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Research article

Relationship between fatigue and quantitative electromyography findings in patients with myasthenia gravis

Didem Savaşcı [a], Metin Mercan [b,*], Vildan Yayla [b]

[a] Basaksehir Cam and Sakura City Hospital, Department of Neurology, Istanbul, Turkey
[b] Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Department of Neurology, Istanbul, Turkey

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ABSTRACT

Background: Fatigue is a common complaint among patients with myasthenia gravis (MG). In this study, we investigated the alterations in muscle morphology in patients with MG experiencing fatigue using quantitative electromyography (QEMG), and explored the relationship between electrophysiological findings and the severity of both fatigue and disease.
Methods: We performed QEMG of the biceps brachii muscle using the peak ratio method and multi-motor unit potential (MUP) analysis across three groups: 18 MG patients with fatigue, 34 MG patients without fatigue, and 33 healthy subjects. Stimulated single-fiber EMG was performed on the frontalis muscle. The severity of perceived fatigue and disease was subsequently assessed using the quantitative myasthenia gravis (QMG) score, the MG-activities of daily living (MG-ADL) profile, self-reported fatigue questionnaires, and handgrip strength measurements.
Results: The QEMG study revealed a reduced mean MUP duration and size index (SI), in addition to an increased peak ratio in patients with MG ($p < 0.05$), which tended to be more pronounced in those experiencing fatigue. Compared to healthy subjects, MG patients with fatigue displayed a myopathic pattern characterised by a high peak ratio, short duration, and small-amplitude MUPs, without any increase in the number of phases or small time intervals. The mean peak ratio was positively correlated with the QMG, MG-ADL, and Fatigue Impact Scale total and physical sub-scores ($p < 0.05$). Further, MG patients with fatigue exhibited reduced maximum grip strength, which was positively correlated with the mean MUP duration, amplitude, SI, and thickness, and negatively correlated with the mean peak ratio ($p < 0.05$). No significant differences were observed in the jitter or block measurements ($p > 0.05$).
Conclusions: The present study investigated electrophysiological findings that were not considered or theorised in prior studies on patients with MG experiencing fatigue. The results of this study suggest that myopathic changes may be a critical pathophysiological component underlying the fatigue associated with MG.

1. Introduction

Myasthenia gravis (MG) is a chronic neuromuscular disorder characterised by an autoimmune response targeting functional molecules in the postsynaptic membrane of the neuromuscular junction [1]. Between 44 % and 89 % of patients with MG report experiencing fatigue, which commonly persists even during periods of remission, and may be disproportionate to the degree of muscle

* Corresponding author. Bakırkoy Dr. Sadi Konuk Training and Research Hospital, Department of Neurology, Bakirkoy, 34147, Istanbul, Turkey.
E-mail address: dr_metin_mercan@hotmail.com (M. Mercan).

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weakness [2–4]. This symptom is associated with a reduced quality of life (QoL) [5]. Fatigue is a subjective phenomenon without any universally agreed-upon definition, encompassing feelings of exhaustion, weariness, depletion of physical and/or cognitive energy, and difficulty in initiating or sustaining voluntary muscle activities [3,6]. Fatigue comprises both physiological and psychological dimensions. Physiological fatigue, also referred to as muscle or physical fatigue, is the decline in maximum voluntary force generation capacity during exercise [7–10]. The terms 'peripheral fatigue' and 'central fatigue' have additionally been proposed to distinguish between different types of physiological fatigue, highlighting their distinct pathophysiological mechanisms in neuromuscular diseases (NMDs) [11–13].

Self-reported multidimensional scales have been developed to assess fatigue and facilitate its recognition in various NMDs [3,5,9,11,14]. Studies employing these questionnaires have identified significant correlations between disease severity and both the degree and frequency of experienced fatigue [4,5,14–17]. Considering that impaired neuromuscular transmission plays a key role in the manifestation of MG symptoms, it is plausible that fatigue perceived by patients may develop as a direct physiological consequence of neuromuscular transmission failure [1,4]. However, current studies have not yet established a clear relationship between neuromuscular transmission failure and fatigue [18,19]. Moreover, impaired neuromuscular transmission is not the sole pathophysiological mechanism involved. Structural changes in limb muscles secondary to chronic disease processes may also be observed in patients with MG [20–22]. However, whether these structural changes contribute to the pathophysiological mechanisms underlying fatigue in patients with MG remains unclear. The primary aim of this study was to determine alterations in muscle morphology in patients with MG experiencing fatigue using quantitative electromyography (QEMG), while the secondary aim was to evaluate the association between electrophysiological findings, fatigue, and disease severity in these patients.

2. Material and methods

2.1. Participants

This prospective study was approved by the local ethics committee, and informed consent was obtained from all participants. We recruited 52 patients with MG who were followed-up at the Neuromuscular Diseases Department of our clinic between 2010 and 2022. All patients were diagnosed with definite MG based on clinical electrophysiological examinations and laboratory findings [1]. The inclusion criteria for patients with MG were as follows: (i) a disease duration >6 months; (ii) clinically stable or asymptomatic status; (iii) a clinical grade of Class I or II on the Myasthenia Gravis Foundation of America classification; and (iv) a score of 16 or less on the quantitative myasthenia gravis (QMG) score during both electrophysiological and clinical examinations. Patients were excluded if they had a QMG score increase of $\geq$2 within the prior 6 months, or if EMG revealed denervation potentials. The control group comprised 33 healthy subjects with no systemic or neurological diseases who did not report experiencing fatigue, as determined based on multidimensional questionnaires. All the participants underwent neurophysiological assessments performed by a senior clinical neurophysiologist blinded to the clinical outcomes. Clinical evaluations were conducted by a different neurologist. The cumulative steroid dose was calculated by reviewing the prescribed oral corticosteroid doses, expressed as prednisone equivalents, in the preceding 6 months [23].

2.2. Clinical examination

2.2.1. Questionnaires

Participants, both with and without fatigue, completed a battery of self-administered questionnaires, including the Chalder Fatigue Scale (CFS), Fatigue Severity Scale (FSS), Fatigue Impact Scale (FIS), Saltin–Grimby Physical Activity Level Scale (SGPALS), and Beck Depression Inventory (BDI). The CFS evaluates psychological and physical fatigue, while the FSS measures the degree of primary physical fatigue experienced within the past week. The FIS separately assesses the cognitive, physical, and psychosocial dimensions of fatigue in daily life. In accordance with previous studies, fatigue was defined as a score of four or higher on both the CFS and FSS. Patients meeting this cut-off value were categorised as the 'MG patients with fatigue' group, while those scoring below 4 were classified as the 'MG patients without fatigue' group [14,24,25]. To further assess the impact of fatigue on QoL, participants completed the MG-QoL-15, a disease-specific instrument designed to evaluate QoL in patients with MG.

2.2.2. Assessment of disease severity

Disease severity and clinical status were assessed using the MG-Activities of Daily Living (MG-ADL) profile and the QMG score, with higher scores indicating greater MG severity. Further, quantitative measurements of the dominant handgrip strength were performed in all participants using a Jamar Hydraulic Hand Dynamometer.

2.3. Electrophysiological studies

2.3.1. QEMG analyses

All electrophysiological tests were performed using the Dantec Keypoint G4 EMG system (Dantec, Skovlunde, Denmark). QEMG analyses were conducted on the unilateral biceps brachii (BB) muscles of all participants, using standard concentric needle electrodes with a recording area of 0.07 mm^2 and a length of 37 mm. The filter settings were maintained between 5 Hz and 10 kHz. The sweep speed was set to 5 ms/div, while the gain was set to 200 μV/div. The motor unit potential (MUP) and interference pattern (IP) data were analysed using the multi-MUP and peak ratio analysis programs.

For multi-MUP analysis, 20–30 individual MUPs were recorded from each BB muscle, with 1–6 MUPs captured from at least 3–4 different insertion sites and depths during sustained slight to moderate levels of voluntary contraction. The needle electrode was positioned to ensure that at least some MUPs exhibited sharp, short rise times, without attempting to optimise the recording electrode position for maximum peak amplitudes. The duration cursors were set automatically, with positional adjustments performed only in exceptional cases, such as when a noisy baseline was present. Potentials with an unsteady baseline in addition to an unclear beginning or end were discarded. MUP parameters, including the duration, amplitude, thickness, size index (SI), and number of phases, were measured and calculated using the EMG system. The multi-MUP analysis results, indicating myopathic changes, were as follows: (i) reduced duration, amplitude, thickness, and SI and (ii) increased number of phases.

IP samples were acquired from ten different sites within the muscle, using at least three needle insertions. Participants were instructed to gradually increase their force from zero to the maximum isometric contraction over a period of 10 s. The ratio of turns to mean amplitude during the gradual increase in force was automatically analysed every 100 ms using a peak ratio analysis program. The evaluated IP parameters included the mean peak ratio, as well as the number of small time intervals of less than 1.5 ms per second [mean NSS/s (log10)]. The participants were allowed to rest for at least 1 min between transitions from one sampling point to the next. Myopathic changes were demonstrated by increases in the mean NSS/s (log10) and mean peak ratio values.

2.3.2. Jitter analyses

Stimulated single-fiber EMG using concentric needle electrodes with a diameter of 0.3 mm and a recording surface area of 0.019 mm^2 was performed on the unilateral frontalis muscle of the patient groups to assess neuromuscular transmission. Patients receiving acetylcholinesterase inhibitors were asked to withhold from taking their medication for at least 12 h prior to the examination. For the jitter measurement, the gain was set to 100–200 μV, while the low-frequency filter was increased to 1 kHz. Stimulation of the temporalis branch of the facial nerve was performed using a monopolar needle electrode, with a stimuli of 0.02–0.05 ms duration and an intensity typically below 2 mA, inducing a visible slight twitch in the frontalis muscle. 'Apparent single-fiber action potentials (ASFAPs)' were recorded with stimulation at 10 Hz, while the stimulation intensity was finely adjusted to avoid submaximal stimulation. Signals displaying parallel rising phases when superimposed, lacked shoulder notches, were larger than 50 μV, and had a fast-rising slope to a well-defined negative peak with a consistent shape across consecutive discharges were accepted for each recording. The individual jitter of each ASFAP was measured from 100 potentials. The average of the individual jitter values from 20 to 30 ASFAPs was defined as the 'mean jitter'. The percentage of abnormal individual jitter (100 × number of ASFAPs with jitter above 28 μs/total number of ASFAPs) and the block percentage (100 × number of blocked ASFAPs/total number of ASFAPs) were also calculated.

2.4. Statistical analysis

Statistical analyses were performed using SPSS software (version 25; Statistical Package for Social Sciences, Chicago, Illinois, USA). Descriptive data were expressed as mean ± standard deviation (mean ± SD) for continuous variables, and as the count (n) and percentage (%) for categorical variables. Continuous variables were tested for normal distribution using the Shapiro–Wilk test. Student's

Table 1
Demographic and clinical data of MG patients with and without fatigue.

	MG patients with fatigue (n = 18)	MG patients without fatigue (n = 34)	P value
Age at onset (years, mean ± SD)	42.7 ± 14.0	45.8 ± 17.6	0.513
Disease duration (years, mean ± SD)	8.53 ± 5.47	6.71 ± 7.9	**0.016**
Disease onset, n (%)			0.982
Early onset MG	10 (55.6 %)	19 (55.9 %)	
Late onset MG	8 (44.4 %)	15 (44.1 %)	
Subgroup by affected muscles, n (%)			0.136
Ocular MG	1 (5.56 %)	9 (26.5 %)	
Generalised MG	17 (94.4 %)	25 (73.5 %)	
Presence of autoantibodies, n (%)			0.111
AChRAb positive	11 (61.1 %)	29 (85.3 %)	
MuSKAb positive	3 (16.7 %)	3 (8.8 %)	
Seronegative	4 (22.2 %)	2 (5.9 %)	
Thymectomy, n (%)	9 (50 %)	15 (44,1 %)	0.686
Thymoma, n (%)	5 (27.8 %)	13 (38.2 %)	0.451
Duration of symptomatic treatment (months, mean ± SD)	89.8 ± 63	57.5 ± 67	**0.016**
Duration of non-steroidal immunosuppressive therapy (months, mean ± SD)	n = 10 67.5 ± 74	n = 16 65.6 ± 70.9	0.792
Duration of steroid treatment (months, mean ± SD)	n = 18 81.2 ± 64.2	n = 31 86 ± 153.7	0.063
Cumulative steroid dose over the last 6 months (mg/kg, mean ± SD)	n = 18 51 ± 40.5	n = 31 41.1 ± 32.1	0.468
Current steroid dose (mg/day, mean ± SD)	n = 18 20.4 ± 15.1	n = 31 18.9 ± 14	0.566

Statistical significance was set at $p < 0.05$.
Abbreviations: AChRAb, acetylcholine receptor antibody; MG, Myasthenia gravis; MuSKAb, muscle specific kinase antibody.

t-test, Mann–Whitney *U* test, and chi-square test (or Fisher's exact test) were applied, as appropriate, to compare the variables between MG patients with and without fatigue. Differences between the MG and control groups were compared using one-way ANOVA with Bonferroni correction for normally distributed continuous variables and the Kruskal-Wallis test with Dunn- Bonferroni adjustment for non-normally distributed continuous variables. Among patients with MG, the Spearman's correlation test was conducted to analyse the relationships between QEMG results, fatigue, disease severity, and jitter measurements. All results were considered statistically significant at $p < 0.05$.

3. Results

The proportion of MG patients with fatigue was 34.6 % (n = 18) of the total MG cohort. MG groups showed no significant differences in gender or age distribution (MG patients with fatigue: 12 women and 6 men, mean age 51.2 ± 11.2 years; MG patients without fatigue: 19 women and 15 men, mean age 52.8 ± 14.6 years). Among the patients with MG, 40 (77 %) were acetylcholine receptor (AChR) antibody-positive, 6 (11.5 %) were muscle-specific kinase (MuSK) antibody-positive, and 6 (11.5 %) had double-negative serology. Disease duration was significantly longer in MG patients with fatigue than in those without fatigue ($p = 0.016$). Furthermore, MG patients with fatigue had a longer duration of symptomatic treatment with pyridostigmine than those without fatigue ($p = 0.016$). However, the durations of non-steroidal immunosuppressive therapy (23 patients on azathioprine and 3 on mycophenolate mofetil) and steroid (prednisolone) treatment were similar between the two groups ($p > 0.05$). Further, no significant differences were observed between the groups in other clinical data, including age at onset, thymus pathology, distribution of muscle weakness, presence of MG-specific autoantibodies, or history of thymectomy (all $p > 0.05$, Table 1).

All fatigue scores, including the physical, cognitive and psychosocial subscores of the FIS, were significantly higher in MG patients with fatigue than in those without fatigue ($p < 0.001$, Table 2). Additionally, questionnaires administered to MG patients with fatigue indicated the presence of depression, a sedentary lifestyle, and reduced QoL ($p < 0.05$, Table 2). QMG and MG-ADL scores were also significantly higher in MG patients with fatigue, indicating a higher disease severity ($p < 0.001$, Table 2). Furthermore, MG patients with fatigue had a lower maximum handgrip strength than those without fatigue ($p = 0.017$). However, no statistically significant differences in the jitter and block measurements were found between the two groups (both $p > 0.05$, Table 2).

In QEMG analyses, multiple comparison tests revealed significant differences in the duration, amplitude, SI, peak ratio, and NSS/s (log10) parameters among the groups ($p < 0.05$, Table 3), while no significant differences were found in the thickness and number of phases ($p > 0.05$, Table 3). In post hoc analyses, the mean MUP duration and SI were significantly reduced, while the mean peak ratio was significantly increased in both patient groups compared with those in the control group ($p < 0.05$). In contrast, the mean MUP amplitude significantly decreased only in MG patients with fatigue ($p = 0.006$), while the mean NSS/s (log10) increased in MG patients without fatigue ($p = 0.008$). QEMG results tended to show more prominent myopathic features in MG patients with fatigue than in those without fatigue. However, these differences were not statistically significant when the two patient groups were compared ($p >$

Table 2
Comparison of clinical and electrophysiological data between MG patients with and without fatigue.

	MG patients with fatigue (n = 18)	MG patients without fatigue (n = 34)	P value
FSS score (mean ± SD)	5.19 ± 0.77	2.82 ± 0.83	**<0.001**
FIS, total score (mean ± SD)	25.2 ± 7.13	8.03 ± 5.28	**<0.001**
FIS, cognitive subscore (mean ± SD)	9.61 ± 3.82	2.15 ± 1.89	**<0.001**
FIS, physical subscore (mean ± SD)	12.9 ± 5.12	5.12 ± 3.52	**<0.001**
FIS, psychosocial subscore (mean ± SD)	2.67 ± 1.41	0.71 ± 0.87	**<0.001**
CFS score (mean ± SD)	7.67 ± 2.35	1.88 ± 1.81	**<0.001**
BDI score (mean ± SD)	12.9 ± 5.89	4.18 ± 5.34	**<0.001**
SGPALS, n (%)			**0.02**
Class 1-2	16 (88.9 %)	18 (52.9 %)	
Class 3-4	2 (11.1 %)	16 (47.1 %)	
MGFA, n (%)			**0.003**
Asymptomatic	3 (16.6 %)	20 (58.8 %)	
1	6 (33.3 %)	10 (29.4 %)	
2A	5 (27.7 %)	4 (11.8 %)	
2B	4 (22.2 %)	0 (0 %)	
QMG score (mean ± SD)	7.39 ± 4.1	3.09 ± 2.31	**<0.001**
MG-ADL score (mean ± SD)	2.56 ± 0.98	1 ± 1.13	**<0.001**
MG-QoL-15 score (mean ± SD)	18.8 ± 8.87	4.68 ± 5.89	**<0.001**
Maximum handgrip strength (KgW, mean ± SD)	24.8 ± 8.39	29.3 ± 7.28	**0.017**
Presence of block, n (%)			0.603
Present	6 (33.3 %)	9 (26.5 %)	
Absent	12 (66.7 %)	25 (73.5 %)	
Mean jitter (μs, mean ± SD)	30.1 ± 19.4	24.8 ± 12.6	0.341
Abnormal individual jitter, (%, mean ± SD)	31.1 ± 33.5	22.5 ± 23.6	0.335

Statistical significance was set at $p < 0.05$.
Abbreviations: BDI, Beck Depression Inventory; CFS, Chandler Fatigue Scale; FIS, Fatigue Impact Scale; FSS, Fatigue Severity Scale; MG-ADL, Myasthenia Gravis Activities of Daily Living; MGFA, Myasthenia Gravis Foundation of America; MG-QoL-15, Myasthenia Gravis Quality of Life 15; SGPALS, Saltin Grimby Physical Activity Level Scale; QMG, Quantitative Myasthenia Gravis.

0.05).

The mean peak ratio showed a significant positive correlation with QMG, MG-ADL, FIS total scores, and FIS physical subscores, along with a negative correlation with maximum handgrip strength ($p < 0.05$). The mean MUP duration, amplitude, SI, and thickness were positively correlated with the maximum handgrip strength ($p < 0.05$). Furthermore, positive correlations were observed between the number of phases and both the QMG and MG-QoL-15 scores ($p < 0.05$). The NSS/s (log10) further showed a positive correlation with both the jitter measurements and MG-ADL scores ($p < 0.05$). The data from the QEMG analyses are presented in Tables 3 and 4.

4. Discussion

The QEMG results demonstrated electrophysiological findings consistent with a myopathic process in the proximal extremity muscles of patients with MG compared with healthy controls. Although the differences between the MG groups did not reach statistical significance, the myopathic changes tended to be more pronounced in MG patients with fatigue. Furthermore, myopathic patterns differed between MG patients with and without fatigue. Further, some myopathic findings were associated with both fatigue and disease severity. MG patients with fatigue exhibited a higher disease severity than those without fatigue. However, no significant differences were observed in jitter or block abnormalities in the frontalis muscle, or in the use of immunosuppressive treatments between the patient groups. MG patients with fatigue also displayed higher levels of depressive mood and a sedentary lifestyle, lower QoL, longer disease duration, and more prolonged use of symptomatic treatment.

Functional and morphological alterations resulting from failure of antibody-mediated neuromuscular transmission in the MG have been discussed for many years [20,26]. The muscle biopsy findings in these patients ranged from nonspecific myopathic changes to mitochondrial alterations, lymphocellular infiltrates, and mild neurogenic features. Among MG patients with MuSK antibodies, myopathic changes, along with prominent mitochondrial abnormalities, have been commonly observed in the affected muscles. Conversely, the most common finding among MG patients with AChR antibodies was muscle fiber (MF) atrophy, which was often accompanied by mild neurogenic features [21,27–29]. Multiple mitochondrial DNA deletions have previously been reported in muscle specimens from seropositive patients, indicating that impaired energy production contributes to muscle atrophy and fatigue [30]. Additionally, autoimmune attacks on MG exert pathogenic effects on muscle-specific stem cells, thus disrupting their muscle regeneration abilities [31]. Experimental studies have further demonstrated that functional denervation or impaired neuromuscular transmission leads to the loss of muscle mass through specific signalling pathways involving atrophy-related genes, such as muscle ring finger 1 and atrogin-1 [26,32,33]. Several QEMG analyses have documented MUP changes indicative of a nonspecific myopathic process characterised by short-duration and small-amplitude MUPs without increased polyphasia [22,34,35]. However, the primary mechanism underlying myasthenic weakness is a reduced density or functionality of receptors in the postsynaptic membrane, resulting in impaired cholinergic transmission between nerve terminals and MFs [1,20]. Consequently, many authors believe that MUP changes in the MG are associated with intermittent blocking of some MF action potentials [36,37]. However, most electrophysiological studies have failed to conclusively demonstrate that the myopathic nature of MUPs in the MG occurs primarily a direct physiological consequence of neuromuscular transmission failure [34,35,38]. The present study revealed prominent myopathic changes in the morphology of the MUP in patients with MG. Furthermore, several MUP parameters were found to be positively correlated with maximum handgrip strength, although our results did not establish an association between these MUP changes and impaired neuromuscular transmission. Given that MUP generation depends on the size, number, and density of MFs, these myopathic findings may result from the functional loss of some MFs within motor units (MUs), or from uniform atrophy of MFs, accompanied by reduced variability in MF diameter.

IP analysis is a QEMG method that can detect myopathic changes with a superior sensitivity to MUP analysis [39]. This technique effectively differentiates between myopathic, neurogenic, and normal muscles. However, studies of IP in patients with MG remain limited. Prior research has identified IP patterns consistent with or suggestive of both myogenic and neurogenic processes in the MG [40,41]. Indeed, one previous IP analysis demonstrated that during maximum force in the BB muscle, patients with MG had a lower mean amplitude and a higher turns/amplitude ratio than controls, both at the beginning and end of the effort, while there was no difference in the number of turns [36]. A subsequent study also reported a myopathic pattern in the facial muscles of approximately half of the patients with MG, characterised by a low mean amplitude and low to normal turns per second, similar to patterns observed

Table 3
Comparison of quantitative electromyography parameters in the biceps brachii muscle.

Parameters	Controls (n = 33)	MG patients with fatigue (n = 18)	MG patients without fatigue (n = 34)	P value
Duration (ms)	10.3 ± 0.91	9.5 ± 0.97*	9.68 ± 1.11*	**0.014**
Amplitude (μV)	383 ± 86	322 ± 41.4**	348 ± 49.9	**0.005**
Thickness (ms)	1.25 ± 0.14	1.19 ± 0.15	1.21 ± 0.15	0.271
Size index	0.28 ± 0.22	0.07 ± 0.20**	0.13 ± 0.23*	**0.002**
Number of phases	2.67 ± 0.30	2.63 ± 0.27	2.56 ± 0.21	0.371
Peak ratio	1.09 ± 0.15	1.28 ± 0.23**	1.21 ± 0.20*	**0.002**
NSS/s (log10)	2.42 ± 0.10	2.47 ± 0.14	2.50 ± 0.13**	**0.009**

Data are presented as the median (mean ± SD). P-values were calculated using the Kruskal-Wallis test or one-way ANOVA. Statistical significance was set at $p < 0.05$. The Dunn-Bonferroni adjustment or Bonferroni correction was applied for two-group comparisons.
MG patients with fatigue vs. control group, *$p < 0.05$ and **$p < 0.01$; MG patients without fatigue vs. control group, *$p < 0.05$ and **$p < 0.01$.
Abbreviations: NSS/s (log10), the number of small time intervals; MG, myasthenia gravis.

Table 4
Associations between quantitative electromyography parameters and patient data.

Patient data	Duration (ms)		Amplitude (μV)		Thickness (ms)		Size index		Number of phases		Peak Ratio		NSS/s (log10)	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
Mean jitter (μs)	−0.176	0.211	−0.068	0.630	−0.086	0.546	−0.233	0.096	0.098	0.489	0.259	0.064	**0.277***	**0.047**
Abnormal individual jitter (%)	−0.155	0.272	−0.027	0.847	−0.052	0.715	−0.211	0.133	0.159	0.259	0.242	0.083	**0.297***	**0.033**
Block (%)	−0.11	0.436	−0.075	0.599	−0.098	0.490	−0.164	0.247	−0.02	0.889	0.208	0.139	**0.322***	**0.020**
FSS score	0.003	0.984	−0.129	0.364	−0.030	0.832	−0.114	0.423	0.231	0.110	0.141	0.318	−0.073	0.605
FIS total score	−0.079	0.577	−0.173	0.219	−0.038	0.788	−0.190	0.177	0.231	0.099	**0.290***	**0.037**	0.112	0.431
FIS cognitive subscore	−0.070	0.623	−0.22	0.117	−0.029	0.838	−0.190	0.178	0.158	0.263	0.199	0.158	0.033	0.817
FIS physical subscore	−0.046	0.748	−0.106	0.454	−0.032	0.821	−0.158	0.264	0.265	0.057	**0.340***	**0.014**	0.151	0.285
FIS psychosocial subscore	−0.193	0.171	−0.264	0.059	−0.099	0.484	−0.238	0.090	0.094	0.507	0.198	0.159	0.067	0.635
CFS score	0.005	0.973	−0.157	0.267	0.037	0.797	−0.038	0.789	0.185	0.190	0.148	0.297	0.000	0.999
MG-QoL-15 score	−0.035	0.808	−0.109	0.440	−0.047	0.743	−0.135	0.339	**0.309***	**0.026**	0.234	0.095	0.085	0.551
QMG score	−0.141	0.319	0.024	0.865	−0.149	0.293	−0.092	0.517	**0.397****	**0.004**	**0.309***	**0.026**	0.217	0.123
MG-ADL score	−0.133	0.346	0.045	0.75	−0.156	0.270	−0.137	0.332	0.256	0.067	**0.339***	**0.014**	**0.275***	**0.048**
Maximum handgrip strength (KgW)	**0.299***	**0.032**	**0.477****	**0.000**	**0.281***	**0.043**	**0.416****	**0.002**	0.120	0.395	**−0.377****	**0.006**	0.069	0.626

Data are presented as Spearman's correlation coefficients. *Correlation significant at the 0.05 level. ** Correlation significant at the 0.01 level.
Abbreviations: CFS, Chandler Fatigue Scale; FIS, Fatigue Impact Scale; FSS, Fatigue Severity Scale; MG-ADL, Myasthenia Gravis Activities of Daily Living; MG-QoL-15, Myasthenia Gravis Quality of Life 15; $r_{s,}$ Spearman correlation coefficient; QMG, Quantitative Myasthenia Gravis.

in patients with myopathic dystrophy [34,42]. Similarly, in the IP analyses conducted alongside multi-MUP analyses, we observed prominent myopathic patterns in patients with MG. Furthermore, the myopathic findings varied between MG patients with and without fatigue. The present study also revealed that the BB muscle in MG patients with fatigue, exhibited a pattern of high peak ratio with short-duration and small-amplitude MUPs compared to healthy subjects, without any increase in the number of phases or NSS/s. In contrast, MG patients without fatigue displayed a myopathic pattern characterised by a high peak ratio and increased NSS/s, accompanied by short-duration MUPs, but without small-amplitude MUPs.

Multi-MUP analysis primarily reflects Type I MF activity, whereas the peak ratio method also captures Type II MF activity, which is involved in larger MUs [39,44,45]. During IP analysis, the maximum peak-to-peak amplitude of the MUPs within the IP increases as the contraction force increases, due to the recruitment of larger MUPs at higher force levels. However, as the maximum contraction force approaches, the NSS generally either remains relatively constant, or slightly decreases owing to MUP summation and cancellation [39, 45]. The peak ratio in the IP analysis represents the maximum ratio of turns per second to the mean amplitude obtained using the mean amplitude per turn as an indicator of force. The NSS reflects the time parameters within the MUPs and is influenced by the number of polyphasic MUPs, individual MUP duration, firing rate complexity, and number of activated MUs constituting the IP [43,45]. In the present study, maximum handgrip strength and FIS physical scores positively correlated with the mean peak ratio; however, no correlation was found with the mean NSS/s. Moreover, in MG patients with fatigue, an increased peak ratio with normal NSS/s was observed, indicating a decrease in mean MUP amplitudes. This likely results from a reduction in the diameter, number, or density of the MFs that generate the MUs. We postulated that the increased peak ratio observed with small-amplitude MUPs is more likely associated with morphological or physiological changes in individual MFs rather than neuromuscular transmission failure. This hypothesis is supported by the lack of any correlation between myopathic findings and jitter or block measurements, despite the significantly higher disease severity and longer disease duration in patients with fatigue than in those without fatigue. However, decreased MUs recruitment, or failure to increase the MUs firing rate may have masked the expected increase in NSS/s in MG patients with fatigue. Consequently, changes in corticomotor output may contribute to a reduced force during maximal contractions, in addition to difficulties in initiating and sustaining voluntary muscle activities [13].

Muscle (or physical) fatigue is a complex phenomenon that arises from both central and peripheral origins, and contributes to the fatigue experienced by NMDs [6,9–11]. The central origin of fatigue is associated with reduced MU recruitment and discharge rates, whereas the peripheral origin of fatigue involves various biological changes in muscle structure, including increases in metabolite concentrations and alterations in neuromuscular transmission and MF morphology [7,9,12]. The power spectrum of the EMG signal indicated significant difference in the trends in muscle fatigue between patients with MG and healthy subjects. In healthy subjects, fatigue is characterised by a continuous increase in low-frequency power with a stable high-frequency power. Conversely, in patients with MG, an initial increase in power across all frequency bands, including high-frequencies, was observed, followed by a marked decrease across the full frequency bands as fatigue developed [46]. However, recent studies using quantitative measures of muscle fatigue have reported that there was no significant difference in muscle fatigue between the two groups, despite a decrease in baseline muscle strength in patients with MG compared to healthy controls [18,19]. In addition, no association was found between impaired neuromuscular transmission and muscle fatigue [19,47].

This study had several limitations. First, our patient cohort was selected from a broad spectrum of patients with MG, without any distinction based on the antibody type. The presence of low-density lipoprotein receptor-related protein 4 (LRP-4) antibodies was not analysed. However, clinicopathological features, muscular pathological changes, and responses to treatment vary considerably among patients with MG, depending on the antibody positivity [1,48]. Our approach may have overlooked the potential differences in fatigue severity and the underlying pathophysiological mechanisms among MG subgroups. Future large-scale studies involving different MG subgroups are therefore required to evaluate the association between fatigue, electrophysiological findings, and antibody types. Second, although current questionnaires are valid tools for identifying fatigue in patients with NMD, self-reported outcomes are prone to bias [5,14,49]. These assessments are influenced by self-perception and tend to reflect subjective experiences of fatigue rather than objective measures of physical fatigue [3,49]. Additionally, the total questionnaire scores often include psychosocial factors such as sleepiness, mental performance, anxiety, and depression. However, the FSS and FIS have been reliably used across several NMDs, particularly in the clinical assessment of physical fatigue [4,11,14]. In addition to self-reported scales, more objective results can be obtained using quantitative neurophysiological techniques developed to measure physical fatigue, such as peak force measurements and power spectral analyses. These methods may aid in elucidating the underlying pathophysiological origins of physical fatigue [11, 13,18,19]. However, these measurements do not always correlate with perceived fatigue, and should therefore be cautiously applied in clinical practice.

In conclusion, our findings further support the presence of morphological alterations in patients with MG, and demonstrate that myopathic patterns differ between patients with and without fatigue. Specifically, MG patients with fatigue exhibited a myopathic IP pattern characterised by a high peak ratio with normal NSS/s in the biceps muscle. This pattern is primarily attributed to the decreased amplitude of activated MUPs due to morphological changes or functional loss of some MFs. Additionally, the impaired central drive resulting from the underlying chronic pathophysiological process may have contributed to the reduced recruitment of large MUs. As such, the pathophysiology of fatigue in the MG appears to be multifactorial, and likely involves both peripheral and central factors. To the best of our knowledge, this is the first study to report the relationship between fatigue and myopathic findings in MG. In this context, therapeutic approaches targeting the underlying pathophysiological mechanisms may be more effective at treating fatigue in MG.

CRediT authorship contribution statement

Didem Savaşcı: Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Data curation. **Metin Mercan:** Writing – original draft, Methodology, Investigation. **Vildan Yayla:** Writing – review & editing, Visualization, Formal analysis, Data curation, Conceptualization.

Data availability

The data used in this study are available from the corresponding author upon reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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