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Neurologic disorders associated with anti-glutamic acid decarboxylase antibodies: A comparison of anti-GAD antibody titers and time-dependent changes between neurologic disease and type I diabetes mellitus

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ABSTRACT

To determine clinical features of neurologic disorders associated with anti-glutamic acid decarboxylase antibodies (anti-GAD-Ab), we examined titers and time-dependent changes of anti-GAD-Ab. Six patients, stiff person syndrome (2), cerebellar ataxia (1), limbic encephalitis (1), epilepsy (1), brainstem encephalitis (1), were compared with 87 type I diabetes mellitus (T1DM) patients without neurologic disorders. Anti-GAD-Ab titers and index were higher in neurologic disorders than in T1DM, suggesting intrathecal antibody synthesis. Anti-GAD-Ab titers in T1DM decreased over time, whereas they remained high in neurologic disorders. Immunotherapy improved neurological disorders and anti-GAD-Ab titers and index provide clinically meaningful information about their diagnostic accuracy.

1. Introduction

Glutamic acid decarboxylase (GAD) is an intracellularly expressed enzyme of central neuronal and pancreatic islet cells that mediates the formation of γ-aminobutyric acid (GABA) from L-glutamic acid. GABA exerts paracrine functions in pancreatic islets and acts as an inhibitory neurotransmitter in the central nervous system. There are two isoforms of GAD, GAD65 and GAD67, which have molecular weights of 65 and 67 kDa, respectively. Pancreatic GAD is mainly GAD65, whereas the brain contains both isoforms. Anti-GAD antibodies have been detected in the early stage of type I diabetes mellitus (T1DM) in up to 80% of patients with the disease (Tuomilehto et al., 1994), as well as in the serum and cerebrospinal fluid (CSF) of patients with neurologic disease such as stiff person syndrome (SPS) (Solimena et al., 1988; Barker et al., 1998). Although anti-GAD antibody titers are usually low (< 100 IU/mL) in patients with T1DM, they are often high in those with SPS (Saiz et al., 2008).

Other than SPS, recent reports have demonstrated that several neurological disorders are associated with anti-GAD antibodies, with these including cerebellar ataxia (Vulliemoz et al., 2007), limbic encephalitis (Matà et al., 2008), autoimmune epilepsy (Peltola et al., 2000), and ocular movement disorder (Dubbioso et al., 2013). However, neurologic disorders with anti-GAD antibodies are rare, and the association between these autoantibodies and the various forms of neurological disease is unclear. Neurologic disorders with anti-GAD antibodies are not recognized as a paraneoplastic nervous system syndrome. However, as previous reports have shown anti-GAD antibody-positive SPS to be associated with neoplasm (Essalmi et al., 2007), it may trigger the autoimmune response in some patients. In SPS, while the efficacy of intravenous immunoglobulin (IVIG) has been established (Dalakas et al., 2001; Dalakas, 2009), there are no randomized controlled trials of IVIG for other neurologic disorders associated with anti-GAD antibodies, and it is unknown whether individual therapies are effective for those neurologic disorders. Furthermore, examination of the association between T1DM and these neurologic diseases or a comparative study of the natural history of anti-GAD antibodies would be intriguing.

The present study examined the clinical entities and features of neurologic disorders associated with anti-GAD antibodies, together with the associated responses to immunotherapy. We also compared the titers and time-dependent changes of these anti-GAD antibodies in patients with T1DM without neurologic disorders.

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2. Materials and methods

The subjects were six patients with neurological disorders associated with anti-GAD antibodies at Seikeikai Hospital and Osaka Medical College. Data were collected on the clinical findings, presence of complications (including autoimmune disease or malignancy), the effectiveness of immunotherapeutic, the cerebrospinal fluid (CSF) results, and the immunological test results (serum anti-GAD, anti-thyroid, and anti-nuclear antibodies). Anti-GAD antibodies were measured using radioimmunoassay (Cosmic Corporation Co., Ltd. Tokyo, Japan). The cut-off value of this test is 1.5 U/mL, and the lower detection limit is 0.11 U/mL.

The index for intrathecal synthesis of GAD antibody was calculated using the following formula: [CSF GAD antibody titer/serum GAD antibody titer]/[CSF albumin/serum albumin] (Saiz et al., 2008). We used the Dalakas criteria for the diagnosis of typical SPS (Alexopoulos and Dalakas, 2013). For comparison, we retrospectively analyzed patients with T1DM who were positive for anti-GAD antibodies detected by screening at Seikeikai Hospital between April 1, 2007, and October 31, 2012. Then, we compared titers and time-dependent changes in anti-GAD antibodies between patients with neurologic disorders and those with T1DM but without neurologic disorders. Differences in mean values between the two groups were examined using the Mann–Whitney *U* test. Differences within the same group between examination times were examined using a paired *t*-test. Statistical significance was set at $p < 0.05$. All analyses were performed using the software Graphpad PRISM, version 5.01 (GraphPad Software, San Diego, CA, USA). Informed consent, according to the requirements of the Declaration of Helsinki, was obtained from all participants.

3. Results

3.1. Clinical characteristics of anti-GAD antibody-positive neurologic disorders patients

We enrolled six patients with neurological disorders who were positive for anti-GAD antibodies; of these, two had SPS, one had cerebellar ataxia, one had limbic encephalitis, one had epilepsy, and one had brainstem encephalitis. The characteristics of these participants are shown in Table 1. None had any magnetic resonance abnormality of the cerebrum, brainstem, cerebellum, or spinal cord. However, all patients had elevated serum anti-GAD antibody titers, ranging from 1440 to 270,000 U/mL (normal ≤ 1). Five patients underwent CSF analyses, and had increased CSF anti-GAD antibody titers ranging from 30 to 770 U/mL. The anti-GAD antibody index in four out of five patients was > 1.0, suggesting that there was intrathecal synthesis of anti-GAD antibodies. While the serum GAD antibody titers tended to be higher for SPS compared with other neurological diseases, the GAD index of SPS tended to be lower. Two patients were positive for anti-nuclear antibodies, one of whom was also positive for anti-Sjögren's-syndrome-related antigen A. Another patient with Graves' disease had anti-thyroid receptor antibodies.

In total, four patients had clinical and laboratory evidence of autoimmune endocrinopathy or disease (two with T1DM, one with T1DM and Sjögren's syndrome, and one with Graves' disease). Interestingly, three of the six patients had associated neoplasms (one breast cancer, one thymoma, and one thyroid cancer/thymoma); each of these patients was treated successfully with surgery, and their neurological symptoms were improved by the combination of surgical treatment and immunotherapy. Finally, five of the six patients were effectively treated with immunotherapy. Two patients with SPS were successfully treated with IVIG, a patient with cerebellar ataxia and a patient with brainstem encephalitis were treated with IVIG and steroid, and a patient with limbic encephalitis was treated with steroid and azathioprine.

3.2. Illustrative cases

Three illustrative cases of neurological disorders associated with anti-GAD antibodies are described below.

3.2.1. Case 1: SPS

A 35-year-old woman experienced thirst and 5 kg weight loss over four months. Three months later, she presented with progressive painful muscle cramp of her lower limbs and walking difficulty. Neurological examination showed no muscular weakness, but she did have muscular rigidity and bilateral contractures in her lower limbs. Her fasting blood glucose was 220 mg/dL, HbA1c was 12.7% (normal < 6.2), and serum anti-GAD antibody level was 190,000 U/mL, so she was diagnosed with T1DM and started on insulin therapy. CSF examination showed normal cell number and protein level, but an

Table 1
Clinical characteristics of anti-GAD antibody-positive neurologic disorders patients.

Case	1	2	3	4	5	6
Age, sex	35, F	53, M	60, F	67, F	37, M	76, F
Disease form	SPS	Cerebellar ataxia	Brainstem encephalitis	SPS	Limbic encephalitis	Epilepsy
Clinical features	Lower legs stiffness	Ataxic gait, diplopia	Trismus, dysphagia, diplopia, Ataxic gait	Lower legs stiffness	Conscious disturbance, convulsion, memory impairment	Convulsion
Course	Acute	Subacute	Subacute	Acute	Acute	Acute
GAD Ab (U/mL)						
Serum	190,000	36,000	2800	270,000	1440	80,500
CSF	770	430	71	700	30	n.d.
GAD Ab index	1.19	2.11	7.6	0.63	2.98	
Other autoantibodies	ANA (+)		TSHR (+)		ANA (+) SS-A (+)	
CSF						
Cells (/mm^3)	1	2	1	1	56	n.d.
Protein (mg/dL)	25.6	37	29	35.1	39	n.d.
IgG index	0.67	0.54	0.82	0.63	0.77	n.d.
OCB	+	−	−	−	−	n.d.
DM	+	−	−	−	+	+
Complications	No	No	Graves' disease	No	Sjögren's syndrome	No
Neoplasm	Breast cancer	No	Thymoma	Thymoma, thyroid cancer	No	No
Immunotherapy	IVIG	IVIG, steroid	IVIG, steroid	IVIG	Steroid, azathioprine	
Pre-treatment mRS	5	5	5	4	5	1
Post-treatment mRS	1	0	2	1	2	1

SPS: stiff person syndrome, Ab antibody, n.d: not determined, ANA: anti-nuclear antibody, CSF cerebrospinal fluid, DM: diabetes mellitus, GAD: glutamic acid decarboxylase, Ig: Immunoglobulin, IVIG: intravenous immunoglobulin, OCB: oligoclonal band, SS-A: Sjögren's-syndrome-related antigen A, TSHR: TSH receptor, mRS: modified Rankin Scale.