



Diagnostic Yield and Sensitivity of Repetitive Nerve Stimulation at Different Frequencies in the Evaluation of Myasthenia Gravis

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Abstract

Background: Myasthenia gravis (MG) is an autoimmune disorder known by fluctuating muscle weakness due to impaired neuromuscular transmission. A decremental response on repetitive nerve stimulation (RNS) represents the electrical correlate of clinical muscle fatigue and weakness in myasthenic patients. While single-fiber electromyography (SFEMG) remains the most sensitive diagnostic test, it is technically demanding and not always available. RNS is a more accessible tool, though its sensitivity can vary depending on stimulation parameters. **Aim:** This study aimed to assess the diagnostic sensitivity of high-frequency (10 Hz) RNS compared to the conventional 3 Hz protocol in patients with myasthenia gravis. **Methods:** A case-control study was conducted on 45 patients with clinically confirmed MG and 45 age and sex-matched healthy controls. Both groups underwent low-frequency (3 Hz) and high-frequency (10 Hz) RNS after withholding anticholinesterase medication for (10-12) hours. Recordings

from the abductor digiti minimi, trapezius, and nasalis muscles were analyzed for decremental responses in both amplitude and area. **Results:** MG patients showed significant decremental responses at both frequencies ($p < 0.005$). High-frequency RNS demonstrated higher sensitivity in detecting neuromuscular transmission defects, particularly in amplitude and area decrements. **Conclusion:** High-frequency RNS enhances the detection of neuromuscular transmission abnormalities in myasthenia gravis. Its improved sensitivity and ease of application make it a valuable addition to the diagnostic workup, especially when SFEMG is unavailable or not feasible.

1. Introduction:

Myasthenia gravis (MG) is an autoimmune condition affecting the neuromuscular junction, leading to muscle weakness that worsens with continuous use and improves with rest [1]. Clinicians typically suspect MG when patients present with fatigable weakness that is more prominent at the end of the day [2]. It is estimated that around 60–85% of patients initially experience ocular symptoms such as ptosis and diplopia, and many eventually develop generalized disease [3].

Simple bedside tests including Cogan's lid twitch, ice pack application, and the curtain sign can support the diagnosis. Although antibody testing (for anti-AChR, anti-MuSK, and anti-LRP4 antibodies) plays a crucial

role, these tests may be negative, particularly in purely ocular forms, and are not always accessible due to financial and logistical limitations [4].

Neurophysiological studies remain essential in confirming MG, with both Repetitive Nerve Stimulation (RNS) and Single Fiber Electromyography (SFEMG) being valuable tools [5]. SFEMG is considered as the most sensitive test, capable of detecting abnormalities in up to 99% of cases. However, its invasive nature, technical demands, and limited availability often restrict its routine use in clinical practice outside specialized centers [6].

RNS, on the other hand, though less sensitive (detecting abnormalities in

approximately 71.6% of generalized MG and 38.5% of ocular MG cases), is a quick, non-invasive, and cost-effective alternative that remains widely used in clinical practice [7]. A decrement of 10% or more in compound muscle action potential (CMAP) amplitude between the first and subsequent responses is considered diagnostic for MG [8].

There are two RNS frequencies can be employed. Low-frequency RNS (2–5 Hz) is commonly used to detect postsynaptic transmission defects in MG, while high-frequency RNS (40–50 Hz) is typically reserved for unresponsive or unconscious patients, particularly in the evaluation of presynaptic disorders such as Lambert-Eaton syndrome [9,10].

Physiologically, these frequencies act differently: during low-frequency stimulation, acetylcholine is gradually depleted from the primary vesicle store, with mobilization from secondary stores compensating after several stimuli, explaining the characteristic decrement seen around the fourth or fifth response. In contrast, high-frequency stimulation induces both rapid vesicle mobilization and calcium accumulation within the presynaptic terminal, which enhances neurotransmitter release [11].

The current study was designed to evaluate the diagnostic yield of a moderately increased frequency (10 Hz RNS) compared to the conventional 3 Hz, aiming to determine whether this adjustment could improve sensitivity in detecting abnormal decrements in patients with MG.

2. Patients and methods:

Patients

This case-control study included 45 patients with a confirmed clinical diagnosis of myasthenia gravis, supported by laboratory and /or neurophysiological findings. Their age ranged from 20 to 40 years. Routine nerve conduction studies and electromyography were performed prior to the study using NIHION KOHDEN device to exclude any neurological disorders that could affect the results of RNS test. Such as peripheral neuropathies, motor neuron diseases or myopathies.

45 healthy controls matched for age and sex were also recruited.

The study was conducted between February and December 2024, with patients recruited from the Neurology Department and outpatient clinic at Beni-Suef University Hospital.

Ethical

Informed consent was obtained from all participants prior to the study, with assurance

of their right to refuse or withdraw without explanation. Confidentiality of all data was maintained. The study received ethical approval from the Faculty of Medicine, Beni-Suef University **under number (FMBUREC/02012024/AbdElatty).**

The included subjects were subjected to RNS testing using two frequencies; Slow frequency (3 Hz) and fast frequency (10 Hz). repetitive nerve stimulation (RNS) tests were conducted on all patients and controls. With taken into consideration that all myathenic patents discontinued their treatment (acetylcholinesterase inhibitors, such as pyridostigmine) , for 10-12 hours prior to testing.

Neurophysiological assessment were performed using NIHOIN KODEN and conducted in the Neuro diagnostic research centre (NDRC), Beni-Suef University Hospital.

During RNS testing, supramaximal stimulation was applied while maintaining skin temperature around 32°C with the subject positioned supine to ensure optimal testing and proper fixation.

Trains of 10 stimuli were delivered sequentially at two frequencies: 3 Hz and 10 Hz. Three motor nerves were stimulated individually : the facial nerve (recorded from nasalis muscle), spinal accessory nerve (

recorded from trapezius muscle) and the ulnar nerve (recorded from abductor digiti minimi muscle).

Amplitude and negative peak area of the successive responses were measured and the percentage of decrementation was calculated comparing the 4th response with the 1st one.

Statistical Analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS), version 22, installed on a Windows 7 operating system (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as numbers and percentages for qualitative variables. For quantitative data, measures of central tendency were expressed as means with standard deviations for normally distributed variables, while medians and ranges were used for non-parametric data.

The normality of quantitative variables within each study group was initially assessed using the One-Sample Kolmogorov-Smirnov test to determine the appropriate subsequent statistical tests. For parametric quantitative data, comparisons between two independent groups were performed using the independent samples T-test. Additionally, the diagnostic performance of the examined test was evaluated through sensitivity and specificity analyses, employing a Receiver Operating Characteristic (ROC) curve to

determine accuracy. A P-value of less than 0.05 was considered indicative of statistical significance.

3. Results:

The patient group included 45 patients (25 males and 20 females) , the mean age was 30.8 ± 3.8 years. The control group included 45 participants (24 males, 21 females), the mean age was 31 ± 3.9 years. There was no statistically significant difference were noted regarding the patients age and sex ($P = 0.96$ and 0.99 respectively).

Comparison of RNS responses between MG patients and healthy controls in 3 Hz & 10 Hz frequencies.

The percentage of area and amplitude decrementation to repetitive nerve stimulation at 3 Hz and 10 Hz stimulation for the spinal accessory, facial and ulnar nerves were studied for the median and IQR .

As regarding Amplitude and area decrementation with 3 Hz & 10 Hz stimulations, MG patients showed a significantly higher percent of decrement for the spinal accessory, facial and ulnar nerves as compared to controls. (P -values < 0.05). (**as shown in Table 1**).

Table 1: comparison between myathenic patients and control as regarding the percent of decrement in area and amplitude to RNS at 3 Hz and 10 Hz stimulation.

Variable	Cases	controls	p-value
Amplitude decrement (%)			
Spinal accessory (3Hz)	8.5	4	<0.001*
Facial nerve (3Hz)	8	3.6	<0.001*
Ulnar nerve (3Hz)	4.5	1.3	<0.001*
Spinal accessory (10Hz)	13	5.3	<0.001*
Facial nerve (10 Hz)	11	4.5	<0.001*
Ulnar nerve (10 Hz)	7.8	3.1	<0.001*
Area decrement (%)			
Spinal accessory (3Hz)	14	5.7	<0.001*
Facial nerve (3Hz)	11.1	5.3	0.005*
Ulnar nerve (3Hz)	5.6	3.2	0.001*
Spinal accessory (10 Hz)	23.6	7.7	<0.001*
Facial nerve (10 Hz)	18.1	6.4	<0.001*
Ulnar nerve (10 Hz)	7	5	0.048*

* Significant p value <0.05

Effect of RNS different frequencies (3 Hz / 10 Hz) on percent of decrementation in amplitude and area in myasthenia gravis patients. (table2).

With spinal accessory nerve stimulation, there was a statistically significant higher median RNS amplitude and area decrement at 10 HZ frequency than 3 Hz with a p-value **(0.030)** and **(0.003)** respectively.

On the other hand, with facial and ulnar nerves, It showed a statistically significant higher median RNS area decrement in 10 HZ frequency than 3 Hz with a p-value (**0.003, 0.007** respectively), while there were no statistically significant difference observed as regarding the median RNS amplitude decrement with a p-value (**0.100, 0.387** respectively) between 3HZ, and 10 HZ frequencies .

Table (2): Effect of RNS different frequencies (3 Hz / 10 Hz) on percent of decrementation in amplitude and area in MG patients.

Nerve	Ulnar RNS			Facial nerve			Spinal accessory nerve		
Variable	Median	(range)		Median	(range)		Median	range	
AMPLITUDE									
3Hz	4.5	(0.2-65.6)	P value 0.387	8	(0.3-56.8)	P value 0.100	8.5	(0.6-55.8)	P value 0.030*
10Hz	7.8	(0.39-64.5)		11	(0.8-60.7)		13	(0.9-61.4)	
Variable	Median	(range)		Median	(range)		Median	range	
AREA									
3Hz	5.6	(0-50)	P value 0.007*	11.1	(0.5-63)	P value 0.003*	14	(0.7-54.5)	P value 0.003*
10HZ	10.7	(0-60)		18.1	(0.2-66.7)		23.6	(4.1-69.3)	

** Significant p value <0.05*

Sensitivity and specificity of RNS different frequencies in MG patients with ROC curve (Table 3).

The sensitivity and specificity of 3Hz and 10 Hz RNS in the diagnosis of MG compared to healthy controls were evaluated using the Receiver Operating Characteristic (ROC) curve.

At 3 Hz stimulation, amplitude decrementation showed the highest sensitivity in the spinal accessory nerve (68.89%), followed by the facial nerve (57.78%), and the lowest in the ulnar nerve (55.56%).

At 10 Hz stimulation, sensitivity improved across all nerves, with the spinal accessory nerve achieving the highest sensitivity (82.22%), followed by both the facial and ulnar nerves (71.11%).

For area decrementation, at 3 Hz stimulation, the spinal accessory nerve showed the highest sensitivity (92.56%), while the facial nerve and ulnar nerve showed lower sensitivities (87.78% and 76.57%, respectively).

At 10 Hz stimulation, the facial nerve demonstrated higher sensitivity (90.58%) compared to the ulnar nerve (83.76%), while the spinal accessory nerve maintained a notably high sensitivity (97.78%).

Table 3: sensitivity and specificity of decrementation in area and amplitude with different frequencies of RNS.

Variable	AUC (%)	Sensitivity (%)	Specificity (%)
Amplitude decrement (%)			
Spinal accessory (3Hz)	73 %	68.89	97.78
Facial nerve (3Hz)	84%	57.78	97.78
Ulnar nerve (3Hz)	77%	55.56	73.33
Spinal accessory (10Hz)	85 %	82.22 %	91.11 %
Facial nerve (10 Hz)	82 %	71.11	97.78
Ulnar nerve (10 Hz)	81 %	71.11	95.56
Area decrement (%)			
Spinal accessory (3Hz)	78	92.56	68.89
Facial nerve (3Hz)	67	87.78	57.78
Ulnar nerve (3Hz)	70	76.57	57.78
Spinal accessory(10 Hz)	88	97.78	82.22
Facial nerve (10 Hz)	79	90.58	71.11
Ulnar nerve (10 Hz)	62	83.76	53.33

AUC: area under the curve.

4. Discussion:

Myasthenia gravis is an autoimmune condition that disrupts the neuromuscular junction in skeletal muscles. Diagnosing MG typically relies on the patient's characteristic clinical presentation together with a range of complementary investigations [12]. Electrodiagnostic studies, such as repetitive nerve stimulation (RNS) and single-fiber electromyography (SFEMG) are essential diagnostic tools to identify myasthenic patients. While SFEMG offers higher sensitivity in identifying MG, its availability remains limited since it is an invasive test that requires experienced personnel and is only available in specialized centers [6].

RNS is a widely used, accessible electrodiagnostic technique for MG, and previous research has reported its sensitivity varying between 30% and 90% for generalized MG, and from 10% to 60% in ocular MG cases [13]. In the current study, we sought to compare the diagnostic yields of the commonly used 3 Hz RNS with a moderately faster frequency (10 Hz) in a group of 45 MG patients, alongside age and sex-matched healthy controls.

With comparing MG patients to controls, our data revealed a statistically significant difference in both amplitude and area decrementation percentages across all tested

nerves — the spinal accessory, facial, and ulnar nerves — this finding is consistent with earlier published work [14,15]. Furthermore, when directly comparing 3 Hz with 10 Hz RNS within the MG group, area decrement values were notably higher across all examined nerves. However, in terms of amplitude decrement, significant differences between 3 Hz and 10 Hz RNS were observed solely in the spinal accessory nerve, with no similar findings for the facial or ulnar nerves.

These observations are compatible with prior studies proposing that higher-frequency RNS, typically between 10 and 15 Hz, could help detection of subtle neuromuscular transmission defects in clinically suspicious cases, even when conventional low-frequency RNS yields normal results. For example, Lo et al. (2003) demonstrated that high-frequency RNS (HFRNS) could detect decremental responses even in mild MG cases, emphasising its potential as a valuable complement to standard protocols [16].

Sun and Lin (2004) further reported that the maximal decremental response in RNS doesn't necessarily appear at the standard 3 Hz but may occur at higher frequencies (higher detection rate at 7 Hz stimulation) [17].

Although higher-frequency RNS is not routinely applied in the evaluation of

congenital myasthenic syndromes (CMS), several studies have explored its diagnostic value and its role in differentiating subtypes, including cases of choline acetyltransferase (CHAT) deficiency [18], GFPT1-related CMS [19], and RAPSN-associated CMS [20]. Notably, 10 Hz RNS over a 5-minute period was shown to reduce endplate potential amplitude by around 50%, with recovery to baseline taking more than 10 minutes ; that is attributed to impaired acetylcholine resynthesis, particularly in congenital myasthenia cases [19].

In SCN4A-related CMS, studies have shown significant decremental responses at both low and high RNS frequencies. Moreover, some cases exhibited decrements exclusively at higher frequencies (10–30 Hz), even when 3 Hz testing remained within normal limits [22]. Supporting this, An et al. noted that increasing stimulation frequency made decrements in CMAP amplitude more evident across patients [19]. This reinforces the notion that certain CMS subtypes, like GFPT1- or RAPSN-related CMS, exhibit distinct electrophysiological characteristics at high RNS frequencies; thereby raise the value of using different frequencies during testing in suspected myasthenic syndromes [19].

Our findings also demonstrated that area decrement is a more sensitive marker than amplitude decrement for detecting neuromuscular transmission abnormalities. That is supported by previous publications, Pavesi et al. (2001) reported that a 10% reduction only in response area in the masseter muscle is considered abnormal [23]. Similarly, Lo et al. (2003) observed that assessing area decrement increased the diagnostic sensitivity of RNS by 5.3%–30% [24]. Costa et al. (2004) evaluated both amplitude and area decrement during RNS and found that area decrement followed a Gaussian (normal) distribution, whereas amplitude decrement demonstrated a non-Gaussian distribution. This is likely attributed to the fact that amplitude values can vary widely between individuals due to differences in muscle size, age, electrode placement, and nerve conduction properties [25].

From a physiological perspective, area measurements are considered more reliable than amplitude for identifying neuromuscular junction failure, particularly since decrements typically occur after the initial stimuli when action potential thresholds are no longer consistently reached. The CMAP's negative peak area reflects the total number of muscle fibers activated, providing a

broader representation of neuromuscular function — especially when waveform duration is abnormally extended. Conversely, amplitude alone may not capture these nuanced alterations in neuromuscular transmission [24].

Given these observations, we applied ROC curve analysis to objectively compare the sensitivity of amplitude and area decrements at both 3 Hz and 10 Hz RNS frequencies.. When stimulating the spinal accessory, facial, and ulnar nerves, the sensitivity of area decrement was higher with 10 Hz RNS than with 3 Hz RNS. Specifically, for the spinal accessory nerve, the sensitivity was 97.78% and 92.56% with 10 Hz and 3 Hz, respectively. For the facial nerve, it was 90.58% and 87.78%, and for the ulnar nerve, 83.76% and 76.57%, respectively.

Similarly, the sensitivity of amplitude decrement was also higher with 10 Hz RNS compared to 3 Hz RNS when stimulating the same nerves. For the spinal accessory nerve, the sensitivity reached 82.22% with 10 Hz RNS and 68.89% with 3 Hz RNS. Regarding the facial nerve, the sensitivity was 71.11% and 55.56%, respectively, while for the ulnar nerve, it was 71.11% with 10 Hz and 57.78% with 3 Hz RNS.

While 10 Hz improved sensitivity overall, its diagnostic yields was not uniform across

all tested nerves, suggesting that certain muscle groups might be more frequency-sensitive, potentially due to differing neuromuscular junction safety factors or muscle fiber composition. This highlights the importance of nerve-specific protocol optimization in MG RNS studies.

The higher sensitivity at spinal accessory and facial nerves suggests using the proximal muscles for RNS may enhance diagnostic accuracy [26]. similarly, An et al. (2022) recommended the trapezius muscle as a favorable target when performing RNS in suspected CMS cases [19].

Moreover, Bou Ali et al. (2017) demonstrated that diagnostic sensitivity improves proportionally with the number of muscles tested. They proposed using bilateral assessment of three muscles — orbicularis oculi, anconeus, and trapezius — to increase the diagnostic yields [27].

Overall, these results emphasize the value of detailed electrodiagnostic testing in patients with suspected myasthenia, using higher stimulation frequencies along with examining several proximal muscles can improve the sensitivity of neuromuscular transmission disorders detection.

5. Conclusion:

High-frequency (10 Hz) repetitive nerve stimulation proved more sensitive than conventional 3 Hz RNS in detecting neuromuscular transmission defects in myasthenia gravis. Given its simplicity, reliability and practicality, 10 Hz RNS may serve as a useful practical alternative to single-fiber EMG in routine clinical practice.

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Conflict of Interest:

Nil

6. Reference:

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