

Relationship between Anti-Acetylcholine Receptor Antibody Titres and Severity of Myasthenia Gravis

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Abstract

Objective: To study the relationship of anti acetylcholine receptor antibody (AChR-Ab) titres with the demographic profile and severity of myasthenia gravis (MG).

Methods: This prospective study was carried out on inpatients and outpatients at the department of Neurology at Pakistan Institute of Medical Sciences, Islamabad. Seropositive cases of myasthenia gravis were collected and were classified as having low AChR-Ab titres (<50nmol/L) and high AChR-Ab titres (>50nmol/L). The comparison of these patients was done using the following parameters: sex, age, clinical presentations, severity of the disease, repeated nerve stimulation test, prostigmine test, the association with thymus status, other autoimmune diseases, and therapeutic outcome.

Results: Out of a total 71 seropositive MG patients enrolled in the study, forty one (57.7%) patients had low titres and thirty (42.2%) had high titres. Their mean age was 33.18±12.99 years (range 13-70) and thirty eight of them were females. The AChR-Ab titers were higher in the younger age group and in women than in men, however, the results were statistically insignificant. The most common presenting symptoms were ocular (91%), followed by generalized weakness with easy fatiguability (57%) and bulbar weakness (46.4%). Majority of the patients at the time of presentation were in Osserman's stage III (43%); while 26% and 19.7% were in stage IIA and IIB respectively. There was no association between the AChR-Ab titers and clinical grades of Osserman's classification.

Conclusion: Serum concentration of anti acetylcholine receptor antibodies do not relate with the clinical severity of myasthenia gravis (JPMA 59:289; 2009).

Introduction

Myasthenia gravis (MG) is an autoimmune disorder that affects the neuromuscular junction at the postsynaptic level. Although the cause of the disorder is unknown, the role of immune responses in its pathogenesis is well established. The condition is caused by sensitized T-helper cells and an immunoglobulin antibody G (IgG) - which attack the nicotinic acetylcholine receptor of the neuromuscular junction (NMJ). A variety of experimental studies support this hypothesis.¹

Anti-acetyl choline receptor antibodies (AChR-Ab titres) are found in nearly 80%-85% of patients with generalised myasthenia gravis and 50%-60% cases of ocular myasthenia gravis. This test is highly specific for MG²

The development of radioimmunoassay test to detect the AChR-Ab has remarkably changed the diagnostic evaluation of myasthenia gravis and is now considered a diagnostic "gold standard." In fact, because of its high specificity, this test has been used in large population based studies to determine both incidence and prevalence of the disease.^{3,4}

If higher titres of antibodies correlate with a more severe disease, then patients with higher titres could be started on more aggressive treatment from the beginning. However, literature reports that antibody titres do not correlate with disease severity across the patient population and mild disease can be associated

with a high titre and a severe disease may be associated with a low titre.² The objective of our study was to find out in our patients the relationship of AChR-Ab titers with demographic profile and severity of myasthenia gravis.

Methods

This prospective study was carried out on inpatient and outpatients of the department of neurology at Pakistan Institute of Medical Sciences, Islamabad.

Each patient was registered with complete information which included name, sex, date of birth, age at onset, diagnosis and main symptoms at onset. History of associated autoimmune disorders such as rheumatoid arthritis, diabetes mellitus, thyrotoxicosis, alopecia areata and vitiligo was taken. Prostigmine test and repetitive nerve stimulation (RNS) was carried out on patients suspected with MG. Definite clinical improvement after intramuscular injection of neostigmine was regarded as positive prostigmine test. Repetitive nerve stimulation (RNS) test was done at stimulation rate of 3 Hz. It was considered suggestive when the decrement exceeded 10% between first and seventh response. If the initial resting RNS test was negative, then post exercise RNS was done.

Other investigations included haematological profile, blood chemistry, thyroid hormone profile, anti-acetylcholine receptor antibody, rheumatoid factor, anti-nuclear antibody,

anti-skeletal muscle antibody and anti double stranded DNA. CT scan thorax was done in 42 patients.

The diagnosis of myasthenia gravis was based on three or more of the following:⁵

- (1) Typical history;
- (2) Clinical evidence of fatigability with recovery on rest;
- (3) Positive prostigmine test;
- (4) Detection of acetylcholine receptor antibodies;

Table: A comparison of the AchR-Ab titers with demographic profile and severity of MG (all p-values >0.05).

	Low Titres (n= 41)	High Titres (n=30)
Age:		
<15 years	2 (4.8%)	1 (3.3%)
15-30 years	14 (34%)	14 (46.7%)
31-45 years	18 (43.9%)	11 (36.7%)
46-60 years	4 (9.7%)	3 (10%)
>60 years	3 (7.3%)	1 (3.3%)
Sex:		
Male	21 (51.2%)	12 (40%)
Female	20 (48.8%)	18 (60%)
Severity of Disease (Osserman's classification):		
I	3 (7.3%)	2 (6.7%)
IIA	14 (34%)	5 (16.7%)
IIB	4 (9.7%)	10 (33.3%)
III	18 (43.9%)	13 (43.3%)
IV	2 (4.8%)	0
Prostigmine Test:		
Positive	41 (100%)	30 (100%)
Negative	0	0
Repeated Nerve Stimulation Test:		
Negative	15(36.5%)	9 (30%)
Positive	26 (63.4%)	21 (70%)
Presence of Other Autoimmune Diseases:		
Present	5 (12.2%)	8 (26.7%)
Absent	36 (87.8%)	22 (73.3%)
Pharmacological Grading:		
I	12 (29.3%)	6 (20%)
II	13 (31.7%)	9 (30%)
III	4 (9.7%)	3(10%)
IV	12 (29.3%)	12 (40%)

- (5) Positive RNS test;
- (6) Exclusion of alternative relevant diagnosis.

Only seropositive patients were included in the study. AchR-Ab titers were classified as low titres: 0.04 - 50nmol/L and high titres: >50nmol/L

The severity of myasthenia gravis in these patients was graded according to Osserman's classification.⁶ Pharmacological grading for severity of MG was done as follows:

- 1) Grade I: Only anticholinesterase drugs (no steroids)
- 2) Grade II: Anticholinesterase drugs plus steroids
- 3) Grade III: Immunosuppressives

4) Grade IV: Plasmapheresis/ Immunoglobulins /mechanical ventilation required at some stage in the disease

The data was entered and analyzed using SPSS version 12.0. Descriptive analysis was done and reported as mean, median and standard deviation for continuous variables and frequencies and percentages for categorical variables. For comparison, univariate analysis was done by using Chi square test, where applicable. p-value <0.05 was taken as statistically significant.

Results

Out of a total 71 seropositive MG patients enrolled in the study, forty one (57.7%) patients had low titres and thirty (42.2%) had high titres. Their mean age was 33.18±12.99 years (range 13-70). Three (4.2%) patients were below 15 years of age while four (5.6%) were above 60 years.

The most common presenting symptoms were ocular (91%), followed by generalized weakness with easy fatigability (57%) and bulbar weakness (46.4%). In 7% patients, the only presenting symptom was weakness of ocular muscles. Difficulty in breathing was seen in 2.8% patients at the onset but 46% patients had respiratory problems at some stage of their disease.

Majority of the patients at the time of presentation were in stage III (43%); while 26% and 19.7% were in stage IIA and IIB respectively.

Overall, 100% of patients were treated with acetylcholinesterase inhibitors and 71.4% received concomitant steroids. 12.7% and 38% required one or more immunosuppressive drugs and plasmapheresis for the control of their symptoms respectively and thymectomy was performed in 29.6% patients.

A comparison of the AchR-Ab titers with demographic profile and severity of MG is shown in Table. The AchR-Ab titers were higher in the younger age group and in women than in men. There was no association between the AchR-Ab titers and grades of Osserman's classification or pharmacological grading.

Discussion

The AchR-Ab Radio Receptor Assay is for in-vitro-diagnostic semi- quantitative determination of autoantibodies against the acetylcholine receptor in human serum and plasma. This assay measures antibodies that precipitate solubilized muscle AChR that has been complexed with iodine labeled alpha- bungarotoxin (α BTX), a snake venom which binds specifically to AChR, and is incubated with serum. Antibodies that bind to the receptor regions that are not sterically blocked by the α BTX are detected.^{6,7}

The present study suggests that antibody titres do not correlate with disease severity across the patient population, so,

mild disease can be associated with a high titre and a severe disease may be associated with a low titre. These findings are consistent with various previous studies.^{2,8-11}

There is abundant evidence that patients with myasthenia gravis have heterogeneous populations of acetylcholine-receptor antibodies and antibody response in MG is polyclonal. Destruction of the AChRs by the antibody is brought about by different mechanisms. i.e. (a) Antibodies cross linking with the AChR with subsequent accelerated endocytosis and degradation of the AChRs by muscle cells; (b) antibody blocking the binding sites of the AChRs; and (c) complement-mediated destruction of junctional folds of the postsynaptic membrane, causing decrease in the number of AChRs at the NMJ and resultant decrease in available surface area for insertion of newly synthesized AChRs.^{2,10,12-14} Interestingly, antibodies from some patients have a more pronounced effect on degradation, whereas others produced more marked blockade of acetylcholine receptors. Not only do the antibodies differ in their functional activities, there are also differences in neuromuscular junctions in different patients, or even in different muscles of an individual patient.¹² This indicates the reason to why antiacetylcholine receptor antibodies vary in their capacity to produce muscle weakness.

However, in an individual patient, the titre does correlate with the disease severity and a decrease in titre means favourable response to the treatment (for example, plasmapheresis). A strong correlation between a change in the anti-AChR concentration and a change in clinical condition was noted during treatment with prednisone or immunosuppression and in the period after thymectomy, whereas no changes in anti-AChR concentrations were found if improvement was caused by the effect of anticholinesterases or if deterioration was caused by infection or emotion.^{2,15}

However, about 10%-20% of patients with acquired myasthenia gravis do not have anti-AChR antibodies detectable by radioimmunoassay. It has been proposed that these patients are likely to have antibodies against components of the neuromuscular junction that are not detected by the current anti-AChR radioimmunoassay e.g. anti muscle specific protein kinase (MuSK) antibody,^{16,17} Striational antibodies like antititin, anti ryanodine¹⁸ and anti-Kv1.4 antibodies.¹⁹ Out of these, striational antibodies can be used as prognostic determinants in MG as higher titers of these antibodies are associated with more severe disease.¹⁸ In future, these antibodies may be useful for predicting the course of the disease in patients with MG and for treatment regimen.¹⁹

Although myasthenia gravis is predominantly antibody mediated, T-cells have also been shown to be important in the pathogenesis of the disease. It is proposed that these T-cells provide help to the B-cells by means of surface molecules and cytokines, resulting in B-cell proliferation and the secretion of AChR specific antibody.^{2,20} Future studies should shed more

light on the exact role of T-cells in the pathogenesis of myasthenia gravis.

Conclusion

Considering the variety of aetiopathogenesis of myasthenia gravis, it is not surprising that serum concentration of anti acetylcholine receptor antibodies do not co-relate with the severity of myasthenia gravis.

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