Blepharospasm with elevated anti-acetylcholine receptor antibody titer

Blefaroespasmo com título elevado de anticorpos antirreceptores de acetilcolina

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Objective: To determine whether serum levels of anti-acetylcholine receptor antibody (anti-AChR-Abs) are related to clinical parameters of blepharospasm (BSP). Methods: Eighty-three adults with BSP, 60 outpatients with hemifacial spasm (HFS) and 58 controls were recruited. Personal history, demographic factors, response to botulinum toxin type A (BoNT-A) and other neurological conditions were recorded. Anti-AChR-Abs levels were quantified using an enzyme-linked immunosorbent assay. Results: The anti-AChR Abs levels were 0.237 ± 0.022 optical density units in the BSP group, which was significantly different from the HFS group (0.160 ± 0.064) and control group (0.126 ± 0.038). The anti-AChR Abs level was correlated with age and the duration of response to the BoNT-A injection. Conclusion: Patients with BSP had an elevated anti-AChR Abs titer, which suggests that dysimmunity plays a role in the onset of BSP. An increased anti-AChR Abs titer may be a predictor for poor response to BoNT-A in BSP.

Keywords: Blepharospasm, hemifacial spasm, botulinum toxin type A.

ABSTRACT

Blepharospasm (BSP) is a focal dystonia involving the orbicularis oculi and accessory muscles, leading to involuntary, inappropriate eyelid closure. The majority of patients with myasthenia gravis (MG), a nerve-muscle junction disease, have generalized fluctuating weakness and ocular muscle fatigued. An increase of the anti-acetylcholine receptor antibody (anti-AChR-Abs) titer is one of the specific tests for MG. Anti-AChR-Abs are highly disease-specific and detected in approximately 80–85% of patients with generalized MG and 40% of patients with ocular MG. However, elevated anti-AChR Abs have been found in patients who have both MG and another disease such as Guillain-Barré syndrome, amyotrophic lateral sclerosis and myotonic dystrophy type 2. BSP and MG are two distinct diseases. The former is distinguished from typical conditions of MG in which eyelid closure is due to weakness of the levator palpebrae muscles. However, Kurlan et al. reported five patients with coexistent Meige’s syndrome and MG. In the current study, we found that anti-AChR Abs levels were elevated in patients with BSP who did not have MG. The aim of this study was to evaluate whether the level of anti-AChR Abs is linked to the clinical parameters of BSP.

Keywords: Blepharospasm, hemifacial spasm, botulinum toxin type A.
METHODS

Study population
Eighthirty-three patients with BSP were enrolled from June 2013 to June 2016 in the Department of Neurology of the First Affiliated Hospital of Zhejiang University. BSP was diagnosed according to published criteria7. These cases were age- and sex-matched to 60 outpatients with hemifacial spasm (HFS; hospital controls) and 58 healthy people (population controls). This study was approved by the ethics committee of the hospital. Written informed consent was obtained from all participants. Clinical outcomes were evaluated in terms of latency, duration of effect, and clinical response, which was evaluated by means of a modified Jankovic Rating Scale. We assessed the patients before receiving a botulinum toxin type A (BoNT-A) injection and one month after the injections, using the Jankovic Rating Scale. We defined poor respondents (treatment failures) as those who, subjectively or from the doctor’s observation, did not achieve adequate symptom relief (primary or secondary non-responders).

Anti-AChR-Abs levels measurement by enzyme-linked immunosorbent assays (ELISA)
Plasma samples from the three groups were collected. All patient samples were tested for antibody binding to human muscle AChR using an ELISA kit (Cusabio; Barksdale, Delaware; USA), following the manufacturer’s instructions8. Samples were placed in a serum separator tube and stored overnight at 4°C to clot. The samples were centrifuged at approximately 3,000 rpm for 15 minutes. Serum was decanted and diluted 101-fold and assayed immediately. Wells were filled with 100 µL of sample. Phosphate buffered saline (pH 7.0–7.2) was used as a blank control. A microplate reader (Biomad, USA) was used to measure the optical density of each sample at 450 nm. Samples were measured in triplicate.

Statistical analysis
Data are expressed as mean ± SD. ANOVA was used to compare averages. Anti-AChR-Abs levels were correlated with other parameters using Pearson’s parametric correlation analysis. A value of p < 0.05 was considered statistically significant.

RESULTS

Demographics of the study subjects.
Sixty women (72%) and 23 men (28%) were enrolled in this study. The mean age of BSP onset was 55 ± 11 years (range, 23–79 years). The percentage of patients with BSP onset varied with age: five (6%) before or at age 40 years, 36 (43.4%) between ages 41 and 50 years, 23 (27.7%) between ages 51 and 60 years, and 19 (22.9%) older than 60 years. For diagnoses, 53 (64%) patients had BSP and 30 (36%) had Meige’s syndrome. Of the 83 patients with BPS, 47 (56.6%) had sensory tricks (Figure 1). The mean duration of the disease was 31 ± 27 months (0.5–180 months). Thirty-five patients, who had abnormal anti-AChR Abs levels (higher than the normal reference range), received a single or a combination of tests related to MG. These tests included a repetitive nerve stimulation test in 34 patients, a neostigmine test in two patients, chest computed-tomography in 30 patients and single-fiber electromyography in 22 patients. All 35 of the patients who underwent the tests related to MG had negative results. The mean duration of the BoNT-A injection treatment was 2.1 ± 0.7 years (range, 0–5 years). The mean number of injections was 1.6 ± 0.3 (range, 0–8). The mean dose per treatment was 36 ± 3 units (range, 28–80 units). The mean duration of response to BoNT-A was 1.6 ± 0.3 months (range, 0.5–8 months).

Serum anti-AChR Abs levels in patients with BSP, HFS and healthy controls
The serum level of anti-AChR Abs was significantly higher (p < 0.001) in patients with BSP compared with patients with HFS and the healthy controls (Table). There was no significant difference (p > 0.05) in serum anti-AChR Abs levels between the BSP group with and without sensory tricks, and the left HFS and right HFS groups (Table).

Correlation between serum anti-AChR Abs levels and sex, age, duration of disease, duration of response to BoNT-A and Jankovic Rating Scale scores in patients with BSP
The level of serum anti-AChR Abs increased with age (Figure 2B), and decreased with the duration of time after the BoNT-A treatment (Figure 2E; r² = 0.2365), which suggests that high levels of anti-AChR Abs indicate a poor response to BoNT-A treatment. One female patient, who had an increased anti-AChR Abs level as high as three times that of the controls, had only two to three weeks of relief after the BoNT-A treatment. This patient had a repetitive nerve stimulation test10 and single-fiber electromyography. The results were normal, and the neostigmine test was negative. The chest computed tomography, free thyroxine, free triiodothyronine, total thyroxine, total triiodothyronine, thyrotropin, the thyroid peroxidase antibody and antinuclear antibody levels were all normal. She underwent an electromyography test of the orbicularis oculi muscle, which revealed continuous motor unit potentials. The patient did not complain of weakness in other muscles, and all the results confirmed the diagnosis of BSP rather than of MG. The patient accepted an injection of BoNT-A at the total dose 50 U. There was no obvious change after the injection.

No relationships were found between the anti-AChR Abs levels and gender (Figure 2A), disease course (Figure 2C), or severity of disease (Figure 2E).
DISCUSSION

Our major finding of this study was that the level of anti-AChR Abs in BSP patients was higher than the levels in patients with HFS and healthy controls. Anti-AChR antibodies are typically IgG1 or IgG3, with a high affinity for AChR, and a dissociation constant of $10^{-10}$ M anti-AChR Abs are specific for patients with MG, and MG and BSP coexist in some patients, suggesting a strong association between BSP and MG. The common features for both MG and BSP include predominant eyelid involvement, ptosis with unequal palpebral fissures, rest benefit, diurnal change, aggravation from reading and fatigue, excessive blinking, and photophobia. The presence of symptoms of ocular surface irritation and concomitant lower facial or cervical dystonia are more common in patients with BSP. One hypothesized mechanism for the coexistence of BSP and MG is that myasthenic ocular muscle weakness produces abnormal feedback to the central nervous system in patients with BSP and MG. Abnormalities in peripheral afferent inputs, such as continual ptosis and eye blinking, may interfere with motor program execution. Another hypothesis is that eye blinking compensates for eye positional errors resulting from a fatigued extraocular muscle. Frequent compensatory blinking may produce abnormal feedback to the central nervous system.

In this study, the BSP patients had increased anti-AChR Abs levels only and had no other clinical signs of MG. None of our patients developed MG or a weakness of other muscles. As well, in this study, some of the BSP patients with high anti-AChR Abs levels had sensory tricks, which are seldom present in MG. Electromyography of the BSP patients' orbicularis showed spasm potentials (Figure 2). Therefore, the AChR antibody was a 'false-positive' test for MG. The presence of autoantibodies against AChR indicate a possible pathogenic relationship with the development of BSP.

Table. Levels of anti-AChR Abs in different groups.

| Group                        | N  | Anti-AChR-Abs (optical density) |
|------------------------------|----|---------------------------------|
| Blepharospasm                | 83 | 0.237 ± 0.022*                  |
| Blepharospasm with sensory trick | 47 | 0.236 ± 0.014**                 |
| Blepharospasm without sensory trick | 36 | 0.239 ± 0.021                   |
| Hemifacial spasm             | 60 | 0.160 ± 0.064                   |
| Left hemifacial spasm        | 30 | 0.173 ± 0.083***                |
| Right hemifacial spasm       | 30 | 0.155 ± 0.083                   |
| Controls                     | 58 | 0.126 ± 0.038                   |

Anti-AChR-Abs: anti-acetylcholine receptor antibodies; All values are presented as mean ± standard deviation; *F value 10.36, p < 0.0001 compared with hemifacial spasm and controls; **F value -0.06, p = 0.9554 compared with blepharospasm without sensory trick; ***F value -0.80, p = 0.4262 compared with right hemifacial spasm.
HFS is another common craniofacial movement disorder, typically caused by vascular compression of cranial nerve VII, leading to involuntary unilateral contractions of the muscles used in facial expression. In our study, there was no difference between the level of anti-AChR Abs in patients with HFS and that in controls, suggesting that BSP and HFS have different etiologies. Moore et al., in their study, found that multiple sclerosis led to BSP and dystonia in a sibling pair. There have been other reports suggesting a relationship between dysimmunity and BSP. We speculate that elevated anti-AChR Abs levels indicate a role for an autoimmune component in the onset of BSP. Further longitudinal studies are needed to confirm this hypothesis.

BSP is a focal dystonia that involves orbicularis oculi and accessory muscles leading to involuntary and inappropriate eyelid closure. An injection of BoNT-A is the first-choice therapy for BSP. However, up to 10% of the patients repeatedly injected with BoNT-A lose response over time. This treatment failure might be associated with several factors, one of which is BoNT-A neutralizing antibodies. In this study, we found that the levels of serum anti-AChR Abs were closely related to the benefit duration after BoNT-A injection. Some of the BSP patients always had a very poor response to BoNT-A starting with the first injection, as opposed to this developing over time, even after increasing the dose of the BoNT-A (up to 80 U). We found that the anti-AChR Abs level increased with age, which is consistent with a previous report. No relationships were found with gender, disease course, sensory trick, or severity of disease.

Figure 2. Correlation between serum anti-AChR Abs levels and sex, age, duration of disease, Jankovic Rating Scale scores and duration of response to BoNT-A in patients with blepharospasm. Parametric Pearson’s correlation analysis results of the correlation between serum anti-AChR Abs levels and sex (A), age (B), duration of disease (C), Jankovic Rating Scale scores (D) and duration of response to BoNT-A (E) in patients with blepharospasm.
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