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Comparison of clinical features between aquaporin-4 antibody seropositive and seronegative patients in neuromyelitis optica and neuromyelitis optica spectrum disorder

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Abstract

Background & Objective: To evaluate the prevalence of aquaporin-4 antibody (AQP-4 Ab) seropositive patients in neuromyelitis optica (NMO) and neuromyelitis optica spectrum disorder (NMOSD) and to compare the clinical, investigation features and treatment outcome between the AQP-4 Ab seronegative and seropositive groups. *Methods:* All NMO and NMOSD patients in Maharat Nakhon Ratchasima hospital during January 2012 to December 2016 were recruited. Demographic, clinical, laboratory and imaging data were collected from the medical records. All data were analysed and compared between seropositive and seronegative groups as appropriates. *Results:* There were 12 (29%) and 30 (71%) NMO and NMOSD patients, respectively. There were 30 (71%) patients who hadAQP-4 Ab seropositive status. Thirty-three (78.6%) patients were female. In seropositive group, there were significantly higher proportion of female patients (87% versus 58%, p=0.04), immunosuppressant treatment (33% versus 0%, p=0.04), patients who had serum albumin less than 4 g/dL (46% versus 0%, p=0.02), cerebrospinal fluid (CSF) pleocytosis (71% versus 17%, p=0.01) and patients with extensive spinal cord involvement (67% versus 25%, p<0.05) than seronegative group. CSF-serum glucose ratio was significantly lower in seropositive group than seronegative group (0.5 ± 0.03 versus 0.7 ± 0.04, p=0.01). *Conclusion:* The prevalence of AQP-4 Ab seropositive patients in NMO and NMOSD was 71%. There were significantly more female patients, immunosuppressant treatment, patients with serum albumin less than 4 g/dL, CSF pleocytosis, low CSF-serum glucose ratio and extensive transverse myelitis than seronegative group.

Keywords: Aquaporin-4 antibody; serologic status; neuromyelitis optica; neuromyelitis optica spectrum disorder; clinical features

INTRODUCTION

Neuromyelitis optica (NMO) and neuromyelitis optica spectrum disorder (NMOSD) are rare neurological conditions which result in permanent neurological deficits causing disability and reduced quality of life. In Thailand, these conditions are more common than multiple sclerosis.[1-3] According to the diagnostic criteria for NMO and NMOSD (revised in 2006 and 2015), the aquaporin-4 antibody (AQP-4 Ab) is an important clinical supplement for diagnosis.[4,5] Some studies showed correlation between serological titer with clinical features, severity, co-existing autoimmunity, disease activity, investigation findings, stabilization and prognosis[2,3,6-12] whereas others did not.[8,13,14] The primary objective of this study was to compare the clinical and investigation features between the aquaporin-4 antibody seronegative and seropositive NMO and NMOSD patients. And the secondary objective to evaluate their AQP-4 Ab serologic status prevalence and treatment outcome.

METHODS

All patients with diagnosis of NMO[4] and NMOSD[5] in Medicine department, Maharat Nakhon Ratchasima hospital during January 2012 to December 2016 were recruited. Their data, including of age, sex, medication, frequency of attacks prior to diagnosis, duration of signs & symptoms, comorbidities, clinical characteristics, Expanded Disability Status Scale (EDSS) (at baseline, post-treatment and follow-up), blood tests, AQP-4 Ab results, sera and cerebrospinal

fluid (CSF), were analysed at Prasat Neurological Institute, Bangkok, Thailand. CSF analyses and imaging results were collected from medical records and imaging reports.

Statistical analysis

The data were analysed using Stata/MP 14, then presented as mean and standard deviation for continuous variables with normal distribution (according to Skewness and Kurtosis test) or median and ranges for the other. The Receiver Operating Characteristic (ROC) analysis was performed to optimize the cutoff point of continuous data. The categorical data were shown as frequency and percentage. Student *t*-test or Mann-Whitney U test (according to data distribution) was used for comparison of continuous variables between AQP-4 Ab seropositive and seronegative groups. Proportions were compared using 2-tailed Chi-square or Fischer-exact test as appropriates. The level of statistical significance was defined when *p*-value was less than 0.05.

RESULTS

From January 2012 to December 2016, there were 12 (29%) and 30 (71%) NMO and NMOSD patients, respectively, 33 (79%) of them were female. Their mean age was 41.0 ± 13.0 years. The number of patients with coexisting disease was 11 (26.3%). There were 3 who had systemic lupus erythematosus (SLE), 1 had old ischemic cerebrovascular disease (CVD) who had fully recovered and the other coexisting diseases were type-2 diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, central retinal artery occlusion and supraventricular tachycardia. The median duration of symptom was 14 days (range 1-1095 days). Most common symptoms were motor weakness (26 or 61.9%) and sensory impairment (21 or 50%). There were 23 (54.8%) myelitis, 11 (26.2%) brainstem encephalitis and 10 (23.8%) optic neuritis patients. The number of patients who had bilateral optic neuritis at onset was 4 (9.5%) and simultaneous myelitis and optic neuritis 3 (7.1%). There were 10 (23.8%) patients treated with steroid and 4 (9.5%) with azathioprine. Physical examination revealed mean muscle power of 3.1 ±1.9 according to Medical Research Council scale. The median of baseline, post-treatment and follow-up of EDSS 7 (range 1-9), 6 (range1-10) and 5 (range 0-10), respectively. The mean follow-up duration was 19 + 8.7 days.

As Table 2, the laboratory investigations showed that 30 (71%) patients were AQP-4 Ab seropositive and mean serum albumin of 4.1 ± 0.4 g/dL. Lumbar puncture was performed in 23 (55%) patients. Two CSF samples of these were tested for AQP-4 Ab and 1 of them was positive. CSF analyses showed that the median white blood cell count and protein level were 7 cells (0-153 cells) and 47 g/dL (10-528 g/dL), respectively, and mean of CSF-serum glucose ratio of 0.6 ± 0.1. From imaging reports, there were 39 patients who had undergone MRI with contrast including 26 (61.9%) brain MRI, 27 (64.3%) spinal cord MRI and 8 (19.1%) orbit MRI. The mean myelitis length was 7.9± 4.3 vertebral bodies.

In the seropositive group, there were significantly higher number of female patients (87% versus 58%, p=0.04), immunosuppressant treatment (33% versus 0%, p=0.04), patients who had serum albumin less than 4 g/dL (46% versus 0%, p=0.02), CSF pleocytosis (71% versus 17%, p=0.01) and patients with extensive spinal cord involvement (67% versus 25%, p<0.05) than seronegative group, as shown in Table 3.CSF-serum glucose ratio was significantly lower in seropositive groupthan seronegative group (0.5 ± 0.03 versus 0.7 ± 0.04, p=0.01). Age, frequency of attacks, presentation syndromes, other laboratory results, other imaging characteristics, follow-up duration and outcome of treatment had no statistically significant difference.

DISCUSSION

The prevalence of AQP-4 Ab seropositive status of NMO and NMOSD patients was 71%. This was compatible with some previous studies (68.6-78.3%).[7,9,10,13] This study revealed that there were significantly more female in AQP-4 Ab seropositive group than negative group, which was same as prior studies.[7,9]

The number of immunosuppressant treatment was significantly higher in AQP-4 Ab seropositive group. This might be correlated with frequency of relapse of immune-related inflammatory demyelinating disease other than NMO and NMOSD such as their comorbid autoimmune diseases (SLE, Rheumatoid arthritis). As previous study, coexisting autoimmune disorder (such as SLE, Sjögren's syndrome, etc.) were more common among seropositive patients.[6,7,15,16] There were some reports that NMO was the first manifestation of SLE and there were AQP-4 Ab existing in the sera for years before the first NMO attack in patients with SLE.[16-18] In the

Table 1: Baseline clinical characteristics of neuromyelitis optica and neuromyelitis optica spectrum disorder patients

Baseline characteristics	N (%) or Mean ± SD	Median (range)
Female	33 (79)	
Age (years)	41.0 ± 13.0	
Coexisting diseases		
SLE	3 (7.1)	
Old ischemic CVD	1 (2.4)	
Others	11 (26.2)	
Symptom duration of the last attack (days)	51.9 ± 174.0	14 (1-1095)
Diagnosis of neuromyelitis optica	12 (29)	
Frequency of attacks prior to diagnosis	1.2 ± 1.2	1(0-5)
Symptoms		
Motor weakness	26 (61.9)	
Sensory impairment	21 (50)	
Visual disturbance	9 (21.4)	
Cranial neuropathy	5 (11.9)	
Consciousness impairment	4 (9.5)	
Syndromes		
Myelitis	23 (54.8)	
Brainstem encephalitis	11 (26.2)	
Optic neuritis	10 (23.8)	
Bilateral optic neuritis	4 (9.5)	
Simultaneous myelitis and optic neuritis	3 (7.1)	
Immunosuppressant treatment		
Steroid	10 (23.8)	
Steroid and Azathioprine	4 (9.5)	
MRC scale of motor power of the last attack	3.1 ±1.9	
Baseline EDSS of the last attack	5.5 ±2.8	7 (1-9)
Posttreatment EDSS	5.3±2.8	6 (1-10)
Follow-up EDSS	4.6 ± 2.8	5 (0-10)
Follow-up duration	19± 8.7	

SD, Standard deviation; SLE, Systemic lupus erythematosus; CVD, Cerebrovascular disease; MRC, Medical Research Council; EDSS, Expanded Disability Status Scale

other hand, SLE could develop overlapping NMO later.[19-21] This study had too small number of SLE patients to show their significant association.

In the seropositive group, we found that there were more patients with serum albumin level less than 4 g/dL which derived from the Receiver Operating Characteristic (ROC) analysis. The reference range of serum albumin level in our laboratory was 3.5-5.5 g/dL. The serum albumin level was in the low normal range probably as a result of acute illness or inflammatory disease.

CSF pleocytosis (CSF white cells >5/mm^3) was found statistically significantly more often in seropositive group as prior study.[9,22,23] In NMO and NMOSD patients, most of the patients had normal CSF glucose level.[24-26] In contrast, this study showed lower CSF-serum glucose ratio (0.5 ± 0.03) in the seropositive group. These findings might be related to severe inflammation of central nervous system[22] causing impairment of active glucose transport across the blood brain barrier and higher metabolic demands of neurons and glial cells.[22,26]

As recent studies[7,10,12], there were more patients who had extensive transverse myelitis in the seropositive group. It was defined that the length of spinal cord involvement were more than 6 vertebral bodies. This optimized cutoff point was compatible with recent study.[7] The explanation might be that the more severe the inflammation, the longer was the spinal cord involvement.

Table 2: Baseline investigation characteristics of 42 neuromyelitis optica and neuromyelitis optica spectrum disorder patients

Baseline characteristics	Mean ± SD or N (%)	Median (range)
Aquaporin-4 antibody seropositive patients	30 (71)	
Positive CSF Aquaporin-4 antibody	1 (50)	
Serum albumin (g/dL)	4.1 ± 0.4	
Serum albumin < 4 g/dL	12 (34.3)	
CSF white cells	17.6 ±32.7	7 (0-153)
CSF pleocytosis(CSF white cells >5 cells/mm^3)	13 (56.5)	
CSF protein	85.0 ± 112.2	47 (10-528)
CSF glucose	67.3 ± 16.6	63 (38-116)
CSF-serum glucose ratio	0.6 ± 0.1	
Abnormal brain imaging		
Brain CT (N=8)	3 (37.5)	
Brain MRI (N=26)	24 (92.3)	
Orbit MRI (N=8)	6 (75.0)	
Spinal cordMRI (N=27)	27 (100)	
Length of myelitis (vertebral bodies)	7.9± 4.3	
Extensive spinal cord involvement (> 6 vertebral bodies)	14 (53.9)	
MRI with contrast	39 (95.1)	

SD, Standard deviation; CSF, Cerebrospinal fluid; CT, Computerized tomography; MRI, Magnetic resonance imaging

Table 3: Comparison of clinical and investigation characteristics according to AQP-4 serologic status

Clinical and investigation features	Seropositive(N=30)	Seronegative(N=12)	*p*-value
Female	26 (86.7)	7 (58.3)	0.04
Age (years)	40.4± 2.5	42.6± 3.2	0.32
Duration of symptom (days)	14 (1-1095)	22 (1-365)	0.42
Diagnosis of NMO	11 (36.7)	1 (8.3)	0.07
Frequency of attacks prior to diagnosis	1 (0-5)	1 (0-2)	0.69
Optic neuritis	6 (20)	4 (33.3)	0.36
Myelitis	18 (60)	7 (58.3)	0.92
Immunosuppressant treatment	10 (33.3)	0	0.04
Motor symptom	20 (66.7)	6 (50.0)	0.31
MRC scale of motor power	3.0 ±0.4	3.6 ±0.5	0.18
Serum albumin < 4 g/dL	12 (46.2)	0	0.02
CSF pleocytosis (CSF white cells >5/mm^3; N=13)	12/17 (70.6)	1/6 (16.7)	0.01
CSF-serum glucose ratio	0.5 ± 0.03	0.7 ± 0.04	0.01
Abnormal brainMRI (N=24)	17/18 (94.4)	7/8 (87.5)	0.54
Length of spinal cord involvement (vertebral bodies)	8.9 + 1.0	5.6+1.2	0.07
Extensive spinal cord involvement (> 6 vertebral bodies; N=14)	12/18 (66.7)	2/8 (25.0)	< 0.05
MRI with Gadolinium enhancement (N=26)	18/29 (62.1)	8/10 (80.0)	0.30
Baseline EDSS of the last attack	7 (1-9)	4.5 (1-8)	0.35
Posttreatment EDSS improvement	8 (26.7)	3 (25.0)	0.91
Follow-up EDSS improvement	11 (39.3)	4 (33.3)	0.72
Follow-up duration	18.0 ± 1.7	22.8 ± 2.1	0.09

Analyses by using student *t*-test or Mann-Whitney U test and Chi-square or Fischer-exact test
NMO, Neuromyelitis optica; MRC, Medical Research Council; CSF, Cerebrospinal fluid; MRI, Magnetic resonance imaging; EDSS, Expanded Disability Status Scale

According to the difference between seropositive and seronegative patients from our study, we suspected that the AQP-4 Ab could be used as an inflammatory marker to evaluate disease severity and prognosis. Large sample size of NMO and NMOSD patients in further studies may be helpful.

The limitations of this study included limited resources that not all patients were investigated with both MRI and AQP-4 Ab, which might lead to an underestimation of NMO and NMOSD prevalence. Neurological imagings were performed according to patients' neurological deficit, which might introduced missing data on the extent of MRI lesions, perhaps including asymptomatic lesions.

There was no statistically significant difference of treatment outcome at post-treatment and short-term follow-up. The long-term outcome might be necessary for further studies.

DISCLOSURE

Conflicts of interests: None

REFERENCES

1. Apiwattanakul M, Kasemsuk C. NMO spectrum disorders comprise the major portion of CNS inflammatory diseases in Thai patients: A cross sectional study. *Mult Scler Relat Disord* 2014; 3:61-6.
2. Siritho S, Nakashima I, Takahashi T, Fujihara K, Prayoonwiwat N. AQP4 antibody-positive Thai cases: clinical features and diagnostic problems. *Neurology* 2011;77:827-34.
3. Apiwattanakul M, Asawavichienjinda T, Pulkes T, *et al*. Diagnostic utility of NMO/AQP4-IgG in evaluating CNS inflammatory disease in Thai patients. *J Neurol Sci* 2012;320:118-20.
4. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; 66:1485-9.
5. Wingerchuk DM, Banwell B, Bennett JL, *et al*. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015; 85:177-89.
6. Estiasari R, Matsushita T, Masaki K, *et al*. Comparison of clinical, immunological and neuroimaging features between anti-aquaporin-4 antibody-positive and antibody-negative Sjogren's syndrome patients with central nervous system manifestations. *Mult Scler* 2012; 18:807-16.
7. Jarius S, Ruprecht K, Wildemann B, *et al*. Contrasting disease patterns in seropositive and seronegative neuromyelitis optica: A multicentre study of 175 patients. *J Neuroinflammation* 2012; 9:14.
8. Siritho S, Apiwattanakul M, Nakashima I, Takahashi T, Fujihara K, Prayoonwiwat N. Features of anti-aquaporin 4 antibody-seronegative Thai patients with neuromyelitis optica spectrum disorders: a comparison with seropositive cases. *J Neurol Sci* 2014; 341:17-21.
9. Sasitorn S, Metha A, Naraporn P. The use of AQP4-antibody testing in diagnosis Thai patients with neuromyelitis optica. *Neurol Asia* 2014; 19:11.
10. Viswanathan S, Arip M, Mustafa N, *et al*. The frequency of anti-aquaporin-4 Ig g antibody in neuromyelitis optica and its spectrum disorders at a single tertiary referral center in malaysia. *Mult Scler Int* 2014;2014:568254.
11. Jarius S, Paul F, Franciotta D, *et al*. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. *Nat Clin Pract Neurol* 2008; 4:202-14.
12. Takahashi T, Fujihara K, Nakashima I, *et al*. Anti-aquaporin-4 antibody is involved in the pathogenesis of NMO: a study on antibody titre. *Brain* 2007; 130:1235-43.
13. Long Y, Lu Z, Hu X. Serum-positive and -negative AQP4 antibody NMO in Chinese patients. *Can J Neurol Sci* 2012; 39:232-5.
14. Melamed E, Levy M, Waters PJ, *et al*. Update on biomarkers in neuromyelitis optica. *Neurol Neuroimmunol Neuroinflamm* 2015; 2:e134.
15. Carvalho DC, Tironi TS, Freitas DS, Kleinpaul R, Talim NC, Lana-Peixoto MA. Sjögren syndrome and neuromyelitis optica spectrum disorder co-exist in a common autoimmune milieu. *Arq Neuropsiquiatr* 2014; 72:619-24.
16. Freitas E, Guimarães J. Neuromyelitis optica spectrum disorders associated with other autoimmune diseases. *Rheumatol Int* 2015; 35:243-53.
17. Karim S, Majithia V. Devic's syndrome as initial presentation of systemic lupus erythematosus. *Am J Med Sci* 2009; 338:245-7.
18. Jacobi C, Stingele K, Kretz R, *et al*. Neuromyelitis optica (Devic's syndrome) as first manifestation of systemic lupus erythematosus. *Lupus* 2006; 15:107-9.
19. Asgari N, Jarius S, Laustrup H, *et al*. Aquaporin-4-autoimmunity in patients with systemic lupus erythematosus: A predominantly population-based study. *Mult Scler* 2017: 1352458517699791.
20. Polgár A, Rózsa C, Müller V, Matolcsi J, Poór G, Kiss EV. Devic's syndrome and SLE: challenges in diagnosis and therapeutic possibilities based on two overlapping cases. *Autoimmun Rev* 2011; 10:171-4.
21. Mehta LR, Samuelsson MK, Kleiner AK, *et al*. Neuromyelitis optica spectrum disorder in a patient with systemic lupus erythematosus and anti-phospholipid antibody syndrome. *Mult Scler* 2008; 14:425-7.
22. Misu T, Takahashi T, Nakashima I, Fujihara K. Biomarkers in neuromyelitis optica. *Brain Nerve* 2012; 64:525-35.
23. Sato DK, Callegaro D, de Haidar Jorge FM, *et al*. Cerebrospinal fluid aquaporin-4 antibody levels in neuromyelitis optica attacks. *Ann Neurol* 2014; 76:305-9.
24. Piccolo G, Franciotta DM, Camana C, *et al*. Devic's neuromyelitis optica: long-term follow-up and serial CSF findings in two cases. *J Neurol* 1990; 237:262-4.
25. Fardet L, Généreau T, Mikaeloff Y, Fontaine B, Seilhean D, Cabane J. Devic's neuromyelitis optica: study of nine cases. *Acta Neurol Scand* 2003; 108:193-200.
26. Cortese I, Calabresi PA. Inflammatory and demyelinating disorders. In: Irani DN, ed. Cerebrospinal fluid in clinical practice. Philadelphia: Saunders; 2009. 217-18.