*18*]. This would cause alteration of the barrier function of the capillary wall [*17*] leading to proteinuria. In our study, TNF did not behave similar to IL-1β in the remission group, where TNF levels decreased to statistically non-significant levels compared to controls while IL-1β levels remained significantly high. This is because TNF is downregulated by corticosteroids [*17*] and also by the shift of CD_4-T cell differentiation to TH_2 instead of TH_1 cells with a consequent reduction of TH_1 cytokine production, especially TNF [*19*]. Moreover, the combined effect of IL-1β and TNF will lead to a more effective local capillary leakage and cell death than with either cytokine alone [*20*]. Therefore, to obtain remission there should be a disengagement of IL-1β and TNF. This emulates

IL-1β and IL-6 in its systemic effects, especially bone marrow stimulation and release of neutrophils [*15*].

While IL-1β and TNF are stimulating mesangial cells to release IL-6 [*17,20*], other sources are activated: TH_2 cells, antigen presenting cells and other somatic cells, especially endothelial cells [*12*]. Concentration of IL-6 was highest in the first presentation group and lowest in the remission group in the INS patients in this study. IL-6 is activated by the high IL-1β and TNF concentrations in the first presentation group, thus IL-6 seems to be able to downregulate TNF, possibly by helping in differentiating CD_4-T cells into TH_2 cells. Consequently, remission will occur and IL-6 will no longer be needed in the remission group and concentration will ultimately decline. This view is supported by the findings that long-term remission of INS is related to the increased TH_2 cytokine production (especially IL-6) and the downregulation of TH_1 cells [*19*]. Proteinuria disappears in remission since it has been shown that TNF alone is the single most important cytokine in pathogenesis in INS [*21*]. Moreover, the use of corticosteroids in the remission group suppressed IL-6 [*22*]. In the relapse group in this study, the proteinuria caused by increased TNF will need a re-increase of IL-6 expression to suppress TNF expression and its proteinuric effect.

In this study, IL-6 combined with other cytokines acted on the bone marrow to increase leukocyte count and haematopoiesis [*12*] as well as megakaryocytes [*23*]. The effect is latent and therefore the platelets increased in the relapse group while the leukocyte count depended more on the effects of other cytokines such as IL-1β and TNF, which can be seen from the results.

The lowest haemoglobin concentration we found was in the first presentation group, possibly as a result of low level of erythropoietin in plasma which can result from increased urinary loss in nephrotic syndrome [*14*]. This may have outweighed the stimulatory effects of erythropoietin production in bone marrow by the high concentrations of IL-1β and IL-6 in the first presentation group, but the reverse occurred in the relapse group.

In this study, the highest increase of urinary excretion of β-2-m was in the first presentation group because of the combined effect of glomerular and tubular dysfunction. It was shown that increased urinary β_2-m excretion is present in massive glomerular proteinuria and in tubular dysfunction, especially the proximal [*24*], with a positive correlation between them independent of the primary renal disease [*25*].

To sum up, significantly high concentrations of IL-Iβ, IL-6 and TNF were uniformly found in the first presentation and relapse INS groups. They were able to positively predict them (100%) and, along with β-2-m, to negatively predict the controls (100%) with fair accuracy. The lower serum values for TNF and urinary β-2m excretion are important factors in defining and predicting remission. IL-1β and TNF usually work in harmony while IL-6 tries to suppress TNF and its harmful effects. It seems that the 3 months remission period used in this study is not a sufficient duration for the return of cytokines and urinary β-2-m to the basal control values. However, the clinical studies on INS in children are quite limited because of its low global incidence (2/100 000 population) [*3*].

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Note from the Editor

We would like to inform our readers that the next special issue of the EMHJ will be on Medical Bioethics. It is scheduled to be Vol. 12 No. 5, 2006.