NKMJ
Namık Kemal Tıp Dergisi
Namık Kemal Medical Journal

Research Article /Araştırma Makalesi
Doi: https://doi.org/10.37696/nkmj.759574 e-ISSN: 2587-0262

CORRELATION OF JITTER PARAMETERS AND ACETYLCHOLINE RECEPTOR ANTIBODY TITERS IN MYASTHENIA GRAVIS

Myasthenia Graviste Jitter Parametrelerinin ve Asetilkolin Reseptör Antikor Titrelerinin Korelasyonu

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Ethics committee approval was obtained from the clinical research ethics committee of Adana City Training and Research Hospital (number: 45/622).

Abstract

Aim: Acetylcholine receptor (AChR) antibody test and single-fiber electromyography (SFEMG) are two important tests for the diagnosis of myasthenia gravis (MG). In this study, it was aimed to find out whether there is a relationship between AChR antibody titers and stimulated SFEMG (SSFEMG) parameter.

Materials and Methods: Patients whose clinical findings, repetitive nerve stimulation (RNS), and AChR antibody test were compatible with MG were included in this retrospective cohort study. The patients had to be newly diagnosed. SSFEMG was applied to the patients and SSFEMG parameters were analyzed.

Results: Sixteen patients (11 males, 5 females) were included in the study. The mean age of the patients was 54.1 ± 15.8 years. AChR antibody positivity, abnormality in RNS and SSFEMG parameters were found in 13 (81%), 9 (56%), 14 (88%) patients, respectively. A significant positive correlation was found between serum AChR antibody level and the percentage of apparent single-fiber action potential (ASFAP) pairs with increased jitter (p=0.019, r=0.639).

Conclusion: This study showed a positive correlation between AChR antibody titers and the percentage of ASFAP pairs with increased jitter obtained by SSFEMG.

Keywords: Acetylcholine receptor antibody, myasthenia gravis, stimulated single-fiber electromyography.

Öz

Amaç: Asetilkolin reseptör (AChR) antikor testi ve tek-lif elektromiyografi (TLEMG), myasthenia gravis (MG) tanısı için önemli olan iki testtir. Bu çalışmada AChR antikor titreleri ve stimülasyonlu TLEMG (STLEMG) arasında bir ilişki olup olmadığının bulunması amaçlanmıştır.

Materyal ve Metot: Klinik bulguları, ardışık sinir uyarımı (ASU) ve AChR antikor testi MG ile uyumlu olan hastalar bu retrospektif kohort çalışmasına dahil edildi. Hastaların yeni tanı almış olması gerekliydi. STLEMG hastalara uygulandı ve STLEMG parametreleri analiz edildi.

Bulgular: On altı hasta (11 erkek, 5 kadın) çalışmaya dahil edildi. Hastaların yaş ortalaması 54.1 ± 15.8 yıl idi. AChR antikor pozitifliği, ASU ve STLEMG parametrelerinde anormallik sırasıyla 13 (%81), 9 (%56) ve 14 (%88) hastada bulundu. Serum AChR antikor düzeyi ve artmış jitterli tek lif aksiyon potansiyel (TLAP) çiftlerinin yüzdesi arasında anlamlı pozitif bir korelasyon bulundu (p=0.019, r=0.639).

Sonuç: Bu çalışma AChR antikor titreleri ve artmış jitterli TLAP çiftlerinin yüzdesi arasında pozitif bir korelasyon olduğunu göstermiştir.

Anahtar Kelimeler: Asetilkolin reseptör antikoru, myasthenia gravis, stimülasyonlu tek-lif elektromiyografi.

INTRODUCTION

Myasthenia gravis (MG) is an immune-mediated neuromuscular junction disorder presenting with fluctuating muscle weakness due to neuromuscular transmission abnormality. In MG, autoantibodies develop against the nicotinic acetylcholine receptor (AChR) on the post-synaptic membrane, and these antibodies can be detected in serum [1,2]. Clinical findings, acetylcholinesterase tests, serum AChR antibody level, repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG) tests are used for the diagnosis of MG [1-3]. Among the diagnostic methods, sensitivities of AChR

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Article History / Makale Geçmişi:

Date Received / Geliş Tarihi: 29.06.2020
Date Accepted / Kabul Tarihi: 28.10.2020

antibody and SFEMG tests are high [2,4-8]. Although AChR antibody titers may correlate with the clinical severity of the disease, SFEMG abnormalities are more associated with the clinical severity of the disease [9-11]. It was reported that AChR antibody levels decreased with treatment and SFEMG abnormalities improved with improvement of the muscle weakness [9-11]. Studies on the correlation of SFEMG abnormalities and serum AChR antibody levels have been previously reported [9,12]. In this study, unlike previous studies, we aimed to find the relationship between serum AChR antibody levels and jitter parameters obtained by using stimulated SFEMG (SSFEMG) instead of voluntary SFEMG. It was also aimed to obtain information about the use of SSFEMG in clinical practice.

MATERIALS AND METHODS

Subjects

Patients diagnosed with MG who applied to our neurology department between September 2018 and December 2019 were analyzed in this retrospective cohort study. All patients had to have fluctuating muscle weakness causing symptoms such as ptosis and had to be newly diagnosed. Other inclusion criteria were that the serum AChR antibody level was high and / or the 4th compound muscle action potential (CMAP) amplitude obtained by RNS at a rate of 2 / 3 / 5 Hz decreased by more than 10% compared to the first CMAP amplitude. Neurological examination, RNS and SFEMG were performed on the same day. In addition, serum samples of the patients were sent to the laboratory for AChR antibody testing on the same day. Patients with neurodegenerative disease, polyneuropathy or myopathy, receiving treatment for MG were excluded from the study. Clinical features and neurological examination findings of the patients were analyzed. Thorax computed tomography (CT) results were recorded. Ethics committee approval was obtained from the clinical research ethics committee of Adana City Training and Research Hospital (number: 45/622).

AChR antibody test

Radioimmunoprecipitation assay was used for AChR antibody testing. The AChRs from human muscle are labelled with ^{125}I-alpha-bungarotoxin which is a snake toxin. AChR antibodies bind to these labelled receptors if they are present in the serum of the patient. The AChR antibody test was evaluated as follows: AChR antibody level <0.25 nmol/L: negative, AChR antibody level 0.25-0.4 nmol/L: borderline positive, AChR antibody level > 0.4 nmol/L: positive.

Repetitive nerve stimulation

RNS were performed using a Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). RNS was performed by recording the abductor digiti minimi and orbicularis oculi muscles. Stimulation and recording were done with surface electrodes. If the extremity temperature was above 32 ºC, RNS test was performed. Cold extremities were heated. The band-pass filter was set at 20Hz-10kHz. The sweep speed and sensitivity were 5 ms / division and 2 mV / division, respectively. Stimulation was performed supramaximally. The basal CMAP amplitude obtained from the ADM muscle and the CMAP amplitude obtained from the same muscle after exercise were compared. It was considered abnormal that the CMAP amplitude obtained from post-exercise increased more than 60% compared to the basal CMAP amplitude. RNS was performed at a rate of 2/3/5 Hz. In addition, RNS at a rate of 50 Hz was performed by recording from ADM muscle. Post-tetanic potentiation and depression were also analyzed. In RNS with low frequency, it was considered

abnormal that the fourth CMAP amplitude decreased by more than 10% compared to the first CMAP amplitude.

Stimulated single-fiber electromyography

SSFEMG were performed using a Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). For this technique, we used the methods previously proposed [13,14]. The band filter is set to 1-10 kHz for SSFEMG. Sweep speed and sensitivity were 0.5 ms / division and 0.5 mV / division, respectively. For stimulation in SSFEMG, a monopolar needle electrode (length=50 mm, diameter=0.50 mm, 25G, Xian Friendship Medical Electronics, Xian, Shaanxi, China) was used as a cathode and a superficial cup electrode as an anode. The monopolar needle electrode was placed at a point over the trace of the temporal branch of the facial nerve, the surface cup electrode placed 2 cm distal to the monopolar needle electrode. The stimulation duration and frequency of the stimulation was 0.04 ms and 10 Hz, respectively. The record was obtained from the frontalis muscle with a concentric needle electrode (length= 25 mm, 28G, Bionen Medical Devices, Florence, Italy). Jitter parameters were obtained by analyzing 50-100 consecutive apparent single-fiber action potentials (ASFAPs). It was considered abnormal when the mean consecutive difference (MCD) of the ASFAPs was > 28μs [13]. If the number of ASFAP pairs with increased jitter is more than 10% of the total number of ASFAP pairs, or if the mean MCD of total ASFAP pairs is > 21μs [13], the SSFEMG test was considered abnormal. Notched potentials were not used for analysis, and care was taken to ensure that the potential may have a rapid rise phase [13-15]. When MCD of the individual ASFAPs < 5 μs, this potential was not taken as it may have been achieved by direct muscle stimulation or intramuscular axonal stimulation. Guidelines were used for other technical errors [13-15].

Statistical analysis

The Shapiro-Wilk test was used to determine the distribution of the data. Pearson's Chi-squared test was used to analyze categorical variables. Spearman's test was used for correlation. Median and mean ± standard deviation (SD) of numeric data were calculated for descriptive statistics. Statistical Package for the Social Sciences (SPSS IBM Corp; Armonk, NY, USA) 22.0 was used to perform the statistical analysis.

RESULTS

Sixteen patients were included in the study. Eleven of the patients (69%) were male. The mean age of the patients was 54.1 ± 15.8 (range 24-80) years. The mean duration of the patients' complaints was 3.5 ± 3.6 (range 1-24) months. Clinical and laboratory findings of the patients are shown in Table 1. All patients had ptosis in one or both eyes or diplopia. Four patients had mild facial muscle weakness or difficulty swallowing. None of the patients received treatment for MG. Thorax CT of one of the patients was compatible with thymoma. The AChR antibody titer was < 0.025 nmol/L in the 3 (19%) patients. The mean of serum AChR antibody levels of the remaining 13 patients was 7.17 ± 6.46 (Range 0.34-17.6) nmol/L. Significant decrement in low frequency RNS test was present in 9 (56%) patients.

The means of the SSFEMG parameters are shown in Table 2. A total of 355 ASFAP pairs were analyzed. The number of 22 ASFAP pairs per patient were examined. The percentage of ASFAP pairs with increased jitter was > 10% in 14 (87.5%) patients. The mean MCD was abnormal in 11(69%) of

the patients. Impulse blocking was observed in 12 (75%) patients. The SSFEMG test of two patients (12.5%) was normal.

Table 1. Clinical and laboratory findings of the patients

Patients	Age / Gen.	Ocular symptoms	Facial or bulbar or limb weakness	AChR antibody titer (nmol/L)	Abn. RNS	Abn. Percentage of ASFAP pairs with increased jitter	Abn. Mean MCD	Impuls blocking
1	**54 / F**	**+**	**-**	1.55	**-**	**+**	**+**	**-**
2	**59 / M**	**+**	**-**	16.80	**+**	**+**	**+**	**+**
3	**73 / F**	**+**	**-**	14.80	**+**	**+**	**+**	**+**
4	**24 / M**	**+**	**-**	0.34	**-**	**+**	**+**	**+**
5	**33 / M**	**+**	**-**	1.33	**-**	**+**	**+**	**+**
6	**64 / M**	**+**	**-**	5.25	**-**	**+**	**+**	**+**
7	**80 / M**	**+**	**-**	17.60	**+**	**+**	**+**	**+**
8	**58 / M**	**+**	**+**	0,43	**+**	**-**	**-**	**-**
9	**62 / F**	**+**	**-**	6.73	**-**	**+**	**+**	**-**
10	**64 / M**	**+**	**+**	3.89	**-**	**+**	**+**	**+**
11	**58 / M**	**+**	**-**	12.57	**+**	**+**	**+**	**+**
12	**59 / F**	**+**	**+**	<0.25	**+**	**+**	**+**	**+**
13	**35 / M**	**+**	**-**	1.70	**-**	**+**	**+**	**+**
14	**46 / M**	**+**	**+**	10.20	**+**	**+**	**+**	**+**
15	**64 / M**	**+**	**-**	<0.25	**+**	**+**	**+**	**+**
16	**32 / F**	**+**	**-**	<0.25	**+**	**-**	**-**	**-**

Abn.: abnormal; AChR: acetylcholine receptor; ASFAP: apparent single fiber action potential; F: female; Gen.: Gender; M: male; MCD: mean consecutive difference; RNS: repetitive nerve stimulation.

Table 2. SSFEMG parameters

SSFEMG parameter	Mean± SD (median)
ASFAP pairs with increased jitter %	57.4 ± 32.6 (59.1)
Impulse blocking %	25.1 ± 24.8 (23.5)
Mean MCD µs	36.7 ± 15.1 (33.5)

ASFAP: apparent single fiber action potential; MCD: mean consecutive difference; SSFEMG: stimulated single-fiber electromyography; SD: standard deviation.

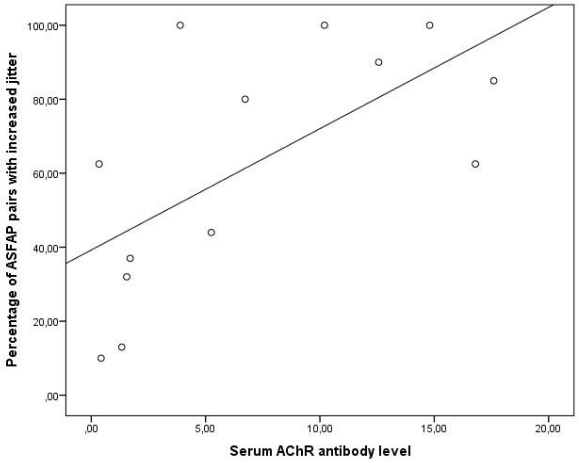
The correlation between SSFEMG parameters and AChR antibody titers is shown in Table 3. Correlation analysis was performed in 13 patients with serum AChR antibody level > 0.25 nmol/L. A positive correlation was found between AChR antibody titer and increased jitter percentage (p= 0.019 r= 0.639, Figure 1).

Table 3. Correlation of SSFEMG parameters and serum AChR antibody levels

SSFEMG parameter	Serum AChR antibody level
ASFAP pairs with increased jitter %	p= 0.019, r=0.639
Blocking %	p= 0.152, r=0,420
Mean MCD µs	p= 0.133, r=0.440

AChR: acetylcholine receptor; ASFAP: apparent single-fiber action potential; MCD: mean consecutive difference; SSFEMG: stimulated single-fiber electromyography.

Figure 1. Correlation of percentage of ASFAP pairs with increased jitter and AChR antibody titers



AChR: acetylcholine receptor; ASFAP: apparent single fiber action potential.

DISCUSSION

SFEMG and AChR antibody tests have very important roles for the diagnosis of MG. These tests may be needed for the diagnosis of MG or they can be used for disease follow-up. The sensitivities of both tests are high for the diagnosis of MG [2,4-8]. Although this study was not sufficient for sensitivity and specificity of the diagnostic tests, percentage of patients with abnormal AChR antibody, RNS, and SSFEMG tests found in this study were similar to previous studies. The sensitivity of AChR antibody titers in ocular MG and generalized MG was approximately 70% and 85% [1,4,6], respectively. In these two MG subgroups, the sensitivity of the SSFEMG test is known as approximately 80% and 95%, respectively [4,5]. The sensitivity of RNS in the ocular and generalized MG was approximately 50% and 75%, respectively [2,4]. In this study, although the patients were not divided into MG subgroups, AChR antibody titer, SSFEMG, RNS were abnormal in 13 (81%) , 14 (88%), 9 (56%) patients, respectively. These findings and previous studies show that using three tests together will increase the sensitivity for the diagnosis of MG [2,4].

It was found in previous studies that there is a relationship between AChR antibody titers and SFEMG parameters [9,12]. This finding may indicate that as AChR antibodies increase, abnormalities in neuromuscular conduction may increase. In this study, a positive similar correlation was found between serum AChR receptor levels and percentage of ASFAP pairs with increased jitter. Unlike other studies [7,9], SSFEMG was applied to patients instead of voluntary SFEMG. Although SSFEMG has more technical requirements than SFEMG, it has some advantages such as not requiring voluntary muscle activation. SFEMG parameters obtained from facial and ocular muscles were reported to be more sensitive for MG diagnosis than SFEMG parameters obtained from forearm muscles such as extensor digitorum communis [7]. Therefore, we obtained ASFAPs from the frontalis muscle. SSFEMG also has disadvantages, one of which is direct muscle stimulation. To prevent this, we used monopolar needle electrodes for stimulation and did not increase the stimulation intensity above 5 mA. The positive correlation between AChR antibody titers and rates of abnormalities in SSFEMG parameters, and the relationship between clinical findings and these tests reported in previous studies show that these two tests can be used in the follow-up of patients [9-12]. The correlation found in this study showed that SSFEMG can be used for the diagnosis and follow-up of MG, just like SFEMG. In addition, as mentioned before, due to some advantages of SSFEMG such as not requiring patient cooperation, it can be used instead of SFEMG when SFEMG cannot be used. Also, in cases where SFEMG and RNS cannot be applied, AChR antibody titers can be used for the diagnosis and monitoring of MG, or vice versa. However, this needs to be confirmed by other studies.

There were some limitations in this study. First, the number of patients was low. The strict inclusion criteria led to a small number of patients included in the study. But it was also important to include newly diagnosed MG patients who did not receive treatment because SFEMG parameters and AChR antibody titers could improve with treatment [9-11]. Secondly, patients were not followed up. For this reason patients were not divided into ocular, bulbar or generalized MG groups. We think that studies involving correlation analysis between titers of AChR or muscle-specific kinase or other antibodies and SSFEMG parameters based on these groups would be interesting.

In conclusion, this study showed a positive correlation between serum AChR antibody level and percentage of ASFAP pairs with increased jitter obtained with SSFEMG. The fact that similar correlation was found using SFEMG in previous studies shows that SSFEMG is an important electrodiagnostic test for the diagnosis of MG just like SFEMG.

Conflicts of interests: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Kaynaklar

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