Clinical characteristics and acetylcholine receptor antibodies in juvenile myasthenia gravis

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Background: **Anti-acetylcholine receptor antibody (AchRab) plays an important role in the pathogenesis of myasthenia gravis (MG). The purpose of this study was to evaluate the clinical characteristics and long-term changes of anti-acetylcholine receptor antibodies in patients with juvenile MG.**

Methods: **The data from 77 patients with juvenile MG, aged from 3 months to 16 years (45 were female and 32 male with a female to male ratio of 1.4:1) treated at Children's Hospital, Fudan University from 1992 to 2002 were reviewed retrospectively. All the patients were confirmed clinically and by the neostigmine test. Information about mode of MG presentation, myasthenia, ocular and systemic involvement, AchRab level, therapy and outcome was collected and evaluated. The serological test was done during follow-up.**

Results: **The onset of MG occurred at age below 3 years. The extraocular muscles were most frequently involved. According to the modified Osserman's criteria, 54 patients (70%) were classified into type I, 21 (27%) type II, and 2 (3%) type III. Of 52 patients, 18 (35%) were positive for AchRab and 16 (31%) were positive for acetylcholine premembrane receptor antibodies. The clinical state of the patients was not clearly correlated with the levels of the antibodies. No significant difference was observed between clinical type and AchRab positive among the 3 groups. Two (11%) of 18 patients were positive for thymona associated antibody (Titinab). Serological test during follow-up showed that 6 (60%) of 10 AchRab seronegative patients turned to be AchRab seropositive. In 85% of the patients, cluster of differentiation (CD) cells were abnormal, most of them showed reduced levels of CD_4^+ or CD_3^+ and CD_8^+. Thymus proliferation was found in 22 patients (42%) by CT and thymoma was confirmed operatively in 2 (4%). In 50% of the patients, electromyography (EMG) showed nothing abnormal. After administration of anticholinesterase drugs and steroids, the prognosis of the patients with MG was fairly good.**

Conclusions: **The age of onset of MG is younger, and the incidence of type II MG is increased. AchRab seronegative patients could turn to be positive. Serological monitoring is helpful to find more AchRab seropositive cases. Steroids have been proven effective and safe in treatment of juvenile MG. Methylprednisolone may produce less side-effects than oral predisone after steroid therapy.**

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Introduction

Myasthenia gravis (MG) is one of the better understood autoimmune diseases, in which target antigen, acetylcholine receptor (AchR), and pathogenic antibody to this receptor are well characterized.[1] Typical symptoms include diplopia, ptosis, dysphagia, and proximal muscle weakness, often with a clear history of fluctuating severity. Acetylcholinesterase inhibitor is used for therapy of MG.[2] MG patients who do not have AchR-specific antibodies (AchRab) are defined as having specific antibody negative MG (SNMG). Those who have AchRab are considered as having specific antibody positive MG (SPMG). Reports have shown that there are different pathological entities for SNMG and SPMG.[3] This study was designed to review retrospectively 77 MG patients who had been diagnosed at Children's Hospital, Fudan University from 1992 to 2002, focusing on the conversion of SNMG and SPMG each other during follow-up.

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Methods

In the 77 patients (45 were female) with MG, aged 1-16 years, the onset of the disease occurred between the ages of 3 months to 16 years, but commonly at an age of 3 years. In this series, 54 patients did not receive any treatment, and 23 received irregular treatment or relapsed after treatment. On admission, weakness of muscles was noted in all patients, one-side ptosis in 34 (44.2%), and two-side ptosis in 37 (48.1%), including 2 patients who suffered from limb weakness. The weakness of limbs was seen in 9 patients, the weakness of muscles of the medulla oblongata including the facial, lingual, throat and masticatory muscles in 7, and the weakness of respiratory muscles in 2. Before admission, the duration of myasthenia in 25 patients lasted for less than 1 month, 19 for 1-3 months, 8 for 3-6 months, 4 for 6-12 months, and 21 for 1-13 years. Most of the patients whose duration of the myasthenia was less than 1 year had not received any treatment. Most of the patients whose duration of the weakness was more than 1 year had received irregular treatment but relapsed thereafter.

In this series, the diagnosis of MG was based on typical clinical features, electromyography (EMG) findings, prostigmine test, and AchRab level. According to the modified Osserman's criteria, the patients were classified into 5 types: type Ⅰ patients (ocular MG); type Ⅱ patients (generalized MG) including type $Ⅱ_A$ patients who had ocular, facial and limb muscle weakness and type $Ⅱ_B$ patients who had bulbar muscle weakness except ocular, facial and limb muscle weakness; type Ⅲ patients who had outbreak generalized weakness and always showed myasthenic crises; type Ⅳ patients whose duration of the disease lasted for 2 years; and type Ⅴ (MG with atrophy of muscles).

The levels of AchRab, PremRab, Titinab and immunoglobin in the sera were estimated. The patients were subjected to CT for thymus, EMG and electrocardiography (EKG)

Prostigmine bromide was given orally to all patients in divided doses of 5-7 mg · kg^{-1} · d^{-1}, and the duration of administration lasted for 2-3 months. At the same time, all patients were given adrenal cortical hormone (prednisone or methylprednisolone). Twenty-one patients were given prednisone in a gradual increasing manner, i.e., it was first given in divided doses of 0.5 mg · kg^{-1} · d^{-1}, then increased from 0.5 mg/kg to 1.5-2 mg · kg^{-1} · d^{-1} every week. When the dose was slowly increased to the maximum effective dose, it began to reduce gradually to 7.5-10 mg/kg on the other day. The duration of treatment lasted for 1.5-2 years. Twelve patients took prednisone in a decreasing manner. Thirty-eight patients received intravenous methylprednisolone and 6 prostigmine bromide 0.5-1 year after thymectomy. Six patients were given intravenous immunoglobulin (IVIG) at a dose of 400 mg · kg^{-1} · d^{-1} for 3-5 days.

Results

Clinical classification

According to the modified Osserman's criteria, type Ⅰ MG was found in 54 patients, type Ⅱ including type $Ⅱ_A$ in 10, and type $Ⅱ_B$ in 11, and type Ⅲ in 2. No type Ⅳ or Ⅴ MG was seen in all patients. A 14-year-old female MG patient showed manifestations of hyperthyroidism after treatment for 3 years. A 2.5-year-old baby with weakness of the extraocular muscles was given oral prednisone for a day in out-patient clinic. He showed extensive weakness of the facial, neck and limb muscles. After hospitalization, acetylcholinesterase inhibitors tests and fatigue tests showed negative results. Two patients with weakness of the limb muscles were misdiagnosed as having psychological fits, and the other 2 patients with myasthenic crises having brain stem encephalitis.

Diagnostic tests

The sera of 52 patients were assayed for the concentrations of AchRab and Premab. Eighteen (35%) of 52 patients were positive for AchRab and 16 (31%) of the 52 patients were positive for acetylcholine PremRab on the initial examination. No difference was observed in each classification.

The sera of 18 patients was assayed for the concentration of Titinab. Two (11%) of the 18 patients were positive for thymoma associated antibody (Titinab). No thymoma was demonstrated by CT imaging.

Twenty patients underwent cluster of differentiation (CD) test and 85% of them showed abnormal results. The levels of CD_4^+ or CD_3^+ and CD_8^+ were reduced. The number of CD_4^+, CD_8^+, CD_3^+, and CD_{19}^+ cells decreased in 15 (88%), in 13 (77%), in 8 (47.1%), and in 6 patients (35.3%), respectively. Immunologlobin test of 20 patients revealed normal levels of IgG, IgA and IgM.

Computed tomography showed hyperplasia of thymus in 22 (42%) of 52 patients, and thymoma in 2 patients (4%) was confirmed operatively. Eighteen patients underwent EMG and EKG. In 50% (9/18) of the patients, EMG showed abnormal results. In all patients, however, EKG showed normal results. Sixteen patients underwent AchRab and PremRab again after they were treated for 7-18 months. Follow-up showed that 60% (6/10) of SNMG patients turned to be of SPMG. Three patients consistently had SPMG at the end of treatment.

Results of treatment

The 2 patients who were treated surgically were lost to follow-up because of long distance from the hospital. Finally 52 patients were followed up prospectively. Increased does of steroid was given to 12 patients, decreased dose of steroid to 12 and intravenous methylprednisolone to 28. The three regimens took effect at 15 (12-19), 7 (1-14) and 2 days (1-12), respectively. The average 9 maximum effect of these regimens was seen at 86 (25-228), 64 (7-165) and 27 days (4-63), respectively. The difference of the three treatments was significant. Twenty-six patients experienced the recurrence of the disease during the treatment. One recurrence took place in 10 patients, 2 recurrences in 10, and 3 recurrences in 4. Six patients were given intravenous injection of immunoglobulin (IVIg) at a dose of 0.4 $g \cdot kg^{-1} \cdot d^{-1}$ for 3-5 days because of exacerbation of weakness. Significant improvement was seen in these patients after 10-15 days, but adverse effects induced by corticosteroids included osteomalacia, hypertension, and gain of body weight, which were monitored during the treatment. Moreover, antacids, potassium and low-salt diet were also given.

Prostigmine bromide combined with corticosteroids made no exacerbation of weakness. Once the condition of the patient was stable for about 3 months at a maximum dose of steroids, or 5 mg (or no more than 20% of the previous dose) per month for several months, the patient was given a maintenance dose for a long period. Most patients had no cushingoid features, hyperglycemia, hypertension, cataracts, and osteoporosis in the treatment of corticosteroids.

Discussion

MG is mostly seen in newborns, children, and adults at the onset age of 20-40 years for females and 40-60 years for males. The patients are usually complicated by thymoma. In 2385 patients with MG reported in China from 1956 to 1998, 56.4% had the onset of the disease in childhood at age of 1-5 years.[4] In this study, the onset age of the patients was mostly below 3 years and female patients were predominant. Most of the patients had ocular MG. About 10%-25% patients were graded as having type Ⅱ disease. Sread's study showed that the onset age of juvenile myasthenia gravis was advanced, but concentrated on 7 years old. 63% of juvenile MG patients had generalized MG and 33% SPMG.[5]

In Hong Kong Chinese, 71% of children with MG had ocular MG[6] in contrast to 70.1% in this study. The majority of MG patients reported abroad had generalized MG.[7] Except for racial and geographical differences, there are other possible causes. (1) At the onset of MG in children, ocular muscles are involved more frequently than other muscles. The mild weakness of the ocular muscle is more visible than that of limb muscle. (2) The weakness of the limb muscle is less prominent but no ocular and bulbar muscle weakness is seen. (3) The patients with facial, lingual, masticatory and bulbar muscles weakness but without ocular muscle weakness are usually misdiagnosed. (4) The children with MG undergoing EMG are less than the adults, and some of them are unable to receive EMG. (5) Follow-up is too short to observe the changes of the disease. Hence the classification should be more exact after the following measures are taken as detailed history taking, fatigue test, EMG and long-term follow-up. In this series, one patient presented with extensive muscle weakness of the face, neck and limbs. Acetylcholinesterase inhibitors and fatigue tests showed negative results. The patient was misdiagnosed as as having brain stem encephalitis. Another patient who had muscle weakness of the limbs was misdiagnosed as being psychological fit. The two patients were subjected to EMG and AchRab. EMG is necessary for the diagnosis of MG.

Spale et al[8] reported that 14.5% patients (112/768) had other autoimmune diseases including rheumatoid arthritis and hyperthyroidism.[8] In our series, a 13-year-old girl with MG relapsed with hyperthyroidism after 3 years of treatment and SNMG turned to be SPMG. The relapsed cases can be divided into two types. One relapses during the treatment, and the other relapses after the treatment. In fact, it is common that the relapse of MG occurs during the treatment.

Because 90% MG adults are SPMG and most children with MG are SNMG, it is not helpful to assess the concentration of AchRab in children in prepuberty.[3] Seropositivity, however, helps to diagnose some patients with MG, who have atypical clinical manifestations or inadequate evidence for diagnosis.

Researchers consider that there are different pathologial mechanisms between SNMG and SPMG.[3] In our series, serological test during follow-up showed that 6 (60%) of 10 SNMG cases turned to be SPMG. Yshikawa et al[9] observed *in vitro* AchRab secreted by lymph nodes from 20 MG patients, who had received extended thymectomy, with special attention to their clinical status and stages. Thymic cells, bone marrow (BM) cells and peripheral blood mononuclear cells (PBMCs) were cultured for 1 week. AchRab and IgG in the culture medium were determined by immunoprecipitation assay and ELISA, respectively. PBMCs secreted AchRAb and IgG most effciently, followed by BM cells and thymic cells. AchRAb secreted by BM cells and PBMCs was significantly related to the levels

of serum AchRab. Thymectomy did not change the secretion of AchRAb or IgG by PBMCs within 3 months. The secretion of AchRab by PBMCs paralleled the clinical status of patients. Some seronegative patients showed positive results in culturing PBMCs, which are the most efficient site for AchRab production. Monitoring AchRab secretion by PBMCs is helpful to estimate the autoimmune activity of MG.[5] Our follow-up study and *in vitro* experiments abroad confirmed that SNMG and SPMG could turn to be each other. Whether SPMG and SNMG are different phases of MG is worth investigating. According to the modified Osserman's criteria, the concentrations of AchRab are consistent with clinical classifications. But other studies showed that the clinical manifestations of MG patients have nothing to do with the concentration of AchRab.[10] In our series, no difference was seen in different types of MG and different concentrations of AchRab.

Titinab is another antibody involving MG of thymoma (MGT). Since the seropositivity of Titinab in MGT is 80%-90%, it is helpful to diagnose MG. The reported concentration of Titinab was highly correlated with the concentration of AchRab and the severity of MGT.[11] In our study, we used ELISA to detect the concentration of Titinab. Because the concentrations of patients with MGT were not all increased, the diagnosis should be confirmed by clinical manifestations and CT. The seropositivity of Titinab was not high in our patients, which was comparable with less thymoma in pediatric patients. But the patients who were positive for Titinab in our series were all positive for AchRab. They did not show any evidence of thymoma in clinical examination and CT. Hence long-term follow-up in patients with a seropositivity of Titinab is required.

MG is an autoimmune disease dependent on T lymphocytes, which are essential to autoimmune response. In our study, 85% patients (17/20) who were subjected to CD cells examination showed abnormal results, and in most patients the levels of CD_3^+, CD_4^+ and CD_8^+ T cells reduced, suggesting that cell immunity is important in the pathogenesis of MG.

Most patients with severe MG are given acetylcholinesterase inhibitors, commonly pyridostigmine, in combination with immunomodulatory therapy.[12] In those with mild MG, pyridostigmine can be prescribed alone. Long-term treatment with ACEIs may induce desensitization and degeneration of the motor endplate associated with morphological changes. Hence acetylcholinesterase inhibitors in combination with glucocorticoids are given to the patients for several months. Single pyridostigmine can not bring about a complete remission.[13]

In the 1960s Brachman suggested a slower injection of steroids to reduce this exacerbation rate.[14] But in this study, this therapy could not relieve and preclude myasthenia but exacerbate myasthenia. With high doses of oral prednisone (e. g. 1 to 1.5 mg · kg^{-1} · d^{-1}), up to 30% of patients experienced an exacerbation of weakness within the first few weeks of the therapy, including myasthenic crises. The following are the advantages of this method: good results in a short period; tapering and stopping use of prednisone in a short period; focusing on the exacerbation of weakness in early period at hospital; and making the patients recover earlier. Intravenous methylprednisolone remains controversial because of concerns over increased weakness after a high dose of therapy, and it is widely used in children, safe and effective. In many studies, the effect of the three methods are similar regardless of the type, onset age and duration of the disease.[14,15] Experiencing exacerbated weakness within the first few weeks of the therapy, the patients should be hospitalized as early as possible. A respective study suggested that myasthenia crises are related to the improper intravenous injection of methylprednisolone in type I patients and those with light dispnea, who are contraindicated for methylprednisolone.[16] Monitoring, prophylaxis and early intervention in treating these side-effects should be emphasized in the management of MG patients on the regimen of prednisone. No cushingoid features, hyperglycemia, hypertension, cataracts and osteoporosis are observed in most patients in and after the treatment of corticosteroids.

During the treatment, exacerbated weakness can be relieved by increased doses of corticosteroids and temporary acetylcholinesterase inhibitors. When the patients are in severe infestation and do not wish to increase the dose of corticosteroids, immunoglobulin (0.4 g · kg^{-1} · d^{-1}) could be given intravenously for 3-5 days. Thymectomy has been widely used since the 1940s as a first-line treatment for MG.[17] Pediatric ocular MG accounts for the most part of the patients. But most of their parents are reluctant to select surgery for their sons or daughters, so only the patients who had been diagnosed as having thymoma are subjected to thymectomy.

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