Renal function in patients with AQP4 antibody-positive neuromyelitis optica spectrum disorder

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Abstract

Background: Increasing evidence has demonstrated that AQP4-IgG can cause damage to kidney in AQP4 antibody-positive neuromyelitis optica spectrum disorder (NMOSD) patients. However, renal impairment in AQP4 antibody-positive NMOSD patients have not been conducted thus far. This study aimed to test and evaluate the changes of estimated glomerular filtration rates (eGFR) in NMOSD patients.

Methods: We performed a cross-sectional study that recruited 93 NMOSD patients, 56 multiple sclerosis (MS) patients and 100 age- and sex-matched healthy controls (HCs), at the Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China during November 2012 and November 2017. eGFR, including $eGFR_{Cr}$, $eGFR_{CysC}$ and $eGFR_{Cr\text{-}CysC}$ were conducted for all study individuals.

Results: NMOSD patients has mildly, but statistically lower $eGFR_{CysC}$ value at both the acute and stable phases than MS patients and HCs, respectively. (NMOSD *vs.* MS: adjusted model: p=0.034 at the acute phase, p<0.001 at the stable phase; NMOSD *vs.* HCs: adjusted model: p=0.001 at the acute phase, p<0.001 at the stable phase). Meanwhile, there is no significant difference of $eGFR_{CysC}$ between MS patients and HCs at both the acute and stable phases. In addition, for NMOSD patients with low-dose glucocorticoid therapy, $eGFR_{CysC}$<90ml/min/1.73 m^2 was more likely to occur at the stable phase, compared to the acute phase (OR =4.68, 95% CI 1.19 to 18.34).

Conclusions: Mildly impaired renal function might occur in patients with AQP4 antibody-positive NMOSD compared to MS.

Background

Neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory disease of central nervous system (CNS) associated with a highly specific serum biomarker and pathogenic autoantibody, aquaporin-4 immunoglobulin G (AQP4-IgG)[1].Recently, increasing evidences have showed that AQP4-IgG could cause damage to the organ outside of CNS[2], such as stomach, airway, muscle, the organ of corti and kidney, which all of them were found the expression of AQP4[3]. However, it is unknown that whether NMOSD patients would have changes of renal function or not.

AQP-4, as known as the membrane bound water channel protein, plays a role in water reabsorption from renal tubular fluid. In 1993, Fushimi K et.al first discovered that the AQP-4 were expressed at the basolateral membrane of collecting duct principal cells in the kidney[4]. After that, reduced urine concentration capacity was found in AQP-4 knockout mice in 1998[5], which this phenomenon was also noticed in NMOSD patients recently[6]. In addition, two case reports have demonstrated that AQP4-IgG could cause damage to kidney[7, 8]. However, it is still unknown about the changes of glomerular filtration rate (GFR) in NMOSD patients.

GFR is universally regarded as the best overall index of renal function. And the equations that estimates GFR were widely recommended to diagnose and evaluate chronic kidney disease[9]. As one of the equations of the estimated GFR (eGFR), the Cockcroft-Gault (CG) formula depends on the patient's age, sex, body weight and serum creatinine (Cr). It can result in the underestimation of renal function, especially in female and underweight person, which might be more obvious in Asian than in non-Asian[10]. Meanwhile, the Modification of Diet in Renal Disease (MDRD) formula is more appropriate for patients with chronic kidney disease, since its main limitation is underestimation of measured GFR at higher levels[11]. However, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) is recommended for general clinical use by the National Kidney Foundation and it could provide more accurate eGFR, especially at higher levels of eGFR[12]. The CKD-EPI Cr equation, which is based on serum Cr levels, is well accepted as the eGFR in clinical study. However, Cr-based eGFR is affected by patient's age, gender, race, weight, height and body surface area, and particularly by muscle mass and dietary intake [13]. In contrast, serum cystatin C (CysC) is not affected by gender and muscle mass, which provides a more accurate measure of renal function [14] and shows a higher correlation with standard measures of GFR [15]. In addition, several reports also demonstrated that eGFR based on the combination of both standardized serum Cr and CysC was more accurate than eGFR based on either marker alone [16], but was still influenced by serum creatinine. Therefore, we chose CKD-EPI formula to estimate GFR from serum cystatin C.

To the best of our knowledge, comparison of eGFR between NOMSD and multiple sclerosis (MS) patients has not been reported yet. The objection of this study is to evaluate eGFR between AQP4 antibody-positive NMOSD and MS patients based on eGFR, estimated using Cr, CysC, and both.

Methods

Study design and samples

This cross-sectional study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University. All study participants gave written informed consent for research and publication.

Figure 1 illustrates the flowchart for the flowchart for the 93 recruited AQP4 antibody-positive NMOSD Chinese Han patients at the acute phase, 56 MS Chinese Han patients at the acute phase without steroids therapy before acute attack from the Department of Neurology and Multiple Sclerosis Research Center, the Third Affiliated Hospital of Sun Yat-sen University during November 2012 and November 2017. Diagnosis criteria for NMOSD and MS patients are based on the 2015 international consensus diagnostic criteria[1] and the McDonald criteria 2010[17], respectively. Experienced neurologists identified patients with NMOSD/MS at the acute phase(Ap-NMOSD/Ap-acute) according to following criteria: worsening of existing neurologic symptoms with an objective change on neurologic examination that lasted for more than 24 hours or a new onset of neurologic symptoms, symptoms and signs attributed to NMOSD/MS instead of other causes, and onset preceded by at least 30 days of clinical stability[18]. Patients with NMOSD/MS at the stable phase(Sp-NMOSD/Sp-MS) meet the following criteria: oral administration of stable-dose immunosuppressive drugs for relapse prevention (Sp-NMOSD patients: the stable-dose of immunosuppressant drug and low dose of prednisone treatment, ranging from 5 to 10 mg/d; Sp-MS patients: the stable-dose of immunosuppressant drugs).

All patients were scored using the Expanded Disability Status Scale (EDSS) [15] and tested for AQP4 antibody using cell-based assay. Among these 93 NMOSD patients at the acute phase, 46 of them had never received glucocorticoid or other disease-modifying immunosuppressive therapy (Ap-NMOSD-nonG) at least three months prior to the admission, and the other 47 patients still had low-dose prednisone treatment (5–10 mg/d) and stable dose of immunosuppressant drugs for relapse prevention (Ap-NMOSD-G). Data at the stable phase were collected from 59 NMOSD with low-dose glucocorticoid therapy and 39 MS patients without steroids therapy. In addition, we also recruited 100 age- and sex-matched healthy Chinese Han controls (HCs) from the Department of Medical Examination Center. None of the study subjects had urinary tract infections, stone of kidney or urinary tract, obviously abnormal thyroid function or smoking history.

Assessment of eGFR

Serum Cr and CysC levels were measured in all NMOSD and MS patients before pulse steroid therapy on the next day after admission. The estimated glomerular filtration rate (eGFR), including $eGFR_{Cr}$, $eGFR_{CysC}$ and $eGFR_{Cr-CysC}$, were calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula[12].

Statistical analysis

Statistical analyses were performed using SPSS 23. Chi-square and Fisher's exact test were used for categorical variables. For continuous variables, Mann–Whitney U test or student T test was used for comparisons among two groups. For comparisons among three groups, Kruskal-Wallis test or one-way Analysis of Variance (ANOVA) was used and then adjusted by Bonferroni. Comparisons using adjusted models were based on linear regression models. Results were considered significant if $p < 0.05$.

Results

Baseline Characteristics

Table 1 summarizes the baseline patient characteristics. According to the core clinical characteristics during the acute phase, 50 (53.76%) NMOSD patients had acute myelitis, 30(32.26%) patients had optic neuritis, and 13 (13.98%) patients had other core clinical characteristics (acute brainstem syndrome or other intracranial lesions). NMOSD patients have longer disease durations than MS patients ($p = 0.020$). The EDSS scores of NMOSD patients were significantly higher than MS patients ($p < 0.001$).

Table 1
Demographic characteristics of patients with NMOSD and MS at the acute phase and HCs

Variables	NMOSD	MS	HCs	*P*
Patient number(n)	93	56	100	-
Sex, female(%)	85(91.49%)	46(82.14%)	83(83.00%)	0.159[e]
Current age, yrs[a]	37.00 (28.00,50.00)	33.00 (24.00, 39.00)	38.00 (32.00, 43.00)	0.003**[f]
Disease duration, median (IQR), y[b]	2.00 (0.69, 4.37)	3.00(0.77,7.75)	-	0.020*[g]
EDSS scores (IQR)	3.5(2.5,5.0)	3.0(2.5,3.5)	-	0.000***[g]
Serum-selected autoantibodies, n(%)[c]				
ANA	17(33.33%)	-	-	
SSA	5(9.80%)	-	-	
SSB	3(5.88%)	-	-	
Core clinical characteristics in the acute phase, n (%)	-	-	-	
Acute myelitis	50(53.76%)	-	-	
Optic neuritis	30(32.26%)	-	-	
Other[d]	13(13.98%)	-	-	

Note: NMOSD, neuromyelitisoptica spectrum disorder; MS, multiple sclerosis; HCs, healthy controls; ANA, antinuclear antibody; EDSS, expanded disability status scale; SSA, Sjogren syndrome A; SSB, Sjogren syndrome B; IQR: interquartile range

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

[a] Age when blood samples were collected.

[b] Disease duration refers to the time between the onset of the first attack and blood collection.

[c] Selected autoantibodies were tested on 51 patients, which included 17 patients with ANA, 5 patients with SSA, and 3 patients with SSB.

[d] Refers to the core clinical characteristics except for acute myelitis and optic neuritis.

[e] Fisher's exact test

[f] Kruskal-Wallis test

[g] Manne Whitney U test

Analysis of the eGFR levels

Comparisons among NMOSD, MS patients at the acute phase and HCs

Serum CysC, serum Cr, $eGFR_{Cr}$, $eGFR_{CysC}$, and $eGFR_{Cr-CysC}$ levels were compared between all of the NMOSD and MS patients at the acute phase, and to HCs. The results are presented in Table 2 and Fig. 2A

Table 2
The comparisons of eGFR levels among HCs, all patients with NMOSD and MS at the acute phase

Variables		Ap-NMOSD[a] (n = 93)	Ap-MS[b] (n = 56)	HCs (n = 100)	*P**	P^1	P^2
CysC(mg/L)[e]	Unadjusted[c]	0.73(0.64,0.81)	0.71(0.64,0.76)	0.73(0.68,0.76)	0.604	-	-
(IQR)	Adjusted[d]	0.73(0.69,0.77)	0.70(0.68,0.72)	0.72(0.70,0.74)	0.023*	0.019*	1
Scr(mmol/L)[f]	Unadjusted[c]	54.00(49.00,61.00)	57.00 (49.00,65.50)	57.00 (50.28,62.85)	0.053*	-	-
(IQR)	Adjusted[d]	53.80(52.22,55.21)	55.64(53.03,57.90)	54.84(54.57,55.53)	0.001**	0.000***	0.001*
$eGFR_{Cr}$(mL/min/1.73 m^2)	Unadjusted[c]	115.20(104.36,126.59)	117.97(110.45,125.60)	115.11(109.45,120.63)	0.490	-	-
(IQR)	Adjusted[d]	116.48(105.21,124.65)	117.54(112.41,123.45)	115.01(110.41,118.08)	0.074	-	-
$eGFR_{CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	111.49(99.64,123.28)	119.50(109.88,125.35)	115.59(108.24,120.23)	0.057	-	-
(IQR)	Adjusted[d]	111.89(102.54,120.26)	118.40(112.46,123.69)	113.99(110.21,120.01)	0.003**	0.002**	0.238
$eGFR_{Cr-cysC}$(mL/min/1.73 m^2)	Unadjusted[c]	115.21± 15.81	120.38 ± 11.51	115.36 ± 7.95	0.180	-	
(mean ± SD)	Adjusted[d]	115.21 ± 12.80	120.38 ± 7.82	115.36 ± 5.77	0.017*	0.023*	1

Note: NMOSD, neuromyelitisoptica spectrum disorder; Ap-NMOSD: NMOSD patients at acute phase; MS, multiple sclerosis; Ap-MS: multiple sclerosis patients oral administration of glucocorticoid for relapse prevention at acute phase; HCs, healthy controls; CysC, Cystatin C; Scr, serum creatinine; $eGFR_{Cr}$, creatinine b estimated glomerular filtration rate; $eGFR_{CysC}$, cystatin C based estimated glomerular filtration rate; $eGFR_{Cr-CysC}$, creatinine and cystatin C based estimated gl filtration rate; IQR, interquartile range;SD, standard deviation; IQR: interquartile range

***P< 0.001, **P< 0.01, *P< 0.05

a and b: The blood samples were collected from patients before pulse steroid therapy on the next day after admission.

[c]: The unadjusted model was calculated using Kruskal-Wallis test/ one-way ANOVA, adjusted by Bonferroni.

[d]: The adjusted model was calculated using a multivariate linear regression model, adjusted by age, sex, diseases duration and EDSS.

[e]: Serum CysC concentrations were tested in 68 healthy controls, 80 acute-phase NMOSD patients and 44 acute-phase MS patients.

[f]: Serum creatinine concentrations were tested in all patients.

*P**= refers to the comparison among NMOSD, MS and HCs

P^1 = refers to the comparison between NMOSD and MS

P^2 = refers to the comparison between NMOSD and HCs

P^3 = refers to the comparison between MS and HCs

In adjusted models, the $eGFR_{CysC}$ level of all NMOSD patients were statistically lower than MS patients at the acute phase (adjusted p = 0.002). There was no difference of $eGFR_{CysC}$ level between NMOSD patients and HCs. The $eGFR_{Cr-CysC}$ levels of NMOSD patients were also statistically lower than MS patients in the adjusted models (adjusted p = 0.023). Additionally, statistically lower of CysC level was observed in all Ap-NMOSD patients than Ap-MS patients (adjusted p = 0.019).

To eliminate the effect of the usage of low-dose corticosteroids, we chose 46 Ap-NMOSD-nonG patients and found that in both the unadjusted and adjusted models, the $eGFR_{CysC}$ level of Ap-NMOSD-nonG patients was statistically lower than Ap-MS patients (unadjusted p = 0.010, and adjusted p = 0.034, Table 3 and Fig. 2B). Compared to HCs, the $eGFR_{CysC}$ level of Ap-NMOSD-nonG patients was also statistically lower than HCs in the adjusted models (adjusted p = 0.001). In addition, $eGFR_{Cr-CysC}$ level was statistically lower in Ap-NMOSD-nonG patients than Ap-MS patients in the adjusted models (adjusted p = 0.011).

Table 3
The comparisons of eGFR levels among HCs, patients with NMOSD-nonG and MS at the acute phase

Variables		Ap-NMOSD-nonG[a] (n = 46)	Ap-MS[b] (n = 56)	HCs (n = 100)	*P**	P^1	P^2
CysC(mg/L)[e]	Unadjusted[c]	0.76(0.68,0.83)	0.71(0.64,0.76)	0.73(0.68,0.76)	0.139	-	-
(IQR)	Adjusted[d]	0.75(0.71,0.79)	0.71(0.68,0.72)	0.72(0.70,0.74)	0.000***	0.000***	0.01
Scr(mmol/L)[f]	Unadjusted[c]	53.75(50.00,61.50)	57.00(49.00,65.50)	57.00 (50.27,62.85)	0.211	-	-
(IQR)	Adjusted[d]	54.56(52.63,56.92)	55.64(53.03,57.90)	54.84(54.57,55.53)	0.145	-	-
$eGFR_{Cr}$(mL/min/1.73 m^2)	Unadjusted[c]	113.35 ± 15.84	117.16 ± 10.96	114.94 ± 7.95	0.211	-	-
(mean ± SD)	Adjusteddd[d]	113.35 ± 12.86	118.01 ± 7.41	114.94 ± 5.83	0.024*	0.024	0.85
$eGFR_{CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	109.75(97.00,118.91)	119.50(109.88,125.35)	115.59(108.24,120.23)	0.013*	0.010*	0.12
(IQR)	Adjusted[d]	110.65 (98.37,117.32)	118.40(112.45,123.69)	113.99(110.21,120.08)	0.000***	0.034*	0.00
$eGFR_{Cr-CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	116.15(101.53,125.54)	120.59(113.40,129.11)	115.52(109.48,120.27)	0.099	-	-
(IQR)	Adjusted[d]	114.85(101.53,123.79)	120.20(1116.12,127.01)	115.55(111.21,118.03)	0.002**	0.011*	1

Note: NMOSD, neuromyelitisoptica spectrum disorder; Ap-NMOSD-nonG: NMOSD patients at acute phase had never received steroids or other disease-modify immunosuppressive therapy at least three months prior to the admission; MS, multiple sclerosis patients without oral administration of glucocorticoid for rela prevention; HCs, healthy controls; CysC, Cystatin C; Scr, serum creatinine; $eGFR_{Cr}$, creatinine based estimated glomerular filtration rate; $eGFR_{CysC}$, cystatin C ba estimated glomerular filtration rate; $eGFR_{Cr-CysC}$, creatinine and cystatin C based estimated glomerular filtration rate; SD, standard deviation; IQR: interquartile

***P< 0.001, **P< 0.01, *P< 0.05

a and b: The blood samples were collected from patients before pulse steroid therapy on the next day after admission.

[c]: The unadjusted model was calculated using Kruskal-Wallis test/one-way ANOVA, adjusted by Bonferroni.

[d]: The adjusted model was calculated using a multivariate linear regression model, adjusted by age, sex, disease duration and EDSS.

[e]: Serum CysC concentrations were tested in 68 healthy controls, 40 acute-phase NMOSD patients and 44 acute-phase MS patients without low-dose glucoco therapy.

[f]: Serum creatinine concentrations were tested in all patients.

*P**=according to Kruskal-Wallis test

P^1 = refers to the comparison between NMOSD and MS

P^2 = refers to the comparison between NMOSD and HCs

P^3 = refers to the comparison between MS and HCs

Comparisons between Ap-NMOSD-G patients and Ap-NMOSD-nonG patients were conducted to investigate the changes of eGFR levels treated by low-dose glucocorticoid. No significant differences were observed in the eGFR levels, CysC and Cr levels between the two study cohorts in the unadjusted model (Table S1 and Fig. 2E). However, higher $eGFR_{CysC}$ levels and lower serum CysC levels were observed in the patients with glucocorticoids therapy in the adjusted model (p = 0.048 $eGFR_{Cys}$, p = 0.002 CysC).

Comparisons among NMOSD, MS patients at the stable phase and HCs

In both the unadjusted and adjusted models, the $eGFR_{CysC}$ level of Sp-NMOSD patients was statistically lower than Sp-MS patients (unadjusted p = 0.013, and adjusted p < 0.001). Compared to HCs, the $eGFR_{CysC}$ level of Sp-NMOSD patients was statistically lower in both the unadjusted and adjusted models (unadjusted p = 0.001, and adjusted p < 0.001).

In addition, the $eGFR_{Cr-CysC}$ level of Sp-NMOSD patients were statistically lower than Sp-MS patients in both the unadjusted and adjusted models (unadjusted p = 0.042, and adjusted p < 0.001, Table 4 and Fig. 2C).

Table 4
The comparisons of eGFR levels among HCs, patients with NMOSD and MS at stable phase

Variables		Sp-NMOSD[a] (n = 59)	Sp-MS[b] (n = 39)	HCs (n = 100)	*P**	P^1	P^2
CysC(mg/L)[e](IQR)	Unadjusted[c]	0.80(0.78,0.85)	0.77(0.69,0.83)	0.73(0.68,0.76)	0.001**	1	0.00
	Adjusted[d]	0.82(0.79,0.86)	0.89(0.65,1.03)	0.72(0.70,0.74)	0.000***	0.899	0.00
Scr(mmol/L)[f](IQR)	Unadjusted[d]	56.00(48.90,60.80)	61.00(53.00,73.00)	57.00 (50.27,62.85)	0.027*	0.022*	0.49
	Adjusted[d]	55.26(54.43, 56.98)	55.88(54.62, 78.43)	54.85(54.56, 55.53)	0.035*	0.212	1
$eGFR_{Cr}$(mL/min/1.73 m^2)	Unadjusted[c]	115.06(105.09,124.72)	117.78(107.71,124.04)	115.11(109.45,120.63)	0.883	-	-
(IQR)	Adjusted[d]	113.76(109.18,109.17)	116.54(110.87, 124.22)	115.01(110.41,118.08)	0.111	-	-
$eGFR_{CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	104.41(88.76,116.95)	116.94(105.67,121.26)	115.59(108.24,120.23)	0.001**	0.013*	0.00
(IQR)	Adjusted[d]	101.14(94.20,105.29)	111.50(103.46, 120.47)	113.98(110.21,120.01)	0.000***	0.000***	0.00
$eGFR_{Cr-CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	111.02(96.86,122.25)	118.67(112.74,125.43)	115.52(109.48,120.27)	0.049*	0.042*	0.52
(IQR)	Adjusted[d]	109.65(96.86,122.25)	117.04(111.34, 126.29)	115.55(111.22, 118.03)	0.000***	0.000***	0.00

Note: NMOSD, neuromyelitisoptica spectrum disorder; Sp-NMOSD, NMOSD patients at stable phase who had the oral administration of low-dose glucocortico dose regimens of immunosuppressant drugs for relapse prevention; MS, multiple sclerosis; Sp-MS: MS patients at stable phase who had the oral administrat dose regimens of immunosuppressant drugs for relapse prevention; HC, healthy control; CysC, Cystatin C; Scr, serum creatinine; $eGFR_{Cr}$, creatinine based esti glomerular filtration rate; $eGFR_{CysC}$, cystatin C based estimated glomerular filtration rate; $eGFR_{Cr-CysC}$, creatinine and cystatin C based estimated glomerular fi SD, standard deviation; IQR: interquartile range

***P< 0.001, **P< 0.01, *P< 0.05

[a] and [b]: The blood samples were collected from patients before pulse steroid therapy on the next day after admission.

[c]: The unadjusted model was calculated using Kruskal-Wallis test, adjusted by Bonferroni.

[d]: The adjusted model was calculated using a multivariate linear regression model, adjusted by age, sex, diseases duration and EDSS.

[e]: Serum CysC concentrations were tested in 68 healthy controls, 40 stable-phase NMOSD patients and 33 stable-phase MS patients.

[f]: Serum creatinine concentrations were tested in all patients.

*P**=refers to the comparison among three groups.

P^1 = refers to the comparison between NMOSD and MS

P^2 = refers to the comparison between NMOSD and HCs

P^3 = refers to the comparison between MS and HCs

Comparisons between acute- and stable-phase patients with NMOSD.

In both the unadjusted and adjusted models, the $eGFR_{CysC}$ level of Ap-NMOSD-G patients was significantly higher than Sp-NMOSD patients (unadjusted p = 0.007, and adjusted p < 0.001, Table 5 and Fig. 2D). In addition, the $eGFR_{Cr-CysC}$ level of Ap-NMOSD-G patients was also statistically higher than Sp-NMOSD patients (unadjusted p = 0.034, and adjusted p < 0.001). Moreover, the mean serum CysC level of Ap-NMOSD-G patients was also significantly lower than the serum CysC level of Sp-NMOSD in both the unadjusted and adjusted model (unadjusted p < 0.001, and adjusted p < 0.001).

Table 5
The comparison of eGFR levels between acute-phase NMOSD-G patients and stable-phase NMOSD patients.

Variables		Ap-NMOSD-G[a] (n = 47)	Sp-NMOSD[b] (n = 59)	*P*
CysC(mg/L)[e]	Unadjusted[c]	0.71 ± 0.11	0.82 ± 0.16	0.000***
(mean ± SD)	Adjusted[d]	0.71± 0.05	0.83 ± 0.07	0.000***
Scr(mmol/L)[f]	Unadjusted[c]	53.92 ± 9.58	55.73 ± 9.68	0.338
(mean ± SD)	Adjusted[d]	53.92 ± 3.50	56.11± 3.08	0.001**
$eGFR_{Cr}$(mL/min/1.73 m^2)	Unadjusted[c]	118.18(105.99,127.78)	115.06(105.09,124.72)	0.470
(IQR)	Adjusted[d]	118.24(107.50,125.25)	113.76(109.18,118.86)	0.079
$eGFR_{CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	114.19 (106.07,125.41)	101.41(88.75,116.94)	0.007**
(IQR)	Adjusted[d]	114.19(104.79,122.46)	101.14(94.20,105.29)	0.000***
$eGFR_{Cr-CysC}$(mL/min/1.73 m^2)	Unadjusted[c]	117.42 ± 16.11	109.25± 17.71	0.034*
(mean ± SD)	Adjusted[d]	117.42 ± 11.79	108.20 ± 6.78	0.000***

Note: NMOSD, neuromyelitisoptica spectrum disorder; Ap-NMOSD-G: NMOSD patients at acute phase received oral administration of low-dose glucocorticoid drugs and stable-dose immunosuppressant drugs for relapse prevention; Sp-NMOSD: NMOSD patients at stable phase received oral administration of low-dose glucocorticoid drugs and stable-dose immunosuppressant drugs for relapse prevention; HC, healthy control; CysC, Cystatin C; Scr, serum creatinine; $eGFR_{Cr}$, creatinine based estimated glomerular filtration rate; $eGFR_{CysC}$, cystatin C based estimated glomerular filtration rate; $eGFR_{Cr-CysC}$, creatinine and cystatin C based estimated glomerular filtration rate; SD, standard deviation; IQR: interquartile range

***P< 0.001, **P< 0.01, *P< 0.05

[a] and [b]: The blood samples were collected from patients before pulse steroid therapy on the next day after admission.

[c]: The unadjusted model was calculated using Mann-Whitney U test/ student T test.

[d]: The adjusted model was calculated using a multivariate linear regression model, adjusted by age, sex, disease duration and EDSS.

[e]: Serum CysC concentrations were tested in 40 acute-phase NMOSD patients with corticosteroids and 32 stable-phase NMOSD patients.

[f]: Serum creatinine concentrations were tested in all patients.

In addition, among the NMOSD patients with low-dose glucocorticoid therapy, 27.5% (11/40) of Sp-NMOSD patients had $eGFR_{CysC}$<90 ml/min/1.73 m^2, compared to 7.5% (3/40) Ap-NMOSD-G patients (OR = 4.678, p = 0.019).

Correlation analysis about eGFR levels among acute-phase NMOSD patients

To determine the association between the clinical characteristics and the glomerular filtration function, unadjusted univariate and adjusted multivariate linear regression models were performed for Ap-NMOSD-nonG patients. Age was observed to be a statistically significant independent predictor of higher serum CysC concentration (p = 0.003), lower $eGFR_{Cr}$ level (p < 0.001), lower $eGFR_{CysC}$ level (p < 0.001), and lower $eGFR_{Cr-CysC}$ level (p < 0.001) in the adjusted analysis among Ap-NMOSD-nonG patients. An older age predicted higher serum CysC concentration (coef. =0.003; 95% CI: 0.001 to 0.006). Lower $eGFR_{Cr}$ (coef. =-0.815; 95% CI: -1.084 to -0.546), $eGFR_{CysC}$ (coef. =-0.793; 95% CI: -1.018 to -0.568) and $eGFR_{Cr-CysC}$ (coef. =-0.951; 95% CI: -1.138 to -0.764) levels were predicted by an older age. Another independent predictor of higher serum Cr concentration that remained statistically significant was sex (p = 0.033), where male patients were predicted to have higher serum Cr (coef. =-8.281; 95% CI: -16.754 to -1.024). However, disease duration, ARR, EDSS scores and serum-selected auto-antibodies (ANA, SSA, SSB) all failed to predict changes in the serum CysC, serum Cr and eGFR levels at the significance level of 0.05.

Discussion

To the best of our knowledge, this study is the first to compare eGFR between AQP4 antibody-positive NOMSD and MS patients. In this study, we found a slight reduction of eGFR (especially $eGFR_{CysC}$) in NMOSD patients, compared to MS patients and HCs, although the average eGFR were within the normal range in all groups.

eGFR is a universal marker of renal function[9], and decreased GFR represents the presence of glomerular small vessel disease [19]. The estimation of renal function using serum CysC levels has clinical advantages as it is not influenced by the blood concentrations, muscle mass, gender, or age [20, 21]. Therefore, to a certain degree, $eGFR_{CysC}$ may be superior to $eGFR_{Cr}$ for evaluating renal function in NMOSD. However, studies have showed that glucocorticoid administration was associated in a dose-dependent fashion with increased CysC values, leading to a dose-dependent underestimation of GFR calculations

based on CysC[22]. Thus, steroid-free status may improve the accuracy of the CysC-based equations. In order to reduce potential bias of results caused by glucocorticoids treatment, we analyzed the serum CysC data from NMOSD patients who had never received steroids or other disease-modifying immunosuppressive therapy at least three months prior to the admission.

The AQP-4 is expressed in principal cells of collecting duct of kidney, where about 15% of tubular fluid was reabsorbed through AQP-2, AQP-3 and AQP-4. Both AQP-4 and AQP-3 are located in the basolateral membrane of principal and responsible for the transportation of water, which is reabsorbed by AQP-2 from tubular fluid into intercellular fluid[23]. If either AQP-4 or AQP-3 was dysfunction, the other one might compensate to keep osmotic pressure in tubular fluid in some degree. And these theories are confirmed by Chou CL et.al, who has reported the mild defect in urinary concentrating ability in AQP-4 knockout mice[5]. Furthermore, it was also discovered that NMOSD patients show a urinary concentrating defect[6]. Thus, the deposition of AQP4-IgG and the loss of AQP4 may potentially lead to damage in kidney. In our study, we found that the mildly lower eGFR was found in NMOSD, but still in the normal range. This phenomenon may be cause by the fact that AQP-4 might play a major role in concentration and dilution of renal tubules, but not in glomerular filtration. Besides, there are so many factors affecting GFR in complex ways, which needs further development.

In a recent study, higher CysC concentration was observed in renal transplant recipients on low-dose prednisone treatment (5–10 mg/d) compared to those on steroid-free immunosuppression [24]. Interestingly, in our study, we did not find higher CysC concentrations in acute-phase NMOSD patients with low-dose glucocorticoid therapy before acute attack, compared to acute-phase NMOSD patients without glucocorticoid therapy before acute attack. The greater increase of CysC in stable-phase NMOSD with glucocorticoid therapy may be explained by a result of the decrease in the inflammatory process. Besides, 27.56% of stable-phase NMOSD patients with low-dose glucocorticoid therapy had $eGFR_{CysC}$< 90 ml/min/1.73 m^2, which implies a substantial loss of glomeruli and impaired renal reserve. In addition, no changes of renal function occurred in MS patients in this study, which was contrary to a previously reported result[25] on progressive type MS. The difference in renal function may be due to the different subtypes of MS. The enrolled MS patients in this study were relapsing-remitting MS, while the previous study was chronic (both primary and secondary) progressive MS patients.

Several limitations need to be addressed in our study. In the present study, we detected differences in eGFR, but results of all groups are in the normal range. However, these still indicated that reduced renal function has occurred in patients with AQP4 antibody-positive NMOSD compared to MS patients and HCs, suggesting that more attention should be paid to protect the renal function throughout the disease stage, especially when applying nephrotoxic drugs. Additionally, the number of patients was relatively small and blood samples were just obtained once. However, to the best of our knowledge, this is the first study to investigate renal function in NMOSD patients. Our hospital is the major research unit focused on this condition in China. More cases will be collected and blood samples will be obtained at different time points for the future investigation. Furthermore, additional measurement of proteinuria and BMI may improve the sensitivity and specificity of our assessment of renal function, which can be investigated in the future.

Conclusion

In conclusion, our results indicate that reduction of eGFR (especially $eGFR_{CysC}$) occurs in patients with AQP4 antibody-positive NMOSD compared to MS patients and HCs. The findings suggest that we may need to pay attention to the protection of renal function and to use nephrotoxic drugs while treating NMOSD.

Abbreviations

NMOSD, neuromyelitis optica spectrum disorder; MS, multiple sclerosis; eGFR, estimated glomerular filtration rate; CNS, central nervous system; AQP4-IgG, aquaporin-4 immunoglobulin G; Ap-NMOSD, NMOSD patients at the acute phase; Ap-Ap-MS, MS patients at the stable phase; Sp-NMOSD, NMOSD patients at the stable phase; Sp-MS, MS patients at the stable phase; CG formula, the Cockcroft-Gault formula; MDRD, Modification of Diet in Renal Disease formula; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CysC, Cystatin C; Cr: creatinine; EDSS, Expanded Disability Status Scale; HCs, healthy Chinese Han controls; ANOVA, one-way Analysis of Variance

Declarations

Authorship contributions Y. J, HI. F., and Y. C. contributed to the conception and design of this study. R. W., R. F., Y. J. and Z. C. collected and organized the data. Y. J., Y. C., J. H., R. W. and R. F. analyzed the data. Y. J., R. W., Y. C., J. H., R. F., R. L., and HI. F. drafted the manuscript. All the authors read and approved the final manuscript.

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Consent for publication: Not applicable.

Competing interests: The authors declare that they have no conflict of interest

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Figures

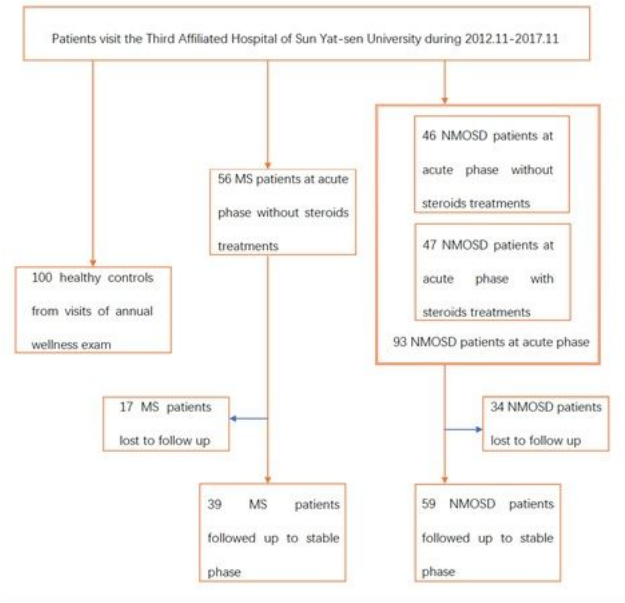

Figure 1

Flowchart for NMOSD and MS cohorts.

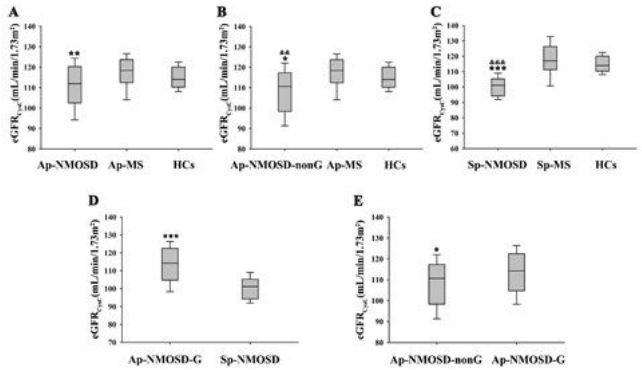

Figure 2

Comparisons of adjusted eGFRCysc levels among NMOSD patients, MS patients and HCs.