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Original Article

Urinary Monocyte Chemotactic Protein -1 in Childhood Nephrotic Syndrome

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Abstract

Background

Childhood nephrotic syndrome is a clinical entity characterized by massive loss of urinary protein (primarily albuminuria) leading to hypoproteinemia (hypoalbuminemia) and edema. The mechanism of development of childhood nephrotic syndrome is not yet clear. Kidney disease involves an interplay between inflammatory and biochemical changes with the development of innate immune response and accumulation and activation of leucocytes, particularly monocytes/macrophages in the kidney. The moncyte chemotactic protein-1 (MCP-1) is one of the CC chemokine family and it plays an important role in the recruitment of monocytes/macrophages into renal tubulointerstitium.

Aim of the Work

The aim was to measure the urinary levels of MCP-1 in children with nephrotic syndrome and compare these levels in remission and relapse as well as in steroid sensitive and steroid resistant cases.

Patients and method

The study included 70 patients with nephrotic syndrome following up in Pediatric Nephrology Clinic, Ain Shams University diagnosed for at least one year and 20 age and sex matched healthy children as control group. Patients with renal impairment and secondary nephrotic syndrome were excluded from the study. Patients were divided into Group A: 35 patients in remission and Group B: 35 patients in relapse. Subgrouping according to the clinical type of nephrotic syndrome was done. Patients were subjected to thorough history taking and careful clinical examination. Routine laboratory investigations were done (s. albumin, s. creatinine, s. cholesterol, complete urialysis and urinary protein/creatinine ratio) together with quantitative determination of urinary monocyte chemotactic protein-1 in cases and controls.

Results

We found a highly significant difference in urinary MCP-1 between group A, group B, and controls, the highest being group B. the levels in patients in remission still exceeded the controls significantly. However, in group B, no significant difference was found between clinical types of the disease in urinary MCP-1. The same was found in group A. no difference was found between males and females in group A as regards urinary MCP-1 levels yet females had significantly higher levels in group B. This difference was not seen in controls.

We found no significant difference in MCP-1 levels in patients receiving different treatment modalities.

Conclusion

Urinary MCP-1 is a highly sensitive and specific biomarker in childhood nephrotic syndrome (98.5% and 100% respectively), being highly elevated in both remission and relapse, but is not a suitable prognostic biomarker being insignificantly different among different clinical types of nephrotic syndrome.

Key words Urinary, Monocyte Chemotactic Protein , Childhood Nephrotic Syndrome

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Introduction

Nephrotic syndrome is defined as heavy proteinuria severe enough to cause hypo-albuminemia, edema, and hypercholesterolemia**.** Although the molecular basis for this is still speculative, there is evidence that nephrotic syndrome may be a consequence of a primary glomerular defect, circulating factors, or an immunological abnormality**.** Most cases of idiopathic NS are steroid sensitive; nevertheless, some cases become steroid-resistant during their clinical course **[1].** The development of kidney disease involves a complex interplay between neurohormonal, inflammatory and biochemical changes which act on renal cells. This can lead to the development of an innate immune response predominantly characterized by the accumulation and activation of leukocytes, particularly monocytes/macrophages, in the kidney. Chemokine-induced recruitment of peripheral leukocytes into tissues is a critical step in the development of inflammatory responses **[2, 3, 4].** Chemokines are classified into 4 families according to the location of cysteine residues. The 4 chemokine groups are CC, C, CXC and CX3C, where C is a cysteine and X is any amino acid residues. **[5].** The monocyte chemotactic protein-1 (MCP-1) is a member of the CC chemokine family, and a potent chemotactic factor for monocyte **[6].** It plays an important role in the recruitment of monocytes/macrophages into renal tubulointerstitium.

Monocyte Chemo t Protein-1 (MCP-1) is produced by a variety of mesenchymal cells, including glomerular cells. Within the glomeruli there is MCP-1 overexpression in both crescent GN and nephrotic conditions. Local recruitment of monocytes is considered the predominant mechanism by which MCP-1 contributes to the renal damage **[7]**. It is also known to be produced by tubular epithelial cells in the kidney and to contribute to renal interstitial inflammation and fibrosis. Protein overload in renal tubular cells is shown to upregulate MCP-1 gene and its protein. These lines of evidence collectively suggest that increased urinary protein excretion probably aggravates renal tubular damage by enhancing MCP-1 expression in tubular cells **[8].**

Detection of MCP-1 in renal tissues as well as in urine samples has been studied in various renal diseases. Monitoring chemokines in urine is a non-invasive procedure and may provide a more dynamic picture of the inflammatory state of the kidney than a biopsy. Urinary chemokines may have a great potential as a biomarker for renal diseases **[4].**

Aim of the Work

The aim was to measure the urinary levels of MCP-1 in children with nephrotic syndrome and compare these levels in remission and relapse as well as in steroid sensitive and steroid resistant cases.

Patients and Methods

A- Patients: The study was conducted in the Nephrology Clinic, Ain Shams University. It included 90 children, 70 of them are nephrotic patients following up at the clinic for at least one year, and 20 age and sex matched healthy children as a control group. The patients were 35 males and 35 females their ages ranged from 1-18 years. The patients were classified into 2 groups:

Group A: This group included 35 nephrotic patients in remission [marked reduction in proteinuria (protein /creatinine ratio ≤ 0.2) or urine albumin dipstick of 0 to trace in association with resolution of edema].

Group B: This group included 35 nephrotic patients in relapse [recurrence of severe proteinuria (protein/creatinine ratio ≥2) or urine albumin dipstick ≥ 2 with a recurrence of edema].

Group C (Control group): This group included 20 age and sex matched healthy children.

B- Methods

Exclusion criteria

- Patients with renal impairment (high serum creatinine level).
- Patients with secondary nephrotic syndrome.

All studied patients had been subjected to the following:

1-Detailed history taking laying stress on: Symptoms of nephrotic syndrome, duration of the disease, response to steroid therapy, other medications and complications of steroid therapy.

2-Careful clinical examination laying stress on: Detection of edema and signs of relapse and signs of therapy complications as cushingoid facies, straiae or hirsuitism.

3-Laboratory investigations including:

A- Routine investigations for nephrotic syndrome including:

- Serum albumin.
- Serum creatinine.
- Serum cholesterol.
- Complete urine analysis.
- Urine protein/ creatinine ratio.

B - Quantitative determination of urinary monocyte chemotactic protein-1 (MCP-1).

Collection of blood samples: Venous blood sample, about 5 cc, was drawn from each subject and the usual precautions for puncture were observed and followed.

a. Blood samples were collected by a syringe with a wide bore needle 5ml.
b. All samples were drawn from each subject under aseptic conditions.
c. Evacuation of the sample after removal of the needle in clot activator test tube.
d. Stored at room temperature for 20 minutes to 2 hours then centrifugation was done for plasma separation.
e. Plasma was then collected at ependorph tube and frozen at -20^{o}C for storage till time of analysis.

Collection of urine sample: Random mid stream urine samples were taken from patients and controls in clean containers at about 10am, complete urine analysis, urinary protein / creatnine ratio were done, and another sample was preserved at -20 °C till time of assay of MCP-1 level in urine.

Detailed laboratory methods:

a. Serum albumin, serum creatinine, serum cholesterol were measured by using Hitachi automatic analyzer 917.
b. Complete urine analysis: dip sticks and microscopic examination.

c. Urine protein /creatinine ratio:

It was estimated on Synchrone CX7 system employing a time end point colorimetric methods.

d. MCP-1 in urine: It was measured by ELISA.

♦ **Test principle for uMCP -1**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MCP-1 has been pre-coated onto a microplate standard and samples are pipetted into the wells and any MCP -1 present is bounded by the immobilized antibody. After washing away any unbound substances, an enzyme linked polyclonal antibody specific for MCP -1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MCP -1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Statistical Methods

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12).

P value >0.05 insignificant- P value <0.05 significant- P value <0.01 highly significant

Results: The children included in the study were divided into 3 groups

Group A: This group included 35 nephrotic patients in remission;15 males and 20 females, their ages ranged from 5-15 years with mean age is (9.2+2.6) years.

Group B: This group included 35 nephrotic patients in relapse; 20 males and 15 females. Their ages ranged from 5-14years with mean age is (9±3) years.

Group C (controls): This group included 20 age and sex matched healthy children; 12 males and 8 females, their ages ranged from 5-15 years with mean age of(8.9+2.8) years.

Group A included 24 steroid responsive nephrotic syndrome [NS] (68.6%) and 11 steroid resistant NS (31.4%), while group B included 28 steroid responsive nephrotic syndrome [NS] (80%) and 7 steroid resistant NS (20%). 40% of patients in group A were treated with steroids alone compared to 45.7% in group B. The other patients used multiple steroid sparing drugs. Both groups A and B had statistically comparable age of onset of the disease and duration of the disease (4.7±2 vs 4.6±2.6 ys for onset, 4.5±2.2 vs 4.3±2.3 ys for duration).

The results are represented in tables (1) to (8) and Figuress (1), (2)

Table 1: Laboratory data of studied groups.

	Group A N=35	**Group C** N=20	**Group B** N=35	**Group C** N=20	**P**	**Least significant difference(LSD) post hoc test**
Pr./cr.	1.06 ± 0.4	0.07 ± 0.03	18.2 ± 7	0.07 ± 0.03	**<0.001 HS**	-Relapse versus remission and versus controls -Reemission versus controls
Cholesterol mg/dl	157 ± 27	87 ± 28	283 ± 26	87 ± 28	**<0.001 HS**	-Relapse versus remission and versus controls -Reemission versus controls
Albumin g/dl	3 ± 0.4	3.6 ± 0.5	2 ± 0.3	3.6 ± 0.5	**<0.001 HS**	-Relapse versus remission and versus controls -Reemission versus controls
Creatinine mg/dl	0.67 ± 0.20	0.50 ± 0.3	0.60 ± 0.3	0.50 ± 0.3	>0.05 NS	

Table 2: uMCP-1 levels in studied groups.

	Group A N=35	**Group B** N=35	**Group C** N=20	**P**	**Post hoc test (LSD)**
Mean+SD ug/dl	102 ± 4.7	322 ± 187	45 ± 20	**<0.001 HS**	-Relapse versus other two groups -Reemission versus controls
Range	91-110	125-900	15-90		

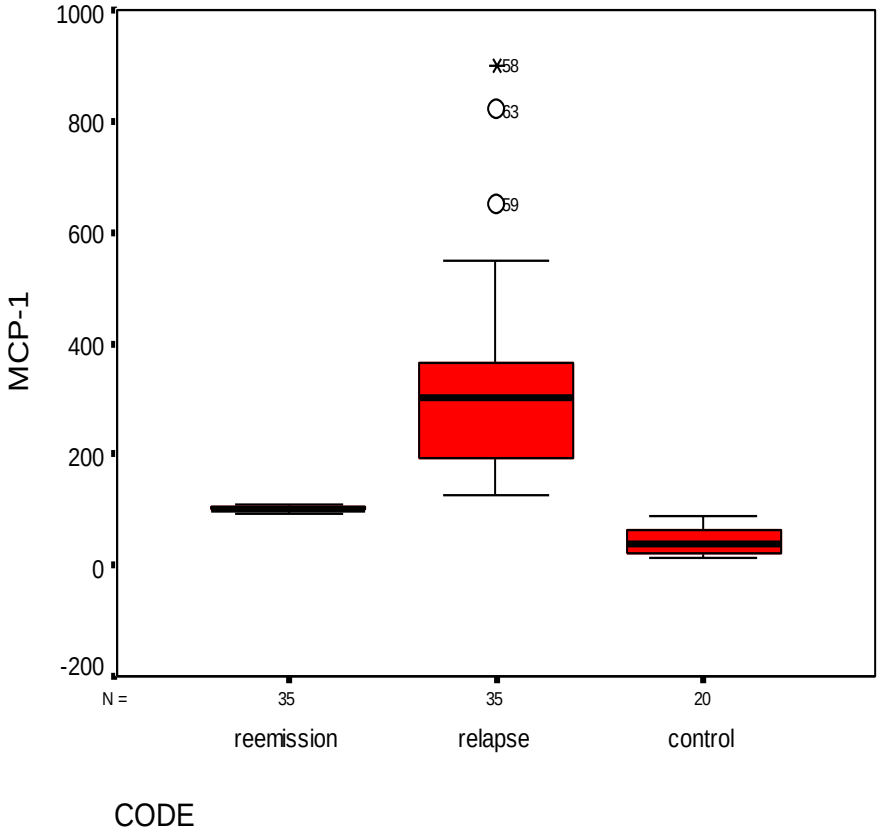

Figure 1: Comparison between the studied groups as regard uMCP-1.

Table 3: Gender difference in uMCP-1 in studied groups.

Mean ± SD	Male	Female	**P**
Group A	103 ± 5	102 ± 4	>0.05 NS
Group B	232 ± 85	441± 219	<0.001 HS
Group C	44.1 ± 20	46.8 ± 21	>0.05 NS

Table 4: uMCP-1 in steroid sensitive and resistant patients in the 2 groups.

Mean ± SD	Steroid sensitive nephrotic syndrome	Steroid resistant nephrotic syndrome	**P**
Group A	103 ± 4.2 (n=24)	93 ± **5.**4 (n=11)	>0.05 NS
Group B	384 ± **22**5 (n 28)	295 ± 165 (n=7)	>0.05 NS

Table 5: uMCP-1 in patients receiving different treatments.

Treatment modality	Mean ± SD	**P**
Steroids	104 ± 6	>0.05 NS
Steroids and single steroid sparing	99 ± 4	
Steroids and multiple steroid sparing	102 ± 3	

Table 6: Correlations of uMCP-1 in group (A).

	uMCP-1	
	r	**P**
Current age	0.11	> 0.05
Age of onset	0.19	> 0.05
Duration	- 0.09	> 0.05
Pr./cr.	0.85	< 0.001HS
Cholesterol	0.70	< 0.001HS
Albumin	- 0.68	< 0.001HS
Creatinine	- 0.04	> 0.05

Table 7: Correlations of uMCP-1 in group(B).

Variables	**uMCP-1** r P	
Current age	0.16	>0.05
Age of onset	0.12	>0.05
Duration	-0.02	>0.05
Pr/cr	0.71	<0.001HS
Cholesterol	0.35	<0.001HS
Albumin	-0.50	<0.001HS
Creatinine	-0.11	>0.05

Table 8: Validity of uMCP in diagnosis of idiopathic nephrotic syndrome.

Variables	**%**
Best cut off=93.5	
Area under the curve (AUC)	0.100
Sensitivity	98.5%
Specificity	100%
PPV	100%
NPV	96%
Accuracy	95%

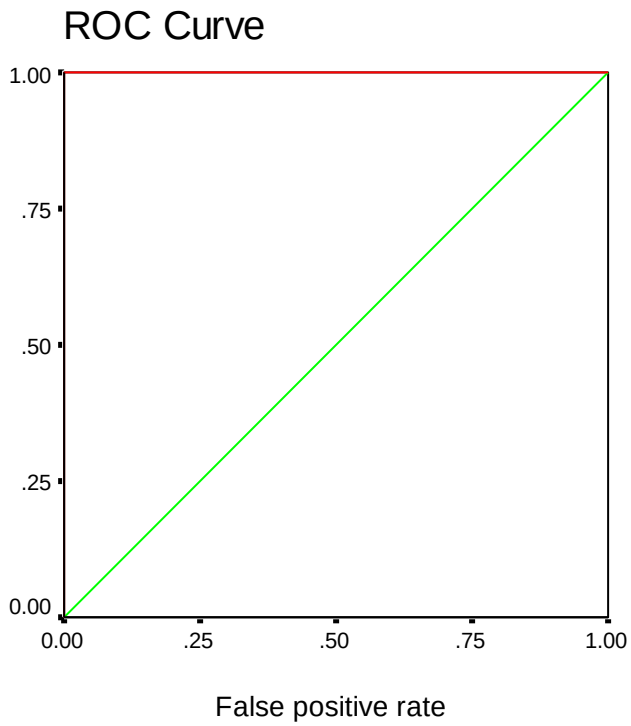

Figure 2: Receiver Operating Characteristic (ROC) curve to define the best cutoff to uMCP-1 patients with nephrotic syndrome.

Discussion

Our study was designed to check urinary MCP-1 level in pediatric patients with idiopathic nephrotic syndrome, effect of remission and relapse on these levels as well as steroid response and to compare our results to the urinary levels of MCP-1 in age and sex matched control group. The current study findings showed that there was highly significant difference in urinary level of monocyte chemotactic protein-1 (MCP-1) between the studied groups where the urinary level of MCP-1 in children with nephrotic syndrome in relapse (group B) (322 ± 187 ug/ml) was markedly higher than patients in remission (group A) (102 ± 4.7ug/ml) and healthy controls (group c) (45 ± 20 ug/ml). Similar findings were also observed in previous studies. **[1, 9]** They observed that urinary excretion of MCP-1was significantly higher in idiopathic nephrotic syndrome (INS).Similarly a study reported high concentrations of MCP-1 in the urine of pediatric patients with minimal change nephrotic syndrome during relapse in comparison to patients in remission and normal healthy control**. [10] , [11].**

On the other hand, another study reported that urinary levels of MCP-1 were not significantly differernt between idiopathic nephrotic patients during remission and in relapse. **[12]** This disagreement may be due to their small sample size or statistical disproportion between the number of relapse and remission cases. Also, in our study we found that MCP-1 level in urine of relapsed nephrotic patients (group B), nephrotic patients in remission (group A) and control subjects (group C) was not significantly correlated with other variables in these groups (serum cr., current age and gender) agreeing with another study that showed no correlation between uMCP-1 and age, gender and race among nephrotic patients and controls **[9, 13].**

Also, we found no significant difference in uMCP-1 levels among patients on different types of treatment. This result agreed with a study reporting that the increase of urinary chemokines (including MCP-1) in INS patients was independent of histology or response to corticosteroid therapy, [12] yet another study stated that the excretion of MCP-1 in children with MCD treated with Cs **[11].**

In the present study, MCP-1 level in urine of relapsed nephrotic patients (group B) was significantly highly positively correlated with Pr/Cr ratio, serum cholesterol and highly negatively correlated with serum albumin. This result is comparable to that observed before **[11]** and those reporting a significant negative correlation between urinary levels of MCP-1 and serum albumin and positive correlation to total serum cholesterol and triglycerides in chronic kidney diseases **[14].**

Also, MCP-1 level in urine of nephrotic patients in remission (group A) was significantly highly positively correlated with Pr/Cr ratio, serum cholesterol and highly negatively correlated with serum albumin. This agreed with previous studies stating that increased urinary protein excretion seems to contribute to the aggravation of renal tubulointerstitial lesions and the progression of renal diseases and increase the level of MCP-1 in the urine **[8].** Similarly many studies showed that uMCP-1 levels positively correlated with albumin excretion rate. **[15, 16, 17].** Previous studies suggested that increased urinary excretion of MCP-1 in the patients with glomerulopathy is probably due to the enhanced production of MCP-1 in renal tubules **[18, 19].** The basolateral secretion of uMCP-1 is expected to be increased simultaneously when urinary MCP-1 excretion is increased

induced by up-regulation of MCP-1 gene and its protein expression due to excessive exposure to plasma protein filtered from the damaged glomeruli **[8].**

Conclusion

Urinary MCP-1 is a highly sensitive and specific marker in INS yet our findings were not conclusive enough to establish a possibility of using urinary MCP-1 as a prognostic marker, pointing out particular steroid response, in INS. Further studies are obviously necessary to address this issue.

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Declaration

Ethics approval and consent to participate

This study protocol and the consents were approved and deemed sufficient by Ethical Committee of Pediatric Department, Faculty of Medicine, Ain Shams University. And informed written consent was obtained in every case from their legal guardians.

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Conflict of interest: No

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