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The Value of Serological Diagnosis in Myasthenia Gravis-Analysis of a Group of People from Algeria

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Abstract

Anti-acetylcholine receptor antibodies (antiAChR Abs) are tested for in presumptive myasthenia gravis (MG) patients using a radio-immunoprecipitation assay (anti-AChR RIPA) and, to a lesser degree, an enzyme-linked immunosorbent assay (anti-AChR ELISA). In this paper, we provide the results of autoantibody identification using several assays in a group of 23 Algerian MG patients. All patients confirmed with generalised MG (16) were positive for anti-AChR by ELISA compared to 87.5% (14) by RIPA. One (14.3%) of the seven (14.3%) ocular MG patients tested positive by ELISA, whereas 28.6% (2) tested positive by RIPA. These findings imply that some of the anti-AChR ELISA results might be false-negatives or false-positives. None of the 23 patients were seropositive for muscle-specific kinase antibodies (antiMuSK abs) by RIPA or by a cell-based test. Overall, the data show that in areas where RIPA is not accessible, anti-AChR ELISA, in combination with clinical and electrophysiological findings, may be effective for MG diagnosis. None of the 23 patients were seropositive for muscle-specific kinase antibodies (antiMuSK abs) by RIPA or by a cell-based test. Overall, the data show that in areas where RIPA is not accessible, anti-AChR ELISA, in combination with clinical and electrophysiological findings, may be effective for MG diagnosis.

Keywords: Anti-acetylcholine; Kinase antibodies; Radio-immunoprecipitation assay; Myasthenia gravis

Introduction

Myasthenia gravis (MG) is an autoimmune illness produced by serum autoantibodies (autoAbs) directed against muscle membrane components at the neuromuscular junction (NMJ). The condition is distinguished by neuromuscular transmission failure and variable, fatiguing muscle weakness [1]. The majority of MG patients present with ocular symptoms. Around 75% of individuals will acquire widespread weakness during the first 2 to 3 years after manifestation. When symptoms are just ocular, the condition is referred to as ocular MG (OMG). The majority of cases of generalised MG (80% to 85%) and half of cases of ocular MG (50%) involve autoAbs directed against the acetylcholine receptor (AChR), but autoAbs directed against other NMJ components, most commonly Muscle Specific Kinase (MuSK), are found in a minority of patients with generalised MG (15% to 20%) [2,3]. The clinical presentation of myasthenia gravis is used to make the first diagnosis [4-6]. The diagnosis is then confirmed by serological testing. The most widely utilised methods for identifying AChR and MuSK autoAbs are radio-immunoprecipitation assays (RIPA). The enzyme-linked immunosorbent assay (ELISA) is less often employed, but it is technically easier and less expensive, which are crucial factors in many regions of the globe. Cell-based assays (CBA) are time-consuming, although they are occasionally employed as a last resort for MG patients when RIPA fails to identify autoantibody [7]. A limited proportion of clinically diagnosed patients are sero-negative for both anti-AChR and anti-MuSK, which might be due to test sensitivity limits or instances caused by antibodies against other synaptic antigens such as agrin and LRP4 [8,9]. We compare the anti-AChR and anti-MuSK status of sera from 23 Algerian MG patients using ELISA and RIPA tests [10].

Materials and Methods

The research comprised 23 Algerian individuals who had a strong suspicion of having MG. Victims were treated at the Ait Idir Neurosurgery Hospital in Algiers, Algeria, the Sidi Belloua Hospital in Tizi Ouzou, Algeria, and the Mustapha Pacha Hospital in Algiers, Algeria (Algiers, Algeria). Displays the demographic and clinical characteristics of the patients. All patients were diagnosed with MG by their neurologists using clinical and electromyography (EMG) criteria. All but three (3/23) showed a decrease in the compound muscle action potential during nerve stimulation. As internal controls, five sera from Algerian individuals with congenital myasthenic syndrome (CMS) were employed. Two sera from healthy Australians were utilised as negative controls for anti-MuSK tests, whereas two sera from anti-MuSK MG patients in Australia were used as positive controls. The serum samples were all kept at +4°C.

Enzyme-Linked Immune-Sorbent Assay (ELISA)

The titres of AChR antibodies were determined using an enzyme-linked immunosorbent assay (ELISA; ElisaRSRTM AChRAb kit, RSR, Cardiff, United Kingdom [11]). This test relies on human anti-AChR Abs competing for binding to the AChR with the two anti-AChR monoclonal antibodies given in the kit, hence suppressing the ELISA signal. According to the manufacturer, only values larger than 0.45 nmol/L were considered positive (RSR, Cardiff, United Kingdom). The study used commercially supplied 125I-AChR and 125I-MuSK, and a RIPA kit that contained supplied standards, following the supplier's recommended protocol (RSR Ltd., Cardiff, UK). For anti-AChR, values ranging from 0 to 0.25 nM were considered negative, while values between 0.25 and 0.4 nM were equivocal, and values above 0.4 nM were considered positive. For anti-MuSK, values between 0 and 0.05 nM were considered negative, while values between 0.05 and 0.09 nM were equivocal, and values greater than 0.09 nM were considered positive. In both cases, a test sample was considered positive if it exceeded the

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mean + 3 standard deviations of the non-myasthenic control values [12]. The positive controls for anti-AChR and anti-MuSK in the kit had values of 1.407 nM and 0.29 nM, respectively [13-17]. The Cell-Based Assay (CBA) is a method used to detect the presence of antibodies against Muscle-Specific Kinase (MuSK) in a patient's blood. This assay involves culturing cells that express MuSK and then incubating the cells with the patient's serum. If the serum contains anti-MuSK antibodies, they will bind to the MuSK receptors on the cells, resulting in a fluorescent signal that can be measured. The CBA is considered a highly sensitive and specific method for detecting anti-MuSK antibodies and is commonly used in the diagnosis of autoimmune diseases such as Myasthenia Gravis. HEK293 cells were cultured in a specific type of medium containing FBS, antibiotic-antimycotic, and glutamine. These cells were then transfected with an expression plasmid containing MuSK-GFP using Lipofectamine LTX® Reagent. After 24 hours, the cells were replated on coverslips and incubated with patient serum at a specific dilution for an hour [18]. The cells were then washed, fixed, and incubated with anti-human IgG-Texas Red for 45 minutes. The cells were permeabilized, counterstained, and mounted on microscope slides. Photomicrographs were collected using a fluorescence microscope and digital camera, and the CBA was performed twice [19,20]. Four raters were asked to score the presence of anti-human IgG immunofluorescence co-localized on the surface of MuSK-GFP-positive cells in each image as either definitely positive, possibly positive, or definitely negative. The scores were converted into percentages, and a test sample was considered positive if it exceeded the mean + 3 SD of the non-null values obtained for the non-myasthenic controls. The purpose of the CBA was to detect the presence of anti-MuSK antibodies in patient serum.

Discussion

The discussion describes the differences observed between the results of ELISA and RIPA tests in the detection of anti-AChR antibodies in the serum samples of Algerian myasthenia gravis patients. The study found that ELISA detected anti-AChR antibodies in fewer GMG patients than RIPA (87.5% vs. 100%, respectively), and that two patients who tested negative by RIPA had relatively high titers by ELISA, suggesting that RIPA may be less sensitive in detecting low-titer/low-affinity antibodies. However, RIPA detected anti-AChR antibodies in two OMG patients who were negative by ELISA. When the patients were classified by EMG decrement and age of onset form, both assays yielded comparable seropositivity ratios, but opposite trends were observed for EOMG and VLOMG seropositivity rates: RIPA showed greater anti-AChR detection rate among patients with VLOMG compared to ELISA, while the latter showed a higher seropositivity rate among patients with EOMG. Overall, the study confirms that the Algerian MG patients' seropositivity ratio is not significantly different from those reported in other populations. The proportion of MG patients who have autoantibodies against MuSK instead of AChR can vary significantly, ranging from 10% to 70% in patients who are negative for anti-AChR antibodies. In this particular study, none of the 23 Algerian MG patients tested positive for anti-MuSK antibodies using the anti-MuSK RIPA assay. Additionally, none of the 12 patient samples that were retested using the CBA assay were positive for anti-MuSK antibodies, despite the fact that serum samples from two Australian anti-MuSK positive control patients showed strong labeling. It is important to acknowledge the limitations of any study, and this study is no exception. The small size of the patient cohort tested is a limitation, as it may not accurately represent the larger Algerian population or other populations with myasthenia gravis. Additionally, the lack of anti-MuSK patients in the cohort is not surprising given the small sample size. The use of clinically positive and negative controls would have provided more information about the sensitivity and specificity of each assay, and an inter-assay sensitivity and specificity analysis could have been performed to better compare the two assays. Finally, the high titers observed in two patients by ELISA could bias the ELISA/RIPA correlation, and a serial dilution of these sera could yield a better quantification and correlation between the two assays.

Conclusion

The use of radioisotopes requires specialized equipment and expertise, which may not be available in all diagnostic laboratories. Therefore, alternative assays such as ELISA have been developed to detect anti-AChR antibodies, which can be performed in most diagnostic laboratories. While RIPA is considered the reference assay for detecting anti-AChR antibodies, the present study showed that the anti-AChR ELISA kit used in the study yielded comparable results to RIPA in detecting anti-AChR antibodies in Algerian MG patients. This suggests that the ELISA assay can be a cost-effective alternative to RIPA in resource-constrained settings. However, further studies with larger sample sizes and sets of positive and negative controls are needed to confirm these findings and assess the sensitivity and specificity of the assays. ELISA is a widely used and accessible assay with high sensitivity and specificity for detecting anti-AChR autoantibodies. In cost-constrained health systems, ELISA can serve as a primary screening test, and samples can be sent for further analysis by RIPA and/or CBA in cases of diagnostic doubt. The availability of commercial anti-AChR ELISA kits and the relative simplicity of its realization make the anti-AChR ELISA assay a useful diagnostic tool when used in combination with clinical observations and EMG. However, it is still recommended to confirm the diagnosis by clinical and electrophysiological criteria in cases of seronegative MG by ELISA.

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Not applicable.

Conflict of Interest

Author declares no conflict of interest.

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