Urinary monocyte chemotactic protein 1 as a predictive marker of steroid responsiveness in children with idiopathic nephrotic syndrome

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ABSTRACT

Background: Idiopathic nephrotic syndrome (INS) is a clinical syndrome characterized by massive loss of urinary protein leading to hypoproteinemia and edema. It is reported that the immune system plays an important role in pathogenesis and pathophysiology of INS. The moncyte chemotactic protein-1 plays an important role in the recruitment of monocytes/macrophages into renal tubulointerstitium.

Aim of the study: The aim of our study was planned to measure the urinary level of monocyte chemotactic protein 1(MCP-1) in children with INS during remission and relapse, and predict steroid responsiveness.

Methods: This prospective study included 50 patients with nephrotic syndrome (NS) following up in Pediatric Nephrology Clinic, Benha University subdivided into 2 groups: group A (cases in remission) & group B (cases in activity). Also, 20 age and sex matched healthy children have been included as a control group.

Results: We found a significant increase in urinary MCP-1 in INS patients as compared to control group (p < 0.001). Also, our study showed that group B had the highest levels of uMCP-1 followed by group A then control group (p<0.001). uMCP-1 levels were significantly higher in the steroid-resistance NS (SRNS) patients than the steroid-sensitive NS (SSNS) patients (p<0.001).

Conclusions: Urinary MCP-1 is highly sensitive and specific biomarker in idiopathic nephrotic syndrome for early detection of disease activity. Urinary MCP-1 can be used as a predictive marker of steroid responsiveness in children with idiopathic nephrotic syndrome.

Key words: Urinary monocyte chemotactic protein-1, Steroid Dependent, Steroid Resistant, Nephrotic Syndrome
Running title: Urinary monocyte chemotactic protein-1in Children with Idiopathic Nephrotic Syndrome

Introduction

Nephrotic syndrome in children is defined as hypoalbuminemia, edema, and proteinuria (protein > 40 mg/m2/h or protein-creatinine ratio > 2000 mg/g (>200 mg/mmol] or protein > 300 mg/dL or 3+ on urine dipstick [in adults, proteinuria > 3.5 g per 24 hours]) [1].

Nephrotic syndrome is a common childhood renal disorder affecting approximately 2–16 per 100,000 children each year and may present at any age during childhood and adolescence [2].

Steroid-sensitive NS is defined as remission within the initial four weeks of corticosteroid therapy [3].

Steroid-resistance NS is defined as the failure to achieve complete remission after 8 weeks of corticosteroid therapy, namely a urinary protein to creatinine ratio <200 mg/g creatinine or <+1 of protein on a urine dipstick for three consecutive days [3].

Although the pathogenesis and pathophysiology of idiopathic NS is not clearly understood, it is reported that the immune system, including activation of lymphocytes, plays an important role. An association among several cytokines and chemokines with idiopathic NS has been reported [4].

Histological reports suggest that macrophages play an important role in the pathogenesis of focal segmental glomerulosclerosis, a form of steroid-resistant NS [5].

Monocyte chemoattractant protein-1(MCP-1) belongs to the CC-chemokine family, is encoded on chromosome 17, and is composed of 76 amino acids. In the kidney, it is produced by mesangial, tubular, epithelial cells, and in smooth muscle. It is mainly expressed by monocytes, activated macrophages, T cells, and NK cells [6].

MCP-1 triggers migration and retention of monocytes and transformation of fibroblasts in the glomeruli, playing an important role in the glomerular inflammation. Urinary MCP-1 was involved in the pathogenesis of renal glomerular and tubular damage [7].

The aim of this study was planned to measure the urinary level of monocyte chemotactic protein 1 in children with idiopathic nephrotic syndrome during remission and relapse as well as in steroid sensitive and steroid resistant cases.

Methods

This prospective study was carried out on 50 children attending nephrology unit and clinic of the pediatric department at Benha University Hospital from January 2021 to July 2021. This study was approved by the ethical committee of the Faculty of Medicine, Benha University. Informed written consents were taken from parents of the included patients. The patients were classified into 2 groups:

Group A: This group included 25 nephrotic patients in remission (marked reduction in proteinuria to < 4 mg/m^2/hr or urine albumin dipstick of 0 to trace for 3 successive days in association with resolution of edema) [3].

Group B: This group included 25 nephrotic patients in activity either with 1st attack or relapsers (severe proteinuria > 40 mg/m^2/hr or urine albumin dipstick ++ or more on 3 successive days, often with association or recurrence of edema) after withdrawal of steroid therapy [8].

Control group: This group included 20 age and sex matched healthy children attending general pediatric clinic at Benha University Hospital.

Inclusion criteria: Patients with idiopathic nephrotic syndrome, children age from 2 years up to 18 years.

Exclusion criteria: Patients with secondary nephrotic syndrome, patients with any manifestations of systemic disease, patients < 2 years or > 18 years.

Ethical consideration: Approval of the study protocol by an ethical committee of Benha University was obtained and informed consent was obtained from the parents before enrollment in the study.

All children will be subjected to the following:
1. Full history taking (including symptoms of nephrotic syndrome, duration of the disease, response to steroid therapy and frequency of relapses).

2. Clinical examination including: a) General examination including: vital signs, pulse, blood pressure, its centile and anthropometric measurements. b) Local examination including: abdominal examination, chest examination, heart examination, neurological examination.

3. Investigations: a) Routine lab investigations: Including complete blood count (CBC), C-reactive protein (CRP), serum creatinine, blood urea, serum albumin, serum total cholesterol, complete urine analysis, and urine protein/ creatinine ratio. b) Specific tests: urinary monocyte chemotactic protein-1 (MCP-1) was done by ELISA technique.

Blood Sampling: Seven ml venous blood sample were withdrawn from all participants in the study (both patients and controls) and divided into two portions: the first portion (2ml) was collected in EDTA tube for CBC. The second portion (5ml) was collected in plain tube and left to clot for 30 minutes then serum was separated for routine biochemical tests.

Urine Sampling: Random mid-stream urine samples was taken from patients and controls in clean containers at about 10 am, and divided into two portions: the first portion for complete urine analysis, urinary protein / creatinine ratio, the

second portion was collected in sterile tube and centrifuged at 1000 RPM for 20 minutes. The supernatant was then collected, aliquoted and immediately frozen at -20°C till the time of assessment of MCP-1 using ELISA technique.

Basic principles of the previous tests:

I. Complete blood count (CBC): was done using fully automated cell counter (Sysmex XS-500i) (Japan).

II. Blood chemistry tests: including (serum creatinine, blood urea, serum albumin, serum total cholesterol) were done by an auto enzymatic colorimetric reaction using a Thermo Scientific Indiko Plus chemistry analyzer (Finland).

III. Urine MCP-1: using a commercially available ELISA kit supplied by Elabscience, Inc, USA, catalog number: E-EL-H6005, sensitivity: 37.5pg/mL, and detection range: 62.5-4000 pg/mL.

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 24. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage. For all analyses, Mann-Whitney U test and Student's t-test were used. P value >0.05 insignificant- P value <0.05 significant- P value <0.001 highly significant.

Results

There were no statistical significant difference between patients and controls as regard age and sex. There were highly statistical significant difference between patients and controls as regard edema and proteinuria, and no statistical significant difference between patients and controls as regard blood pressure, pyuria and hematuria. There were no statistical significant difference patients and controls as regard serum creatinine, blood urea and CRP **(table 1)**. There were highly statistical significant difference between group A and group B as regard protein/creatinine ratio, albumin and cholesterol **(table 2)**.

Table 1: Demographic and Laboratory data of studied groups

Groups / Parameters		Group A (n = 25)		Group B (n = 25)		Control Group (n = 20)		Stat. test	P value
Age (years)	Mean±SD	8.6±2.2		8.3±2.9		9.0±2.4		F=0.33	0.720 NS
Sex	Male	11	44%	12	48%	8	40%	X²=0.28	0.865 NS
	Female	14	56%	13	52%	12	60%		
Edema	Absent	25	100%	6	24%	20	100%	X² = 46.9	< 0.001 HS
	Present	0	0%	19	76%	0	0%		
Proteinuria	Negative	15	60%	0	0%	20	100%	X² = 86	< 0.001 HS
	Trace	10	40%	0	0%	0	0%		
	++	0	0%	4	16%	0	0%		
	+++	0	0%	21	84%	0	0%		
Serum creatinine (mg/dl)	Mean±SD	0.6±0.2		0.5±0.2		0.5±0.2		F=0.93	0.399 NS
Blood urea(mg/dl)	Mean±SD	25.6±8.0		24.2±6.7		28.3±9.6		F=1.37	0.260 NS
CRP(mg/L)	Median	3.0		2.7		2.7		F=68.9	< 0.001 HS
	IQR	1.4		1.0		1.4			

CRP: c-reactive protein; SD: standard deviation; IQR: interquartile range; X^2: Chi-square test; F: F value of ANOVA test; NS: non-significant; HS: highly significant.

Table 2: Comparisons between studied groups as regard protein/creatinine ratio, albumin and cholesterol

Groups / Parameters		Group A (n = 25)	Group B (n = 25)	Control Group (n = 20)	Stat. test	P value
protein/creatinine ratio (mg/g Cr)	Mean±SD	556.2±227.7	6899.8±4288.8	44.2±10.6	F=52.7	< 0.001 HS
Serum albumin(g/dl)	Mean±SD	3.0±0.3	2.3±0.4	4.1±0.5	F=104.2	< 0.001 HS
Serum total cholesterol(mg/dl)	Mean±SD	160.8±21.3	241.9±65.1	95.5±16.7	F=68.9	< 0.001 HS

F value of ANOVA test was used

There was highly statistical significant difference in uMCP-l levels between the patients and control group ($p < 0.001$). Also, we found that group B had the highest levels of uMCP-1 followed by group A then control group. The difference between the three groups was highly statistical significant ($p < 0.001$) **(table 3) (figure 1)**.

uMCP-1 levels were significantly higher in the SRNS patients than the SSNS patients ($p<0.001$) **(table 4) (figure 2)**. It was shown that uMCP-1 can be used to discriminate between SRNS and SSNS groups in all studied patients at a cut off level of > 68.7 pg/ml, with 88.9% sensitivity, 93.8% specificity, 93.5% PPV and 89.4% NPV (AUC = 0.96 & $p < 0.001$).
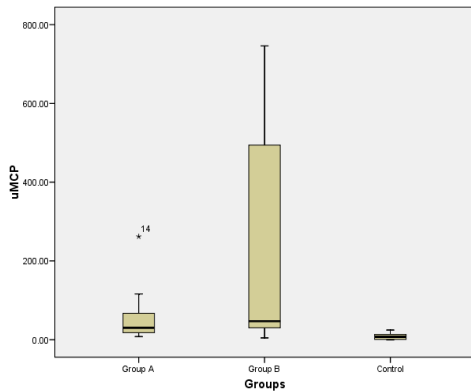

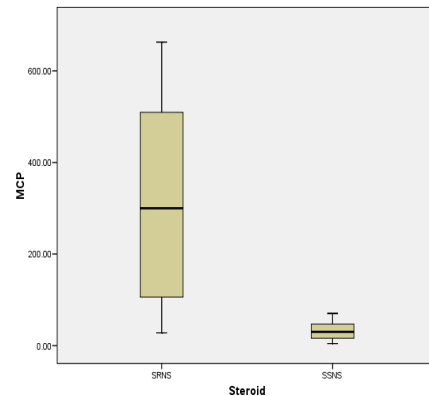**Figure 1:** Comparison between the studied groups as regard uMCP-1.

Figure 2: Comparison between SRNS and SSNS as regard uMCP-1.

Table 3: uMCP-1 levels in studied groups

		Group A (n = 25)	Group B (n = 25)	Control Group (n = 20)	KW	P-value
uMCP-1 (pg/ml)	Median	30.1	46.9	7.2	31.9	< 0.001 HS
	IQR	16.2 - 74.8	29.01 - 501.7	0.57 - 13.9		

Kruskal Wallis (KW) test was used

Table 4: uMCP-1 levels in steroid-resistance NS, steroid-dependent NS patients

		SRNS (n = 18)	SSNS (n = 16)	Stat. test	P-value
uMCP-1 (pg/ml)	Median	300	30.1	Mw = 10	< 0.001 HS
	IQR	104.4 - 528.1	15.3 - 46.9		

Mann Whitney U test was used

Our study showed that uMCP-1 level in group B and group A were highly positively correlated with protein/creatinine ratio and serum cholesterol and highly negatively correlated with serum albumin.

Discussion

Our study revealed highly statistical significant difference between patients and control group (p < 0.001), and highly statistical significant difference between group B (patients in activity) & group A (patients in remission) (p < 0.001) as regard protein/creatinine ratio.

Our study was consistent with Wasilewska et al., [9] study that observed higher protein/creatinine ratio in patients with MCD with proteiuria than control group which is statistically significant.

In our study, group B had the highest level of serum cholesterol followed by group A then control group, the differences between the three groups were highly statistical significant (p < 0.001), and that is in agreement with Bakkaloglu et al., [10] who observed significant differences in serum cholesterol level in INS patients, consistent with their disease activity, in comparison with to INS patients in remission and controls, and in contrast with Filha et al., [11] who found no significant difference between INS patients and controls as regard serum cholesterol.

In our study, group B had the lowest level of the serum albumin followed by group A then control group, the differences were highly statistical significant between the three groups (p < 0.001), and that is in agreement with Wasilewska et al., [9] who found that group IA (MCD with proteinuria) had the lowest level of the serum albumin followed by group IB (MCD after proteinuria regression) then control group, the differences were highly statistical significant between the three groups, and in contrast with Filha et al., [11] who found no significant difference between INS patients and controls as regard serum albumin.

In this study, we found that urinary MCP-1 was elevated in children with NS, suggesting involvement of immune cells in the development of NS. Urinary MCP-1was elevated significantly during relapse than remission.

Our study was consistent with Cho et al., [12] study that reported high concentrations of IL-8 and MCP-1 in the urine and plasma of pediatric patients with minimal change nephrotic syndrome (MCNS) during activity in comparison to patients in remission and healthy controls.

Also, Woroniecki et al., [13] study observed that urinary excretion of MCP-1 was significantly higher in active idiopathic nephrotic syndrome patients as compared to patients in remission and controls.

Also, this study was in agreement with the study done by Wasilewska et al., [9] that showed uMCP-1 in children with minimal change disease in relapse (IA) was significantly higher than in group IB (MCD after proteinuria regression) and controls (p < 0.05).

Also, Vianna et al., [14] study revealed that urinary concentrations of MCP-1/CCL2 were significantly higher in INS patients in comparison to the healthy controls.

Moreover, Simões e Silva et al., [15] study found that patients with active proteinuric forms of chronic glomerulonephritis have higher urine excretion of CCL2/MCP-1 than healthy controls. This study pointed to a potential role for CCL2/MCP-1 in glomerular inflammation.

Furthermore, our study is in agreement with Matsumoto et al., [4] study that found urinary MCP-1 was significantly higher in INS children at onset compared with at remission and compared with the control group.

Similarly, Mariani et al., [16] study found that urinary MCP-1 level was higher in children with active nephrotic syndrome, compared to those in remission and controls.

Also, this study was consistent with the study done by Filha et al., [11] that found urinary concentrations of MCP-1/CCL2 were

significantly higher in INS patients when compared with the control group (p = 0.015).

Moreover, our study is in agreement with Angela [17] study that reported a statistically increase in the level of urinary MCP-1 in all groups of patients (SRNS, SSNS), compared to the control group. The concentration of urinary MCP-1 in the groups of patients with SSNS and SRNS, during the clinical manifestations and remission period exceeded 2.0 – 2.4 times the values of the control group which is statistically significant.

Our study found that uMCP-1 was elevated significantly in the SRNS patients than the SSNS patients, suggests that urinary MCP-1 could be a predictive biomarker of steroid responsiveness in INS.

Matsumoto et al., [4] study concluded that urinary MCP-1 level was significantly elevated in the SRNS patients compared with SSNS patients. Increased urinary excretion of MCP-1in children with SRNS is a potential predictive biomarker of steroid responsiveness in idiopathic NS.

Also, our study was consistent with the study done by Besbas et al., [18] that found urinary levels of MCP-1 were significantly higher in SRNS versus SSNS and healthy controls.

In another study, Khatibi et al., [19] reported higher level of uMCP-1 in the SRNS patients compared with SSNS patients and thus predict resistance to glucocorticoids.

Also, our study was matched with that reference of Agrawal et al., [20] which found that uMCP-1 was able to discriminate children with SRNS versus SSNS at the time of initial disease presentation.

Moreover, Angela [17] noted that the group of patients with SRNS showed higher mean values of MCP-1 in the urine compared to those in the group with SSNS (p < 0.001).

On the other hand, Souto et al., [21] did not find any statistically significant differences between steroid-sensitive and steroid-resistant patients or between patients in relapse and remission as regard uMCP-1.

The differences between this study and the study done by Souto et al., was that in this study number of patients was (50) compared to (32) in Souto et al's study and number of controls in this study was (20) compared to (12) in Souto et al's study.

Also, The differences between this study and the study done by Souto et al., was that in this study number of patients in relapse was (25) compared to (21) in Souto et al's study and number of patients in remission in this study was (25) compared to (11) in Souto et al's study.

As regard geographical distribution, Souto et al's study was done in Brazil while this study was done in Egypt.

In conclusion, Urinary MCP-1 is highly sensitive and specific biomarker in childhood nephrotic syndrome for early detection of disease activity as its level is markedly elevated early in the course of the disease. Moreover, urinary MCP-1 can be used as a predictive marker of steroid responsiveness in children with idiopathic nephrotic syndrome.

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