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ORIGINAL COMMUNICATION

Quantification of saccadic fatigability and diagnostic efficacy for myasthenia gravis

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Abstract

Background and Objectives The diagnostic challenge of myasthenia gravis (MG) is exacerbated by the variable efficacy of current testing methodologies, necessitating innovative approaches to accurately identify the condition. This study aimed to assess ocular muscle fatigue in patients with MG using video-oculography (VOG) by examining repetitive saccadic eye movements and comparing these metrics to those of healthy control participants.

Methods This prospective, cross-sectional study was conducted at a tertiary care center and involved 62 patients diagnosed with MG (48 with ocular MG and 14 with generalized MG) and a control group of 31 healthy individuals, matched for age and sex. The assessment involved recording saccadic eye movements within a $\pm 15°$ range, both horizontally and vertically, at a rate of 15 saccades per minute over a 5-min period, resulting in 75 cycles. Participants were afforded a 3-min rest interval between each set to mitigate cumulative fatigue. The primary outcome was the detection of oculomotor fatigue, assessed through changes in saccadic waveforms, range, peak velocity, latency, and the duration from onset to target, with a focus on comparing the second saccade against the average of the last five saccades.

Results In the evaluation of repetitive saccadic movements, patients with MG exhibited a reduced saccadic range and a prolonged duration to reach the target, compared to healthy subjects. Furthermore, a significant elevation in the frequency of multistep saccades was observed among MG patients, with a marked rise observed over consecutive trials. Receiver operating characteristic (ROC) analysis revealed the discriminative performance of multistep saccade frequency, in conjunction with variations in saccadic range and duration from onset to target achievement between the second saccade and the mean of the final five saccades, as effective in distinguishing MG patients from healthy subjects. Although alterations in peak saccadic velocity and latency were less pronounced, they were nevertheless detectable.

Discussion The utilization of VOG for repetitive saccadic testing in the diagnosis of MG has demonstrated considerable diagnostic precision. This methodology affords significant accuracy in evaluating ocular muscle fatigue in MG patients, providing class III evidence supportive of its clinical application.

Keywords Myasthenia gravis (MG) · Saccade · Saccade quantification · Oculomotor fatigue · Video-oculography (VOG) · Diagnostic implications

Introduction

Myasthenia Gravis (MG) is an autoimmune disorder characterized by impaired neuromuscular transmission, resulting in fatigable, fluctuating muscle weakness [1]. It represents the most prevalent disorder of neuromuscular transmission, primarily affecting adults, with an incidence rate ranging from 0.3 to 2.8 per 100,000 individuals [2]. The onset of symptoms often initially affects ocular muscles, manifesting as double vision or ptosis, and may extend to impact facial, neck, bulbar, limb, and respiratory muscles [3]. The diagnostic process for MG encompasses a

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comprehensive medical history, clinical examination, and specific laboratory tests, including autoantibody detection and electrophysiologic and pharmacologic assessments [3, 4]. While serology for acetylcholine receptor (AChR) and muscle-specific kinase (MuSK) antibodies is pivotal in raising suspicion for MG, its diagnostic efficacy is tempered by variable sensitivity and specificity, alongside the delay in seroconversion, which compels the consideration of alternative diagnostic strategies [1]. The heterogeneity in antibody detection rates, notably for AChR and MuSK, contributes to a subset of patients being classified as seronegative [4, 5]. The sensitivity for newly recognized antibodies, such as those against lipoprotein-receptor-related protein 4 (LRP4), is currently limited to advanced research facilities. The diagnosis of ocular MG, where symptoms are confined to the extra-ocular muscles, poses additional challenges. While recent advances in non-invasive diagnostic techniques, like repetitive ocular vestibular-evoked myogenic potential (oVEMP), show promise for detecting decreased extra-ocular muscle activity in MG, they are restricted by the need for specialized equipment [6]. Furthermore, although single-fiber electromyography (SFEMG) exhibits high sensitivity for MG detection, its specificity is influenced by a range of factors [7, 8].

In response to the need for improved diagnostic methodologies for MG, our latest research underscores the importance of diminished oculomotor range following repetitive saccades as an indicator of MG-related oculomotor fatigue [9]. Employing three-dimensional video-oculography (VOG), this study aims to precisely assess the impact of fatigue on saccadic eye movements in MG patients relative to healthy individuals. Prior investigations have identified reductions in oculomotor range post-repetitive saccadic movements; the current study seeks to further explore detailed saccadic parameters. The primary objective is an in-depth analysis of saccadic eye movements using three-dimensional VOG, striving to distinguish MG patients from healthy controls effectively.

Methods

Study design and participants

This cross-sectional study was conducted at the Jeonbuk National University Hospital, South Korea, from August 2022 to September 2023, adhering to the Declaration of Helsinki. All participants provided written informed consent, with the study receiving approval from the Institutional Review Board of Jeonbuk National University Hospital (Approval No. 2022-04-044-001). Patients diagnosed with MG were identified in the Neurology Department, with diagnoses confirmed by clinical evidence of fluctuating muscle weakness and supportive tests, including serological tests for Acetylcholine Receptor (AchR) or Muscle-Specific Kinase (MuSK) antibodies, the neostigmine test, and/or significant decrement in repetitive nerve stimulation (RNS). Patients were categorized as having ocular MG (OMG) if symptoms were limited to ocular muscles, or generalized MG (GMG) if symptoms extended to facial, bulbar, neck, or limb muscles. Age- and sex-matched healthy controls (HCs) without neuromuscular or neurological disorders were also included.

Quantitative measures of saccades

A single video-oculography (VOG) session was conducted for each participant by a trained examiner in a dimly lit room, using a three-dimensional VOG system (SMI, Teltow, Germany), capturing eye movements at a 60 Hz sampling rate and 0.1° resolution during saccadic tasks. To ensure optimal testing conditions, participants were asked to avoid caffeine, alcohol, acetylcholinesterase inhibitors for 24 h, and corticosteroids for 72 h before the test. The session included a comprehensive five-point calibration for both eyes, followed by saccadic tasks covering ± 15° in both horizontal and vertical directions, with targets alternating every second at a frequency of 15 saccades per minute (0.25 Hz) across 75 cycles. To reduce the impact of cumulative fatigue, a 3-min rest was allowed between trials. A forehead bar stabilized participants' heads at a viewing distance of 1.5 m, with custom software facilitating calibration, target presentation, and blink-related deviation filtering.

Data analysis, performed using MATLAB, focused on saccadic range, peak velocity, latency, time to target, and saccade waveform (Fig. 1). Saccadic onset and end were determined based on eye velocity thresholds of 30°/sec, and the end of a saccade was marked when it dropped below this threshold [10, 11]. The duration from saccade onset to target was calculated from the onset time to the time of eye's arrival at the target. Peak velocity was identified as the highest velocity during the saccade [11], and latency measured the time from target presentation to saccade initiation [12, 13].

The analysis aimed to identify the pulse and step components of saccades, assessing their accuracy in achieving a normometric refixation. The saccadic waveforms were categorized as 'single-step' or 'multiple-step' based on eye position and velocity time series analyses (Fig. 1). Multiple-step saccades characterized by distinctive velocity profiles suggestive of premature saccade termination. This classification into hypometric or hypermetric saccades within the multiple-step category relied on analyzing their segmented refixation patterns. In contrast to the straightforward trajectory of single-step saccades,

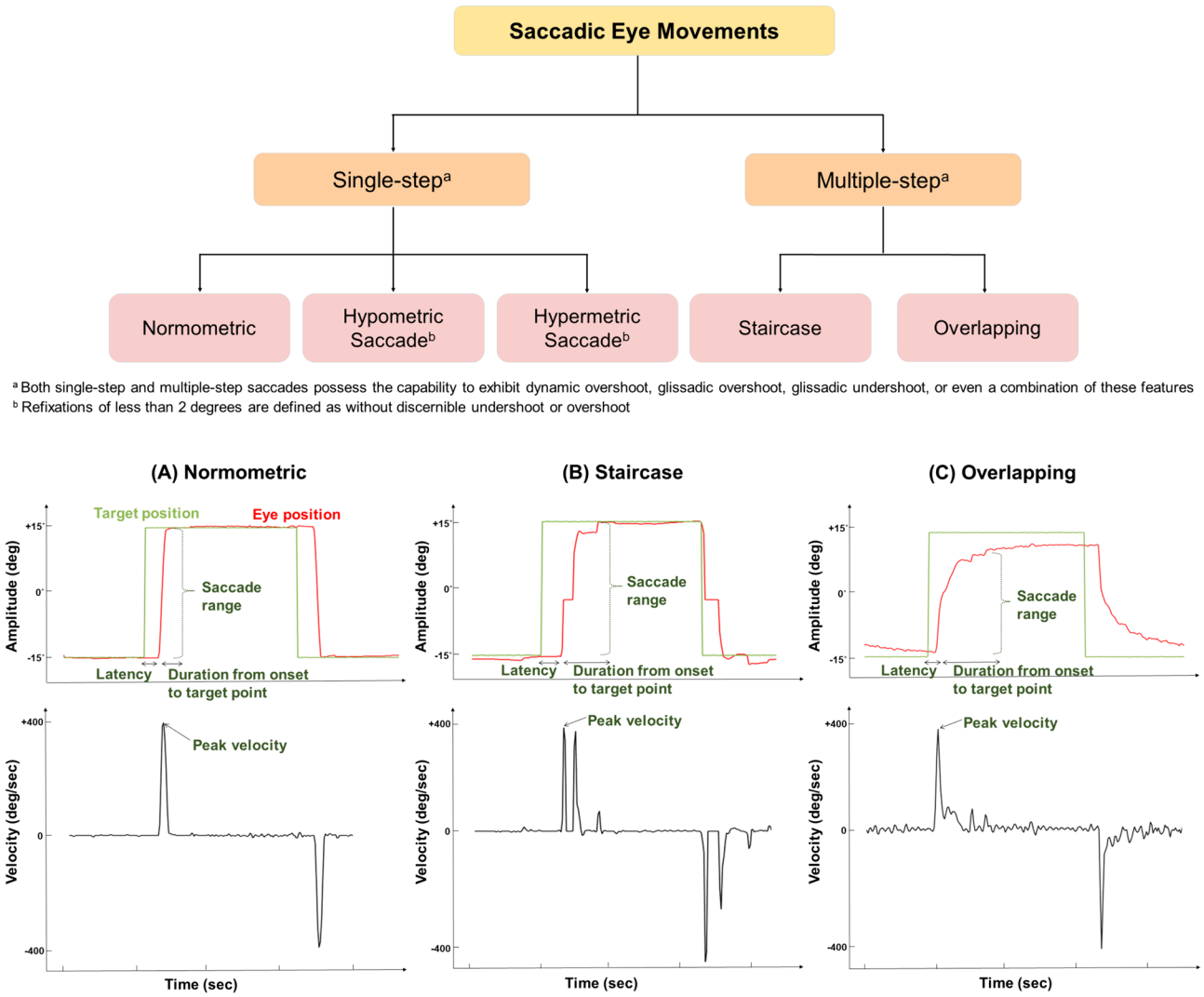


Fig. 1 Classification of saccadic eye movements. **A** Normal saccadic eye movement dynamics. This panel illustrates the trajectory of a typical saccadic eye movement as the participant's gaze sequentially follows a moving target. The green line denotes the position of the target, while the red line represents the participant's eye movement. Key metrics quantified include latency (the time interval between target onset and the beginning of the saccade), peak velocity (maximum velocity during saccade execution), range (total degree of eye movement as the participant follows the target position), and duration from onset to target (the total duration of the participant's eye movement while tracking the target). **B** Depicts staircase-type saccades in patients with MG. Here, the eye velocity trace drops to zero after reaching the first peak, indicating an intersaccadic interval, followed by several small discrete saccades. **C** Demonstrates that MG patients also exhibit multiple hypometric saccades. These occur with no latency or intersaccadic interval between them, forming an overlapping type of multiple-step saccadic pattern

multiple-step saccades featured several distinct saccadic pulses. These were further categorized into 'staircase' or 'overlapping' types, based on their velocity profiles and patterns of refixation [14]. 'Staircase' saccades were marked by successive short-latency intervals (Fig. 1B), whereas 'overlapping' saccades occurred with negligible latency periods between movements (Fig. 1C) [15, 16]. Both saccade types were observed to potentially exhibit dynamic, glissadic overshoots, undershoots, or a mix of these behaviors. This classification provided insights into ocular motor function dynamics and the physiological basis of saccadic refixations, typically involving minor corrections for overshoots or undershoots [14]. A significant refixation discrepancy was defined as movements exceeding 2° in amplitude [17].

Oculomotor performance fatigability was assessed by comparing the second saccade's performance to the average performance of the last five saccades. The second saccade was used as a representative baseline measure, reflecting the initial state of the oculomotor system before significant fatigue onset. Using the second saccade minimizes the influence of initial adjustments that often impact the first saccade, thereby enhancing consistency and reliability

by reducing variability associated with initial subject acclimatization. The average performance of the last five saccades served as an indicator of accumulated fatigue effects over the testing period, capturing the progressive decline in saccadic performance—a critical marker of oculomotor fatigability in MG patients. Averaging these saccades reduces the impact of any single anomalous saccade, thus providing a more robust measure of fatigability.

Statistical analysis

The analysis was performed utilizing SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, USA) and R software version 4.3.0. Categorical data were summarized as frequencies and proportions, while continuous variables, not adhering to normal distribution, were presented as median values with 95% confidence intervals (CIs). Group comparisons, both between MG patients and HCs and within MG subgroups (ocular vs. generalized MG), were conducted using Fisher's exact test for categorical variables and the Mann–Whitney U test for continuous variables. A p-value of less than 0.05 was considered statistically significant. The ability of saccadic parameters to discriminate MG patients from HCs was evaluated through receiver operating characteristic (ROC) analysis using the R package pROC, with the analysis yielding the area under the ROC curve (AUC), sensitivity, specificity, predictive values, and the statistically significant cutoff value for optimal discrimination.

Data sharing statement

The raw data for this study were collected at Jeonbuk National University Hospital. The derived data supporting the conclusions of this investigation are available from the corresponding author [S.-Y Oh], upon reasonable request.

Results

Study population

This cohort consisted of 62 patients diagnosed with MG, including 48 individuals (77%) with ocular MG (OMG) and 14 (23%) with generalized MG (GMG). Additionally, age- and sex-matched 31 HCs were recruited for comparative analysis. Table 1 delineates the comprehensive demographic and clinical characteristics. No statistically significant differences were noted between the OMG and GMG groups regarding sex, age, and duration of disease. The prevalence of ptosis was significantly higher in the OMG group (91.7%) than in the GMG group (57.1%) ($p = 0.006$, Mann–Whitney U test). AchR antibodies were elevated in 43 participants (69.4% of the study population), with a higher incidence observed in the GMG subgroup (92.9%) compared to the OMG subgroup (62.5%) ($p = 0.046$, Mann–Whitney U test). MuSK antibodies were identified in a single patient (1.6%). Repetitive nerve stimulation (RNS) tests indicated abnormalities in 24 patients (38.7%), more frequently in the GMG group (78.6%) versus the OMG group (27.1%) ($p = 0.001$, Mann–Whitney U test). The edrophonium test showed abnormal results in 35 participants (56.4%), with no significant difference between MG subgroups ($p = 0.76$, Mann–Whitney U test). Thymoma was detected in 8 patients (12.9%), and thymic hyperplasia was observed in 11 individuals (17.7%). Eligibility for the study required at least one abnormal test result among these evaluations, thereby excluding individuals with normal findings in all tests.

Quantitative analysis of saccades

Our study undertook a comprehensive evaluation of saccadic parameters, including saccadic range, peak velocity, latency, and time from onset to target, alongside the classification of saccade waveforms. Initial comparisons for the second saccade revealed no significant differences in horizontal and vertical saccadic ranges and peak velocities between MG patients and HCs (Table 2). However, MG patients exhibited longer times from onset to target for both horizontal (130.1 ms vs. 110.9 ms; $p = 0.069$) and vertical saccades (130.4 ms vs. 122.4 ms; $p = 0.14$), although these differences were not statistically significant. Subsequent analysis of the average of the last five saccades indicated a considerable reduction in saccadic range for MG patients compared to HCs in both horizontal and vertical planes (23.9° vs. 27.0° and 23.0° vs. 25.6°, respectively; $p < 0.001$ for both) and significantly extended durations from saccade onset to target (horizontal: 227.8 ms vs. 128.9 ms; vertical: 226.4 ms vs. 136.6 ms; $p < 0.001$ for both). A decrease in vertical saccadic peak velocity was noted for MG patients (355.8°/s vs. 400.9°/s; $p = 0.02$), with no significant differences in horizontal peak velocities. Latencies for horizontal and vertical saccades between MG patients and HCs did not differ significantly (Table 2).

Comparative analysis from the second to the average of the last five saccades within MG patients showed marked declines in saccadic ranges and increased durations from onset to target, with vertical saccadic latency notably prolonged; however, no significant changes were observed in peak velocities or horizontal saccadic latency (Fig. 2 and Table 2). Subgroup analysis comparing OMG and GMG revealed no significant differences in saccadic measurements. Supplementary Table 1 that divides the parameters for the more and less affected eyes, reveals that significant reductions in saccade range and increases in

Table 1 Clinical characteristics of myasthenia gravis (MG) patients and healthy control (HC) group

	MG (n = 62)				HC (n = 31)	p-value^{F}
	Ocular MG (n = 48)	Generalized MG (n = 14)	p-value^{F,M}	Total (n = 62)		
Age						
< 50 years, n (%)	18 (37.5)	7 (50)	0.538	25 (40.3)	16 (51.6)	0.232
≥ 50 years, n (%)	30 (62.5)	7 (50)		37 (59.7)	15 (48.4)	
Gender						
Female, n (%)	18 (37.5)	7 (50)	0.538	25 (40.3)	17 (54.8)	0.352
Male, n (%)	30 (62.5)	7 (50)		37 (59.7)	14 (45.2)	
Clinical presentation						
Ptosis, n (%)	44 (91.7)	8 (57.1)	**0.006**	52 (83.8)	–	–
Diplopia, n (%)	45 (93.8)	11 (78.6)	0.12	56 (90.3)	–	–
Bulbar symptom, n (%)	–	7 (50)	–	9 (14.5)	–	–
Limb weakness, n (%)	–	12 (85.7)	–	12 (19.4)	–	–
Disease duration (month), mean ± SD	52.46 ± 80.21	45.4 ± 46.4	0.09	50.87 ± 73.67	–	–
Antibody positivity						
AchR antibody, n (%)	30 (62.5)	13 (92.9)	**0.046**	43 (69.4)	–	–
Musk antibody, n (%)	–	1 (7.1)	–	1 (1.6)	–	–
AchR antibody titer (nM), mean ± SD	2.2 ± 3.88	7.49 ± 6.7	**0.003**	3.4 ± 5.1	–	–
Repetitive nerve stimulation test positivity, n(%)	13 (27.1)	11 (78.6)	**0.001**	24 (38.7)		
Edrophonium test positivity, n (%)	28 (58.3)	7 (50)	0.76	35 (56.4)	–	–
Chest CT						
Thymoma, n (%)	7 (14.6)	1 (7.1)	0.851	8 (12.9)	–	–
Thymic hyperplasia, n (%)	8 (16.7)	3 (21.4)	0.75	11 (17.7)	–	–
Not tested, n (%)	1 (2.1)	0 (0)	–	1 (1.6)	–	–

AchR, acetylcholine receptor; *F*; Fisher's exact test; IQR, interquartile range; M, Mann–Whitney *U* test; MG, myasthenia gravis; SD, standard deviation

The bold indicates the section headings within the table

duration from onset to target were observed in both the more and less affected eyes (Supplementary Table 1).

Regarding saccadic waveforms, MG patients frequently exhibited multiple-step saccades characterized by distinctive velocity profiles suggestive of premature saccade termination (Fig. 1). Over a session of 75 saccadic cycles, the occurrence of both 'staircase' and 'overlapping' saccade types was significantly higher in MG patients than in healthy controls (HCs) for both horizontal (39.1% vs. 29.9%, $p < 0.001$) and vertical saccades (45.6% vs. 24.6%, $p < 0.001$), as shown in our results. No significant differences were found between MG subgroups in these patterns (Table 3).

Receiver operating characteristic (ROC) curve analysis

The discriminative ability of the frequency of multistep saccades, along with changes in saccadic range and time from onset to target between the second saccade and the average of the last five saccades, to distinguish MG patients from HCs was assessed using ROC curve analysis (Fig. 3A–C). For the proportion of multistep saccades, the area under the curve (AUC) was 0.788 for horizontal saccades (95% CI 0.676–0.901; $p < 0.001$), with an optimal cutoff value of 31.82%, achieving a sensitivity of 80.6% and specificity of 83.9%. In vertical saccades, the AUC was 0.822 (95% CI 0.728–0.916; $p < 0.001$), with a best cutoff value of 33.7%, yielding a sensitivity of 82.3% and specificity of 74.2% (Fig. 3A). For the decrement in saccadic range, the AUC values were 0.702 for horizontal saccades (95% CI 0.587–0.817; $p < 0.001$), with a best cutoff value of − 8%, corresponding to a sensitivity of 64.5% and specificity of 90.3%, and 0.772 for vertical saccades (95% CI 0.655–0.890; $p < 0.001$), with a cutoff value of − 6.6%, leading to a sensitivity of 75.8% and specificity of 83.9% (Fig. 3B). The analysis of increased duration from onset to target yielded AUC values of 0.795 for horizontal saccades (95% CI 0.701–0.888; best cutoff value, 49.2%; sensitivity 71.0%, specificity 96.8%) and 0.792 for vertical saccades (95% CI, 0.699–0.884; best cutoff value, 57.7%; sensitivity 56.5%, specificity 96.8%) (Fig. 3C).

Table 2 Saccadic parameter analysis comparing the second saccade with the average of the last five saccades in both eyes of MG patients and the healthy control group

	MG (n = 62)				HC (n = 31)	p-value^{M} (MG vs. HC)
	Ocular MG (n = 48)	Generalized MG (n = 14)	p-value^{M}	Total (n = 62)		
The second saccade (horizontal)						
Ranges, deg, median (95% CI)	27.2 (24.8, 29.5)	27.9 (26.7, 29.1)	0.46	27.8 (26.7, 28.8)	28.5 (26.9, 30.2)	0.5
Peak velocity, deg/s, median (95% CI)	419.5 (397.9, 441.0)	416.3 (364.9, 467.8)	0.78	418.8 (399.2, 438.3)	410.6 (382.8, 438.3)	0.63
Latency, ms, median (95% CI)	251.7 (237.4, 266.1)	262.7 (229.3, 296.1)	0.96	254.2 (241.2, 267.2)	226.0 (214.2, 237.8)	**0.008**
Duration from onset to target point, ms, median (95% CI)	129.2 (114.3, 144.1)	133.1 (105.0, 161.1)	0.78	130.1 (117.3, 142.8)	110.9 (99.7, 122.2)	0.069
Mean of the last 5 saccades (horizontal)						
Ranges, deg, median (95% CI)	22.4 (19.7, 25.2)	24.3 (22.9, 25.6)	0.22	23.9 (22.7, 25.1)	27.0 (25.1, 29.0)	**0.001**
Peak velocity, deg/s, median (95% CI)	408.2 (381.2, 435.1)	380.9 (327.5, 434.4)	0.16	402.5 (370.7, 434.3)	402.0 (378.5, 425.6)	0.4
Latency, ms, median (95% CI)	258.6 (241.5, 275.7)	258.6 (221.3, 295.9)	0.92	258.6 (243.4, 273.7)	235.3 (219.5, 251.1)	0.49
Duration from onset to target point, ms, median (95% CI)	230.2 (209.7, 250.7)	219.5 (186.3, 252.7)	0.62	227.8 (210.6, 244.9)	128.9 (121.5, 136.4)	**< 0.001**
Difference between the second saccade and the mean of the last 5 saccades						
Ranges, deg, median (95% CI)	− 4.8 (− 8.1, − 1.4)	− 3.7 (− 4.8, − 2.5)	0.67	− 3.9 (− 5.0, − 2.8)	− 1.5 (− 3.4, 0.39)	**0.002**
Peak velocity, deg/s, median (95% CI)	− 16.3 (− 42.7, − 23.7)	− 26.4 (− 73.7, 20.8)	0.29	− 18.6 (− 41.0, 3.7)	− 22.6 (− 46.6, 1.5)	0.5
Latency, ms, median (95% CI)	9.8 (− 3.5, 23.0)	8.3 (− 6.2, 22.7)	0.46	9.4 (1.2, 20.0)	15.4 (0.67, 30.2)	0.33
Duration from onset to target point, ms, median (95% CI)	101.0 (81.7, 120.4)	86.4 (41.4, 131.4)	0.6	97.7 (80.2, 115.2)	17.9 (9.9, 26.1)	**< 0.001**
The second saccade (vertical)						
Ranges, deg, median (95% CI)	27.9 (26.7, 29.1)	26.7 (23.2, 30.3)	0.88	27.6 (26.4, 28.8)	27.6 (25.5, 29.7)	0.71
Peak velocity, deg/s, median (95% CI)	371.2 (246.6, 395.7)	374.9 (314.7, 435.2)	0.50	372.0 (349.6, 394.5)	389.9 (258.7, 421.2)	0.45
Latency, ms, median (95% CI)	249.7 (235.3, 264.0)	256.7 (221.1, 292.2)	0.58	251.3 (238.0, 264.4)	250.5 (231.1, 269.9)	0.50
Duration from onset to target point, ms, median (95% CI)	127.1 (113.3, 140.9)	142.0 (118.1, 165.8)	0.33	130.4 (118.7, 142.2)	122.4 (107.7, 137.0)	0.14
Mean of the last 5 saccades (vertical)						
Ranges, deg, median (95% CI)	23.1 (21.7, 24.4)	22.9 (19.8, 25.9)	0.96	23.0 (21.8, 24.2)	25.6 (23.3, 27.9)	**0.01**
Peak velocity, deg/s, median (95% CI)	356.5 (331.3, 381.7)	353.3 (292.3, 414.3)	0.82	355.8 (332.8, 378.8)	400.9 (369.9, 431.9)	**0.02**
Latency, ms, median (95% CI)	266.5 (249.8, 283.2)	273.6 (236.5, 310.6)	0.71	268.1 (253.2, 283.0)	250.1 (234.5, 265.6)	0.18

Table 2 (continued)

	MG (n = 62)				HC (n = 31)	p-value^{M} (MG vs. HC)
	Ocular MG (n = 48)	Generalized MG (n = 14)	p-value^{M}	Total (n = 62)		
Duration from onset to target point, ms, median (95% CI)	225.7 (206.1, 245.4)	228.8 (189.6, 267.9)	0.93	226.4 (209.4, 243.5)	136.6 (128.5, 144.7)	**< 0.001**
Difference between the second saccade and the mean of the last 5 saccades						
Ranges, deg, median (95% CI)	− 4.8 (− 5.9, − 3.8)	− 3.9 (− 6.0, − 2.1)	0.47	− 4.6 (− 5.5, − 3.7)	− 1.9 (− 3.9, − 0.03)	**< 0.001**
Peak velocity, deg/s, median (95% CI)	− 37.5 (− 62.5, − 12.6)	− 7.8 (− 32.4, 16.9)	0.10	− 30.8 (− 50.8, − 10.8)	− 0.7 (− 33.4, 32.1)	0.06
Latency, ms, median (95% CI)	16.1 (1.0, 31.3)	10.1 (− 20.1, 40.3)	0.47	14.8 (1.6, 27.9)	2.38 (− 13.9, 18.7)	**0.002**
Duration from onset to target point, ms, median (95% CI)	98.7 (74.2, 123.2)	86.8(47.5, 125.9)	0.55	95.9 (85.5, 116.4)	14.3 (0.3, 28.2)	**< 0.001**

The bold indicates the section headings within the table

CI, confidence interval; M, Mann–Whitney *U* test; MG, myasthenia gravis

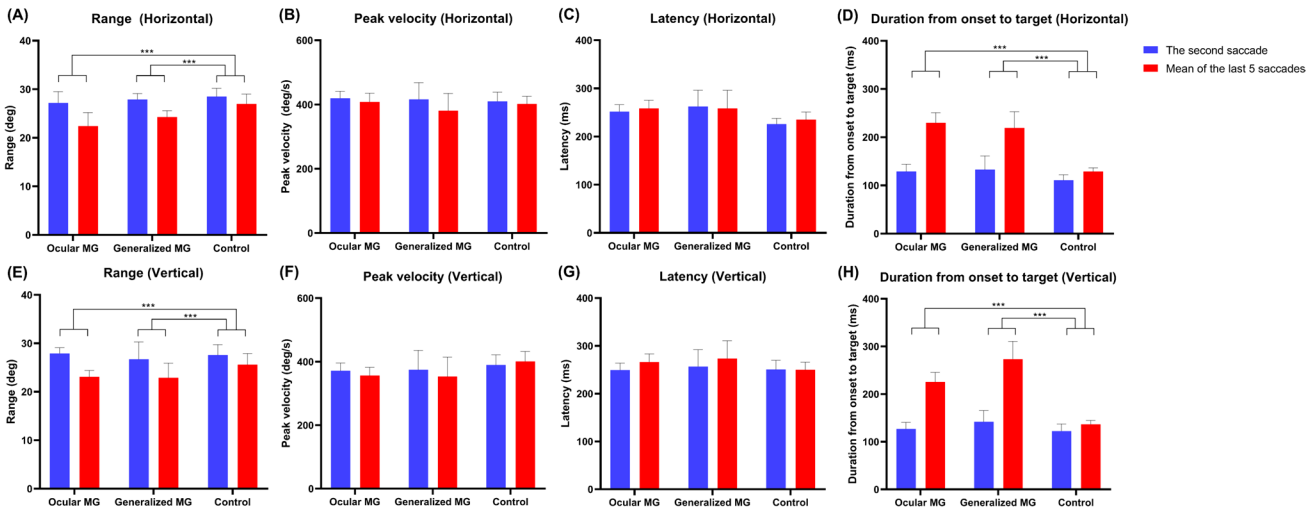


Fig. 2 Analysis of saccadic parameters. Analysis of various saccadic parameters for the second saccade (blue) and the mean of the last 5 saccades (red) in ocular MG, generalized MG, and control groups for horizontal saccades (**A–D**) and vertical saccades (**E–H**). The difference between the second saccade and the mean of the last 5 saccades, reduced range, and increased duration from onset to the target point was significant in MG patients compared to HCs. Subgroup analysis comparing ocular MG and generalized MG did not reveal a significant difference

Discussion

This investigation aimed to determine if VOG quantification of saccadic eye movements could distinguish MG patients from HCs by identifying signs of oculomotor fatigue. Building upon prior research, this study incorporated a larger cohort and examined a broad array of saccadic parameters, including latency, peak velocity, saccadic range, duration from onset to target, and waveform patterns. Our analyses revealed marked differences in saccadic range and the time from onset to target between the second saccade and the average of the last five saccades, suggesting oculomotor fatigue. Notably, the frequency of multistep saccades was significantly higher in MG patients. The ROC curve analysis validated the utility of these saccadic parameters especially the frequency of multistep saccades, reduced saccadic range, and extended saccade duration from onset to target, as robust metrics for differentiating MG patients from healthy subjects.

Table 3 Saccadic wave analysis of MG patients and control group

	MG (n = 62)				HC (n = 31)	p-value^{M} (MG vs. Control)
	Ocular MG (n = 48)	Generalized MG (n = 14)	p-value (OMG vs. GMG)	Total (n = 62)		
Horizontal saccades type (%)						
Single-step, median (95% CI)	60.6% (57.6, 63.7%)	64.1% (57.6, 70.6%)	0.252	61.4% (58.7, 64.1%)	72.0% (67.3, 76.8%)	**< 0.001**
Multiple-step, median (95% CI)	40.0% (36.7, 43.3%)	35.8% (29.4, 42.3%)	0.181	39.1% (36.6, 41.9%)	29.9% (26.8, 32.7%)	**< 0.001**
Staircase, median (95% CI)	37.3% (34.0, 40.5%)	31.4% (23.1, 39.7%)	0.170	36.0% (32.9, 39.0%)	27.9% (23.2, 32.7%)	**< 0.001**
Overlapping, median (95% CI)	2.7% (0.8, 4.6%)	4.4% (0.0, 10.5%)	0.800	3.1% (1.2, 5.0%)	0.04% (0.0, 0.1%)	**0.012**
Vertical saccades type (%)						
Single-step, median (95% CI)	55.1% (50.6, 59.6%)	55.0% (44.9, 65.1%)	0.711	55.1% (51.0, 59.1%)	75.3% (69.3, 81.3%)	**< 0.001**
Multiple-step, median (95% CI)	45.7% (41.4, 50.1%)	45.0% (34.8, 55.1%)	0.590	45.6% (41.6, 49.5%)	24.7% (18.7, 30.7%)	**< 0.001**
Staircase, median (95% CI)	38.4% (33.1, 43.7%)	40.1% (28.1, 52.2%)	0.953	38.8% (34.1, 43.5%)	24.4% (18.4, 30.4%)	**0.001**
Overlapping, median (95% CI)	7.3% (4.1, 10.6%)	4.8% (0.0, 10.3%)	0.514	6.8% (4.0, 9.5%)	0.3% (0.0, 0.6%)	**< 0.001**

The bold indicates the section headings within the table

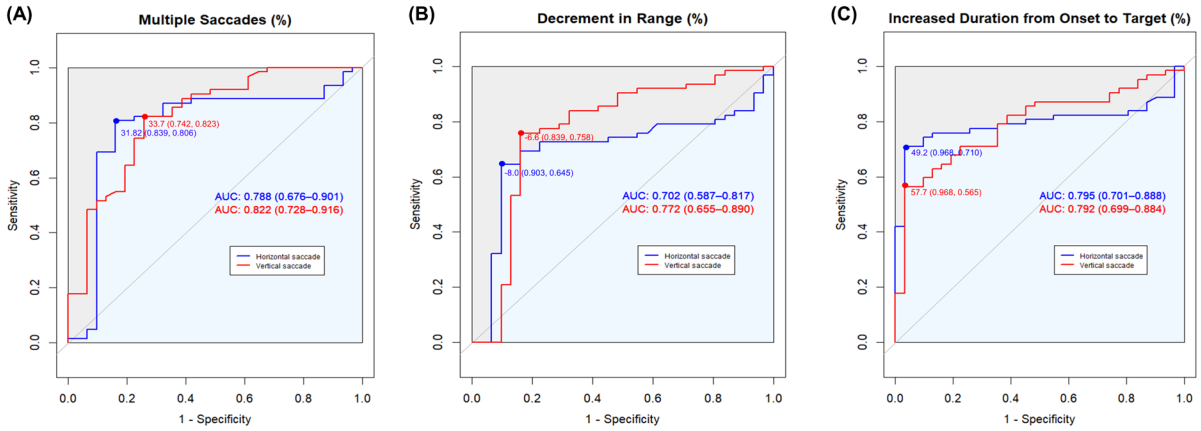
CI, confidence interval; GMG, generalized myasthenia gravis; M, Mann–Whitney *U* test; MG, myasthenia gravis; OMG, ocular myasthenia gravis

Saccades are rapid eye movements that transition the gaze between fixation points [11], requiring precise coordination of the burst and step phases for initiation and stabilization, respectively [18]. The examination of these movements is crucial for understanding ocular myasthenia's pathogenesis and contributing to its differential diagnosis [19–22]. Changes in saccadic properties, such as gain, accuracy, velocity, and latency after extended saccadic tasks, have been implicated in indicating fatigue [23, 24]. The vulnerability of extraocular muscles (EOMs) to neuromuscular transmission failure is heightened due to their reduced safety factor, exacerbated by exercise-induced stress [19, 25–29]. Additionally, the oculomotor system's central control mechanisms may face challenges in quickly compensating for asymmetries and varying deficits in EOMs, which heavily depend on visual feedback.

Our research explored the decline in saccadic ranges with consecutive 30° saccades over a series of 75 cycles. In a controlled comparison, individuals with MG demonstrated a notable diminution in saccadic range during a 10° downward vertical saccade relative to healthy participants [30]. This finding is consistent with a noticeable decline in saccadic range after three minutes of continuous 30° saccades [31], reinforcing the concept of oculomotor fatigability in MG as previously identified in our and other research. These observations of reduced oculomotor range, both in saccadic and smooth pursuit movements after sustained eye activity, serve as markers of oculomotor fatigue in MG [9, 32].

Studies examining saccadic peak velocities in MG patients have yielded varied results [31]. Some studies report a reduction in peak velocity following repetitive eye movements, indicating variable signs of fatigue among MG patients, which are not consistently observed across the spectrum [20–22, 31, 33]. Noteworthy is the use of binocular phase plane analysis to uncover disconjugacy in saccade velocities, a feature initially absent in MG patients who later exhibit this characteristic, contrasting with conditions such as internuclear ophthalmoplegia or cranial nerve palsy [33]. The decrement in velocity observed in a subset of MG patients suggests a relationship with the amplitude of the target and the influence of the orbit's restricting forces on maintaining normal saccade velocity [34]. Our study aligns with these findings, observing a trend towards reduced mean peak velocity in the latter saccades, although these differences were not statistically significant.

Saccadic latency, the time elapsed from stimulus presentation to the initiation of eye movement, is generally around 200 ms [35]. Previous research indicated no



	Cut-off values (%)	Sensitivity,% (95% CI)	Specificity,% (95% CI)	PPV,% (95% CI)	NPV,% (95% CI)	AUC (95% CI)	*p-value*
Multistep saccade, % (Horizontal)	31.82	80.6 (70.8-90.5)	83.9 (70.9-96.8)	90.9 (83.3-98.5)	68.4 (53.6-83.2)	0.788 (0.676-0.901)	<0.001
Multistep saccade, % (Vertical)	33.70	82.3 (72.7-91.8)	74.2 (58.8-89.6)	0.864 (77.7-95.2)	67.6 (51.9-83.4)	0.822 (0.728-0.916)	<0.001
Decrement of range, % (Horizontal)	-8.0	64.5 (52.6-76.4)	90.3 (79.9-100)	93.0 (85.4-100)	56.0 (42.2-69.8)	0.702 (0.587-0.817)	0.001
Decrement of range, % (Vertical)	-6.6	75.8 (65.1-86.5)	83.9 (70.9-96.8)	90.4 (82.4-98.4)	63.4 (48.7-78.2)	0.772 (0.655-0.890)	<0.001
Increment of duration from onset to target , % (Horizontal)	49.2	71.0 (59.7-82.3)	96.8 (90.6-100)	97.8 (93.5-100)	62.5 (48.8-76.2)	0.795 (0.701-0.888)	<0.001
Increment of duration from onset to target , % (Vertical)	57.7	56.5 (44.1-68.8)	96.8 (90.6-100)	97.2 (91.9-100)	52.6 (39.7-65.6)	0.792 (0.699-0.884)	<0.001

Fig. 3 The receiver operating characteristic (ROC) analysis. The ROC analysis in our study produced a fitted ROC curve, illustrating the effectiveness of multistep saccades and the differences in range and duration from onset to target between the second saccade and the average of the last five saccades in distinguishing between MG patients and HCs. **A** The area under the curve (AUC) for the proportion of multistep saccade was 0.788 for horizontal saccades and 0.822 for vertical saccades, indicating that vertical saccades may be a more reliable predictor of MG diagnosis. With the decrease in range (**B**) and increase in duration from onset to target (**C**), the area under the curve (AUC) for vertical and horizontal saccades were depicted. The blue circle shows the optimal cutoff value for horizontal saccades, while the red circle indicates the optimal cutoff value for vertical saccades

significant difference in saccadic latency for 10° saccades between MG patients and controls, but an increased latency was observed in MG patients during 20° saccades [22]. This suggests that although MG pathophysiology might not directly influence saccadic latency, the cumulative fatigue associated with repetitive ocular movements in MG could lead to a slight latency delay. Our investigation aligns with these observations, revealing a modest increase in horizontal saccadic latency among MG subjects [30].

The typical duration of a saccade ranges from 80 to 120 ms. However, our data reveal prolonged saccadic durations within the MG group, often marked by premature cessation or temporary deceleration [10, 20]. Saccades characterized by multiple steps and exceeding 2° in refixation displacement are defined as "fragmented" or "stepwise," [17] a pattern previously linked to cerebellar disorders and increasingly observed in neurological conditions such as Parkinson's disease [15, 17, 36, 37]. The genesis of these multistep saccades likely involves a complex interaction among lower and higher oculomotor structures, including the cerebellum, basal ganglia, and cerebral cortex, along with midbrain elements like omnipause and burst neurons crucial for eye movement control [38, 39]. In the context of MG, computational models have highlighted the role of EOM tonic fiber dysfunction and the resultant hypometric response due to paresis. A compensatory central proprioceptive adjustment seems to modify the pulse-step neural command, triggering repeated, corrective saccades until the target is fixated [40, 41]. Additionally, the high motor neuron firing rates required during saccades in MG may lead to variability in motor commands and subsequent saccadic fragmentation as individual motoneurons lose synchrony [42]. While neuromuscular transmission deficits are evident in MG, an observed adaptive extension in the burst phase of central neural firing, particularly with multistep saccades, suggests a primary central rather than peripheral mechanism. Therefore, the saccadic behavior in MG reflects an intricate balance of central adaptations and peripheral neuromuscular challenges, manifesting in extended saccadic durations and the distinct occurrence of multistep saccades [39, 42].

Fatigue within the saccadic system, manifesting as reduced saccadic range, prolonged duration, and the emergence of multistep saccades, is notably exacerbated by repetitive saccadic activities [29]. These effects illustrate the significant impact of fatigue on the precision and

efficiency of ocular motility in MG. Previous investigations have identified saccadic subtypes displaying intrasaccadic anomalies indicative of fatigue, including a stutter and decrescendo pattern characterized by multiple saccadic fragments in MG patients [39, 43]. Intriguingly, such abnormal saccadic patterns have been observed even in eyes not directly afflicted by MG, which corroborates our findings. The identification of these specific intrasaccadic variations may facilitate the differentiation of MG from other neurological disorders associated with brainstem lesions [44]. Based on our analysis, it is possible that the most diagnostically useful parameter is the frequency of multistep saccades. The frequency of multistep saccades demonstrated the highest diagnostic value, with a ROC analysis showing an AUC of 0.822 for vertical saccades and 0.788 for horizontal saccades. Multistep saccades are indicative of impaired neuromuscular transmission and increased fatigability, which are hallmark features of MG. This parameter effectively captures the cumulative impact of fatigue on the oculomotor system, providing a clear distinction between MG patients and healthy individuals. While the decrement in saccadic range was slightly less effective than the frequency of multistep saccades, it also provided valuable diagnostic insights with an AUC of 0.772 for vertical saccades and 0.702 for horizontal saccades. The parameter of increased duration from onset to target showed significant diagnostic potential, with an AUC of 0.795 for horizontal and 0.792 for vertical saccades, further supporting the identification of MG-related oculomotor fatigue. In addition, our study found that vertical saccades are more affected than horizontal saccades in MG patients (Fig. 3). This difference is likely due to the unique anatomical and physiological characteristics of the EOMs. Notably, a study by Cleary et al. observed that elevator muscle weakness, particularly in the superior rectus and inferior oblique muscles, was more prevalent in MG patients compared to controls [45]. This finding aligns with our previous research, where decrements in repetitive vertical saccades showed the highest diagnostic value, with an AUC of 0.91 ($p < 0.001$; cutoff value, 6.401%; sensitivity, 78.3%; specificity, 95.8%) [9]. Vertical saccades require more precise control and neuromuscular coordination due to gravitational effects and the necessity for maintaining binocular alignment. Therefore, vertical muscles may have a higher proportion of fibers susceptible to fatigability, leading to greater impairment in vertical saccades. Clinically, this underscores the importance of assessing both vertical and horizontal saccades in the diagnostic evaluation of MG. Vertical saccadic impairment may be a more sensitive indicator of oculomotor fatigability.

The limitations of this research warrant consideration. This study's scope, confined to a single tertiary care center, may not encompass the entire spectrum of MG manifestations. Future investigations should adopt a multi-centered approach, encompassing a broader demographic of MG patients and including individuals with ophthalmoplegic conditions not attributed to MG. Additionally, examining patients pre- and post-treatment could further validate our findings.

In summary, our research underscores the diagnostic utility of video-oculography (VOG) for quantifying saccadic eye movements as a non-invasive method for detecting MG. This study represents a significant step forward in quantifying a comprehensive array of saccadic parameters and patterns, offering vital diagnostic insights, particularly when conventional diagnostic tests are inconclusive. The application of eye movement recording, a technique proven in other neurological disorders, holds promise for enhancing early detection and management of MG, with the potential to substantially improve patient outcomes.

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Data availability All individual data of the participants that underlie the results reported in this article will be available after de-identification (manuscript, tables, and figures).

Conflicts of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical standard statement All methods in this study involving human subjects were carried out in compliance with the institutional and/or national research committee's ethical standards, as well as the 1964 Helsinki Declaration and its subsequent revisions or comparable ethical standards.

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