Seroprevalence of NMO-IgG Antibody in Neuromyelitis optica (NMO) and Its Specificity in Differentiating NMO from Other Demyelinating Diseases with Overlap Symptoms: An Iranian Experience

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ABSTRACT

Neuromyelitis optica is an inflammatory demyelinating disease (IDD) of the CNS, which mainly affects optic nerve and spinal cord. Autoantibodies against aquaporin-4 also known as NMO-IgG have been implicated in the pathogenesis of NMO. We evaluated the sensitivity and specificity of NMO-IgG assay for diagnosing NMO patients and differentiating them from MS patients and those with undifferentiated IDD with overlap symptoms.

Eligibility of patients with demyelinating disorders was evaluated based on physical examination, laboratory and imaging studies. Thirty four definite NMO patients (disregarding NMO-IgG status), 34 multiple sclerosis (MS) patients with a history of optic neuritis (ON) or myelitis that were matched for age and disease activity and 44 patients with ON or myelitis attacks fulfilling neither criteria of MS or NMO (NMO spectrum) were selected as undifferentiated group. NMO-IgG was measured in the serum of the included patients by cell-based indirect immunofluorescence assay (IFA).

NMO antibody was positive in 11 (32.3%), and 4 (9.09%) patients in NMO and undifferentiated groups, but was undetctable in MS patients. NMO antibody was 32% (95%Cl: 19-49%) sensitive in detecting NMO patients. Its specificity in differentiating NMO from MS subjects was 100 %(95% Cl: 90-!00%). NMO antibody was 95% (95% Cl: 0.88-0.98) specific in differentiating NMOs from other demyelinating diseases.

Our results showed that although NMO antibody is highly specific for NMO, current method of measuring it with cell-based IFA is not highly sensitive for diagnosing NMO patients.

Keywords: Demyelinating disorders; Devic disease; Multiple sclerosis; Neuromyelitis optica

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INTRODUCTION

Neuromyelitis optica (NMO) or Devic's disease is an immune mediated demyelinating and necrotizing disease of central nervous system. It typically manifests as recurrent episodes of optic nerves or spinal cord involvement leading to irreversible damage and permanent disability.[1,2] Despite its tendency to involve primarily optic nerves and spinal cord, symptomatic or asymptomatic brain lesions are frequently seen in confirmed NMO patients.[3-6]

The spectrum of inflammatory demyelinating diseases (IDD) of the central nervous system (CNS) could be defined based on clinical course, severity and chronicity of the disease.[7,8] NMO may account for 1 % of CNS IDDs among Caucasians. Considerable overlap between these diseases can lead to diagnostic uncertainty in some cases. Distinction between these entities would be possible on the basis of clinical history, para-clinical investigations including imaging and laboratory tests as well as exclusion of other etiologies.[9] Some electrophysiological tools such as evoked potentials, sympathetic skin responses and some biochemical studies have shown to be useful in diagnosis and determining the severity and chronicity of demyelinating disorders.[10-11]

Although the nature of NMO has not been elucidated yet, evidence suggests B cells and humoral immune system involvement in the pathogenesis of the disease. In this regard, NMO-IgG autoantibody is believed to target aquaporin-4 water channels leading to cascades of inflammatory responses and tissue destruction.[12] Other than in CNS, Aquaporin-4 is also expressed in kidney and stomach. However, NMO autoantibody specifically affects astrocyte foot processes in CNS while the kidney and stomach are spared.[13] Discovery of this highly specific antibody significantly improved our understanding of NMO pathophysiology and made it as a separate entity from multiple sclerosis (MS) as a prototype of IDDs. NMO-IgG positivity has been considered as one of the supporting criteria in the revised diagnostic criteria for adult [14] and pediatrics NMO.[15] However, NMO-IgG might be positive in several other CNS autoimmune disorders that do not fulfill the criteria of NMO, such as longitudinally extensive transverse myelitis, recurrent isolated optic neuritis (ON) and bilateral ON. These disorders are suggested to be grouped under the NMO spectrum disorders entity[2]. Several studies assessed the

sensitivity and specificity of NMO-IgG in the diagnosis of NMO in Europe,[16,17] Americas [18] and south East Asia.[19] Despite some variability in sensitivity and specificity, these studies showed promising role for NMO-IgG antibody in the diagnosis of NMO. As a result, the recent diagnostic criteria for NMO considers NMO-IgG antibody as one of the non-absolute criteria.[14,20]

Several methods for identifying NMO antibodies have been proposed. The most used methods are indirect immunofluorescence (IF), cell based assays, radioimmunoprecipitation assays and fluoroimmunoprecipitation assays. The gold standard test has not been elucidated yet.[21]

In this study, we examined the applicability of the NMO-IgG test with cell based indirect immunofluorescence method in Iran and determined the sensitivity and specificity of this test for differentiating NMO from other IDD with overlap symptoms.

PATIENTS AND METHODS

Patients

Patients were consecutively recruited in the study from December 2009 to June 2011. All patients who were referred to the MS and demyelinating diseases clinic in our center and had Magnetic resonance imaging(MRI) at the onset of their symptoms were screened. Following thorough physical examination and assessment of expanded disability status scale score, complete blood tests were requested to assess the likelihood of other diagnoses as the source of symptoms and/or coexistence of other autoimmune diseases. These included, but not limited to, basic laboratory tests, B12 serum level, Anti-neutrophil cytoplasmic, antinuclear, anti-dsDNA, and anticardiolipin (IgG and IgM) antibodies, as well as serum human T-lymphotropic virus (HTLV-1 and 2) antibodies. Enrolled patients were divided into three groups. The NMO group consisted of patients with clinical diagnosis based on the revised diagnostic criteria for NMO disregarding the NMO-IgG status. The MS group included patients with clinically definite MS based on 2010 revised McDonald criteria.[22] In order to have a clinically relevant comparison, only MS patients with a prior history of ON or myelitis were included. Each MS patient was selected to match an NMO patient in gender and current disease activity (see

below). The third group included patients with demyelinating myelitis and/or ON, hence not fulfilling criteria of NMO or MS, and not matched to other groups. Patient's disease was considered active if an attack of either ON or myelitis had occurred within 30 days from sampling day. Age of symptom onset and age at enrollment, gender, activity of the disease, NMO antibody positivity, type and number of attacks, plus information needed to assess patients based on Wingerchuk et al criteria [14] were recorded. Cerebrospinal fluid oligoclonal band result was available for a subset of patients (seven NMO patients and 15 in ON/Myelitis group) who had underwent lumbar puncture prior to enrollment.

Written informed consent was obtained from all patients. Ethics board of Tehran University of Medical Sciences approved the protocol and ethics of the current study.

Antibody Detection

NMO-IgG (aka anti-AQP4) antibody was detected by cell-based indirect IF method using a commercially available Anti-AQP4 kit (Euroimmun AG, Lübeck, Germany). In brief, EDTA blood was collected and plasma was separated within 6 hours. Plasma was then stored in two separate aliquots up to two weeks at 4°C and diluted with phosphate buffered saline (1:9) for the test. The plasma was incubated with recombinant AQP4 expressed on HEK293 cells on slide. Then, it was further incubated in dark with fluorescein-labeled anti-human antibody conjugate using provided slide. The slide was washed and mounted. Results were read by 40X magnification with an IF microscope. Fluorescent membranous staining of some of HEK293 cells was considered positive. In each slide, positive

and negative controls were included.

Statistical Analysis

Mann-Whitney non-parametric comparison was used to compare continuous variables. Fisher exact test was used for dichotomous variables. Two sided analysis applied to all tests and the level of significance was considered as $p<0.05$. SPSS version 16 (Chicago, IL) was used for all analyses including descriptive analysis, group comparisons and deriving sensitivity, specificity and their confidence intervals. Sensitivity and specificity were calculated with following formula TP/TP+FN and TN/TN+FP, respectively (TP=true positive and TP= True negative, FN=false negative and FP= false negative).

RESULTS

A total of 34 patients with NMO, 34 with MS and 44 with NMO spectrum (undifferentiated group) were identified. The mean ages of NMO patients, MS patients and undifferentiated group were 36.21±10.04, 27.62±7.65, and 31.14 ±0.85 years, respectively. Females/male ratios in NMO and MS group were 25/8 and was 31/13 in undifferentiated group. There was no difference between the age of male and female NMO patients at the onset of disease ($p>0.05$). NMO patients had mean recurrent attack rate of 1.13 per year while 12 patients had no attack recurrences. Table 1 and 2 show characteristics of enrolled patients. Baseline tests including renal function test were normal in all cases.

In 34 NMO patients, NMO antibody was positive in 11 patients (32.3%). Even though NMO antibody had higher rate of positivity in patients with active disease (9patients/ 23patients, 39.13%) compared with patients

Table 1. Baseline characteristics (part 1) of participants

Patients	N (female)	Age ‡	Age at the onset of symptoms ‡	Duration of symptoms ‡, §	Attack per year‡	Number of optic neuritis attacks ‡	Number of myelitis attacks ‡
NMO*	34 (25)	36.21(35)±10.04	30.53(29.5)+/-9.91	5.10(2.50)±6.51	1.13(0.65)±1.09	3.32 (3.0)±1.49	2.0 (2.0)±1.34
MS †	34 (25)	27.62(30)±7.65	23.54(23)±7.04	4.08(3)±3.77	1.29(1.08)±0.97	1.0 (1.0)±1.09	2.40 (1.50)±2.01
Undifferenti Ated group	44 (31)	31.14(30.5)±0.85	26.78 (25)±7.82	4.35(3) ±4.25	3.07 (0.8)±0.91	1.64 (1)±1.08	4.82(3.78)±5.21

* Neuromyelitis optica

†Multiple sclerosis

‡ Mean(median)+/-SD

§ Calculated in patients with more than one year interval from the onset of their disease

Table 2. Baseline characteristics (part 2) of participants

	EDSS score ††	Segments of spinal cord involved in MRI		Brain MRI	NMO IgG Ab (positive)	OCB ‡‡ (positive)	Optic nerve involvement
NMO*	Mild: 9 (26.5%)	> 3 seg	34 (100%)	Normal: 15 (44.1%)	11(32.3)	Positive: 2(28.5%)	Left: 3 (8.0%)
	Moderate: 20 (58.8%)			Plaque positive: (Barkhof negative)		Negative: 5 (71.5%)	Right: 4 (11.7%)
	Severe: 5 (14.7%)			19(55.9%)			Bilateral: 27 (79.3%)
MS †	Mild: 9 (26.5%)	0 seg	23(67.7%)	Barkhof positive: 34 (100%)	0 (0%)	-	None: 7 (20.5%)
	Moderate: 23 (67.5%)	< 3 seg	8 (23.5%)				Left: 7 (20.5%)
		4 seg	3 (8.8%)				Right: 17 (50.0%)
	Severe: 2 (6.0%)						Bilateral: 3 (9.0%)
Undifferen Tiated Group	Mild: 17 (38.6%)	0 seg	8(18.1%)	Normal: 14 (31.8%)	4 (9.09%)	Positive: 12 (80%)	Left: 4 (9.09%)
		1 seg	17 (38.6%)				Right: 4 (9.09%)
	Moderate: 23 (52.2%)	2 seg	2 (4.5%)			Negative: 3 (20%)	Bilateral: 6 (13.6%)
		3 seg	17(38.6%)	Plaque positive: (Barkhof negative)			
	Severe: 4 (9.09%)			30 (68.1%)			

* Neuromyelitis optica

† Multiple sclerosis

†† Expanded disability status scale

‡‡ Oligoclonal band

who had non-active disease (2 patients /11 patients, 18.18%) or recurrent attacks (7 patients /17 patients, 41.17%) compared with single attack (4 patients /17 patients, 23.5%), these differences were not statistically significant (*p* values: 0.271 and 0.465, respectively). NMO antibody was equally positive (*p*=0.718) in patients with brain MRI abnormality (5/19, 26.31%) and patients with normal MRI (5/15, 33.3%). Of these 34 NMO patients, seven underwent lumbar puncture. In all of them NMO-IgG were negative in the serum. Two of them had oligoclonal bands in their CSF.

Out of 44 patients in undifferentiated group that did not fulfill either MS or NMO criteria, 30 had pure demyelinating myelitis. Of these 30 patients, 17 had extensive longitudinal myelitis in the spinal cord MRI. Out of remaining 14 patients in this group, 13 were patients with ON and myelitis, and one patient had isolated ON. Of these 44 patients, four had positive NMO antibody (3 with only myelitis attacks [2/3 had extensive longitudinal lesions], 1 with ON and myelitis). Two patients had progressive symptoms after myelitis without any subsequent attack. Anticardiolipin antibody was positive in one case without any other evidence of antiphospholipid syndrome.

Sensitivity of NMO antibody for detecting clinically diagnosed NMO patients was 32% (95% CI: 19-49%). The specificity was 100% (95% Cl: 0.90-1.00) and 95% (95% CI: 0.87-0.98) for differentiating clinical NMO patients from MS patients and other NMO spectrum diseases, respectively.

DISCUSSION

Our study estimated that in a population of Iranian patients, NMO-IgG test sensitivity is about 32.3%. Specificity of 100% in differentiating MS from NMO patients is reassuring its usefulness in differentiating NMO from its most common and important differential diagnosis and underlines the pathophysiological differences of these two entities. NMO-IgG was also highly specific (95%) for differentiating NMO from other demyelinating diseases with overlap symptoms.

The rate of NMO-IgG seropositivity in our patients with NMO was within the range reported in other studies performed on Caucasian populations reporting a seropositivity of 30–73%.[23] However, this range is wide and different studies reach to variable results. For example a study on Caribbean demonstrated 33.3 % sensitivity of NMO antibody [24] while another study in Japan showed 94% sensitivity to detect NMO patients.[25] The variable sensitivity across studies might be explained by different ethnic groups examined, characteristics of patients (e.g. recurrence and activity of the disease), and more importantly as found by Pisani et al, as a result of not using appropriate method of measurements.[26]

Various methods, including testing CSF for NMO-IgG has been proposed to increase the sensitivity of NMO antibody assay. Klawiter et al [27] reported three cases of clinical NMO with negative serum and positive CSF NMO-IgG. They suggested that combination of CSF and serum NMO antibody testing could increase the sensitivity of the test. Subsequent study by Jarius et al[17] on 37 patients with NMO spectrum disease did not support that hypothesis.

The sensitivity of the test varies with the technique used to detect the antibody. Various studies in different centers around the world compared IF (used in this study), immunoprecipitation and cell based assay with different head to head comparison results.[28-30] A review article by waters et al did not find different sensitivity between these three methods.[31] We used IF to achieve results more comparable to the revised diagnostic criteria[14] in order to look for some ethnic and center-specific differences. The setting used in this study increases the sensitivity of the IF assay by using transfected cells expressing large amounts of AQP-4 antigen. However, it is not clear which of these methods could be considered as a "gold standard". Newer methods of detecting NMO antibody using cell-based assay has been also proposed.

In contrast to sensitivity, specificity of the test was highly similar in multiple studies and was over 90 % in most studies.[13, 25, 30, 32-33] Our results showed 100 % specificity which was comparable to those results.

In our study, 55.9% of NMO patients had non-specific plaques in their brain at the onset of the disease and three (15.7%) fulfilled Barkhof criteria consequently (later in their disease course, not in time of their initial manifestation), close to a similar study[3]. The frequency of oligoclonal band in NMO ranges from 0% to 37% in different studies;[21] and we observed it in 28.5% of our NMO patients. Renal function test was normal in all of the NMO patients which was consistent with previous studies[13] and further suggested that NMO antibodies were directed only against astrocyte foot processes at the blood brain barrier.

A pervious study on 96 samples from NMO-IgG positive patients suggested that NMO-IgG serum levels correlated with disease activity and recurrence.[35] Even though we did not measure the antibody quantitatively; we saw a trend of higher percentage of patients with positive antibody in active and multiple attacks subgroups. However, the difference did not reach the significance level. Our study may lack the power to detect it because of not measuring the antibody levels quantitatively.

Our results suggested that serum NMO-IgG assay is a highly specific yet not enough sensitive test, and should be used in combination with clinical and imaging data for diagnostic purposes. NMO-IgG Antibody allows earlier diagnosis of NMO and differentiating it from other central nervous system demyelinating diseases with overlap symptoms. Future works should be directed to identify the best method, single test or combination of tests, for detecting NMO antibodies and the exact factors responsible for great variation among different studies as well as determining why NMO antibody is negative in a fraction of NMO patients. It is also worth identifying the clinicopathological relevance of brain lesions in patients with NMO.

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