Original Article

International Journal of Neurology and Neurosurgery
Volume 9 Number 2, July - December 2017
DOI: http://dx.doi.org/10.21088/ijnns.0975.0223.9217.6

Neostigmine Test: It's Role in the Diagnosis of Ocular Myasthenia Gravis

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Abstract

Introduction: Ocular myasthenia gravis (OMG) is a most common focal presentation of myasthenia gravis (MG). Most of the recent studies on myasthenia gravis including both generalized and ocular myasthenia used Edrophonium in the diagnosis. As per our knowledge after reviewing available literatures, no study mentioned about the Neostigmine test in a systematic manner. Neostigmine has some advantages over Edrophonium. Present study focused on Neostigmine test in a systematic manner in the diagnosis of OMG *Methods:* Twenty seven patients of suspected ocular myasthenia underwent clinical examination, electrophysiological (RNS and SFEMG) tests, ice pack test and neostigmine test in a systematic manner. After recording baseline data, 0.6mg of atropine was given intramuscularly and 15min later 0.03mg/kg of neostigmine was given intramuscularly. Subjective and objective improvement in clinical parameters was assessed at 15 minute intervals after Neostigmine injection for up to 1.5 hours. *Results:* Of 27 patients, 20 were diagnosed to have ocular myasthenia gravis based on clinical criteria and electrophysiological tests. Out 20 OMG patients, 11 (55%) had shown unequivocally positive results and equivocal result seen in 6 (30%) of patients. 2 patients with restricted extraocular movements without baseline diplopia developed diplopia at 30-44 minutes of the test. All seven non myasthenia patients had shown negative results. It has 85% sensitivity, 100% specificity with 100% positive predictive value and 70% negative predictive value. *Conclusion:* The lower dose (0.03mg/kg) of neostigmine appears to be associated with fewer side effects compared to earlier literature mentioned dose (0.04mg/kg) with equal sensitivity. Improvement in ptosis was more frequent than diplopia. The time interval for maximal improvement in different clinical features varies in a given patient. Therefore, it is necessary to observe the patient for up to 60 minutes during Neostigmine test.

Keywords: Diplopia; Ptosis; Ocular; Neostigmine.

Introduction

Myasthenia gravis (MG) is the well known autoimmune disease caused by autoantibodies against the acetylcholine receptor (AChR) at the neuromuscular junction, subsequently leading to abnormal fatigability and weakness of skeletal muscle. Ocular myasthenia gravis (OMG) is a most common focal presentation of myasthenia gravis (MG), affects extraocular muscles, levator palpebrae superioris and/or orbicularis oculi. Extraocular muscle weakness with ptosis and diplopia is present in about 90% of MG patients, being the initial complaint in about 50%. In about 20% of the patients the disease will always be restricted to the extraocular muscles [1].

The neuromuscular junction became the focus of the disease in 1935, when Mary Walker discovered that physostigmine and later neostigmine, both inhibitors of the enzyme acetylcholinesterase, provided effective temporary relief of the symptoms of MG [2]. Acetyl choline esterase inhibitors (AChEI) are being used in the diagnosis of myasthenia gravis. Acetyl cholinesterase inhibitors (AChEI) acts by inhibiting acetyl cholinesterase in the synaptic cleft, the life span of acetylcholine transmitter released from the presynaptic terminal is prolonged, increasing the probability of interaction with the postsynaptic

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Received on 19.09.2017, **Accepted on** 23.10.2017

receptor. Administration of AChEI will increase the availability of ACh at the neuromuscular junction, and overcome the neuromuscular transmission block, thereby improving the patient's signs and symptoms. This principle is the basis of edrophonium test and neostigmine test.

This test is often regarded as the mainstay of the office diagnosis of OM. The most important technical aspect of the test is the need for an unequivocal endpoint to judge the success or failure of the test. For test result to be considered positive, a dramatic, unequivocal improvement in muscle function should be observed directly by the examiner. The Neostigmine test is most reliable when the patient has ocular muscle weakness or ptosis. Resolution of eyelid ptosis or the direct observation of the strengthening of at least a single paretic EOM, can be taken as the only reliable endpoints. Improvement in ptosis is the most reliable sign [3]. For the extra-ocular muscles, assessment of ocular alignment has been used frequently. However, the ocular alignment in up to 25% of patients with myasthenia will paradoxically worsen with edrophonium [4] and increased deviations can also be found in patients with other ocular motor palsies [5]. The major problem is that changes in alignment may not only reflect increased strength but also increased weakness after edrophonium, which can occur in normal muscles and nonmyasthenic weakness [6]. Therefore, the measure of edrophonium effect is best served by observation of increased strength of a single muscle rather than changes in the relative strength of two muscles (i.e., ocular alignment).

The sensitivity of the edrophonium test is about 95% in generalized myasthenia [7] and reportedly similar in ocular myasthenia with ptosis [8]. The neostigmine test has a relatively high sensitivity, 94% to 100% for generalized MG and 69% to 91% for ocular MG [9]. The evaluation of AChE inhibitors effects in patients with OMG showed 97% sensitivity and 83% specificity [10]. However, diplopia fails to respond in about a third of patients, and long-standing myasthenia may not respond at all. False-positive results have been described in other neuromuscular junction disorders like Lambert-Eaton syndrome (37% positive), Botulism (27% positive), Congenital myasthenia (End-plate acetylcholine receptor deficiency), neuropathic illnesses like Guillain-Barré syndrome, Amyotrophic lateral sclerosis and central conditions, including cerebral aneurysms, brain stem gliomas and other tumors (Pseudomyasthenia) [11,12]. Vision changes, stomach upset, flushing, flatulence, dizziness, drowsiness, muscle cramps, muscle twitching or increased urination may occur. A caution should be exercised in case of chronic airway diseases, seizures, cardiac illness, thyroid disorders, intestinal disorders, ulcers, urinary problems, allergies (especially allergy to bromides).

Why this study?

1. The most important diagnostic test is the detection of serum antibodies against AChR which is positive in 90% of patients with generalized MG, but only in 65% with purely ocular MG [1].
2. Single-fiber electromyography (SFEMG) of the orbicularis oculi muscle has high sensitivity (97%) and specificity (92%) for the diagnosis of ocular myasthenia [13]. But it is a very technical demanding test, needs experienced electrophysiologist and not easily accessible in developing countries like India.
3. Most of the recent studies on myasthenia gravis including both generalized and ocular myasthenia used Edrophonium to assess the response to acetylcholine esterase inhibitors in the diagnosis.
4. Patients who do not respond to edrophonium may respond to intramuscular neostigmine, which has a longer duration of action. This is particularly useful in infants, children and in patients with minimal or highly variable signs whose response to edrophonium may be too brief for adequate observation. Longer duration of action of neostigmine allow orthoptic measurements of ocular alignment [14].
5. As per our knowledge after reviewing available literatures, no study mentioned about the Neostigmine test in a systematic manner. Neostigmine has some advantages over Edrophonium.

Present study focused on Neostigmine test in a systematic manner in the diagnosis of OMG.

Materials and Methods

This is a prospective hospital based study conducted between October 2010 to October 2013 in National Institute of Mental Health and Neurosciences, Bangalore. Twenty seven patients with age group of [3] 10 years of suspected ocular myasthenia gravis referred from various parts of the India were evaluated with systematic clinical examination, ice pack test and electrophysiological tests - Repetitive Nerve Stimulation (RNS) and Single Fiber Electromyography (SFEMG) tests. All 27 patients underwent Neostigmine test in a systematic manner

and data entered using a predetermined proforma. After recording baseline data, 0.6mg of atropine was given intramuscularly and 15min later 0.03mg/kg of neostigmine was given intramuscularly. Subjective and objective improvement in ptosis, diplopia, ocular movements, and facial muscle weakness were assessed at 15 minute intervals after Neostigmine injection for up to 1.5 hours.

Criteria for clinical diagnosis of OMG (Kupersmith MJ, 2003) [15];

1. Ptosis of one or both eyelids not due to local eyelid disease, preferably that could fatigue or improves with rest.
2. Extra ocular muscles weakness and/or diplopia with clear cut fatigability or recovery.
3. Weakness of one or both orbicularis oculi.
4. Fatigue of affected muscle with clear cut worsening of ptosis after upward gaze for 30-60seconds or worsening of monocular duction after 120sec of gaze in the direction of action, recovery of ptosis after 30 to 5min eye closure or recovery monocular duction after 120sec of gaze in the direction antagonist muscle.
5. No pupillary abnormality.

Diagnosis of ocular myasthenia gravis was made based clinical criteria (Kupersmith MJ, 2003) and one of following.

1. Repetitive nerve stimulation (RNS) studies with more than 10% decremental response.
2. Single fiber electromyography(SFEMG) with abnormal jitter and normal fibre density.
3. Presence of acetylcholine receptor antibody (AChR-Ab).

Test results were divided into 3 categories as following,

1. Unequivocally positive - a dramatic, unequivocal improvement in muscle function should be observed directly by the examiner.
2. Equivocally positive - any improvement, not to the full extent which is supported by good subjective improvement noticed by patient.
3. Negative - no objective improvement.

Results

Based on above criteria, patients were divided into two groups: 1. Ocular myasthenia gravis group. 2. Non myasthenia group. Twenty patients fulfilled the criteria for the ocular myasthenia and remaining seven patients were labeled as non myasthenia patients. Among 20 confirmed ocular myasthenia patients, 17 (85%) were male and 3 (15%) were female. Age of onset varied from 14-74years with the mean age onset is 40.05±17.36. Of these patients, 19 (95%) had ptosis, 12 (60%) had diplopia, 17(85%) had diurnal variation and worsening of symptoms on visual strain, 16 (80%) had improving symptoms with afternoon and 6 (30%) had history suggestive of relapses and remission. No patient had afternoon ectropion or seasonal variations.

Among seven patients of non myasthenia group, 2 (25%) were male and 6 (75%) were male. The mean age of onset of disease was 36.6myears (SD, 17.6). All 7 (100%) patients had ptosis with left sided onset was seen in 5 (71%). At the time of presentation 3 (42%) had only left side ptosis and 4 (57%) had bilateral ptosis. Diurnal variation in ptosis was seen in 2 (28%) patients. No patient had fatigable ptosis, Cogan's lid twitch sign or Enhanced ptosis sign. Only one patient had diplopia in horizontal gaze with mild diurnal variation of 3 months duration. 3 (37.5%) patients had restricted ocular movements.

The results of neostigmine test of twenty ocular myasthenia gravis patients given Table 1.

Results of Neostigmine Test of Ocular Myasthenia Gravis Group

In 52.9% of the patients, maximal improvement was observed at 45 minutes. However, the maximal improvement was seen at 15 minutes in 11.1% of patients of ptosis, 20% of patients of restriction of ocular movements, 11.1% of patients for diplopia and 25% of patients for orbicularis oculi. On contrary, maximal improvement was seen at 23.5% of patients with respect to PFW and ptosis time, 66% patients with respect to diplopia and 10%of patients with ocular movement restriction at 60 minutes.

11/20 (55%) had shown unequivocally positive results and equivocal result seen in 6/20 (30%) of patients. 2 patients with restricted extraocular movements without baseline diplopia developed diplopia at 30-44 minutes of the test. Commonly observed neostigmine related adverse effects like nausea, vomiting, abdominal discomfort and giddiness were seen in 6/20 (30%) patients and 2 patients had florid fasciculation.

Results of Neostigmine Test in Non Myasthenia Group

None of the seven non myasthenia patients had shown positive result in the test. Five patients underwent muscle biopsy, 2 patients biopsy showed

Table 1: Palpebral fissure width (PFW) assessment

No	Baseline		15 min		30 min		45 min		60 min		90 min	
	R	L	R	L	R	L	R	L	R	L	R	L
1	5	6	5	6	7	7	9	9	9	9	8	9
2	7	8	7	8	12	13	13	14	13	14	11	12
3	5	8	5	9	7	9	8	9	10	10	10	10
4	9	13	9	13	12	13	13	14	13	14	13	14
5	8	12	10	12	12	13	13	13	13	13	12	13
6	13	10	13	11	13	12	13	13	13	13	13	11
7	8	7	10	9	11	11	11	11	11	11	9	9
8	12	10	12	10	12	12	13	13	13	13	12	12
9	14	0	14	0	15	12	15	15	15	15	15	12
10	13	13	13	13	13	13	13	13	13	13	13	13
11	8	6	15	12	12	8	12	8	10	8	8	6
12	6	10	6	10	6	10	6	10	6	10	6	10
13	4	8	4	8	6	10	10	12	12	13	12	13
14	10	12	12	13	12	13	12	13	12	13	10	12
15	3	3	5	5	8	9	8	9	6	7	6	9
16	8	11	11	12	12	12	13	12	13	12	12	12
17	10	11	12	12	13	12	13	13	13	13	11	12
18	10	10	10	10	10	10	10	10	10	10	10	10
19	0	0	0	0	0	0	2	3	6	8	8	10
20	6	11	10	12	12	12	13	11	12	11	11	11

Parameters entered are width of palpebral fissure width in millimeter at baseline and 15 minutes interval after neostigmine injection till 90 minutes.

Table 2: Ptosis time assessment

No	Baseline		15 min		30 min		45 min		60 min		90 min	
	R	L	R	L	R	L	R	L	R	L	R	L
1	25	20	25	20	40	35	55	50	60	60	50	50
2	6	7	8	12	20	20	20	20	20	20	15	15
3	15	35	15	35	20	40	20	45	20	45	20	40
4	10	60	10	60	20	60	25	60	30	60	30	60
5	20	60	30	60	30	60	35	60	35	60	30	60
6	60	30	60	40	60	40	60	45	60	40	60	35
7	10	15	10	15	15	20	15	20	15	10	15	10
8	60	30	60	30	60	40	60	60	60	60	60	50
9	60	0	60	10	60	45	60	60	60	45	60	30
10	60	60	60	60	60	60	60	60	60	60	60	60
11	40	30	60	40	60	40	40	30	40	30	40	30
12	40	60	40	60	40	60	40	60	40	60	40	60
13	20	30	20	30	30	35	50	60	60	60	60	60
14	10	60	30	60	40	60	40	60	40	60	20	60
15	20	20	30	30	30	30	30	30	30	30	25	25
16	35	60	40	60	40	60	40	60	40	60	40	60
17	40	45	40	45	60	50	60	60	60	60	60	50
18	60	60	60	60	60	60	60	60	60	60	60	60
19	0	0	0	0	0	0	0	0	30	20	30	20
20	30	60	30	60	60	60	60	60	60	60	60	60

Parameters entered are ptosis time in seconds at baseline and 15 minutes interval after neostigmine injection till 90 minutes.

No	Baseline		15 min		30 min		45 min		60 min		90 min	
	R	L	R	L	R	L	R	L	R	L	R	L
1	0	0	0	0	0	0	3	3	3	3	3	3
2	2	2	2	2	1	1	1	1	0	0	0	0
3	3	3	3	3	3	3	3	3	3	3	3	3
4	3	3	3	3	3	3	3	3	3	3	3	3
5	1	0	1	0	0	3	3	3	3	3	3	3
6	3	2	3	1	3	1	3	0	3	0	3	1

7	1	1	1	1	0	0	0	0	0	0	0	1
8	1	1	1	1	0	0	3	3	3	3	3	3
9	3	3	3	3	3	3	3	3	3	3	3	3
10	3	3	3	3	3	3	3	3	3	3	3	3
11	2	2	1	1	1	1	1	1	1	1	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0
13	1	1	1	1	1	1	0	0	3	3	3	3
14	3	3	3	3	3	3	3	3	3	3	3	3
15	2	2	1	1	1	1	1	1	1	1	2	2
16	3	3	3	3	3	3	3	3	3	3	3	3
17	3	3	3	3	3	3	3	3	3	3	3	3
18	3	3	3	3	3	3	3	3	3	3	3	3
19	1	1	1	1	0	0	3	3	3	3	3	3
20	3	3	3	3	3	3	3	3	3	3	3	3

Parameters entered are severity of restriction of extraocular movements at baseline and 15 minutes interval after neostigmine injection till 90 minutes. Severity of the restriction of extraocular movements is graded as follow;
0- Mild, restriction in one direction in one eye
1- Moderate, restriction in one direction in two eyes or in two directions in one eye
2- Severe, restriction in multiple directions
3- No restriction

Table 4: Diplopia assessment

No	Baseline	15 min	30 min	45 min	60 min	90 min
1	3	3	3	3	3	3
2	3	3	3	2	3	3
3	3	3	3	1	0	3
4	3	3	3	3	3	3
5	2	1	0	0	3	3
6	1	0	0	0	1	1
7	1	1	1	1	1	1
8	3	3	3	3	3	3
9	3	3	3	3	3	3
10	1	1	1	1	1	1
11	3	3	1	0	0	0
12	0	0	0	0	0	0
13	0	0	0	3	3	3
14	0	0	0	0	0	0
15	0	0	0	3	3	3
16	3	3	3	3	3	3
17	1	1	0	3	3	3
18	1	1	1	0	3	3
19	2	2	1	0	3	3
20	3	3	3	3	3	3

Parameters entered are diplopia in different gazes at baseline and 15 minutes interval after neostigmine injection till 90 minutes. Severity of the diplopia is graded as follow;
0- Mild, present in one gaze
1- Moderate, present in 2 gaze
2- Severe, present in more than 2 gaze
3- No diplopia

Table 5: Weakness of Orbicularis oculi assessment

No	Baseline		15 min		30 min		45 min		60 min		90 min	
	R	L	R	L	R	L	R	L	R	L	R	L
1	3	3	3	3	3	3	3	3	3	3	3	3
2	1	1	1	1	1	1	0	0	0	0	0	0
3	0	3	3	3	3	3	3	3	3	3	3	3
4	3	3	3	3	3	3	3	3	3	3	3	3
5	3	3	3	3	3	3	3	3	3	3	3	3
6	3	3	3	3	3	3	3	3	3	3	3	3
7	3	3	3	3	3	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3	3	3	3	3	3
9	0	2	1	2	0	1	0	0	0	0	0	0

10	1	3	3	3	3	3	3	3	3	3	3	3
11	3	3	3	3	3	3	3	3	3	3	3	3
12	3	3	3	3	3	3	3	3	3	3	3	3
13	3	3	3	3	3	3	3	3	3	3	3	3
14	1	1	1	1	0	0	0	0	0	0	0	0
15	3	0	0	0	0	0	0	0	0	0	0	0
16	3	3	3	3	3	3	3	3	3	3	3	3
17	3	3	3	3	3	3	3	3	3	3	3	3
18	3	3	3	3	3	3	3	3	3	3	3	3
19	1	1	1	1	1	1	0	0	0	0	0	0
20	3	3	3	3	3	3	3	3	3	3	3	3

Parameters entered are severity of weakness of orbicularis oculi at baseline and 15 minutes interval after neostigmine injection till 90 minutes. Severity of the weakness of orbicularis oculi is graded as follow;
0- Mild, able to bury the eyelashes but evident on attempting to open with maximal force by examiner
1- Moderate, unable to bury the eyelashes and/or weakness is evident on attempting to open with minimal force by examiner
2- Severe, unable to approximate eyelids with an attempt of forceful closure by patient
3- Absent, able to bury the eyelashes

Table 6: Maximum improvement timing

Time	PFW, N=17	Ptosis time, N=17	EOMS restriction, N=10	Diplopia, N=9	Orbicularis oculi weakness, N=4
15 min	2	0	2	1	1
30 min	2	5	1	0	1
45 min	9	7	5	2	2
60 min	4	4	2	4	0

PFW-palpebral fissure width; EOMS-extraocular movements;

Table 7: Neostigmine test in ocular myasthenia

Results	N = 20
Unequivocally positive	11
Equivocal	6
Negative	3

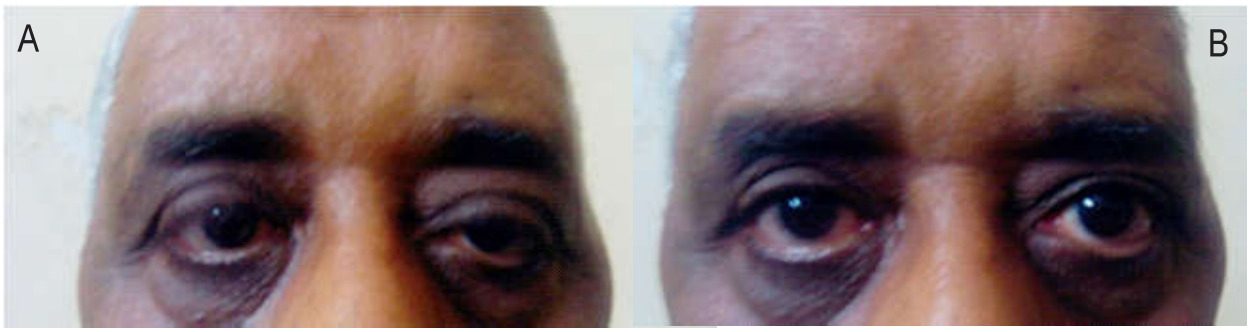

Fig. 1: A. Baseline - moderate ptosis on left side. Palpebral fissure width (Left more than Right). **B.** At 45 min after neostigmine injection, left sided ptosis had improved fully.

features of mitochondrial cytopathy and 3 patients biopsy was normal. One patient was not willing for biopsy and another patient did not return for further evaluation. After 2 years of initial evaluation, we were able to contact six patients to collect the clinical information regarding disease course through phone call conversation as they did not turn up for follow up. Among these six patients, 2 patients with the diagnosis of mitochondrial cytopathy had clinical stable course with multivitamin treatment. Remaining 4 patients did not report any change in their symptoms. Based on the above results in our study, neostigmine test has 85% sensitivity, 100% specificity, 100% positive predictive value and 70% negative predictive value.

Discussion

Most of the recent studies on myasthenia gravis including both generalized and ocular myasthenia

used Edrophonium to assess the response to acetylcholine esterase inhibitors in the diagnosis. As per our knowledge after reviewing available literatures, no study mentioned about the Neostigmine test in a systematic manner. Neostigmine has some advantages over Edrophonium.

As dose of neostigmine 0.04mg/kg intramuscular as mentioned in previous literatures was known to cause more adverse effects based on our own experience in patients with myasthenia gravis, the dose was reduced to 0.03mg/kg.

We observed susceptibility to neostigmine varies from individual to individual and muscle to muscle in patients with ocular myasthenia. Even though 52.9% patients had maximal improvement at 45 minutes, significant number of patients with ptosis (11.1%), with restriction of ocular movements (20%) and orbicularis oculi (25%) had maximal improvement at first 15 minutes and 23.5% of patients with respect to PFW, 66% patients with respect to diplopia and 10% of patients with ocular movement restriction maximal improvement at 60 minutes. All of our patients with ptosis and orbicularis oculi weakness had improvement at different timings with maximal at 45 minutes and 7/9 (77.7%) of patients with diplopia had improvement with maximal at 60 minutes. Among 8 patients with both ptosis and diplopia, 2 (25%) had improvement only in ptosis and none of these had improvement only in diplopia.

Fresh diplopia without baseline diplopia may occur after neostigmine injection as a positive response we observed in 2 of our patients. This may be explained by varying degree of affection and/or susceptibility of different ocular muscles.

Beekman R et al in 1997 (9) reported that neostigmine test has sensitivity of 69% to 91% for ocular MG. We found 85% patients with ocular myasthenia had positive response to neostigmine with unequivocal improvement was seen in 55% patients. It has 100% percent specificity with 100% positive predictive value and 70% negative predictive value.

Neostigmine related adverse effects like nausea, vomiting, abdominal discomfort and giddiness were seen in very few patients. 2 patients had florid fasciculation appeared at 15min and disappeared by 60min.

Conclusion

The lower dose of neostigmine appears to be associated with fewer side effects. Because of longer duration of action, Neostigmine provides adequate time for assessment of all the parameters. Improvement in ptosis was more frequent than in diplopia. In different patients, maximal improvement in ptosis, restricted eye movements, diplopia, and orbicularis oculi weakness was present at different time intervals. Therefore, it is necessary to observe the patient for up to 60 minutes during Neostigmine test.

Abbreviations

PFW-palpebral fissure width;

EOMS - extraocular movements;

RNS - Repetitive nerve stimulation;

SFEMG - Single fiber electromyography;

AChR – Ab - Presence of acetylcholine receptor antibody;

AChEI - Acetyl choline esterase inhibitors

Acknowledgement

I would like to thank all the patients and the family members for their extreme co-operation and I am grateful to NIMHANS for giving me permission to conduct this study.

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