

Urinary CD80 as a Replacement for Renal Biopsy for Diagnosis of Pediatric Minimal Change Disease

Heba Mostafa Ahmed,¹ Dina Ahmed Ezzat,¹ Noha A Doudar,² Mai Adel¹

¹Departement of Pediatrics, Faculty of Medicine, Beni-Suef University, Egypt

²Department of Clinical and Chemical Pathology, Faculty of Medicine, Beni-Suef University, Egypt

Keywords. minimal change disease, focal segmental glomerulosclerosis, CD80, child

Introduction. Early diagnosis of minimal change disease (MCD) is challenging in nephrotic children. CD80 is a protein expressed on the surface of podocytes associated with nephrotic syndrome and it is implicated in the induction of proteinuria. This study aimed to investigate the use of urinary CD80 for the diagnosis of MCD. **Materials and Methods.** Urinary CD80 levels were evaluated in 36 children with nephrotic syndrome and normal glomerular filtration rate. They were divided into three groups of MCD (n = 21), focal segmental glomerulosclerosis (n = 9), and other glomerulopathies (n = 6). The MCD group was subdivided into 2 of those with remission (n = 11) and those in the active stage (n = 10). Forty healthy children were included as controls.

Results. The urinary CD80 level was significantly higher in the MCD group (3.5 ± 2.1 ng/mg creatinine) than in the focal segmental glomerulosclerosis group (1.2 ± 0.5 ng/mg creatinine, $P < .001$), the other glomerulopathies group (1.4 ± 0.7 ng/mg creatinine, $P < .001$), and the control group (0.7 ± 0.2 ng/mg creatinine, $P < .001$), while it showed no significant difference among the non-MCD groups. There was no significant difference between MCD in remission and MCD in relapse, either. A urinary CD80 cutoff value of 1.5 ng/gm creatinine showed a sensitivity of 100% and a specificity of 86% for diagnosis of MCD.

Conclusions. Urinary CD80 levels were significantly higher in the children with MCD than in the controls and patients with other causes of nephrotic syndrome.

IJKD 2018;12:107-11
www.ijkd.org

INTRODUCTION

Nephrotic syndrome is one of the most common pediatric kidney disorders, characterized by proteinuria, hypoalbuminemia, and generalized edema.^{1,2} The incidence of nephrotic syndrome is ranging between 2 to 7 newly diagnosed cases per 100000 children per year, and the prevalence is about 16 cases per 100000 children.³ Minimal change disease (MCD) is the most common cause of idiopathic childhood-onset nephrotic syndrome. It accounts for about 85% of cases, followed by

focal segmental glomerulosclerosis (FSGS), in almost 10% of cases.⁴

The majority of children with MCD who present with nephrotic syndrome respond to corticosteroid therapy, but some are steroid dependent or steroid resistant, while the majority of patients with FSGS are steroid resistant with a high possibility to progress to end-stage renal disease.⁵ Other glomerular diseases (such as mesangiocapillary proliferative glomerulonephritis [MPGN] and immunoglobulin A nephropathy) may present

with the picture of nephrotic syndrome due to associated massive proteinuria.⁶ The majority of patients with steroid-resistant nephrotic syndrome (SRNS) are treated with second-line agents, such as calcineurin inhibitors and other immunosuppressant medications. There is a highly variable response. This variability in responding to calcineurin inhibitors likely reflects in part in differences in the underlying histopathology of the SRNS, with MCD more likely to respond than FSGS.⁷

Renal biopsy is the only available method of diagnosis of the underlying pathology of nephrotic syndrome, especially in SRNS, but it is invasive and has many complications.⁸ Cluster of differentiation 80 (CD80) is a protein found on the surface of activated B lymphocytes and monocytes that helps T-cell activation.⁹ Podocytes are highly specialized cells of the kidney glomerulus that wrap around capillaries and that neighbor cells of the Bowman capsule,¹⁰ and they can be induced to express CD80, causing rearrangement of the T cell actin cytoskeleton and proteinuria.^{11,12} Some recent studies have documented that urinary CD80 increases in MCD rather than in other renal glomerular disorders.^{10,13} The aim of this study was to investigate the possibility of using urinary CD80 levels for the diagnosis of MCD.

MATERIALS AND METHODS

This study included children with nephrotic syndrome, diagnosed and followed up in the period from January 2014 to January 2017, and age- and sex-matched healthy children as controls. Patients younger than 1 year or older than 18 years and those with renal impairment were excluded from the study. All children included in the study were subjected to a written consent from their parents.

The patients were classified according to renal pathology into 3 groups of MCD, FSGS, and MPGN. They were also subdivided into 2 groups according of those in remission stage (urine protein-creatinine ratio < 0.2) and those in active stage (urine protein-creatinine ratio > 40).

Age of onset of the disease, medical treatment, and number of relapses were documented. Complete clinical examination included body weight, height, body mass index, blood pressure, and presence or absence of edema. Laboratory investigations included serum urea, creatinine, sodium, potassium, albumin, and cholesterol, and

urine protein-creatinine ratio.

The samples were obtained in early morning, voided directly into a sterile container. Samples were centrifuged for 20 minutes at the speed of 2000 rpm to 3000 rpm to remove supernatant and were stored at -20°C. Quantitative determination of urinary CD80 was done applying a sandwich enzyme-linked immunosorbent assay technique (Lifespan BioSciences, USA).

Statistical analysis was done using the SPSS software (Statistical Package for the Social Sciences, version 22.0, IBM Corp, New York, NY, USA). Data were described in the form of mean \pm standard deviation for quantitative data, and frequency and proportions for qualitative data. A *P* value less than .05 was considered significant. Differences were analyzed between the groups by the Student *t* test as regards normally distributed data; otherwise, the Mann-Whitney *U* test was used. Correlations were analyzed using the Spearman correlation coefficient test. The receiver operating characteristics curve analysis was performed to identify the optimal cutoff value for CD80 to distinguish between MCD and other renal pathologic findings.

RESULTS

This study included 76 children, 36 of whom with nephrotic syndrome (21 boys and 19 girls) and 40 were healthy controls. The patient group included 21 with MCD, 9 with FSGS, and 6 with MPGN. The indications of renal biopsy in the patients were SRNS in 6 patients, drug-sensitive nephrotic syndrome in 8, nephritic nephrotic syndrome in 4, and age at onset of greater than 9 years in 3.

The three groups of the patient and the controls were not significantly different in age, sex, and weight. Serum albumin and cholesterol and urine protein-creatinine ratio were significantly higher in the patients groups as compared to the controls. However, there were no significant differences between the three patients groups as regarding these parameters (Table 1). Most of the patients in the three experimental groups had taken prednisone or other immune suppressant therapies.

The concentration of urinary CD80 was significantly higher in the MCD group (3.5 ± 2.1 ng/mg creatinine) than in the FSGS group (1.2 ± 0.5 ng/mg creatinine, *P* < .001), MPGN group (1.3 ± 0.7 ng/mg creatinine, *P* < .001), and control group (0.7 ± 0.2 ng/mg creatinine, *P* < .001).

Table 1. Characteristics of Patients With Minimal Change Disease (MCD), Focal Segmental Glomerulosclerosis (FSGS), and Other Glomerular Diseases, Compared With Controls

Characteristic	Children With Nephrotic Syndrome			Control Group	P
	MCD	FSGS	Other Glomerulopathies		
Number of children	21	9	6	40	
Weight, kg	30.2 ± 10.4	22.6 ± 11.6	20.5 ± 6.0	21.5 ± 6.0	.29
Age, y	8.4 ± 3.2	6.6 ± 3.4	5.5 ± 4.2	6.3 ± 2.6	.31
Sex					
Male	12 (57.1)	3 (33.3)	3 (50)	32 (80)	
Female	9 (42.9)	6 (66.7)	3 (50)	8 (20)	.24
Serum albumin, g/dL	3.9 ± 0.9	3.2 ± 1.0	3.4 ± 1.0	4.9 ± 0.1	.001
Urine protein-creatinine ratio	1.4 ± 1.8	2.7 ± 2.8	3.5 ± 3.5	0.5 ± 0.2	.04
Serum cholesterol, mg/dL	203.0 ± 61.4	200.4 ± 60.7	262.0 ± 131	139.7 ± 11.1	.03
Urinary CD80, ng/L	1030.4 ± 241.1	504.8 ± 217.3	536.0 ± 205	488.8 ± 53.7	< .001
Urinary CD80, ng/mg creatinine	3.54 ± 2.10	1.18 ± 0.51	1.26 ± 0.69	0.75 ± 0.20	< .001
Prednisone, n (%)	16 (76.2)	7 (77.8)	6 (100)	0	...

The concentration of urinary CD80 showed no significant difference among the FSGS patients, MPGN, and controls (Tables 1 and 2). There was no significant difference between MCD in remission

and MCD in relapse as regarding urinary CD80 levels, either (Table 3). There was no significant correlation between urinary CD80 and the degree of proteinuria.

Table 2. Comparison of Median Urinary CD80 Values*

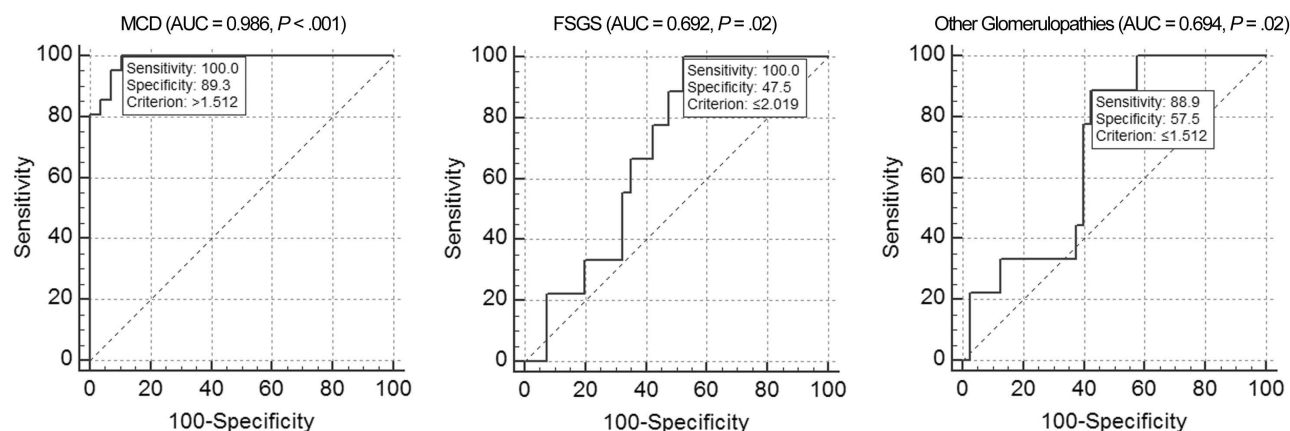
Group	Median, ng/L	95% Confidence Interval
Minimal change disease	814.40	901.51 to 979.16
Focal segmental glomerulosclerosis	554.70	239.73 to 665.22
Other glomerulopathies	635.35	401.33 to 784.72
Controls	679.00	734.43 to 852.23

*P < .001

Receiver operating characteristics curves were used to assess the possible efficacy of urinary CD80 for diagnosis of MCD. The area under the receiver operating characteristics curve of urine CD80 levels for the diagnosis of MCD was 0.977 (95% confidence interval, 0.899 to 0.999). The analysis rendered an optimal cutoff value of 1.5 ng/mg creatinine, corresponding to 100% sensitivity and 86% specificity (Figure).

Table 3. Urinary CD80 in Patients With Minimal Change Disease in Remission and Active Relapse

Parameter	Remission	Relapse	P
Urinary CD80, ng/L	859.05 ± 243.47	675.66 ± 489.93	.13
Urinary CD80, ng/mg creatinine	3.43 ± 2.20	4.03 ± 1.85	.62



Receiver operating characteristics curves for urinary CD80.

DISCUSSION

There is an urgent need for a precise biomarker that can differentiate MCD from FSGS and other glomerular diseases rather than the invasive renal biopsy. In this study, results showed that using a cutoff value of 1.5 ng/mg creatinine, urinary CD80 can discriminate MCD from FSGS and other glomerulopathies with an area under the receiver operating characteristics curve of 0.977, and an optimized sensitivity of 100% and specificity of 86%. This notifies that urinary CD80 can be a suitable biomarker for the diagnosis of MCD, confirming findings previously reported by other studies.^{10,13,14} In addition, the absence of significant difference between the three patient groups in protein-creatinine ratios together with the nonsignificant value of urinary CD80-creatinine ratios between patients with MCD in relapse and patients in remission signifies that urinary CD80 is related to the underlying renal pathology rather than disease activity and proteinuria. However, other studies have reported that urinary CD80 levels are higher in MCD patients in relapse than in MCD patients in remission and FSGS patients.^{10,13,15} Limitations of the study done by Garin and colleagues included that the MCD and FSGS patient groups were significantly different in age and sex distribution.^{10,13}

CD80 is a protein expressed on the surface of several antigen-presenting cells, and it was recently found to be expressed on podocytes as well.^{6,11} Recently, a role for podocyte CD80 has been shown in several experimental models of proteinuria.¹⁴ Increased expression of CD80 in podocytes was found in genetic, immune-mediated, drug-induced, and bacterial toxin-induced investigational kidney diseases with nephrotic syndrome.¹² In turn, CD80 expression in cultured podocytes has resulted in a reduced expression of nephrin, which is critical to preserve the glomerular capillary barrier to protein.¹⁶ Normally, the expression of factors like (cytotoxic T-lymphocyte-associated-4, interleukin-10, and probably transforming growth factor- β) that downregulate the podocyte CD80 response resulting in limitation of the proteinuria. However, in MCD patients, there is a defect in CD80 podocyte autoregulation. This defect causes persistent CD80 expression and persistent proteinuria.¹⁴

CONCLUSIONS

Our study demonstrated that urinary CD80 levels

were significantly higher in patients with MCD than in patients with other conditions or in healthy controls. There was no significant differences in urinary CD80 between patients in remission and patients in relapse. A cutoff value of 1.5 ng/mg creatinine corresponding to 100% sensitivity and 89% specificity signified that urinary CD80 could be used as a reliable diagnostic biomarker for MCD in patients with SDNS or FRNS despite that renal biopsy is still the golden route for identifying the underlying renal pathology.

CONFLICT OF INTEREST

None declared.

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Correspondence to:

Heba Mostafa Ahmed, MD
Department of Pediatrics, Beni-Suef University, Beni-Suef, Egypt
Tel: +20 100 151 6641
E-mail: heba_most@yahoo.com

Received June 2017

Revised December 2017

Accepted December 2017