Relationship between Low and High Anti-acetylcholine Receptor Antibody Titers and Clinical Severity in Myasthenia Gravis

Ubaid Ullah, Sadaf Iftikhar and Muhammad Athar Javed

Department of Neurology, King Edward Medical University/Mayo Hospital, Lahore, Pakistan

ABSTRACT

Objective: To determine the frequency of low and high anti-AChR (acetylcholine receptor) antibody titers and to evaluate their relationship with clinical severity in myasthenia gravis.

Study Design: Cross-sectional, observational study.

Place and Duration of Study: Department of Neurology, King Edward Medical University/Mayo Hospital, Lahore from April 2017 to March 2018.

Methodology: Fifty-six seropositive patients, aged between 18-75 years, were included. A blood sample was obtained from each patient to assess for the anti-AChR antibody titers by enzyme-linked immunosorbent assay (ELISA) technique and classified as low (0.4-<50 nmol/L) and high AChR antibody titers (≥50 nmol/L). Clinical severity was graded according to the Osserman's classification.

Results: Out of 56 patients, 51.79% (n=29) were males and 48.21% (n=27) were females, and mean age was 32.73 ±8.48 years. Mean anti-AChR antibody titer was found 40.45 ± 13.54; 60.71% (n=34) had low and 39.29% (n=22) had high titers. Upon grading the severity, 1.79% (n=1) had grade I, 25% (n=14) had grade IIa, 26.79% (n=15) had grade IIb, 37.5% (n=21) had grade III, and 8.93% (n=5) had grade IV. These grades were significantly associated with high/low titers of anti-AChR antibody (p<0.001) but no significant association was found with age and gender (p=0.39 and 0.19 respectively).

Conclusion: Serum concentration of anti-AChR antibodies has significant association with the clinical severity in myasthenia gravis.

Key Words: *Anti-acetylcholine receptor antibody, Myasthenia gravis, Neuromuscular junction diseases.*

How to cite this article: Ullah U, Iftikhar S, Javed MA. Relationship between Low and High Anti-acetylcholine Receptor Antibody Titers and Clinical Severity in Myasthenia Gravis. *J Coll Physicians Surg Pak* 2021; **31(08)**:965-968.

INTRODUCTION

Myasthenia gravis (MG), the most common of neuromuscular junction disorders, is an acquired, predominantly antibody-mediated autoimmune disease in which antibodies are targeted against nicotinic acetylcholine receptor (AChR) at the neuromuscular junction, resulting in an overall reduction in the number of AChRs and damage to the postsynaptic membrane. Clinical hallmark of MG consists of fluctuating fatigability and weakness affecting ocular, bulbar and proximal skeletal muscles.[1]

Correspondence to: Dr. Ubaid Ullah, Department of Neurology, King Edward Medical University / Mayo Hospital, Nila Gumbad, Anarkali, Lahore, Pakistan
E-mail: ubaidullah0264@gmail.com

Received: October 15, 2019; Revised: December 25, 2020; Accepted: February 24, 2021
DOI: https://doi.org/10.29271/jcpsp.2021.08.965

In generalised MG, AChR antibodies are detected in up to 85% of patients, whereas in purely ocular MG, only about 50% of patients are antibody positive.[2] Three subtypes of AChR antibodies have been identified: binding, blocking, and modulating. These lead to AChR loss on the postsynaptic membrane *via* accelerated receptor degradation or receptor blockade. AChR modulation is caused by antibodies that cross-link AChRs and facilitate endocytosis, resulting in receptor loss on the postsynaptic membrane. In addition, complement-mediated damage to the postsynaptic membrane results in fewer membrane folds.[3]

Presence of these anti-AChR antibodies are specific for MG.[4]It is observed that patients with severe generalised MG and/or thymoma tend to have higher titers of AChR antibodies and greater activity of AChR modulating antibodies.[5] However, some patients with severe muscle weakness have low titers of antibodies, and some patients with only ocular MG or in remission have high titers. These paradoxical findings reflect heterogeneity in the specificities, affinities and idio-types of anti-AChR

antibodies.In general, the AChR antibody titer is directly proportional to the disease severity, high AChR antibody titers are associated with progression from ocular to generalised MG [6,7] and more propensity for myasthenic crisis.[8] In a study done by Somnier,[9] anti-AChR antibody titres were found to correlate with the clinical severity, gender and thymus pathology. Similarly, Heldal *et al*.,[10] also found a positive association between AChR antibodies concentration and clinical severity of the disease. Contrarily, various studies have found no or only weak association between AChR antibody titers and clinical severity in MG.[11-14]

As the literature provides conflicting evidence of the relationship between anti-AChR antibody titers and the disease severity in MG, more research is a need of the hour for better understanding of this relationship and subsequent modification in the management strategies. Furthermore, the only local study by Aurangzeb *et al*. [11] conducted a decade ago, also failed to establish any kind of relationship between these two entities. So, this study was aimed at determining the frequency of low and high anti-AChR antibody titers and evaluating their potential relationship with the clinical severity in myasthenia gravis.

METHODOLOGY

This cross-sectional study was conducted in the Department of Neurology, King Edward Medical University/Mayo Hospital, Lahore, from April 2017 to March 2018. After getting approval from the Institutional Review Board, 56 newly-diagnosed seropositive MG patients of both genders aged between 18-75 years were included. Sample size was calculated with 95% confidence interval, 13% margin of error; and expected percentage of anti-AChR antibody titer as 42.2%.[11]MG was defined as having signs and symptoms with fatigability, positive stigma test, positive repetitive nerve stimulation (RNS) and positive anti-AChR antibody titer (>0.4 nmol/L). Seronegative MG and patients with thymoma, Lambert-Eaton myasthenic syndrome, muscular dystrophies, thyrotoxicosis, polymyositis, rheumatoid arthritis, spinal muscular atrophy, congenital myasthenic syndrome and familial MG, all were excluded from the study.

A blood sample of 2 ml was taken from each patient in red-top tube to assess the anti-AChR antibody titers by the enzyme-linked immunosorbent assay (ELISA) technique at the time of admission, before starting the immunosuppressive therapy, and were classified as low (0.4-<50 nmol/L) and high AChR antibody titers (≥50 nmol/L) because the reported average titer is about 50 nmol/L for the generalised MG.[15] Clinical severity was graded according to the Osserman's classification of myasthenia gravis: Grade I, focal disease (restricted to the ocular muscle); grade II, generalised disease that is either mild (IIa) or moderate (IIb); grade III, acute severe generalised disease with respiratory failure; and grade IV, severe generalised disease with respiratory failure (progression within 2 years).[16] Verbal informed consent was taken from each patient before enrollment in the study; and all data were collected on a pre-designed proforma.

The data were analysed by Satistical Package for Social Sciences (SPSS) version 22 software. Means and standard deviations were calculated for quantitative variables like age and anti-AChR antibody titer. Percentages and frequencies were calculated for qualitative variables like gender, high/low antibody titer and grades of severity. Relationship between the clinical severity of MG and anti-AChR antibody titer was found. Data was stratified for age and gender to address effect modifiers. Post-stratification Chi-square test was applied to look for the significance, p-value <0.05 was considered statistically significant.

RESULTS

Out of 56 seropositive MG patients, age distribution showed 78.57% (n=44) were between 20-40 years; and 21.43% (n=12) were between 41-51 years, mean and SD was calculated as 32.73 ± 8.48 years. Gender distribution showed 51.79% (n=29) were males and 48.21% (n=27) were females. Mean anti-AChR antibody titer was calculated as 40.45 ± 13.54 nmol/L. Frequency of low/high anti-AChR antibody titer showed 60.71% (n=34) had low and 39.29% (n=22) had high antibody titers.

Table I: Comparison of frequency of grades of severity in myasthenia gravis with high/low Anti-AChR antibody titers (n=56).

Grades of severity in myasthenia gravis	Anti-AChR antibody titers			
	High	Low	Total	
I	0	1 (2.94%)	1 (1.79%)	p<0.001
IIa	0	14 (41.17%)	14 (25%)	
IIb	0	15 (44.12%)	15 (26.79%)	
III	17 (77.27%)	4 (11.76%)	21 (37.5%)	
IV	5 (22.72%)	0	5 (8.93%)	
Total	22 (39.29%)	34 (60.71%)	56	

p-value < 0.05 was considered significant.

Table II: Stratification for frequency of high/low Anti-AChR antibody titers with regards to age and gender (n=56).

		High/Low Anti-AChR antibody titers		Total
		High	Low	
Age (years)	20-40	16 (72.72%)	28 (82.35%)	44 (78.57%)
	41-51	6 (27.27%)	6 (17.65%)	12 (21.43%)
Total		22 (39.29%)	34 (60.71%)	56
p=0.39				
Gender	Male	9 (40.91%)	20 (38.24%)	22 (39.29%)
	Female	13 (59.09%)	14 (61.76%)	34 (60.71%)
Total		22 (39.29%)	34 (60.71%)	56
p=0.19				

**p-value <0.05 was considered significant.*

Frequency of the grades of clinical severity in myasthenia gravis patients showed that 1.79% (n=1) had grade I, 25% (n=14) had grade IIa, 26.79% (n=15) had grade IIb, 37.5% (n=21) had grade III, and 8.93% (n=5) had grade IV. Comparison of frequen-

cies of clinical severity grades in myasthenia gravis in patients with low and high anti-AChR antibody titers was done that showed significance ($p<0.001$, Table I).

Cross-tabulations of age, and gender with high/low anti-AChR antibody titers were found insignificant ($p=0.39$ and 0.19 respectively, Table II).

DISCUSSION

This study showed that out of 56 seropositive MG patients, 60.71% (n=34) had low and 39.29% (n=22) had high anti-AChR antibody titers; and these titers have significant association ($p<0.001$) with the clinical severity in MG. However, no association was found with age and gender ($p=0.39$ and 0.19, respectively). These findings are consistent with that of Somnier,[9] who also found a positive correlation between anti-AChR antibodies titers and clinical severity in MG. In addition, he also reported that pathology of thymus and gender has significant relationship with anti-AChR antibody titers; though that is not seen in this study ($p=0.19$).

Strong association between anti-AChR antibodies and clinical severity was also demonstrated by Tindall studied patients in remission or with only ocular symptoms, who differed significantly from those with generalised disease ($p<0.01$ and <0.05 respectively).[17] Similarly, Heldal *et al.* also found positive association between the concentration of anti-AChR antibodies and Myasthenia Gravis Foundation of America (MGFA) score that was used to grade the clinical severity in MG.[10] This scoring system is similar to Osserman's classification, which is used in this study. She reported that anti-AChR antibody titers reflect the degree of medical response and assist in the decision of modifying or keeping the immunosuppressive therapy unchanged regarding the therapy and its dose. She also observed that the association between changes in anti-AChR antibody titers and MGFA score had no longer remained significant after three years of the disease probably, due to long-term immunosuppression.

Several possible explanations exist for the disparity between absolute titer and the disease severity, one or more of which may be responsible, *i.e.* presence of more than one idio-type of the antibodies within each patient; differences in the immunoglobulin class; variation of the complement fixation and/or increased cross-linking, and direct interference with the acetylcholine binding sites. All these factors lead to paradoxical findings of low antibody titers in some patients of severe muscle weakness, and high antibody titers in some patients in remission or having only ocular symptoms.

Several studies have found no or only weak association between AChR antibody titers and clinical grades of severity in MG. Lindstrom *et al.* found that neither the presence of anti-AChR antibodies nor its titer has correlation with age, gender, duration of the disease and immunosuppressive treatment,[18] contrary to the results reported by Heldal *et al*.[10] However, Limburg *et al*. and Mantegazza *et al*. both provided evidence for an association between anti-AChR antibody titer, and age at onset and duration of the disease.[13,19] Their findings are not consistent with the present as there was no significant relationship between anti-AChR antibody titers and age of the MG patients ($p=0.39$).

Sanders *et al*.[14] demonstrated that change in anti-AChR antibody levels correlates only weakly with change in clinical severity of MG. Eymard also concluded that neither anti-AChR antibody level nor antigenic repertoire correlates with the disease severity.[12] Similarly, in the only related study done in Pakistan by Aurangzeb *et al*. about a decade ago,[11] also failed to establish any relationship between serum concentration of anti-AChR antibodies and clinical severity in MG; whereas, we found significant correlation between these two entities ($p<0.001$). The present results are also consistent with a recent study done in Taiwan,[20] which has suggested that anti-AChR antibodies are not only a diagnostic marker but also an acceptable predictive marker for the outcome in patients with myasthenia gravis.

This study has a few limitations; firstly, it is a single-centre study; secondly, due to inclusion of only seropositive MG patients, the heterogeneous characteristics of seronegative MG patients could not be assessed. Lastly, being a government hospital-based study conducted on the patients belonging to the lower socio-economic status, the results cannot be generalised.

CONCLUSION

Anti-AChR antibody titers have a significant association with the clinical severity in myasthenia gravis. So, it can be recommended to monitor the serum concentration of these antibodies for better optimisation of immunosuppressive therapy in these patients. Thus, further studies are needed for the formal development of rigorous clinical practice guidelines.

DISCLOSURE:
This article is extracted from the dissertation of corresponding author, submitted to CPSP as requirement for fellowship.

ETHICAL APPROVAL:
This study was approved by Institutional Review Board of King Edward Medical University/Mayo Hospital, Lahore.

PATIENTS' CONSENT:
Informed consents were obtained from the patients to publish the data.

CONFLICT OF INTEREST:
Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
UU: Data acquisition and analysis, interpretation, drafting, integrity of the work.
SI: Conception and design, interpretation, critical appraisal, revision and final approval.
MAJ: Critical appraisal and final approval.

REFERENCES

1. Melzer N, Ruck T, Fuhr P, Gold R, Hohlfeld R, Marx A, *et al.* Clinical features, pathogenesis, and treatment of

myasthenia gravis: A supplement to the guidelines of the german neurological society. *J Neurol* 2016; **263(8)**: 1473-94. doi: 10.1007/s00415-016-8045-z.

2. Padua L, Stalberg E, LoMonaco M, Evoli A, Batocchi A, Tonali P. SFEMG in ocular myasthenia gravis diagnosis. *Clin Neurophysiol* 2000; **111(7)**:1203-7. doi: 10.1016/s1388- 2457(00)00307-2.
3. Howard FM Jr, Lennon VA, Finley J, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis. *Ann N Y Acad Sci* 1987; **505**:526-38. doi: 10.1111/j.1749-6632.1987.tb51321.x.
4. Meriggioli MN, Sanders DB. Muscle autoantibodies in myasthenia gravis: Beyond diagnosis? *Expert Rev Clin Immunol* 2012; **8(5)**:427-38. doi: 10.1586/eci.12.34.
5. Huang GZ, Lo YL. Correlation between acetylcholine receptor antibody levels and thymic pathology in myasthenia gravis: A review. *J Clin Neuromuscul Dis* 2013; **14(4)**:209-17. doi: 10.1097/CND.0b013e31828a0090.
6. Peeler CE, De Lott LB, Nagia L, Lemos J, Eggenberger ER, Cornblath WT. Clinical utility of acetylcholine receptor antibody testing in ocular myasthenia gravis. *JAMA Neurol* 2015; **72(10)**:1170-4. doi: 10.1001/jamaneurol.2015.1444.
7. Chithra P, Iype T, Bhagya S, Geetha S. Clinical-Immunological profile of myasthenia gravis-a tertiary care centre experience. *JMSCR* 2017; **5(4)**:20130-34.
8. Bindu PS, Nirmala M, Patil SA, Taly AB. Myasthenia gravis and acetylcholine receptor antibodies: A clinico immunological correlative study on South Indian patients. *Ann Indian Acad Neurol* 2008; **11(4)**:242-4. doi: 10.4103/0972-2327.44560.
9. Somnier FE. Clinical implementation of anti-acetylcholine receptor antibodies. *J Neurol Neurosurg Psych* 1993; **56(5)**: 496-504. doi: 10.1136/jnnp.56.5.496.
10. Heldal AT, Eide GE, Romi F, Owe JF, Gilhus NE. Repeated acetylcholine receptor antibody-concentrations and association to clinical myasthenia gravis development. *PLoS One* 2014; **9(12)**:e114060. doi: 10.1371/journal.pone.0114060.
11. Aurangzeb S, Tariq M, Irshad M, Badshah M, Khan RS. Relationship between Anti-acetylcholine receptor antibody titres and severity of myasthenia gravis. *J Pak Med Assoc* 2009; **59(5)**:289-92.
12. Eymard B. Antibodies in myasthenia gravis (French). *Rev Neurol (Paris)* 2009; **165(2)**:137-43. doi: 10.1016/j.neurol.2008.11.020.
13. Limburg PC, The TH, Hummel-Tappel E, Oosterhuis HJ. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 1. Relation to clinical parameters in 250 patients. *J Neurol Sci* 1983; **58(3)**:357-70. doi: 10.1016/0022- 510x(83)90095-3.
14. Sanders DB, Burns TM, Cutter GR, Massey JM, Juel VC, Hobson-Webb L. Does change in acetylcholine receptor antibody level correlate with clinical change in myasthenia gravis? *Muscle Nerve* 2014; **49(4)**:483-6. doi: 10.1002/mus.23944.
15. Lindstrom J, Shelton D, Fuji Y. Myasthenia gravis. *Adv Immunol* 1987; **42**:233-84.
16. Osserman KE, Kornfeld P, Cohen E, Genkins G, Mendelow H, Goldberg H, *et al.* Studies in myasthenia gravis: Review of two hundred eighty-two cases at the Mount Sinai hospital, New York City. *AMA Arch Intern Med* 1958; **102(1)**:72-81. doi: 10.1001/archinte.1958.00260190074008.
17. Tindall RS. Humoral immunity in myasthenia gravis: clinical correlations of anti-receptor antibody avidity and titer. *Ann N Y Acad Sci* 1981; **377**:316-31.
18. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. *Neurology* 1976; **26(11)**:1054-9. doi: 10.1212/wnl.26.11.1054.
19. Mantegazza R, Pareyson D, Baggi F, Romagnoli P, Peluchetti D, Sghirlanzoni A, *et al.* Anti AChR antibody: Relevance to diagnosis and clinical aspects of myasthenia gravis. *Ital J Neurol Sci* 1988; **9(2)**:141-5. doi: 10.1007/BF02337460.
20. Chou CC, Su IC, Chou IJ, Lin JJ, Lan SY, Wang YS, *et al.* Correlation of anti-acetylcholine receptor antibody levels and long-term outcomes of juvenile myasthenia gravis in Taiwan: A case control study. *BMC Neurol* 2019; **19(1)**:170. doi: 10.1186/s12883-019-1397-0.