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Repetitive Nerve Stimulation (RNS) As a Tool in the Diagnosis of Ocular Myasthenia-A Study from A Tertiary Care Centre in India

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Abstract

Myasthenia gravis is an autoimmune postsynaptic disorder affecting the neuromuscular junction of skeletal muscle. It is characterized by a wide variety of signs and symptoms. Ocular Myasthenia is a form of the disease in which the disease is limited to the ocular muscles. Ocular myasthenia is diagnosed by typical clinical features and response to neostigmin. A large number of tests and investigations like Icepack test, Repetitive nerve stimulation, and Single fiber EMG etc are available for the diagnosis. This is a cross sectional record based study using the copies of discharge cards of patients with symptoms suggestive of ocular myasthenia admitted in neurology ward and outpatient ticket details of the similar patients attending in the neurology OPD from June 2020(01/06/2020 to June 2023 (01/06/2023). Patients were divided into two groups. (1) Patients who had ptosis, ocular symptoms,signs and clinical features suggestive of ocular myasthenia and positive response to neostigmin. Patients who had ptosis and ocular findings, but without clinical featuresof myasthenia and without any response to neostigmin. Patients in both groups were subjected to Repetitive Nerve stimulation test. Both patent groups were further followed upwith relevant investigations. Ocular myasthenia is more common in young females, there is a single peak of incidence (30-40yrs) in the females and 60-70 yrs in males. Diurnal variation and fatigability were hallmarks of the disease .Ptosis followed by diplopia were the common symptoms. Repeptitive nerve stimulation is a useful test which can be done as an outpatient procedure for the diagnosis of ocular myasthenia. The sensitivity and specificity of the RNS test in our study was 90.00% & 94.29% respectively (with 95% CI). The accuracy of the test was 92.14% .All this values are significant when compared to other studies. RNS has to be used judiciously and the procedure should be planned meticulously to produce better yields.

OPEN ACCESS

Key Words

MG(myasthenia gravis), RNS (repetitive nerve stimulation), achrab (acetylcholine receptor antibody, hrct(high resolution computerized tomogram), MRD (marginal reflex distance)

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INTRODUCTION

Myasthenia gravis is an autoimmune disease affecting the neuromuscular junction of Skeletal muscles. Ocular myasthenia is a common form characterised by ocular symptoms. Myasthenia is diagnosed by typical clinical features and response to neostigmen[1]. A variety of tests and investigations helpful for diagnosis like ice test, neostigmin test and Repetitive Nerve Stimulation test (RNS). Single fiber EMG also has described as a modern reliable Investigation, but it is available only in few centers. But there are few studies regarding the utility of these investigations.

Brief Review of Literature: Myasthenia gravis is a relatively rare disorder of the neuromuscular junction. There is Defective neuromuscular transmission due to the action of auto antibodies against Acetyl choline receptor antibodies at the post synaptic membrane in most of the patients[2]. All Forms of myasthenia are characterized by fatigability of muscles. Usual muscles involved are Ocular, bulbar and limb muscles. Diurnal variation is another hall another hall mark of this disease[3].

Disease course is long and variable ranging from mild cases to frequent life threatening exacerbation. Myasthenia can be of two forms 1) Congenital, 2) Acquired. Acquired Myasthenia can present in two forms. 1) Ocular, 2) Generalized. Ocular symptoms of MG are caused by weakness of levator palpabrae, orbicularis oculi and other external ocular muscles. Unilateral or bilateral ptosis, horizontal or vertical diplopia, blurring vision and weakness of eye closure are common symptoms. When Myasthenia generalized. Other muscles like facial, palatal, pharyngeal, proximal limb muscles and even respiratory became affected[4]. Diagnosis of Myasthenia is based on clinical findings and response to cholinesterase inhibitors (edrophonium or neostigmine)[1]. Other tests which support the diagnosis of ocular myasthenia are 1) Presence of acetylcholine receptor antibody (AChR-Ab), 2) HRCT chest to exclude thymoma, 3) single fiber EMG.

Repetitive Nerve Stimulation is performed in neurophysiology lab to assess the integrity of Neuromuscular junction. An active electrode is placed over a target muscle and a reference elecctrode over a distal target tendon. It can be done at slow frequencies, (2-3 Hz) or at fast frequencies (30Hz or more) and it measures the resulting compound muscle action (CMAP) potential. CMAP is the sum action potentials from many muscle fibres. RNS is done to look for decremental response of greater than 10% in the baseline to peak amplitude between the first and the fourth or fifth response[6]. A decremental response of more than 10% is considered as positive. RNS is classically done at 4 muscles, orbicularis occuli, nasalis, Abductor digiti minimi and trapezius[4].

Utility of RNS in facial and thenar muscles has been stressed in the study. Post exercise RNS are particularly useful in patients with suspected ocular myasthenia which show only equivocal CMAP decrement at rest. Done at fast rates can be used for diagnosing presynaptic neuromuscular disorders like Lambert Eaton Myasthenic syndrome in which after post exercise testing there is post exercise facilitation and an incremental pattern can be observed. RNS has also been described as an important test in the case of sero negative myasthenia also[7].

Ki Hoon Kim Et al has predicted that positive RNS test may me useful in predicting the conversion of ocular myasthenia to generalized myasthenia7. Sensitivity and specificity of RNS has been described as 70%8 and specificity varies in many studies. There are only few studies especially from India, regarding the utility of investigations for the diagnosis ocular myasthenia especially about Repetitive nerve stimulation (RNS). Early diagnosis and prompt treatment will help in decreasing the burden of morbidity and mortality due to the disease. This is record based study to find out the diagnostic value of Repetitive nerve stimulation (RNS) in the early diagnosis of ocular myasthenia.

Objectives: To study the specificity and sensitivity of Repetitive nerve stimulation (RNS) in the diagnosis of ocular myasthenia from a tertiary care centre in India

MATERIALS AND METHODS

Study Design: Cross sectional study-Record based

Study Setting: GMC, Department of Neurology Thrissur
Study participants

This is a cross sectional record based study using the copies of discharge cards of patients with symptoms suggestive of ocular myasthenia admitted in neurology ward and out patient ticket details of the similar patients attending in the neurology OPD from June 2020 (01/06/2020 to June 2023 (01/06/2023). The investigations and tests done on the patients are analyzed.

Patients were divided into two groups. (1) Patients who had ptosis, ocular symptoms, signs and clinical features suggestive of ocular myasthenia and positive response to neostigmin.{2} patients who had ptosis and ocular findings, but without clinical features of myasthenia and without any response to neostigmin. Control group consisted of patients with 3rd nerve palsy, muscle diseases, cerebrovascular accidents, toxins and cases of trauma etc Patients in both groups were subjected to Repetitive Nerve stimulation test. Both patent groups were further followed up with

relevant investigations. All recorded investigations were analysed. A positive RNS test was described as one in which there as a decremental response of more than 10% amplitude between the first and the fourth or fifth response, when a train of impulses are transmitted to selected muscle[6].

RNS is Performed as Per the Following Protocol[9]:

- Extremities should be warmed; concerned muscles should be immobilized as best as possible.
- Routine motor nerve conduction studies are performed first ensures that the nerve is normal.
- RNS is performed at 3Hz, at rest for 5-10 impulses (stimulated supra maximally) and repeated three times, 1 minute apart. Normally there will be a < 10% decrement between the first and fourth responses.
- If >10% decrement occurs and is consistently reproducible:
- The patient is asked to do maximal voluntary exercise for 10 seconds.
- To demonstrate post exercise facilitation, immediately repeat 3Hz RNS post exercise to demonstrate and see the repair of the decrement.
- If <10% decrement or no decrement occurs:
- Patient is asked to perform maximal voluntary exercise for 1 minute, then perform 3Hz RNS immediately and for 1 minute, then do 3 Hz RNS immediately and 1, 2, 3 and 4 minutes after exercise to show post exercise exhaustion.
- If a significant decrement occurs, have the patient perform maximal voluntary exercise again for 10 seconds and immediately repeat 3Hz RNS to demonstrate repair of the decrement.
- RNS is usually performed on the Nasalis, Orbicularis Oculi, Ulnar and Spinal accessory nerves.

Technical Factors to be Considered in Doing RNS: 1) Immobilize the recording electrode over the muscle .If is not done, it may lead to defective configuration of CMAP.2) Stimuli should be supra maximal.3) Limbs should be warmed adequate at 33 degree Celsius.4) Acetylcholine esterase inhibitors should be withheld prior to the study.5) Nerves selected usually are orbicularis oculi, nasalis, spinal accessory and ulnar.

Inclusion criteria: All the patients in the study are cases of ptosis and ocular symptoms suggestive of myasthenia. These patients were divided into two groups. (1) patients who had ptosis, ocular symptom, signs and clinical features suggestive of ocular myasthenia and positive response to edrophoniunm.{2} patients who had ptosis and ocular findings, but without clinical features of myasthenia and without any response to edrophoniunm.

Exclusion Criteria:

- Cases of congenital myasthenia,
- Patients with eye lid abnormalities and pseudo ptosis conditions simulating ptosis but dropping is not due to weakness of levator palpabrae superioris.
- Study period-2 weeks
- Simple size. All cases during the period 01/06/2020 to 01/06/ 2023 with ptosis and ocular symptoms ,simulating ocular myasthenia will be included.
- Data collection tool-structured proforma
- Data collection Record based Analysis. Secondary data of patients with of patients with myasthenia are collected using a structured proforma. Data on clinical presentation, age of onset, gender, clinical tests performed and diagnostic tests employed are collected.

Variables included are Age of onset, Presentation (clinical presentation), Gender, and Investigations.

Data Analysis: Data was entered in MS excel and analysed using appropriate software like Epi info. Qualitative data was analyzed using proportions. Will be tabulated and discussed.

Ethical Consideration: This is a record based study. It was started after getting the approval from the research and Ethical committee. Confidentiality of data was maintained.

Study Methods: This is a retrospective study using the medical case sheet of oculomysathenia patients admitted in neurology ward and outpatient tickets of myasthenia patients attending in Neurology OPD from June 2022- June 2023

RESULTS AND DISCUSSIONS

There were 70 patients, 28 males and 42 females, as shown in figure no:1

Age Distribution: Age wise distribution of the patients are shown in table no:1 and fig no:2 respectively.No patients in the age group 0-10 and 70-80yrs grou Figure no:2

In the control group, there were 20 males and 50 females

Control Group AGE Distribution: Age wise distribution of the cases in the control group are given in table no: 2

PTOSIS: In our study 29 patients had unilateral ptosis (30%), while in the control group 25 patients had unilateral ptosis. In the study group out of the 49

patients 42 had unequal involvement of one eye (49%). In the control group 34 out of the 45 patients had unequal involvement of one eye >the other. Extent of ptosis was graded using the Marginal reflex distance (MRD) as mild, moderate and severe in both patient and control groups as given in figure no:3

Diplopia: In our study, 28 patients (40%) had diplopia.In the control group 35 patients had diplopia, show in figure no. 4

ICE Pack Test Positivity: Ice pack test was done in all the patients of study and control group and the results are given below. Measure the degree of ptosis by the MRD. Ice pack is applied firmly to the ptotic eye and maintained there firmly for 2 minutes, again the MRD is measured and icepack positivity ascertained. Results in the control and study group are given in figure no: 5 and table no:3.

ACH Receptor Antibody: Figure no:6 shows the results of the acetylcholine receptor antibody test for the study and control groups.

Presence of Thymoma: HRCT Chest was done in all the patients of study and control group, and the results are given in the figure no: 7.

Repetitive Nerve Stimulation Test: Repetitive nerve stimulation was done for all the patients in the study and control group and the results as shown in the figure no: 8 and table no:4. A decremental response after 4th or 5th stimuli is considered as RNS positive Shown in figure no. 8 and Table no. 4

The sensitivity, specificity and other values regarding the accuracy of the test are given in table no:5

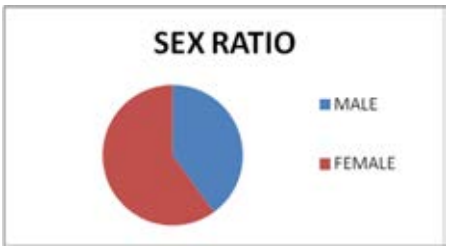

Fig. 1: Sex distribution of Cases

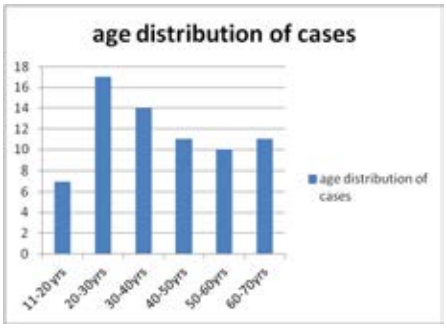

Fig. 2: Age distribution of cases

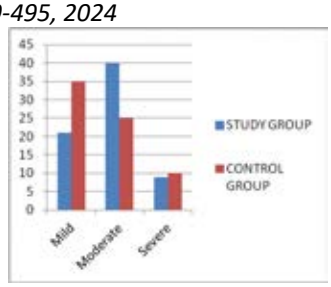

Fig. 3: Grading of Ptosis in study and control groups

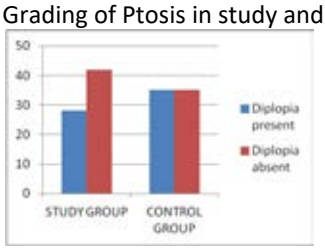

Fig. 4: Presence and absence of Diplopia in study and control groups

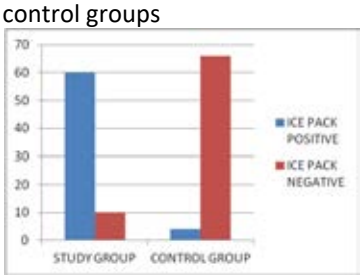

Fig. 5: Ice pack positivity and negativity in patients and control group

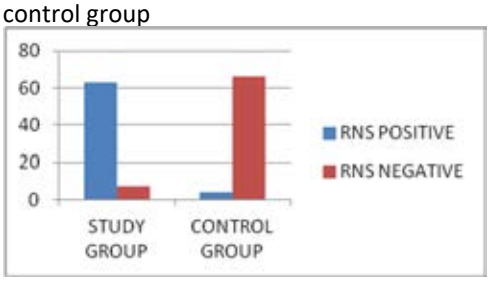

Fig. 6: Acetylcholine receptor antibody positivity and negativity in study and control groups

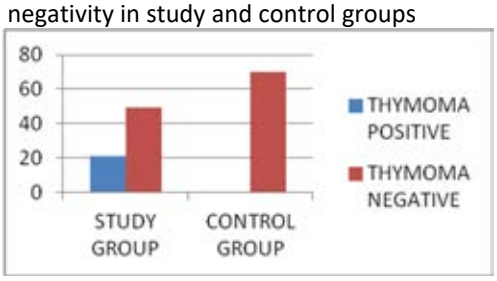

Fig. 7: Presence and absence of thymoma in study and control group

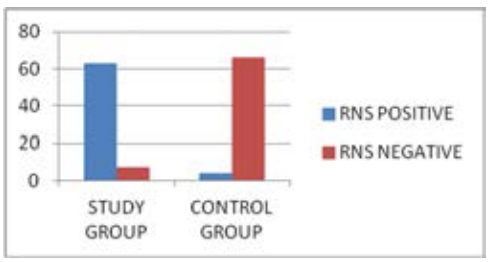

Fig. 8: RNS positivity and negativity in study and control groups

Table: 1

AGE Groups					
11-20 yrs	20-30yrs	30-40yrs	40-50yrs	50-60yrs	60-70yrs
3M+4F=7	4M+13F=17	4M+10F=14	4M+7F=11	4M+6F=10	9M+2F=11

Table no: 2

AGE Groups					
11-20 yrs	20-30yrs	30-40yrs	40-50yrs	50-60yrs	60-70yrs
1M + 3F =4	4M+16F=20	5M+10F=15	1M+11F=12	2M+7F=9	7M+3F=10

Table: 3

Total no of pateints	**Study group**	**Control group**
ICE PACK POSITIVE	60 patients (85.7%)	4 patients (5.7 %)
ICE PACK NEGATIVE	10 patients (14.2 %)	66 patients (94.3%)

Table: 4

Total no of pateints	**Study group**	**Control group**
Rns decremental response positive	63patients (90%)	4 patients (5.7%)
Rns decremental response negative	7 patients (10%)	66 patients (94.3%)

Table: 5

Statistic	**Value**	**95% CI**
Sensitivity	90.00%	80.48%-95.88%
Specificity	94.29%	86.01%-98.42%
Positive Likelihood Ratio	15.75%	6.06-40.92
Negative Likelihood Ratio	0.11%	0.05-0.21
Disease prevalence(*)	50.00%	41.44%-58.56%
Positive Predictive Value(*)	94.03%	85.84%-97.61%
Negative Predictive Value(*)	90.41%	82.33%-95.02%
Accuracy(*)	92.14%	86.38%-96.01%

Sex Ratio: Usually, Myasthenia is considered as a disease of young females. In our study group, the incidence of male and female patients was 1:1.5. Poulas et al has described an incidence of 1:1.4 and Robertson et al has reported an incidence of and 2:1 sex ratio. In their studies[10,11]

Age Group: In our study there was a single peak in the females between 20-30 years and in the males between 60 and 70 years. This single peak in females between 20 and 30 is in concordance with studies of Poulas et al and Saha[10,12] .A similar single peak in the males between 60 and 70 years has been described by Poulas et al and Singhal[1,10]. In our control group also, Male: Female sex ratio was 1:2.5.Fatigability and diurnal variation are considered hallmarks of the disease and was present in all the patients in the study group.

Ptosis: In our study 29 patients had unilateral ptosis (30%), while in the control group 25 patients had unilateral ptosis. In the study group out of the 49 patients 42 had unequal involvement of one eye (49%). In the control group 34 out of the 45 patients had unequal involvement of one eye more than the other.

Diplopia: In our study, 28 patients (40%) had diplopia Leeamornsiri S et al has described an incidence of 30% of diplopia (Reference no:12).Twitch fibres in Extraocular Muscles (EOM) have an increased frequency of synaptic firing compared to other limb muscles. This will render them more prone to fatigue.There are fewer no: of AcH receptors in EOM and the differential expression of AcH receptors in these muscles make them more vulnerable to fatigue then other muscles.

Ice Pack Test Positivity: Ice pack test was done in all the patients of the study and control group. Ice pack test was positive in 60 patients (85.7%).In the study group and 4 patients in the control group.It is postulated that cooling reduces cholinesterase activity, which increases the availability of acetylcholine. Cooling will also promote the efficiency of acetylcholine in eliciting depolarization at the motor end plate.Ertas et al reported an ice pack test positivity of 80% in his study[14] and Tabassi et al., have reported a 100% sensitivity and specificity for the test in his study[15]

Ach Receptor Antibody: Acetylcholine receptor antibodies were positive in 21 patients in our studies but it was negative in all the patients of control groups.Meriggioli et al hasa reported positivity of 40-50% for the AChR-Abs (Ref no: [16])

Presence of Thymoma: HRCT Chest was done in all the patients of study and control group.It was positive in 21 patients (30%)of the study group. Zhi-Feng Mao et al has reported an incidence of 21% of thymoma in his study.

Repetitive Nerve Stimulation Test: In Neuromuscular junction, neurotransmitter acetylcholine (ACH) is packaged as vesicles in presynaptic terminal in discrete units known as quanta. These quanta are located in three separate stores namely, a) Primary or immediately available store b) Secondary or mobilization store and
c) Tertiary store or reserve store[3]

When a nerve action potential depolarizes the presynaptic junction, there will be activation of voltage gated calcium channels and influx of calcium which will results in the release of acetylecholine from the presynaptic terminal. ACH then diffuses across the synaptic cleft and binds to the Acetylcholine receptors (ACHRs) on the post synaptic muscle membrane. This will result in a local depolarization,the end plate

potential(EPP).The size of the EPP is proportional to the amount of ACH that binds to ACHRs[3].

If the EPP depolarizes the muscle membrane above threshold, an all-or-none muscle fibre action potential (MFAP) is generated and propagated. Under normal circumstances, the EPP always rises above threshold resulting in a muscle fibre action potential. The amplitude of EPP above threshold value needed to generate a muscle fibre action potential is called the safety factor[9].

During Slow RNS (2-3Hz) in normal subjects, ACH quanta are progressively depleted from the primary store, and fewer quanta are released with each successive stimulation. The corresponding EPP falls in amplitude, but because of the normal safety factor, it remains above threshold to ensure generation of a muscle fibre action potential with each stimulation. After the first few seconds,the secondary (mobilization) store begins to replace the depleted quanta with subsequent rise in EPP[4].

In Myasthenia, the safety factor is reduced (i.e., baseline EPP is reduced but still above threshold). This occurs as a result of fewer ACHRs and, accordingly less binding of ACH. The decreased safety margin, combined with the natural decrease in neurotransmitter supply, causes subsequent End Plate Potentials (EPPs) to drop below the threshold, leading to a failure in generating corresponding muscle fiber action potentials. As the quantity of individual Muscle Fiber Action Potentials (MFAPs) decreases, there is a decline in both the amplitude and area of Compound Muscle Action Potential (CMAP). This decrement is due to fewer numbers of EPPs reaching threshold and reduced number of MFAPs adding to the CMAP. Often, after-the fifth or sixth stimulus, the secondary store are mobilized and there will not be any further loss of MFAP. This will result in stabilization or slight improvement or repair of CMAP decrement after the fifth or sixth stimulus giving the characteristic 'U' shaped decrement[9].

Effects of exercise can be demonstrated on RNS. After 10 seconds of voluntary muscle contraction, RNS is again done. Brief exercise can repair EPPs that have been developed by slow RNS. If EPPs are facilitated above threshold, muscle fibre action potentials will be generated that were not present previously. As a result of this, the decrement of CMAP amplitude and area that has developed during slow RNS may be repaired[19].

Post exercise exhaustion, can be demonstrated by exercise the muscle actively for 1 min. Then Slow RNS is performed at 1, 2, 3 and 4 minutes later. In patient with normal safety factor, EPP never falls below threshold and CMAP amplitude and area remain stable[9]. In patients with NMJ disorders, the decrement in CMAP amplitude and area in response to slow RNS becomes more between 2-4 minutes after prolonged exercise RNS was done in all the patients of the study and control group. It was positive in 63 patients (90%) in the study group and 4 patients in the control group (5.7%).The sensitivity and specificity of the RNS test in our study was 90.00% and 94.29% respectively(with 95% CI) The test had a positive likelihood ratio of 15.75and negative likelihood ratio of 0.11.The positive predictive value of RNS was 94.03% and a negative predictive value of 90.41%.The accuracy of the test was 92.14% .All this values are significant when compared to other studies .RNS positivity was reported to be 76.5% and 79.5% respectively in the studies of singhal[1] and Leeeamornsiri[12]

CONCLUSION

Myasthenia gravis tends to be more prevalent among young females. There is a single peak of incidence in both the sexes (female population between 30-40 years), and in the (males between 60-70years).Diurnal variation and fatigability are hallmarks of the disease Ocular symptoms are more common in our study population. Ptosis followed by diplopia is the commonest symptom. RNS is a useful test for the diagnosis of ocular myasthenia. In our study, RNS tests have a high specificity and sensitivity. The accuracy of the test is also significant when compared to other tests. The results are quickly available and the procedure is easy when compared with test like Acetylcholine receptor antibody test and single fiber EMG. It can be done as an outpatient procedure and doesn't produce any harmful effects on the patients. But it should be judiciously used on suspicious patients. Meticulous selection of the sites of the nerves and carefully done procedures will give better results.

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