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Original article

Coexisting neuronal autoantibodies among children with demyelinating syndromes

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Abstract

Objectives: To determine the incidence and clinical relevance of neuronal autoantibodies in children with demyelinating syndromes.

Methods: We conducted a prospective study including 31 consecutive children with demyelinating syndromes. Four patients with N-Methyl-D-aspartate receptor (NMDAR) encephalitis, 32 patients with Guillain-Barre syndrome, 13 children with benign childhood epilepsy, and 28 healthy children were used as controls. Prior to initiating immunomodulatory therapy, serum samples were tested for antibodies against NMDAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) 1, AMPAR2, leucine-rich glioma-activated protein 1, contactin-associated protein 2, gamma-aminobutyric acid B receptors, paraneoplastic ma antigen 2 (PNMA2/Ta), Yo, Ri, Hu, CV2, amphiphysin, and aquaporin-4 by indirect immunofluorescence assays.

Results: Three anti-neuronal antibodies were detected; NMDAR antibody in one with multiple sclerosis, PNMA2/Ta antibody in one with multiple sclerosis, and Yo antibody in one with clinically isolated syndrome. The positivity rate of neuronal autoantibodies in demyelinating syndrome was 10%. All seropositive patients were found to be negative for tumor screening. None of these patients exhibited symptoms of encephalitis.

Conclusion: Children with demyelinating syndromes without symptoms of encephalitis can be positive for anti-neuronal antibodies.

Keywords: Demyelinating; Multiple sclerosis; Neuronal autoantibody; NMDAR

1. Introduction

Pediatric demyelinating syndromes are a heterogeneous group of disorders with variable clinical course, magnetic resonance imaging (MRI) findings, and response to treatment [1,2]. Multiple mechanisms in disease pathogenesis may be responsible for the different phenotypes of demyelinating syndromes [1,3]. New diagnostic and therapeutic approaches were developed by improved understanding of antibody-mediated pathogenesis. In this regard, antibodies against aquaporin-4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG) were associated with demyelinating syndromes. A positive test for AQP4 antibody has been determined as a diagnostic criterion for neuromyelitis

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optica (NMO), and antibody to MOG were correlated with specific MRI pattern and good outcomes in patients with acute disseminated encephalomyelitis (ADEM) [4,5].

Antibodies against neuronal cell-surface and intracellular antigens were primarily detected in central nervous system autoimmune disorders as autoimmune encephalitis and paraneoplastic neurological syndromes [6,7]. After then, N-Methyl-D-aspartate receptor (NMDAR) antibody was subsequently identified in patients with overlapped NMDAR-encephalitis and demyelinating syndrome [8–11]. Therefore, it has been hypothesized that neuronal antibodies may have a role in demyelination processes.

This study was planned to determine the incidence and clinical relevance of neuronal autoantibodies in demyelinating syndromes by conducting a prospective study.

2. Materials and methods

We performed a prospective case-control study between February 2012 and February 2016 in the Istanbul Faculty of Medicine, Department of Pediatric Neurology, including children with demyelinating syndromes. Demyelinating syndromes were diagnosed according to International Paediatric Multiple Sclerosis Study Group (IPMSSG) consensus definitions, and McDonald's 2010 dissemination in space and time criteria [12,13]. Patients with clinical follow-up of less than 18 months were excluded. In our study, 31 consecutive pediatric patients with demyelinating syndromes (18 girls, male-to-female ratio 1:2.1) were included. Clinically isolated syndrome (CIS) in 11 patients, multiple sclerosis (MS) in 13 patients, ADEM in 6 patients, and NMO in 1 patient were diagnosed. Four patients with NMDAR encephalitis, 32 patients with Guillain-Barre syndrome (GBS), 13 children with benign childhood epilepsy, and 28 healthy children were used as controls.

Serum samples from 108 treatment-naïve patients and controls were analyzed for the presence of IgG antibodies against NMDAR; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) 1; AMPAR2; leucine-rich glioma-activated protein 1 (LGI1); contactin-associated protein 2 (CASPR2); gamma-aminobutyric acid B receptors (GABARB1/B2); and AQP4 by commercial cell-based indirect immunofluorescence assay (Euroimmun, Luebeck, Germany). All samples were also tested for antibodies against paraneoplastic ma antigen 2 (PNMA2/Ta), Yo, Ri, Hu, CV2, and amphiphysin using 3 tissue-based indirect immunofluorescence assays including nervus suralis, cerebellum, and intestinal tissue sections from monkey (Euroimmun), and positive results were confirmed in a commercial immunoblot test (Euroimmun) in the Neuroimmunology Laboratory of Istanbul Faculty of Medicine. AQP4 antibody was not detected in any of the subjects. Patients with antibodies against intracellular antigens underwent tumor screening (complete blood count, smear of peripheral blood, erythrocyte sedimentation rate, neuron-specific enolase, human chorionic gonadotropin, alpha-fetoprotein, urine vanilmandelic acid and homovanillic acid, chest X-ray, abdominal ultrasound), and were treated based on their demyelinating phenotype. Demographic characteristics and anti-neuronal antibody status were recorded. All MRI scans had T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR) and gadolinium-enhanced T1 sequences in two different planes.

All statistical analyses were applied using SPSS Statistics for Windows version 16.0 (IBM Corp., Armonk, NY, USA). Data are presented as median, minimum, maximum, frequency, and percentage. Ethical approval was granted by Istanbul Faculty of Medicine Ethics Committees. All patients and/or their legal representatives provided a written informed consent.

3. Results

The median age at presentation was 13 years (range 1.5–17.5 years, mean 11.2 years, SD 4.66). NMDAR (n = 1/31), Yo (n = 1/31), and PNMA2/Ta (n = 1/31) antibody were determined in one each patient with demyelinating syndrome. The positivity rate of neuronal autoantibodies in demyelinating syndrome was 10%. These antibodies were detected in patients with MS and CIS. Patients with ADEM, NMO, GBS, benign childhood epilepsy and healthy controls were negative for all of the antibodies tested. NMDAR antibody was detected in all patients with NMDAR encephalitis. AMPA1, AMPA2, LGI1, CASPR2, GABAR B1, Ri, Hu, CV2.1, and amphiphysin antibodies were not detected in any patients. Demographic data of patients and controls are shown in Table 1.

NMDAR antibody was detected in a girl aged 14 years with MS. She presented with isolated hemiparesis. Brain MRI showed a large confluent lesion accompanied by solitary parietal lesion [Fig. 1]. Remission was observed after high-dose methylprednisolone therapy. After six months, she has accrued new asymptomatic lesions, fulfilling McDonald criteria [13]. She was diagnosed as having MS, and achieved complete remission after high-dose methylprednisolone treatment. She took no disease-modifying therapy and had no relapse at the 3-year follow-up. She did not develop NMDAR encephalitis-related symptoms such as amnesia, behavior disturbance or encephalopathy during follow-up.

Yo antibody was found in a girl with CIS who presented with unilateral central facial palsy at the age of 16 years. Brain MRI revealed multiple T2 lesions in

Table 1
Demographic data of 31 children with demyelinating syndrome and controls.

	CIS	MS	ADEM	NMO	GBS	NMDAR-E	HC	BCE
n	11	13	6	1	32	4	28	13
Female, n (%)	9 (82)	5 (38)	4 (67)	0 (0)	11 (34)	3 (75)	15 (53)	5 (38)
Median age, y (range)	11 (2–17)	14 (13–17)	8 (3–14)	7	10 (7–14)	5 (2–8)	12 (2–18)	10 (4–14)
Follow-up duration, y (range)	3 (1.5–4)	2 (1.5–4)	3 (1.7–3.3)	4	–	–	–	–

Abbreviations: ADEM = acute disseminated encephalomyelitis; BCE = benign childhood epilepsy; CIS = clinically isolated syndrome; GBS = Guillain Barre syndrome; HC = healthy controls; MS = multiple sclerosis; NMDAR-E = NMDA receptor encephalitis; NMO = neuromyelitis optica.

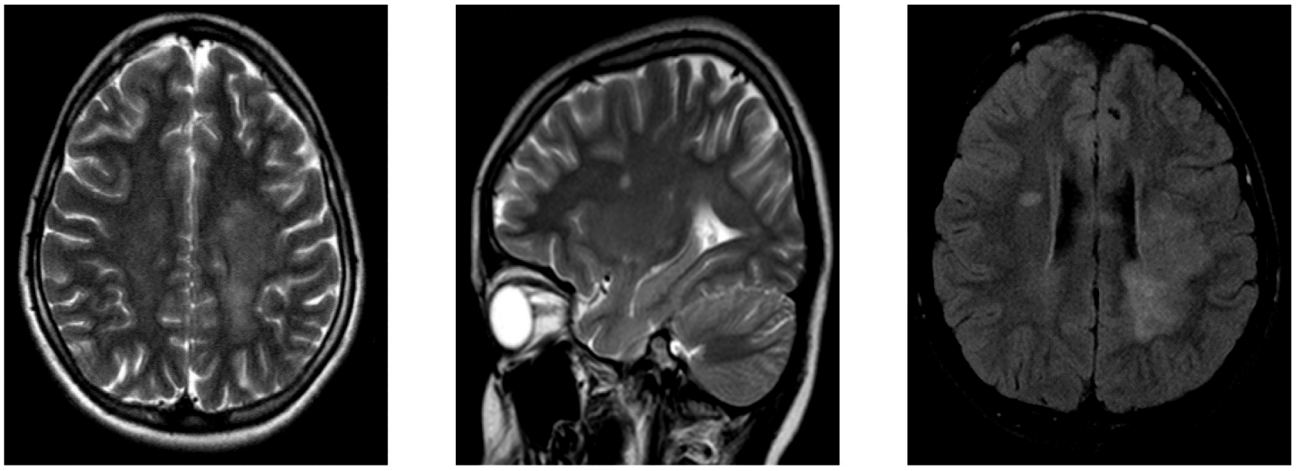

Fig. 1. Imaging of patient with MS and NMDAR antibody: At admission, T2-weighted images revealed (A) a large confluent lesion in the left parietal area, and (B) periventricular lesions. (C) Six months later, a new lesion emerged on FLAIR sequence in the right frontocentral area.

supratentorial and infratentorial location. She had complete remission after high dose methylprednisolone therapy, and has been in follow-up for 3 years without relapse.

A boy aged 10 years was positive for PNMA2/Ta antibody. His clinical feature was unilateral ptosis accompanied by multiple T2 lesions in periventricular, juxtacortical, and intracallosal location. After 23 months, he developed monoparesis and new demyelinating lesions on MRI. He was diagnosed as having MS and disease-modifying therapy (interferon beta-1a) was started. He had a relapse characterized by unilateral blurred vision after 13 months under disease-modifying therapy. Spinal MRI showed cervical T2 lesions in addition to previous lesions on brain MRI. He responded to high-dose methylprednisolone treatment on each event. He had no further relapse in 1 year under interferon beta-1a treatment. Seropositive patients with demyelinating syndrome did not have symptoms seen in encephalitis, and they remained negative for tumor screening.

4. Discussion

Herein, we present the association of childhood demyelinating syndromes with anti-neuronal antibodies. Although neither anti-MOG nor anti-glycine receptor antibody testing were performed in our study, 3 antibodies were detected in the serum of different patients with demyelinating syndrome. These antibodies were against neuronal cell-surface antigens in 1 patient with MS and intracellular antigens in 2 patients with MS and CIS; NMDAR, PNMA2/Ta, and Yo antibody, respectively. The positivity rate of anti-neuronal antibodies in demyelinating syndrome was 10%. All anti-neuronal antibodies remained negative in the control group, which allowed an evaluation of the assays used. A patient who was NMDAR antibody positive exhibited a distinct phenotype based on the demyelination feature. However, patients who were PNMA2/Ta and Yo antibody positive presented with classical symptoms and MRI findings for demyelinating syndromes. The follow-up of these patients was longer than the average follow-up intervals reported in a previous study [14].

Antibody to NMDAR was seen in a patient with MS using cell-based assay which has been recommended as a confirmatory test based on its high specificity (100%) and sensitivity (86.3%) for the detection of serum antibodies against neuronal cell-surface antigens [15,16]. A NMDAR antibody-positive patient with MS had none of the neuropsychiatric symptoms typical for

anti-NMDAR-encephalitis throughout the entire study period. Most of the previous reports indicated that anti-NMDAR-encephalitis and demyelinating syndromes can overlap [8–11]. However, serum NMDAR antibody positivity in patients with demyelinating syndrome without encephalopathy has previously been reported, in line with our finding [14].

The other two detected antibodies were against PNMA2/Ta and Yo antigens which are localized intra-neuronal, and the positivity of these antibodies was verified with a second assay as recommended [15]. A PNMA2/Ta antibody-positive patient with MS had exhibited a relapsing-remitting MS pattern, and Yo antibody-positive patient presented with CIS. Limbic encephalitis-related symptoms did not emerge during the follow-up period. We determined no neoplastic disorders in patients with PNMA2/Ta and Yo antibodies. These findings are in favor of the hypothesis that intracellular antibodies originate in released intracellular proteins during the primary destruction of neuronal cells in demyelinating syndromes [17].

This study has some limitations. Although, previous reports showed that MOG antibodies are detected among a variety of pediatric demyelinating syndromes with distinct clinical and MRI features, we could not test for MOG antibodies if the antibody positive patients with demyelinating lesion had MOG antibody too [18]. It would have been better if cerebrospinal fluid had been tested for the anti-neuronal antibodies, especially in seropositive patients. The investigation of antibodies to neuronal surface antigens only in serum is an important limitation as false positive and false negative results have been reported when only serum is analyzed [16]. However, high specificity and sensitivity of serum tests were taken as reference in this issue [15,16]. Finally, number of antibody positive patients was small which leads to problems in statistical analysis. Despite these limitations, we think that this study demonstrated that the positivity rate of anti-neuronal antibodies in demyelinating syndrome is not low (10%).

In conclusion, NMDAR, PNMA2/Ta and Yo antibodies can be detected in children with demyelinating syndromes, even in the absence of symptoms of encephalitis. Further research is required to establish their clinical relevance.

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