Acetylcholine Receptor Antibody in Thai Generalized Myasthenia Gravis Patients

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The authors studied acetylcholine receptor antibody (AChR Ab) in twenty-six Thai patients diagnosed as having generalized myasthenia gravis and fifteen control cases. AChR Ab assay was done by radio-immunoassay technique and reported by titer in nmole/L. The positive result was defined by titer more than 0.5 nmole/L. In the myasthenia gravis group, age ranged from 18 to 64 years old with mean of 34 years old. The female: male ratio was 4.2:1. Duration of disease before taking blood sample ranged from 1 month to 14 years with a mean of 3.9 year. The AChR Ab could be detected in 21 out of 26 patients (80.7%). In the control group, tests were all negative. The results of the test made the sensitivity of 80.7% and specificity of 100%. The positive predictive value was 100%, the negative predictive value was 75%, and the prevalence was 60.3%. There was no correlation between AChR Ab titer and clinical features. This test is a very valuable test in case of uncertainty in the diagnosis of myasthenia gravis.

Keywords: Acetylcholine receptor antibody, Myasthenia gravis, Generalized, Radioimmunoassay

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University of Oxford, Oxford, United Kingdom. The methods for AChR Ab assay are described as follows:

**Standard anti-acetylcholine receptors antibody assay**

Myasthenia gravis sera were incubated at 1, 2.5 and 10 ul with 10-20 fmole of \( ^{125} \text{I-a-BuTx} \) binding sites (labeled with \( ^{125} \text{I-a-BuTx} \) to about 80% saturation) in a volume of 75 ul. For the two smaller volumes, sera were diluted 10 in 20 in PTX buffer (20 mM phosphate, pH 7.4, 0.1% triton x 100) and 20 and 50 ul added to 25 ul labeled muscle extract. The 10 ul assay was set up by adding 10 ul directly to the labeled extract. The volumes were made up with PTX buffer. After two to four hours at room temperature anti-human IgG (Seward Laboratories Ltd; 15-30 ul diluted 1:3 in PTX) was added to the 1 and 2-5 ul assays and the tubes left overnight at 4 c. The precipitates were pelleted, washed and counted. To the 10 ul assay an equal volume of 16% polyethylene glycol was added and after overnight incubation and centrifugation the pellets were washed twice very briefly with 1 ml of PTX buffer\(^{(6)}\) and counted.

Control incubations were performed with sera from normal healthy persons or neurological controls. The mean results from three control incubations were subtracted low titre (0.5-1.0 nmole/L), serum were included as positive controls. Results were expressed as nmole of \( ^{125} \text{I-a-BuTx} \), binding sites precipitated/litre of serum.

The results were given as positive only if all three tests (1, 2.5 and 10 ul serum) were positive and consistent with each other. If there were inconsistent, or the values obtained were less than 1.0 nmole/L, the serum was retested. Titres greater than 0.5 nmole/L were given as positive and based on the value obtained with 1 ul and 2.5 ul of serum\(^{(9)}\). In the case of relatively low titres, for example < 2.0 nmole/L with 1 ul of serum, often precipitated most or all of the available ACh R in which case the titer was given as a minimum value.

The mean cpm of three control sera was subtracted from these values, which in some tests sera gave negative results. Sera from other normal controls or non-myasthenic neurological patients gave values between -0.3 and 0.3 nmole/L (based on 2.5 ul serum). However, on repeat testing no control serum consistently gave values over 0.2 nmole/L\(^{(9)}\). Thus, sera whose values repeatedly fell in the range 0.2 to 0.5 nmole/L were designated as equivocal.

**Results**

Twenty-six patients with generalized myasthenia gravis and fifteen control sera were studied for clinical and AChR Ab. In the myasthenia gravis group, age ranged between 18-64 years old with the mean of 34 years old. There was a female predominance, the female: male ration was 4.2:1. Duration of disease before taking a blood sample ranged from one month to 14 years with a mean of 3.9 years. Fourteen of 26 patients were treated by thymectomy. The mean age of control cases was 26 years old (range 18-44). The AChR Ab could be detected (titer > 0.5 nmols/L) in 21 from 26 patients (80.7%) in the myasthenia gravis group. AChR Ab level varied from 4-7588 nmole/L. On the other hand, in the control group, tests were all negative (Table 1). From the results, sensitivity, specificity, positive predictive value, negative predictive value, and prevalence of this test were 80.7%, 100%, 100%, 75% and 60.3, respectively. AChR Ab titers were also calculated against age at onset and duration of disease by using log scale. There was no correlation between AChR titers and those two variables. While the titer difference between sex, and thymectomized patients could not be analyzed because of the small sample size.

**Discussion**

Myasthenia gravis is an acquired neuromuscular junction disorder. It presents in two main age groups; women between second and third decades and men over the age of 60. The diagnostic approaches are based on clinical presentations with the laboratory confirmation such as the tensilon test, Acetylcholine receptor antibodies (ACh R Ab), or electromyography with repetitive nerve stimulation.

AChR Ab, autoantibodies against the acetylcholine receptor, plays both a pathogenetically important role and diagnostic tool. These antibodies are found in 93, 88, and 71 percent of individuals with moderate to severe generalized MG, mild generalized MG, and ocular MG, respectively\(^{(10)}\). It can be found in some disorders that are not usually confused with MG (eg, primary biliary cirrhosis, tardive dyskinesia.

**Table 1.** The number of myasthenia gravis group (MG) and control group correlate with the ACh R test

<table>
<thead>
<tr>
<th></th>
<th>MG (N(%)</th>
<th>Control (N(%)</th>
<th>Total (N)</th>
</tr>
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<tbody>
<tr>
<td>ACh R test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21 (80.8)</td>
<td>0 (0)</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (19.2)</td>
<td>15 (100.0)</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100.0)</td>
<td>15 (100.0)</td>
<td>41</td>
</tr>
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</table>

Note: ACh R means Acetylcholine receptor antibody
autoimmune thyroiditis, systemic lupus erythematosus, thymoma without myasthenia, and amyotrophic lateral sclerosis. The results of the present study showed, for the first time, sensitivity and specificity of AChR Ab in Thai patients with MG. The sensitivity was 80.7% which is within the same range as the previous reports in Caucasian 73-90%, Jamaican 71.4%, and Chinese 71%. Approximately 10-20 percent of MG patients have a negative test on AChR Ab test by standard method or antibody negative myasthenia. Some of these patients have antibodies directed against a muscular-specific receptor tyrosine kinase (MuSK) that are not found in cases who are AChR Ab positive. The positivity of AChR Ab was uniformly negative in all cases or 100% specificity. Regarding specificity, one must keep in mind that the sample size was small, it may not be able to detect any false positive in the general population if the incidence is low. There was no correlation between AChR Ab titer and clinical features in the presented patients. These findings confirmed previous reports that age, sex, duration of disease, and treatment did not influence the concentration of AChR Ab in MG patients. Because the present study was a cross sectional study, it certainly did not provide the relationship between clinical course and AChR Ab in individual patients. It has been reported that the majority of patients with a prolonged improvement have a 50 percent or greater decrease in AChR Ab concentration regardless of type of therapy. In conclusion, AChR Ab test is sensitive and specific for the diagnosis of MG in Thai patients. It may not be very helpful in a typical prostigmine positive case but it certainly can be helpful to diagnose atypical, unresponsive to prostigmine cases.

References


Acetylcholine receptor antibody ในผู้ป่วยชาวไทยที่เป็นโรค myasthenia gravis ชนิดทั้งตัว

สุทธิพันธ์ จิตพิมลมาศ, สมศักดิ์ เทียมเก่า, วิรจิตต์ โชติมงคล, กิตติศักดิ์ สวรรยาวิสุทธิ์, Angela Vincent, John Newsom-Davis

คณะผู้ศึกษาได้ตรวจวัดระดับ Acetylcholine receptor antibody (ACh R Ab) ในผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรค myasthenia gravis ชนิดทั้งตัวจำนวน 26 รายและผู้ป่วยที่ไม่เป็นสุสานจำนวน 15 ราย โดยวิธีทางภูมิคุ้มกันรังสีและหาตรวจพบระดับที่สูงกว่า 0.5 nmole/L ถือว่าผลลบ ภายในผู้ป่วยที่ไม่เป็นโรค myasthenia gravis มีอายุระหว่าง 18 ถึง 64 ปี ผลส่วนใหญ่จะอยู่ต่ำกว่า 4.2: 1 ระหว่างเวลาที่เป็นโรคตอนตรวจระดับ ACh R Ab ตั้งแต่ 1 เดือนถึง 14 ปี (เฉลี่ย 3.9 ปี) ระดับ ACh R Ab สามารถตรวจพบในผู้ป่วยที่เป็นโรค 21 รายจาก 26 ราย (80.7%) ในกลุ่มควบคุมพบว่าผลการตรวจในกลุ่มควบคุมมีระดับ ACh R Ab ต่ำกว่า 100 และไม่มีความสัมพันธ์ระหว่างระดับ ACh R Ab กับอาการทางคลินิก.