

Value of urinary CD80 in differentiating minimal change disease from focal segmental glomerulosclerosis

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ABSTRACT

Background. Minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) are two common forms of childhood nephrotic syndrome (NS). Separating MCD from FSGS is essential to determine the treatment therapy and the prognosis. Cluster of differentiation 80 (CD80) in urine is now considered a valuable marker for detecting MCD. Therefore, we conducted this research to evaluate the role of urinary CD80 concentrations in differentiating MCD from FSGS.

Methods. A cross-sectional descriptive study was performed on 67 children with NS who underwent kidney biopsy and 67 children in the control group.

Results. MCD accounted for the majority (61.2%), while FSGS accounted for 38.8% with an MCD/FSGS ratio of 1.6:1. There were no significant differences in age, gender, hematuria, hypertension, urinary protein-creatinine ratio, and albumin concentrations between the two study groups ($p > 0.05$). There were statistically significant differences in

glomerular filtration rate (GFR) between the two study groups with $p < 0.05$. The urinary CD80/creatinine ratio in the MCD group was 25.6 (5.4-38.3) $\text{pg}/\mu\text{mol}$, which was significantly higher than that of the FSGS group (3.6 (1.9-6.5) $\text{pg}/\mu\text{mol}$) with $p < 0.05$. The area under the ROC curve for urinary CD80/creatinine ratio in the diagnosis of MCD with FSGS was 0.87 (95% CI 0.79-0.95) at a cut-off value of 4.6 $\text{pg}/\mu\text{mol}$, with a sensitivity of 85.4% and specificity of 73.1%, $p < 0.05$.

Conclusion. The urinary CD80/creatinine ratio was useful in differentiating between nephrotic syndrome with MCD and FSGS.

Keywords: nephrotic syndrome, minimal change disease, focal segmental glomerulosclerosis, Cluster of differentiation 80 (CD80)

INTRODUCTION

Nephrotic syndrome is a common glomerular disease in children, with a frequency of 2-7 per 100,000 children under 16 years old. Its clinical features include edema, increased urinary protein, hypoalbuminemia, and hyperlipidemia [1]. The two most common forms of NS in children are MCD, accounting for 77%, and FSGS, accounting for 8% [2]. The response to therapy varies depending on the histopathological injury. The majority of MCD responds well to corticosteroid treatment. On the other hand, primary FSGS is a frequently relapsing disease, resistant to corticosteroids, and associated with a high risk of progression to end-stage kidney disease [1]. Accurate diagnosis of these common histologic types will aid clinicians in accurately diagnosing the disease, selecting appropriate treatment, and predicting patient outcomes. Kidney biopsy is a procedure used to obtain tissue samples to diagnose these histologic types; however, it is invasive and not widely available. Researchers have been trying to identify circulating factors in the blood or body fluids that can help identify histopathological forms of the disease without the need for kidney biopsy. One of these factors is CD80 (Cluster of differentiation 80), which has been suggested to be associated with the pathogenesis of MCD. It is present at high concentrations in the urine of active MCD, while serum CD80 concentrations remain relatively unchanged [3]. Studies on urinary CD80 have

demonstrated usefulness in diagnosing MCD and differentiating treatment responses to corticosteroid therapy [4-8].

Therefore, this study was performed to investigate the value of urinary CD80 in distinguishing between MCD and FSGS.

MATERIAL AND METHODS

Subjects

A cross-sectional descriptive study was conducted on 67 pediatric patients with NS who underwent kidney biopsy from April 2018 to November 2023, and 67 healthy children without renal disease as the control group.

Inclusion criteria were children under 16 years old with NS who had had a kidney biopsy with the following lesions: MCD or FSGS.

A control group was established including patients who did tests for urinary CD80, urinary excretion creatinine, and proteinuria, and collected information on age, gender, urinary CD80, and urinary excretion creatinine.

An NS group with biopsy-proven who met the inclusion criteria was selected for this study. Urinary CD80 and urinary excretion creatinine were collected at the time when the patient relapsed close to the time of the kidney biopsy procedure. Variables included age, gender, hematuria, hypertension, creatinine, GFR, proteinuria, urinary excretion creatinine, urinary CD80, and kidney histologic lesions.

Quantification of urinary CD80 used chemical kits from Abbexa, Cambridge, United Kingdom.

Data processing method

Data was analyzed using SPSS software. Comparison of the differences between the ratios used test χ^2 . Comparison of the mean values used the Student's t-test. Determined the value of urinary CD80 concentration in diagnosing histologic lesions and calculated the sensitivity and specificity corresponding to the cut-off point of this biomarker using the ROC curve.

RESULTS

This study was conducted on 67 children in NS group and 67 children in control group. The mean age of the NS group was 9.0 ± 3.9 years, and the mean age of control group was 7.4 ± 3.6 years; there was no significant difference in age between the two study groups with $p > 0.05$. The male-to-female ratio of NS and control group were 3.2:1 and 2.5:1, respectively, with no statistical difference, $p > 0.05$.

MCD accounted for the majority (61.2%), while FSGS accounted for 38.8% with an MCD/FSGS ratio of 1.6/1 (Table 1). There were no remarkable differences in age, gender, hematuria, hypertension, urinary protein-creatinine ratio, and serum albumin concentrations between the two study groups with $p > 0.05$. However, there were statistically significant disparity in GFR between MCD and FSGS, $p < 0.05$ (Table 2). The urinary CD80/creatinine ratio was significantly higher in the NS group compared to the control group with $p < 0.05$ (Table 3). The urinary CD80/creatinine ratio was significantly higher in the MCD compared to the FSGS, $p < 0.05$ (Table 4). The area under the receiver operating characteristic curve (AUC) for the urinary CD80/creatinine ratio to diagnose MCD was 0.87 (95% confidence interval: 0.79-0.95) at a cut-off value of $4.6 \text{ pg}/\mu\text{mol}$, with a sensitivity of 85.4% and specificity of 73.1% ($p < 0.05$) (Table 5 and Figure 1).

DISCUSSION

General characteristics

In this study, we quantified urinary CD80 for the patients with NS who underwent the kidney biopsy and the control. The control group had a similar age and gender distribution to the NS group.

The mean age in the NS group was 9.0 ± 3.9 years, which was higher than the age reported in the study by Anochie [9], and inconsistent with the literature that primary NS affected children aged 1-6 years [1]. Our study included children with indications for kidney biopsy, who often had been treated for a longer period and progressed to drug resistance, hence the higher age compared to other studies was appropriate.

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The male-to-female ratio in the study group was 3.2:1, indicating a male predominance. This was consistent with the study by Kumar [10] and the literature on gender distribution in NS patients, where males are more commonly affected than females [11].

Regarding the distribution of histologic lesions in NS

In our study, MCD accounted for the majority (61.2%), followed by FSGS with 38.8%. A study of Asian researchers about kidney biopsies over 27 years presented the results that IgA nephropathy was predominant at 27.9% in primary glomerular disease, followed by MCD (21.3%), and FSGS (3.4%) [12]. A international study conducted in a large population (512 children with NS) indicated that the MCD accounted for 77% and FSGS was 7% [2]. Similarly to above studies, our research proved that MCD was outweighed by FSGS in childhood NS.

Regarding the clinical and laboratory characteristics of the study groups

Table 2 showed significant differences in GFR between the MCD and FSGS. Characteristics such as age, gender, hematuria, hypertension, the urinary protein-creatinine ratio, and serum albumin did not differ between these two histologic lesions. We investigated the age distribution in two study groups, Table 2 showed that the age distribution among age groups was not different within each histologic lesion. There was no notable disparity in age between the two histologic forms. Similarity to our study, a research about the age distribution in childhood NS performed by Vivarelli showed that the peak of MCD was 5 to 6 years, and it accounted for more than 50% of histological forms, while FSGS distributed evenly low, it was around 15% [13].

In Kumar's study, the male-to-female ratio was 3.1:1 for MCD and 2.7:1 for FSGS [10]. Alshami's study also did not find any difference in gender between the histological groups [14]. Therefore, gender difference does not aid in distinguishing MCD from FSGS.

Besides, Kumar showed that hematuria mainly occurred in FSGS and not in MCD, and this difference was statistically significant [10]. Our study recorded that gross hematuria occurred in MCD with a low incidence. Some studies have reported that gross hematuria could also happen in MCD and FSGS. For example, Alshami's study reported one case

of gross hematuria (2%) in the MCD group and two cases of hematuria (10%) in the FSGS group [14].

There was no statistical difference between histological types regarding hypertension. According to Alshami, hypertension could occur in 36% of MCD and 60% of FSGS, and the difference was no significant in hypertension between the two categories with $p=0.06$ [14]. Kumar's study found that 11 out of 95 children with MCD had hypertension, accounting for 11.6%. In the group without MCD, hypertension accounted for 32.8%, and there was a significant difference in hypertension between the two groups [10]. Overall, hypertension symptoms can occur in all histological types.

There was no difference in urinary protein and serum albumin concentrations between the histological types. The reason may be that these tests were parts of the diagnostic criteria for both groups, so the difference was not statistically significant.

Table 2 showed a significant difference in the symptoms of decreased GFR between MCD and FSGS. According to the literature, FSGS has a higher risk of progressing to end-stage kidney disease than MCD despite getting the appropriate therapy [15]. Therefore, determining FSGS is essential to establish the appropriate treatment therapies and give the long-term prognosis for patients.

Regarding the urinary CD80 concentration

In Table 3, urinary CD80 concentrations were represented by urinary CD80/creatinine ratio with $\text{pg}/\mu\text{mol}$ units. Our research would adjust urinary CD80 concentration to urinary creatinine excretion to reduce the influence of glomerular filtration rate on urinary CD80 concentration. As the results are shown in Table 6, the researchers used CD80 chemicals from separate brands, such as Bender Medsystem, Lifespan BioSciences, and Fisher Scientific. Hence, the results of the studies were different. Our research used chemicals from Abbexa, UK, a research chemical that has not yet been marketed. Therefore, analyzing the value of CD80 studies should focus on the role of urinary CD80 in diagnosis and treatment prognosis rather than comparing its absolute concentrations.

In Tables 3 and 4, the urinary CD80/creatinine ratio was prominently higher in the NS group compared to the control group. This result was consistent with the studies listed in Table 6, such as Ling, Ahmed, and Gonzalez.

Based on the description of Shimada's study, MCD was hypothesized to result from the "two-hit" of immune podocyte dysfunction. The first effect was the activation of CD80 in podocytes in response to T-cell cytokines or microbial products. This partly explained why NS recurred after an infection. The second effect was an inadequate response to CTLA-4 due to T-regulatory cell abnormalities [16].

Value of urinary CD80 in the diagnosis of histologic types

AUC for urinary CD80/creatinine ratio in diagnosing MCD was 0.87 (95% CI 0.79-0.95) at a cutoff point of 4.6 pg/ μ mol, with a sensitivity of 85.4% and specificity of 73.1% ($p < 0.05$). These results were similar to the studies of Garin [5], Ling [7], Ahmed [17] and Gonzalez [18] on the value of urinary CD80 in recognizing MCD.

Confirming the role of CD80 in MCD contributes to the diagnosis and opens the way for future research on targeted therapies. According to the most recent studies, abatacept was a class of drugs investigating and testing on CD80. Abatacept (CTLA4- Ig) is an immunoglobulin that inhibits CD80 receptors directly and indirectly. In 2015, a small study was performed with one MCD, one FSGS, and 3 recurrent FSGS patients after kidney transplantation; researchers found that proteinuria had reduced significantly and urinary CD80 was non-quantifiable in patients with MCD after abatacept treatment, while in the rest of patients, proteinuria remained significantly high, and urinary CD80 concentrations were still detectable. In 2019, a study of MCD with frequent relapses showed a prolonged remission after the use of abatacept [19]. The trials above revealed positive results for the efficacy of abatacept therapy in NS, further supporting the association of CD80 with MCD.

Our study has some limitations: (1) the sample size of FSGS was relatively small. (2) The urinary CD80 measurement might not be optimal, because it might be influenced by the drug dosage. However, performing kidney biopsy for children with pre-treatment NS is not indicated. Therefore, the measurement of urinary CD80 at the time of kidney biopsy is worth it. It is explained that when a nephrologist wants to perform a kidney biopsy to determine the kidney histologic lesion, measuring urinary CD80 will help determine the MCD lesion and avoid an invasive procedure. This is also necessary for the health center where the kidney biopsy can not be performed.

CONCLUSION

The urinary CD80/creatinine ratio in the NS with MCD was 25.6 (5.4 – 38.3) $\text{pg}/\mu\text{mol}$, which was significantly higher than that in the NS with FSGS. The urinary CD80/creatinine ratio helps differentiate MCD from FSGS.

Informed Consent: Written informed consent was obtained from the caregivers of the participants.

Declaration of Interests: The authors declare that they have no conflict of interest with respect to the author or publication of this article.

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Table 1. Histopathological findings of the two groups MCD and FSGS.

Histopathological findings	Frequency (n)	Percentage (%)
Minimal change disease	41	61.2
Focal segmental glomerulosclerosis	26	38.8
Total	67	100

Table 2. Clinical and laboratory characteristics of MCD and FSGS

Characteristics		Minimal change disease (n=41) n (%)	Focal segmental glomerulosclerosis (n=26) n (%)	p
Age group (years)	1-6	14 (34.1)	6 (23.1)	0.5
	7-10	13 (31.8)	8 (30.8)	
	11-15	14 (34.1)	12 (46.1)	
	Mean	8.7 ±3.8	9.7± 3.9	
Gender	Male	31 (75.6)	20 (76.9)	0.9
	Female	10 (24.4)	6 (23.1)	
Hematuria	Yes	1 (2.4)	3 (11.5)	0.3
	No	40 (97.6)	23 (88.5)	
Hypertension	Yes	7 (17.1)	5 (19.2)	0.8
	No	34 (82.9)	21 (80.8)	
Low glomerular filtration rate (GFR<90ml/p/1.73m ²)	Yes	6 (14.6)	11 (42.3)	0.01
	No	35 (85.4)	15 (55.7)	
Urinary protein-creatinine ratio (mg/mmol)	Median (25 th -75 th)	645.5 (293.2-1314.0)	636.5 (332.8-1040.1)	0.9
Serum albumin (g/l)	Median (25 th -75 th)	20.1 (17.4-28.9)	24.0 (16.4-32.2)	0.8

Glomerular filtration rate (ml/m/1.73m ²)	Mean±SD	143.3±56.7	113.4± 57.0	0.04
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Table 3. Urinary CD80 concentrations in the study groups.

Concentration	Nephrotic syndrome group (n=67)		Control group (n=67)		p
	Median	25th-75th	Median	25th-75th	
Urinary CD80/creatinine ratio (pg/μmol)	7.9	3.7-30.5	1.8	0.2-3.1	<0.001

Table 4. Urinary CD80/creatinine ratio in NS according to histopathological forms.

Urinary CD80/creatinine ratio (pg/μmol)	Median	25th-75th	p
Minimal change disease (n=41)	25.6	5.4-38.3	<0.001
Focal segmental glomerulosclerosis (n=26)	3.6	1.9-6.5	

Table 5. Area under the ROC curve in the diagnosis of MCD

AUC	95%CI	p	Cut-off value	Sensitivity	Specificity
0.87	0.79-0.95	<0.05	4.6 pg/μmol	85.4%	73.1%

Table 6. The research on urinary CD80

Year	Author	Case	Age	Group	Urinary CD80/creatinine ratio	Conclusion	Chemical brands
2010	Garin [5]	37	6.1±1.2	MCD (n=15)	524±86 (ng/g)	Urinary CD80 could be useful in distinguishin	Bender MedSystems,
			35.8±3.8	FSGS (n=22)	57±9 (ng/g)		

						g MCD from FSGS	Burlingame, CA, USA
2015	Ling [7]	165	5.9±3.9	MCD (n=37)	689.66±378.21 (ng/g)	Urinary CD80 was available in diagnosing MCD with a cut-off point of 328,98 ng/g with a sensitivity of 81.1% and a specificity of 94,4%	Bender MedSystems, Burlingame, CA, USA
			6.8±3.8	FSGS (n=27)	123.49±167.88 (ng/g)		
			7.8±3.4	Others (n=30)	152.37±220.14 (ng/g)		
			6.1±3.8	Control (n=71)	81.83± 23.01 (ng/g)		
2018	Ahmed [17]	36	8.4 ± 3.2	MCD (n=21)	3.54 ± 2.10 (ng/mg)	The threshold value of urinary CD80/creatinine ratio at 1.5ng/mg had a sensitivity of 100% and a specificity of 89% for determining the diagnosis of MCD compared to	Lifespan BioSciences, USA
			6.6 ± 3.4	FSGS (n=9)	1.18 ± 0.51 (ng/mg)		
			5.5 ± 4.2	Other kidney disease (n=6)	1.26 ± 0.69 (ng/mg)		
			6.3 ± 2.6	Control (n=40)	0.75 ± 0.20 (ng/mg)		

						other glomerular lesions such as FSGS.	
2020	Gonzalez [18]	210	17 (7, 49)	² MCD (n= 53)	139 (79, 221) (ng/g)	CD80 was highest in the MCD group and helped distinguish MCD from other causes of NS such as Lupus, FSGS, IgA...	Fisher Scientific, Pittsburg, USA
			36 (17, 52)	² FSGS (n= 43)	66 (29, 98) (ng/g)		
			67 (59, 75)	Diabetic nephropathy (n =106)	63 (37, 124) (ng/g)		
			44 (39, 50)	IgA (n=28)	19 (7, 52) (ng/g)		
			59 (51, 68)	MN (n = 54)	69 (45, 96) (ng/g)		
			35 (23, 48)	Lupus nephritis (n =31)	90 (19, 395) (ng/g)		
			55 (48, 66)	Polycystic kidney disease (n = 9)	31 (14, 44) (ng/g)		
			72 (55, 80)	Pyuria (n = 19)	42 (33, 90) (ng/g)		

			55 (41, 72)	Control (n = 34)	36 (22, 57) (ng/g)		
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Figure legend:

Figure 1. Area under the ROC curve in the diagnosis of MCD