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Study on the clinical and electrophysiological characteristics of nerve function in myasthenia gravis patients in Vietnam

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Abstract

Background: In Vietnam, there is limited research on the role of nerve conduction in myasthenia gravis and its association with clinical features.

Objective: This study aims to describe the electrophysiological features in patients with myasthenia gravis.

Methods: This descriptive study was conducted from September 2019 to December 2021. The study included 33 myasthenia gravis patients who sought medical consultation or received inpatient treatment during this period. The Myasthenia Gravis Foundation of America classifies myasthenia gravis into five groups: I, IIa, IIb, IIIa, IIIb, IVa, IVb, and V. Notably, Group I involves pure ocular weakness, whereas Group a primarily impacts limb and axial muscles, and Group b mainly affects bulbar and respiratory muscles.

Results: The study revealed that motor and sensory nerve conduction in the upper and lower limbs were within normal limits for the patient group under evaluation. Repetitive nerve stimulation testing at a frequency of 3 Hz showed positive results in 66.7% of myasthenia gravis patients. Myasthenia gravis patients displayed distinct clinical symptoms, with ptosis being the most common (87.9%). Myasthenia Gravis Foundation of America classification indicated the highest proportion in subgroup IIa (24.2%), with myasthenia gravis predominating in limb and axial muscles (Group a) observed in 51.5% of cases. Needle electromyography showed no abnormalities in myasthenia gravis patients. There was an association between acetylcholine receptor antibody titers and the results of the 3 Hz repetitive nerve stimulation test in myasthenia gravis patients, with a significance of $p = 0.002$.

Conclusion: Nerve conduction studies should be performed in patients with suspected neuromuscular disorders to aid in differential diagnosis and definitive diagnosis of myasthenia gravis.

Keywords

Electrophysiological characteristics, nerve function, myasthenia gravis, Vietnam

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Introduction

Myasthenia gravis (MG) is an autoimmune disease that causes fluctuating weakness and fatigue in the skeletal muscles, primarily due to the attack of antibodies on the neuromuscular junction, specifically targeting the nicotinic receptors for acetylcholine. Approximately 80%–90% of MG patients have detectable serum autoantibodies against acetylcholine receptors (AChRs).[1–3] MG incidence and prevalence rates, documented over 70 years through European and U.S. epidemiological studies, estimate new cases at 5–30 per 1,000,000 individuals annually and prevalence at 10–20 per 100,000 individuals.[4] MG rates are expected to rise. It commonly affects individuals aged 30–50, with 10% starting before 18. Prevalence is higher in females than males.[1,3,5]

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MG is an autoimmune disorder primarily controlled by B lymphocytes and T lymphocytes, which produce autoantibodies affecting the postsynaptic membrane structure and impairing nerve conduction to muscles.[6,7] MG is primarily caused by defects in AChR structure or reduced receptor quantity in the postsynaptic membrane. Autoantibodies targeting AChR, MuSK, or LRP4 lead to receptor destruction or blockade.[1,8] This results in a decreased number of nicotinic receptors, flattening of postsynaptic membrane folds, and impaired signal transmission from nerve to muscle, causing muscle weakness.[1,6,8]

In Vietnam, the advantages and drawbacks of serological and electrophysiological diagnostic methods for MG are influenced by various factors. Serological testing, detecting antibodies, offers non-invasiveness and accessibility, especially for autoimmune markers like AChR antibodies. However, its limited sensitivity (about 20% of cases lack detectable AChR antibodies) reduces effectiveness. Electrophysiological tests directly assess neuromuscular function, aiding MG confirmation, but in Vietnam, challenges arise due to variable healthcare resources and expertise, requiring specialized equipment and trained professionals, leading to potential diagnostic delays and limited accessibility.

Biomedical science advancements aid early MG diagnosis, prognosis, and severity assessment. In Vietnam, limited use of quantitative autoimmune antibody testing is reported. Around 20% of MG patients lack AChR antibodies, affecting diagnostic accuracy.[9] Therefore, the role of nerve conduction studies, particularly repetitive nerve stimulation (RNS) techniques, is considered crucial for MG diagnosis. The positive results of RNS in MG patients have varied in different studies[10–16] with rates reported as 70.3% by Sadri Y and 73.7% by Zahra Vahabi. In Vietnam, publications discuss clinical characteristics and AChR antibody levels in MG patients. Limited research explores the role and diagnostic value of nerve conduction studies, and the associations between RNS and clinical features of MG are unclear. This study aims to describe electrophysiological features in MG patients.

Methods

Study design

The study was designed as a descriptive research method (Figure 1). The subjects were selected for the study using a consecutive sampling method. The study locations included: (i) Can Tho City General Hospital, where the subjects participated in the study and underwent inpatient neurophysiological measurements; (ii) Department of Immunology and Allergy, Hanoi Medical University, where human leukocyte antigen-B (HLA-B) and human leukocyte antigen-DRB1 (HLA-DRB1) testing was performed using the polymerase chain reaction with sequence specific primers (PCR-SSP) method; and (iii) Medic Hoa Hao Hospital in Can Tho, where quantification of AChR antibody was conducted. The study was conducted from September 2019 to December 2021. In our study, the sampling method was adapted to accommodate the extended sampling period caused by the rarity of the condition. This involved employing a flexible approach based on patient availability, rather than fixed time intervals, due to the limited number of cases.

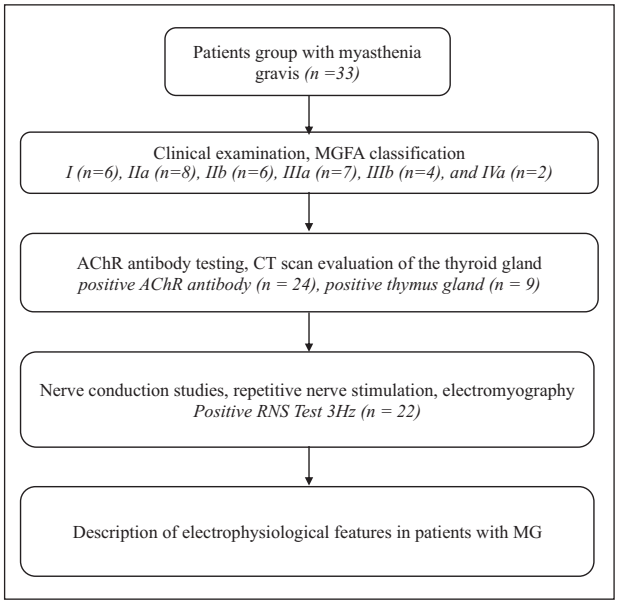

Figure 1. Study design.

Study population

The study was conducted on 33 MG patients who sought medical consultation or received inpatient treatment at Can Tho City General Hospital from September 2019 to December 2021. The patients were diagnosed with MG and classified according to the Myasthenia Gravis Foundation of America (MGFA) disease severity classification.[17] The diagnoses were made by specialized physicians who independently assessed the patients' clinical conditions. The patients presented with specific clinical manifestations as follows[18]: (i) fatigable weakness of muscles, exacerbated during strenuous activities, more severe at the end of the day, and mildly relieved with rest or the use of cholinesterase inhibitors; (ii) atypical distribution of muscular involvement, typically involving ocular and bulbar muscles; and (iii) no sensory disturbance or pain. After the initial assessment, these patients continued to receive treatment and were regularly monitored to track the study parameters.

Inclusion criteria included patients diagnosed with MG and the voluntary consent of the patient or their legal representative to participate in the study, as evidenced by signed informed consent forms. Exclusion criteria encompassed congenital myasthenic syndromes of genetic origin, Lambert–Eaton myasthenic syndrome, botulism-induced

myasthenia, and patients without a sufficient quantity of high-quality whole blood samples for HLA-B and HLA-DRB gene typing.

Evaluation and collection

This study collected data including gender (male, female), age at onset (under 45 or 45 and above) following the guidelines of the British Neurological Society,[19] and disease duration (calculated from the time of diagnosis to study participation). The reasons for seeking medical attention related to MG symptoms such as ptosis, diplopia, limb weakness, dysphagia, dysarthria, and respiratory difficulty were also recorded.

Clinical symptoms were assessed, including ptosis, diplopia, limb weakness, dysarthria, dysphagia, symptoms worsening with activity and improving with rest, and response to anticholinesterase medications. The AChR antibody test was considered positive with a value of $\geqslant 0.52$ nmol/L and negative with a value <0.52 nmol/L. Thymus gland involvement was categorized into having or not having thymic abnormalities. The classification of MG according to the MGFA was divided into five groups: I, IIa, IIb, IIIa, IIIb, IVa, IVb, and V.[20] Classification based on the affected muscle groups included Group I (pure ocular weakness), Group a (predominantly affecting limb and axial muscles), and Group b (predominantly affecting bulbar and respiratory muscles). The classification based on disease severity includes mild (Group I, Group II), moderate, and severe cases (Group III, Group IV, Group V) according to MGFA criteria.[17]

Nerve conduction in motor nerves of both upper and lower limbs, including between, trunk, stump, and deep muscles, were assessed through the following parameters: (i) Distal Motor Latency; (ii) Motor Conduction Velocity; and (iii) Amplitude of the muscle action potential. The sensory nerve conduction in both upper and lower limbs, including between, trunk, superficial, and calf nerves, was evaluated through the following parameters: (i) Distal Sensory Latency; (ii) Sensory Conduction Velocity; and (iii) Amplitude.

The results of the 3 Hz RNS test were interpreted according to the guidelines of the American Association of Electrodiagnostic Medicine (AAEM).[21] A positive result was defined as a decrease in the amplitude of the fourth or fifth compound muscle action potential (CMAP) by more than 10% compared to the first CMAP, observed in at least one muscle. A negative result was determined when there was no decrease in amplitude of the fourth or fifth CMAP by more than 10% compared to the first CMAP. The 3 Hz RNS test assessed the positive rate in muscle groups like orbicularis oculi, extensor digitorum, and abductor pollicis brevis. A positive result occurred when the fourth or fifth CMAP's amplitude dropped over 10% compared to the first CMAP, whereas a negative result meant no such drop exceeding 10%. Amplitude decrement in these muscle groups during the 3 Hz RNS test was calculated as a percentage decrease in the fourth or fifth CMAP compared to the first CMAP. The 30 Hz RNS test results were interpreted as per the AAEM guidelines.[21] A positive result was defined by an amplitude increase between the tenth and first CMAP exceeding 200%, whereas a negative result meant no increase above 200%.

In spontaneous needle electromyography (EMG), we observed various waveforms like fibrillation potentials, positive sharp waves, complex repetitive discharges, and myotonic discharges. We also evaluated motor unit potential (MUAP) recruitment. In mild muscle contractions, approximately 1 MUAP with a firing rate of 5–7 Hz (type I muscle fibers) was involved. With increased muscle contraction, more MUAPs participated, a phenomenon known as recruitment. Typically, less than 20 MUAPs per second were observed in normal situations, but the rate decreased when it exceeded 20 MUAPs per second. Normal MUAPs had a duration of 5–15 ms, an amplitude between 100 μV and 2 mV, and approximately 2–3 phases. Any deviations beyond these values indicated abnormalities. Neurophysiological measurements were performed following the guidelines for recording nerve conduction in individuals suspected of abnormal synaptic transmission according to Preston's protocol in 2013.[22] Neurophysiological recordings were conducted in accordance with the guidelines provided by authors Ralph M. Buschbacher and Preston David, as well as the AAEM in 2001.

Ethics in research

This study obtained ethical approval from the Biomedical Research Ethics Council of Hanoi Medical University, certified by number NCS: 04/DMUHM-ERC, dated 29 March 2019. Participants and their legal representatives were fully informed about the procedures, purposes, risks of the study, and provided written informed consent prior to any interventions. All activities conducted in this study adhered to the regulations and ethical principles for biomedical research in Vietnam and internationally. All participants voluntarily participated in the study after receiving comprehensive counseling. This was a descriptive study without any interventions; thus, it did not impact the participants' treatment methods or timelines. Written consent was obtained from the participants or their legally authorized representatives for this study

Statistical analysis

Data analysis was performed using the Statistical Packages for Social Sciences (SPSS) software, version 22.0 (IBM Corp., Armonk, N.Y., USA). Descriptive statistics, including mean, standard deviation, median, minimum value, maximum value, percentages, and frequencies, were used to summarize the variables. The normality of quantitative variables was assessed using Q–Q plots and the Shapiro–Wilk test.[23] Statistical tests were employed to compare means between two groups, specifically the *t*-test for normally distributed variables and non-parametric tests (Kruskal–Wallis[24] and Mann–Whitney *U*) for variables that did not follow a normal distribution. The chi-square test (or Fisher's exact test when

Table 1. Demographic characteristics and electrophysiological features of myasthenia patients.

Variables		Patient group ($n = 33$)
Demographics		
Gender, *n* (%)	Male	14 (42.4)
	Female	19 (57.6)
Age (years), mean ± SD		52.76 ± 10.75
	Male	53.6 ± 9.1
	Female	51.5 ± 12.8
Onset age group, *n* (%)	<45 (early)	9 (27.3)
	⩾45 (late)	24 (72.7)
Decreased amplitude of 4th/5th CMAP compared to the first on 3 Hz RNS test		
Orbicularis oculi muscle, mean ± SD	Right	6.73 ± 4.82
	Left	7.18 ± 5.03
Trapezius muscle, mean ± SD	Right	10.27 ± 5.6
	Left	9.27 ± 5.09
Extensor digitorum muscle, mean ± SD	Right	5.03 ± 4.06
	Left	5.12 ± 3.82
Characteristics of needle electromyography recordings		
Spontaneous electrical activity, *n* (%)	Present	3 (9.1)
	Absent	30 (90.9)
MUAP morphology, *n* (%)	Normal	33 (100)
	High-amplitude-polyphasic	0 (0)
MUAP recruitment, *n* (%)	Normal	29 (87.9)
	Reduced	4 (12.1)

appropriate) was used to compare proportions. The study used odds ratios and 95% confidence interval (95% CI) to evaluate the relationship between the results of the 3 Hz RNS test and gender, age, thymus, MGFA classification, and AChR antibody levels. Statistical significance was defined as $p < 0.05$, indicating a significant difference.

Results

During the period from September 2019 to December 2021, a total of 33 MG patients participated in the study (Table 1). Among them, 19 patients were female, accounting for 57.6% of the total. The average age of the MG patients was 52.76 ± 10.75 years, with the youngest patient being 33 years old and the oldest being 74 years old. The majority of patients (72.7%) had a late onset of symptoms at ⩾45 years of age.

In terms of low-frequency RNS test at 3 Hz, 22 MG patients had positive results, accounting for 66.7%, whereas 11 patients had negative results, accounting for 33.3%. Three patients (9.1%) exhibited spontaneous electrical activity, and four patients (12.1%) had reduced motor unit action potential recruitment. No abnormalities were observed in the morphology of motor unit action potentials in the MG patients.

The average duration of illness among the MG patients in the study was 2.52 ± 1.39 years, with the shortest duration being 1 month and the longest duration being 60 months. The average duration of illness in patients with thymic tumors (3.33 ± 1.41 years) was higher than that in patients without thymic tumors (2.21 ± 1.27 years), and the difference was statistically significant with a *p*-value of 0.047. The average duration of illness was higher in the male gender group compared to the female gender group, and in the late onset group (⩾45 years old) compared to the early onset group (<45 years old), but these differences were not statistically significant.

The most common reasons for seeking medical attention among the MG patients were ptosis (87.9%), followed by swallowing difficulties (57.6%), diplopia (54.5%), limb weakness (51.5%), and respiratory distress (42.4%), with the lowest rate being dysarthria (33.3%). Among the MG patients, 30.3% presented with two symptoms, whereas the lowest rate (9.1%) was observed in patients with only one symptom or all six symptoms (Figure 2).

Ptosis was the most prevalent symptom, occurring in 29 MG patients, accounting for 87.9%. Diplopia was observed in 22 patients, accounting for 66.7%. Clinical manifestations affecting the respiratory and throat muscles were seen in 20 patients (60.6%) with swallowing difficulties, 14 patients (42.4%) with respiratory distress, and 12 patients (36.4%) with dysarthria. Weakness in limb muscles and the trunk was present in 21 patients (63.6%). Increased symptoms with activity and improvement with rest were reported by 25 patients (75.6%), and 93.9% of patients experienced symptomatic improvement with the use of cholinesterase inhibitors (Figure 3).

According to the MGFA classification, there were eight patients with MG in subgroup IIa, accounting for the highest proportion of 24.2%; two patients in subgroup IVa, accounting for the lowest proportion of 6.1% (Table 2). Among the MG patients, six cases were classified as Group I, accounting for 18.2%; there were 17 cases (51.5%) of MG predominating in limb and axial muscles (Group a), and 10 cases (30.3%) predominating in throat and respiratory muscles (Group b). Patients in subgroup IIIa had a higher incidence of thymus gland involvement (5/9 cases) and AChR antibody positivity (7/24 cases) compared to the other subgroups.

The highest reduction in CMAP amplitude from the first to the fourth/fifth stimulus was observed in the trapezius muscle (right: 10.27% ± 5.6%; left: 9.27% ± 5.09%), whereas the lowest reduction was seen in the extensor digitorum muscle (right: 4.73% ± 3.28%; left: 5.12% ± 3.82%). Comparing the three patient groups, the degree of reduction in CMAP amplitude was higher in Group A patients with myopathy compared to the other two groups, and this difference was statistically significant in the left orbicularis oculi muscle and right extensor digitorum muscle with $p < 0.05$ (Table 3).

Age of onset ($p = 0.033$) and AChR antibody ($p = 0.002$) were found to be associated with the 3 Hz RNS test (Table 4). Specifically, positive 3 Hz RNS results were significantly higher in late-onset MG patients aged 45 and above (79.2%)

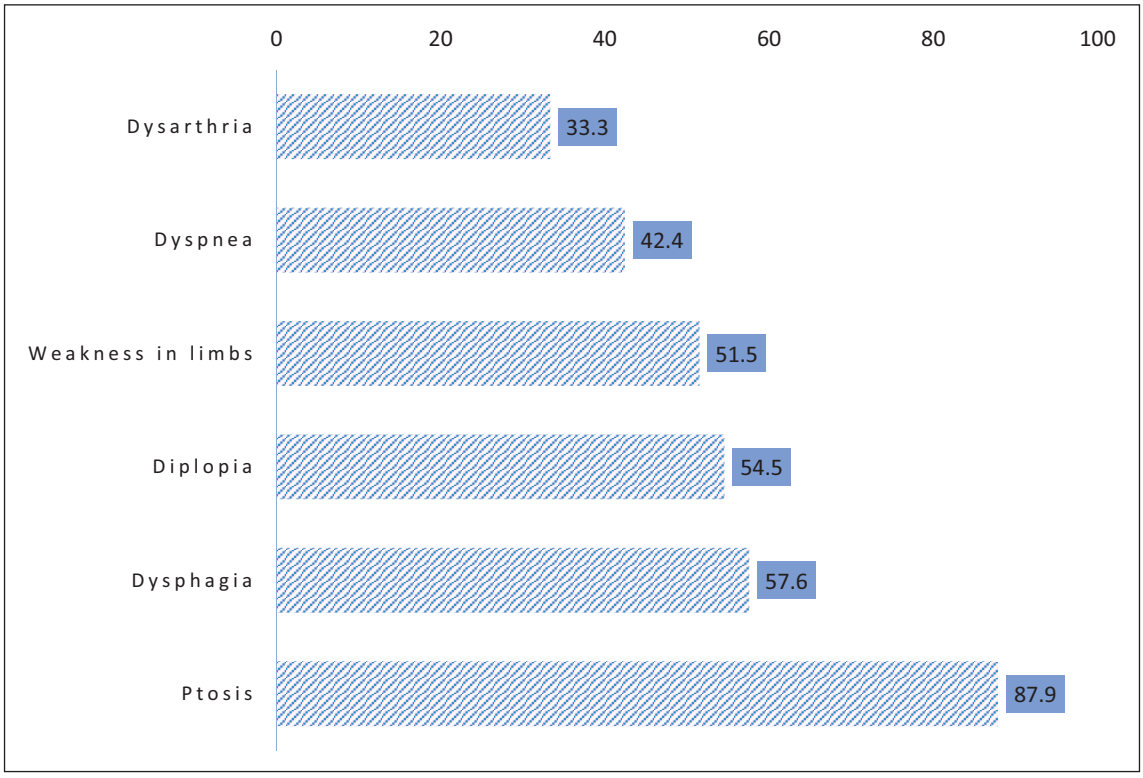

Figure 2. Reasons for patients' medical visits.

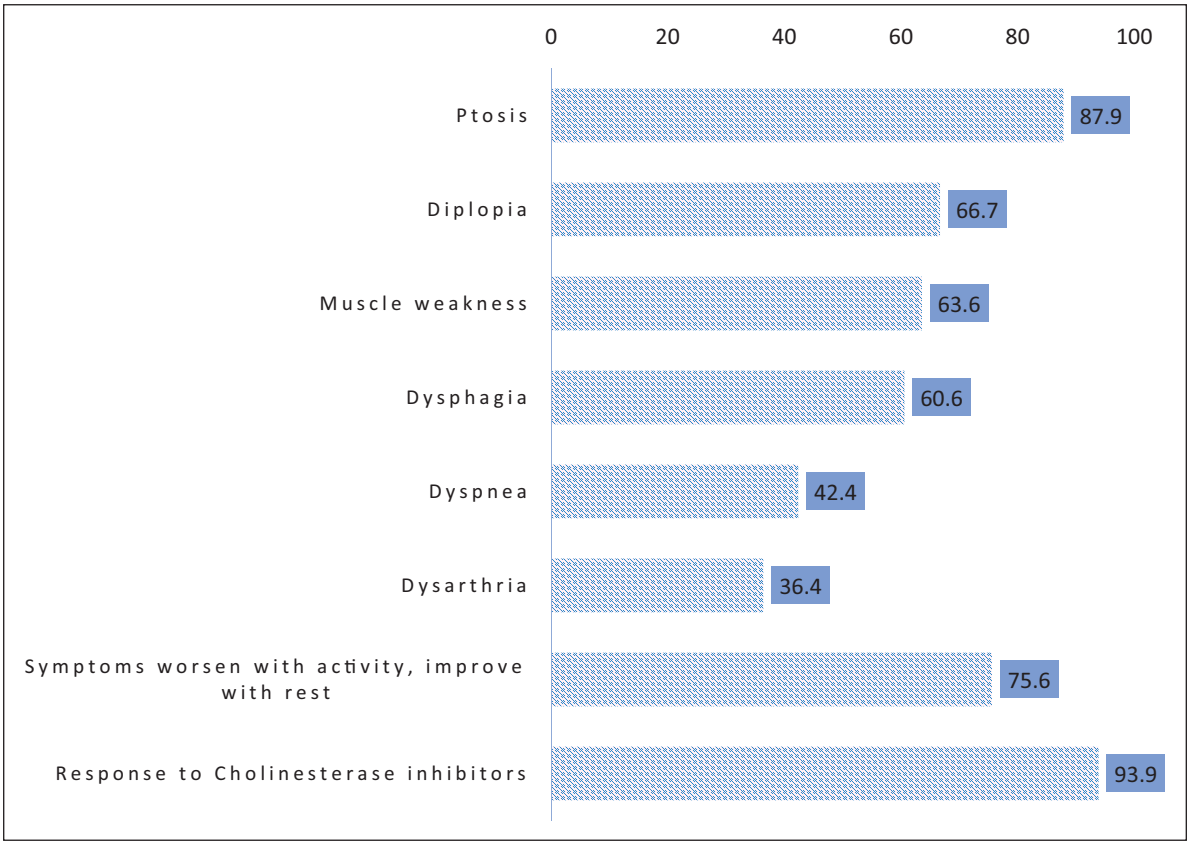

Figure 3. Clinical manifestations of symptoms ($n = 33$).

Table 2. Characteristics of gender, age, anti-AChR antibodies, and thymus gland in MG patients according to MGFA classification.

MGFA classification subgroups	Gender (male:female)	Average age (years)	Patients with anti-AChR antibodies	Patients with thymus gland
I ($n=6$, 18,2%)	4:2	48.17 ± 13.14	3	1
IIa ($n=8$, 24,2%)	2:6	53.88 ± 8.39	5	1
IIb ($n=6$, 18,2%)	2:4	51.5 ± 8.94	4	0
IIIa ($n=7$, 21,2%)	3:4	49.29 ± 11.29	7	5
IIIb ($n=4$, 12,1%)	3:1	61 ± 8.41	4	2
IVa ($n=2$, 6,1%)	0:2	61.5 ± 17.68	1	0
Total	33	–	24	9

Table 3. Degree of reduction in amplitude of the 4th or 5th CMAP compared to the 1st CMAP on 3 Hz RNS test in patients classified according to MGFA.

Muscle groups for performing RNS test	Subgroups	Reduction in CMAP amplitude of the 4th/5th compared to the 1st CMAP amplitude (%)				p (Kruskal–Wallis)
		I ($n=6$)	a ($n=17$)	b ($n=10$)	$n=33$	
Orbicularis oculi muscle	Right	3.17 ± 4.54	8.59 ± 4.65	5.7 ± 4.08	6.73 ± 4.82	0.070
	Left	3.17 ± 6.08	9.35 ± 3.82	5.9 ± 4.7	7.18 ± 5.03	0.027
Trapezius muscle	Right	6.5 ± 5.58	11.47 ± 4.9	10.5 ± 6.26	10.27 ± 5.6	0.201
	Left	6.0 ± 6.39	9.82 ± 4.17	10.3 ± 5.46	9.27 ± 5.09	0.221
Extensor digitorum muscle	Right	2.5 ± 3.89	6.06 ± 2.05	3.8 ± 3.85	4.73 ± 3.28	0.041
	Left	3.67 ± 5.09	6 ± 2.76	4.5 ± 4.55	5.12 ± 3.82	0.163

compared to early-onset patients under the age of 45 (33.3%). Furthermore, MG patients with positive AChR antibody had a significantly higher likelihood of positive 3 Hz RNS results compared to those without AChR antibody presence. However, no significant associations were found between gender, thymus gland, MGFA classification, and the 3 Hz RNS test.

Discussion

Electrophysiological characteristics of the nervous system in patients with MG

MG is a neuromuscular disorder that affects the neuromuscular junction through immune-mediated mechanisms, primarily affecting the axial muscle groups, respiratory muscles, and ocular muscles. These clinical manifestations are similar to those seen in the Lambert–Eaton syndrome (an autoimmune disorder involving presynaptic junctional attack) and botulism (a toxin-mediated condition). Therefore, in order to make a definitive and differential diagnosis in patients with abnormal clinical manifestations at the neuromuscular junction, David C. Preston recommends conducting nerve conduction studies, RNS tests, and EMG needle examinations on these myasthenic patients for accurate diagnosis and exclusion of other conditions.[22]

This study found that nerve conduction parameters, including latency, amplitude, and velocity, were within the normal range in peripheral nerves of patients with muscle weakness based on reference values.[25] This aligns with previous studies indicating intact myelin and axons in these patients.[22,26] The findings suggest structural changes in the postsynaptic membrane, potentially affecting synaptic transmission. Reduced postsynaptic receptors in muscle weakness may lead to increased spatial summation during electrical stimulation, resulting in higher CMAP amplitudes.[27] Additionally, sensory nerves exhibited prolonged latencies and slower conduction velocities in patients with muscle weakness, indicating early demyelination. Timely intervention is crucial to address this demyelination. Further research on nerve conduction in muscle weakness patients is needed to explore the association between nerve conduction results and the condition.

The positive rate of RNS 3 Hz test in the muscle weakness group was 66.7%. This difference was statistically significant with $p < 0.00$. The positive rate of RNS 3 Hz test varied among different MGFA muscle weakness subgroups, with 16.7% in Group I, 88.2% in Group a, and 60% in Group b. These results are consistent with studies conducted by João Costa[10] (98%), Bou Ali[28] (89%), and Witoonpanich[29] (80%). However, in our study, the group of patients with pure ocular muscle weakness (Group I) had a lower positive rate of 16.7%. This rate is similar to the findings of Sadri[12] (30%) and Zahra Vahabi[13] (normal) for pure ocular muscle weakness group.

Based on AAEM guidelines, abnormal 3 Hz RNS results on a muscle indicate myasthenia and have diagnostic significance. Our study found higher positive 3 Hz RNS results in

Table 4. Association between the results of 3 Hz RNS test with gender, age, thymus, MGFA classification, and AChR antibody.

Variables	RNS test 3 Hz		Total	OR (95% CI)	*p* (Fisher)
	Positive	Negative			
	n (%)	*n* (%)			
Gender					
Male	8 (57.1)	6 (42.9)	14	0.48	0.459
Female	14 (73.7)	5 (26.3)	19	(0.11–2.07)	
Onset age					
⩾45 years old	19 (79.2)	5 (20.8)	24	7.60	0.033
<45 years old	3 (33.3)	6 (66.7)	9	(1.38–41.61)	
Thymus					
Yes	8 (88.9)	1 (11.1)	9	5.71	0.212
No	14 (58.3)	10 (41.7)	24	(0.61–53.22)	
MGFA classification					
A	15 (88.2)	2 (11.8)	17	5.00	0.153
B	6 (60.0)	4 (40.0)	10	(0.72–34.92)	
AChR antibody					
Positive	20 (83.3)	4 (16.7)	24	17.50	0.002
Negative	2 (22.2)	7 (77.8)	9	(2.61–117.37)	

the trapezius muscle (82.4%) for Group a and in the orbicularis oculi muscle (60%) for Group b. However, there were no statistically significant differences between tested muscles and MGFA subgroups. These findings align with Costa et al.'s[10] publication, which showed higher positive 3 Hz RNS results in the trapezius muscle for Group a and in the orbicularis oculi/levator palpebrae muscles for Group b, with statistical significance. Our study, along with global publications, provides guidance for electromyographers in performing the 3 Hz RNS test for myasthenia diagnosis: focus on the trapezius muscle for Group a and the orbicularis oculi muscle for Group b.[10,29,30]

Different muscle groups (trapezius, orbicularis oculi/levator palpebrae, and extensor digitorum) exhibited varying degrees of CMAP amplitude reduction. The trapezius muscle showed the highest reduction, whereas the orbicularis oculi and extensor digitorum muscles had lower reductions.[10] Theoretical explanations suggest that damage to the postsynaptic membrane due to AChR reduction may contribute to CMAP amplitude reduction.[31] These results emphasize the need for further in-depth studies to understand the association between CMAP reduction in the 3 Hz RNS test, muscle groups, and postsynaptic membrane damage.

The literature suggests that patients with MG have normal EMG results. However, EMG needle examinations are recommended to rule out conditions such as botulism and to exclude combined injuries from motor neuron diseases, polyneuropathies, or genetic muscle disorders.[22] The EMG results from 33 MG patients showed that 90.9% had no spontaneous potentials, 87.9% had normal motor unit recruitment, and 100% had no abnormalities in motor unit shape. Only three patients had spontaneous potentials, and four patients showed reduced recruitment. According to Preston, a small percentage of normal individuals may exhibit spontaneous potentials during EMG, characterized by muscle fiber twitching and occasional sharp waves. In some severe cases of MG, reduced recruitment can be observed.[22,32]

Factors associated with RNS testing

The study found that males had a 57.1% positive result on 3 Hz RNS testing, whereas females had a 73.7% positive result. However, there was no significant association between gender and 3 Hz RNS test results ($p = 0.459$). These findings support previous research that also did not find a gender association with RNS 3 Hz testing in patients with neuromuscular transmission abnormalities. Therefore, RNS 3 Hz testing remains a valuable diagnostic tool for both males and females in clinical practice.[33]

The findings revealed that in the late-onset group, the percentage of patients with positive 3 Hz RNS test results (79.2%) was higher than those with negative results (20.8%). In contrast, in the early-onset group, the percentage of patients with positive 3 Hz RNS test results (33.3%) was lower than those with negative results (66.7%), and this difference was statistically significant ($p = 0.033$). The increased likelihood of positive results in the 3 Hz RNS test among patients with late-onset neuromuscular disorders suggests caution in performing the technique and interpreting the results for patients with early-onset conditions. Our research results differ from the report by Shang et al.,[33] who found no association between age and 3 Hz RNS test outcomes.

MG patients with thymic tumors had a positive 3 Hz RNS test rate of 88.9%, whereas those without thymic tumors had

a rate of 58.3%. Among patients with a negative 3 Hz RNS test, 11.1% had thymic tumors, compared to 41.7% without thymic tumors. Thymic tumors release mature T cells into the blood, activating the production of disease-causing autoantibodies.[34] This may explain why MG patients with thymic tumors are more sensitive to the 3 Hz RNS test. However, no significant association was found between MG patients with thymic tumors and the 3 Hz RNS test results ($p = 0.212$).

In the diagnostic process of MG, AChR antibody testing is commonly used. However, approximately 20% of MG patients do not have detectable AChR antibodies. Nerve conduction studies play an important role in diagnosing MG[18] when AChR antibody testing is not available. Among MG patients, those with AChR antibodies had a positive 3 Hz RNS test rate of 83.3%, whereas those without AChR antibodies had a rate of 22.2%. There was a significant association between AChR antibody presence and the 3 Hz RNS test results ($p = 0.002$). This could be due to the presence of AChR antibodies activating the immune response, influencing nerve conduction as measured by the 3 Hz RNS test. These findings suggest that both criteria can be used interchangeably in healthcare facilities where one of the techniques is unavailable.

The data hold particular significance for the context of Vietnam. The study highlights the unique characteristics of MG within the Vietnamese population and underscores the crucial role of electrophysiological tests in diagnosis and differentiation. Although nerve conduction parameters were generally within the normal range in peripheral nerves of individuals with muscle weakness, the high positive rate of the 3 Hz RNS test in muscle weakness patients further emphasizes its diagnostic relevance, particularly in specific muscle groups. Additionally, the study considers factors such as age, gender, the presence of thymic tumors, and AChR antibodies, offering valuable insights that are directly applicable to healthcare practices in Vietnam, reinforcing the importance of comprehensive diagnostic approaches, especially in cases where certain antibodies may not be detectable.

Limitations

The study has some limitations. First, the study sample was limited to a few healthcare facilities in Can Tho, which may affect the representativeness of the results. The study duration was short and may not have allowed for long-term monitoring of patient conditions and the effectiveness of treatment was also not assessed. Additionally, the study did not perform a sample size calculation, which could potentially impact the statistical power and generalizability of the findings from the current study. Furthermore, this study did not document the number of cases falling under the exclusion criteria.

Conclusion

In conclusion, the study findings suggest that motor and sensory nerve conduction in the upper and lower limbs were within normal limits. RNS testing at a frequency of 3 Hz showed that 66.7% of MG patients had positive results. The positive results were higher in the trapezius muscles for Group a (predominantly limb and axial muscles) and in the orbicularis oculi muscles for Group b (predominantly respiratory and throat muscles). There was a significant decrease in compound muscle action potential amplitude in the fourth or fifth stimulation compared to the first stimulation, with the highest decrease observed in Group a and the lowest in Group I (pure ocular myasthenia). Needle EMG revealed no abnormalities in the MG patients. There was an association between AChR antibody titers and the results of the 3 Hz RNS test in MG patients with a significance of $p = 0.002$. Electrophysiological nerve testing should be performed in patients with suspected myasthenic symptoms for differential diagnosis and definitive diagnosis of myasthenia. For patients in Group A (predominantly limb and axial muscles) with myasthenia, the RNS test should be initially conducted on the nerve–muscle pair of the trapezius muscle. For patients in Group B (predominantly respiratory and throat muscles), the test should be initially conducted on the nerve–muscle pair of the orbicularis oculi muscle. In cases of isolated ocular myasthenia, additional clinical evaluations should be combined with RNS testing. Electrophysiological nerve testing offers advantages for facilities without quantitative testing for specific antibodies like Acetylcholine or others such as MuSK, LRP4. The RNS test can be employed for MG diagnosis. However, the diagnostic yield of nerve conduction studies varies based on clinical subgroups, being more sensitive in generalized MG and less sensitive in patients with isolated ocular myasthenia. A novel finding of the study reveals that the diagnostic sensitivity of the RNS test varies depending on the subgroup of MG patients. Therefore, it is recommended for clinicians to consider this variability when using RNS for diagnosing suspected MG patients.

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Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of conflicting interests

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Institutional review board statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Can Tho University of Medicine and Pharmacy.

Ethical approval

Ethical approval for this study was obtained from the Biomedical Research Ethics Council of Hanoi Medical University (Approval Number NCS: 04/DMUHM-ERC).

Informed consent

Informed consent was obtained from the patient or their legal representative to participate in the study. For participants aged 18 years and older, written informed consent from legally authorized representatives was obtained.

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