Interventional Effect of Jianpi Bushen Granule Combined with Western Medicine on the Level of Serum Acetylcholine Receptor Antibodies in Myasthenia Gravis Patients

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ABSTRACT

Objective: This study is aimed to investigate the clinical significance of regulating effect of Jianpi Bushen Granule (JBG) combined with Western medicine on the level of serum acetylcholine receptor antibodies (AchRAb) in myasthenia gravis (MG) patients, so as to offer a theoretical basis of the targeted therapy with AchRAb.

Methods: We detected the level of anti-AchRAb with enzyme linked immunosorbent assay (ELISA) in the sera from 60 cases of MG patients (randomly divided into the trial group 30 cases, control group 30) for before and after treatment 6 months, and the clinical evaluation carried out by the Quantitative score of myasthenia gravis (QMG) for before and after treatment 3,6 months.

Results: (1) Comparison of the level of serum AchRAb: All included MG patients, the positive rate of serum AchRAb was about 81.67% (49/60), and the trial group was 83.33% (25/30), the control group was 90% (27/30), and the serum AchRAb levels of the trial group (0.994 ± 0.417) was lower than that of the control group (1.068 ± 0.358), but there was no significant difference between the two groups before treatment (P > 0.05). After 6 months of treatment, the serum AchRAb levels of the trial group (0.721 ± 0.280) was also significantly lower than that of the control group (0.907 ± 0.387), and the serum AchRAb levels was decreased dramatically compared with the same group before treatment, there was significant difference between the two groups (P < 0.05). (2)Comparison of QMG scoring:QMG scoring was decreased dramatically in the trial group compared with that of the control group after treatment, there was significant difference (P < 0.05), moreover, the total effective rate in the trial group (50%,86.67%) was significantly higher than those in the control groups (23.33%, 40%) after 3,6 months treatment (P < 0.05). There was also significant difference in the clinical evaluation by the QMG between the two groups before and after treatment. (3) Adverse reactions: no obvious adverse reactions appeared among the two groups.

Conclusions: JBG combined with Western medicine therapy on MG by regulating the level of patients’ serum AchRAb shows definite effects, and it is worthy of further studying.

Key Words: Myasthenia gravis, JBG, AchRAb, ELISIA

Abbreviations: JBG: Jianpi Bushen Granule; AchRAb: acetylcholine receptor antibodies; MG: myasthenia gravis; ELISA: enzyme linked immunosorbent assay; QMG: Quantitative score of myasthenia gravis; AchR: acetylcholine receptor; N2AchRab: nicotinic acetylcholine receptor; TCM: Traditional Chinese Medicine; MuSK: muscle-specific kinase; NMJ: neuromuscular junction

INTRODUCTION

Myasthenia gravis (MG) is a kind of autoimmune disease, it mainly involves the acetylcholine receptor (AchR) on the postsynaptic membrane of neuromuscular junction, and mediated by the specific nicotinic acetylcholine receptor (N2AchRab), as well as the complement participates its process. The latest epidemiological data shows that the incidence rate of MG is 8-20/100,000, the number of people contracting this disease in the United States are about 53000–59900, of contracting this disease are about 600,000 in China[1]. According to the theory of immunology, MG patients’ serum acetylcholine receptor antibodies (AchRAb) may appear to increase. Moreover, further clinical study also found that the AchRAb were detected in the peripheral blood of MG patients.

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up to 85%–90%, the clinical symptoms of MG patients could be improved significantly by taking the plasmapheresis to remove AchRAb, which might be due to the dual role of acetylcholine receptors included autoantigen model and neuromuscular receptors model, and the studies on antigenic structure and channel function also attracted the attention of many scientists worldwide. However, MG is a cosmopolitan stubborn disease, which may be involved in multiple mechanisms and regulated by multiple genes. Unfortunately, current evidence indicates that the exact pathogenesis of MG was still unclear, and the existing western treatment for MG had different degree of side effects. In addition, alternative treatment with Chinese characteristics has been shown to be effective in MG for many years, and particularly in ocular MG or generalized MG.

Therefore, to develop a strong effective but less side effects of traditional Chinese medicine compound is the urgent mission, developed by Pro. Jingsheng Zhang under the guidance of TCM PIJISHEN theory, and had obtained manifest therapeutic effects on whether the clinical or experimental studies till now. Then, based on our previous studies, it is necessary to further explore to the internal mechanism of effectively improve the clinical treatment of MG patients by the use of JBG combined with conventional Western medicine, can regulate the patient’s immune imbalance through reduced serum AchRAb level in blood, so as to improve the clinical symptoms of patients with MG. The study is reported as follows.

MATERIALS AND METHODS

1. Objects

The 60 patients enrolled were outpatients of special MG clinics of the Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (TCM) and the China Affiliated Hospital of Liaoning University of TCM from November 2010 to April 2012. The age distribution of the objects was defined in 14–75 years old. And the objects were recruited to randomly assigned to 2 groups: the trial group (30 cases) and the control group (30 cases) and the trial group (12 female, 18 male, age 48.70 ± 16.45 y, disease duration 28.03 ± 23.83 mo [mean ± SD]) the controls group (13 female, 17 male, age 49.03 ± 15.26 y, disease duration 30.43 ± 26.69 mo), controls with no history of autoimmune disease were recruited. Statistical analysis showed that the baseline data of the two groups of patients by age, sex, duration, no significant difference, was comparable (P > 0.05). All objects were informed, voluntary and signed informed consent.

2. Inclusion and Exclusion Criteria

Inclusion criteria: (1) in line with diagnosis of MG based on the Western medicine diagnostic criteria for autoimmune myasthenia gravis, including symptoms of fluctuating muscle weakness supported by an electromyographic pattern of neuromuscular transmission dysfunction by repetitive stimulation, belonging to the modified Osseman A, AA,AB type; (2) patients with Chinese medical diagnosis of flaccidity fitting to the differentiation standard of spleen-kidney deficiency type syndrome, Where have the one of the main symptoms and secondary symptoms of more than one, or both, see tongue, pulse can be included; (3) patients were between 14 and 75 years old patients were voluntary; (4) the subjects were informed voluntary and written informed consent to participate the study.

Exclusion criteria: (1) Osseman III, IV, V; (2) Familial MG, congenital myasthenic syndrome and drug (D-penicillamine, interferon induced by MG, etc.); (3) patients who were nursing or pregnant; (4) patients who had comorbidity diseases such as cerebrovascular diseases, renal insufficiency, hematopoietic system diseases, and psychiatric diseases; (5) allergies (allergic to more than 2 kinds of food or medicine); (6) The last 1 months participated in other clinical trials of drugs; (7) Once plasma exchange or intravenous gamma globulin in treatment patients in the past three months. In accordance with these exclusion criteria were excluded.

This study was approved by the ethics examination committee of the Longhua hospital and written permission was obtained from all who participated in the study. (The ethic approval number: 2010LCSY016, and trial registration number: ChiCTR-TRC-13004125)

3. Drugs Preparation

JBG was provided by Benxi chinese medicine factory Co.Ltd. (Liaoning Province, China). The preparation was a mixture of 11 Chinese herbal medicines shown in Table 1. In brief,

Table 1. The ratio of the components in JBG.

<table>
<thead>
<tr>
<th>Chinese name</th>
<th>Pharmaceutical name (Latin name)</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huangqi</td>
<td>Dried root of Astragalus membranaceus (Fisch.) (Radix Astragali)</td>
<td>50</td>
</tr>
<tr>
<td>Taizishen</td>
<td>Dried root of Pseudostellaria heterophylla (Miq.) (Radix Pseudostellariae)</td>
<td>25</td>
</tr>
<tr>
<td>Baihu</td>
<td>Dried root of Atractylodes macrocephala Koidz. (Rhizoma Atractyloides)</td>
<td>15</td>
</tr>
<tr>
<td>Zhike</td>
<td>Dried immature fruit of Citrus aurantium L. (Fructus Auranti)</td>
<td>15</td>
</tr>
<tr>
<td>Shengma</td>
<td>Dried root of Cimicifuga foetida L. (Rhizoma Cimicifugae)</td>
<td>15</td>
</tr>
<tr>
<td>Kuncao</td>
<td>Dried or fresh aerial parts of Leonurus japonicus Houtt. (Herba Leonuri)</td>
<td>30</td>
</tr>
<tr>
<td>Fangfeng</td>
<td>Dried root of Saposhnikovia divaricata (Turcz.) (Radix Saposhnikoviae)</td>
<td>10</td>
</tr>
<tr>
<td>Danping</td>
<td>Dried root of Angelica sinensis (Oliv.) Diels (Radix Angelicae Sinensis)</td>
<td>10</td>
</tr>
<tr>
<td>Gouqi</td>
<td>Dried ripe flesh of Lycium barbaryum L. (Fructus Lycii)</td>
<td>15</td>
</tr>
<tr>
<td>Heshouwu</td>
<td>Dried root of Polygonum multiflorum Thunb. (Radix Polygoni Multiflori)</td>
<td>15</td>
</tr>
<tr>
<td>Shanyourou</td>
<td>Dried ripe flesh of Cornus officinialis Sieb (Fructus Corni)</td>
<td>15</td>
</tr>
<tr>
<td>Total amounts</td>
<td></td>
<td>215</td>
</tr>
</tbody>
</table>
these were extracted with 1 L of boiled water twice for 1 hr. Poaching liquid was mixed two times. The dregs of the decoction were removed after filtering. The filtered liquid was lyophilized and crushed into a thin powder. The yield of the dried extract was about 38%. JBG was prepared and stored in a granular form before administration to patients. The Drug specifications was 10 g per bag, including 7.4 g of crude drug. Olfactory pyridostigmine tablets (an effective drug generally accepted in MG clinical trials) were provided by Chinese Shanghai Sunway pharmaceutical Co. Ltd., China (No. H31020867). The Drug specifications was 60 mg per tablet.

4. Administration

The trial group was received JBG (take a package each time and was given orally two times per day) combined with olfactory pyridostigmine tablets (60 mg/times, 4 times/day), according to the patient’s symptoms was improved, gradually tapering to a relatively low maintenance dose reference to some literature[11]. The control group was only received olfactory pyridostigmine tablets (the same as the trial group). The entire course of treatment was maintained for six months. The dosages of western medicines were gradually reduced to a relatively low maintenance level (olfactory pyridostigmine), but alteration of the study herbal medications was not allowed during the study period. To the end of the trial, we can use the number of JBG recovery to ensure the compliance of the patients taking Chinese herbal and the quality of this study.

5. Sample collection and Determination of Serum AchRAB

Serum samples (5 ml fasting venous blood of each sample) were collected early in the morning after the first (Enrollment) and second (6 mo), rapidly separated serum, were saved at −70°C in order to be measured. The serum samples in our laboratory was coated onto a 96-well microtiter plate (Fudan University neuropathy Institute (20030801) with 0.1 M carbonate bicarbonate buffer (pH9.6) overnight at 4 °C. The plates were blocked with 2% BSA in PBS at room temperature for 30 min. Serum samples diluted 1/800 in PBS/0.05% Tween 20 were added and incubated at 37 °C for 90 min. After four washes, HRP-conjugated goat anti-mouse IgG isotypes (IgG, IgG1, IgG2b (Caltag, San Francisco, CA)) diluted 1/800 in PBS/0.05% Tween 20 were added and incubated at 37 °C for 90 min. Subsequently, ABTS (indicator) solution in 0.1 M citric buffer, pH 4.3, in the presence of H2O2 was added, and color was allowed to develop at room temperature in the dark. Absorbance values were measured at a wavelength of 492 nm, usig a Dynatech ELISA reader, and the results were expressed as OD values (P/N > 2.5, positive).

6. The Quantitative score of myasthenia gravis (QMG) Evaluation

Symptoms were observed and recorded by means of the QMG at 3 times during the study: initiation day, end of the 3.6 months (discrepancy within 2 days was allowed), respectively. This scale had 13 items, used to evaluate the fatigue state in patients with MG, (the left, the right score), a total of 6 points, with a four-level scoring system: (1) normal (0points); (2) mild, sometimes (1points); (3) moderate, always (2points); (4) severe, nearly substantially completely unable to be fit to do some action (3points)[12]. Every item was scored and the total points were calculated.

7. Therapeutic Effect Criteria

The therapeutic effect criteria were formulated according to references[13]. These were as follows: Clinical relative scoring= ( absolute score of Pre-treat —Absolute scores of post-treat ) / absolute score of Pre-treat ×100%. (1) Cure rate: ≥95%, (2) Basic cure rate:81%~95%, (3) Markedly effective:51%~80%, (4) Improvement rate: 26%~50%, (5) ineffective:≤25%. The total effective rate = (Cure rate + Basic cure rate + markedly effective + Improvement rate. The efficacy of QMG was compared before and after treatment 3, 6 months.

8. Safety Evaluation

We examined the breathing, heart rate and blood pressure once a week, and examined routine blood tests, routine urinalysis, routine stool, hepatorenal function, and electrocardiogram before and after treatment.

9. Statistical Analysis

All the data are analyzed with the SPSS 15.0 software (produced by SPSS Corporation, Chicago, IL, USA). For the measurement data, the paired sample t-test was used for the comparison of the variables in each group before and after treatment; comparison of the variables in groups was done using One-way variance analysis and the q test; and categorical variable analysis was performed with the χ2 test. Values of P < 0.05 were considered significant.

RESULTS

1. Effect of JBG on the level of Serum AchRAB

Twenty-five male and 35 female MG patients were enrolled in this study, 49 patients’ serum AchRAB showed positive (81.67%), 25 cases (83.33%) for trial group,27 patients (90%) for the control group, there was no significant difference between the two groups before treatment (P > 0.05), was comparable; after 6 months treatment, there were 21 case’s serum AchRAB in the trial group showed positive (70%), 25 cases (83.33%) in the control group, we do statistical analysis using the χ2 test, χ2 = 0.001, P=0.970 > 0.05, means that the patients’ serum AchRAB positive between the two groups rate was no significant difference before and after treatment, while the patients’ serum AchRAB levels in the trial group were significantly lower than that of the control group before and after treatment, and there was significant difference between the two groups, with statistical significance (P < 0.05). Moreover, the patients’ serum AchRAB levels in the two groups were significantly lower than that of the same group before treatment, there was also significant difference (P < 0.05) (Table 2, Fig 1.)
2. Comparison of the QMG Scoring and Efficacy between the Two Groups Before and After Treatment

After 3 and 6 months of treatment, the QMG scores of the trial group and the control group were lower than the same groups before treatment, there was a difference between the two groups ($P < 0.05$). Moreover, the QMG scores of the trial group were apparently lower than that of the control group ($P < 0.05$), there was also obvious difference ($P < 0.05$, Table 3).

After 3 months of treatment, the improvement rate, ineffective rate, and the total effective rate of the trial group were $50\%$ (15/30), $50\%$ (15/30), and $50\%$ (15/30), respectively; of the control group, $23.33\%$ (7/30), $76.67\%$ (23/30) and $23.33\%$ (7/30), respectively. The improvement rate and the total effective rate in the trial group were significantly higher than those in the control group ($P < 0.05$). After 6 months of treatment, the cure rate ($3.33\%$), markedly effective rate ($30\%$), and improvement effective rate ($53.33\%$) in the trial group were significantly higher than those in the control group ($0\%$, $13.33\%$, and $26.67\%$, respectively), there was also significant difference ($P < 0.05$). In addition, the improvement rate, ineffective rate, and the total effective rate of the two groups were obviously better than the same group before treatment, there was significant difference ($P < 0.05$, Fig 2).

3. Safety

No obvious adverse reactions appeared among the two groups during the treatments.

**DISCUSSION**

Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction usually caused by antibodies to the nicotinic acetylcholine receptor (AChR) and occasionally to muscle-specific kinase (MuSK)[14]. To some extent, acute exacerbation of generalized MG can cause swallowing impairment, respiratory failure, or death and has already deeply caused much hazards to human health, as well as also often lead to high medical costs[1,15]. With the development of research, it has been recognized that AChRAb was mainly circulating antibodies which targeted at the target organ neuromuscular junction (NMJ) postsynaptic membrane AChR, can launch a specific attacks against the postsynaptic membrane unless complements includes, made the postsynaptic membrane simplistic, reduced the NMJ receptor and

<table>
<thead>
<tr>
<th>Item</th>
<th>Pre-treat</th>
<th>6 months</th>
<th>Pre-treat</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial group (n = 30)</td>
<td>Control group (n = 30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AChRAb positive</td>
<td>25(83.33%)a</td>
<td>21(70%)bc</td>
<td>27(90%)</td>
<td>25(83.33%)</td>
</tr>
<tr>
<td>AChRAb levels</td>
<td>0.994 ± 0.417a</td>
<td>0.721 ± 0.280bc</td>
<td>1.068 ± 0.358</td>
<td>0.907 ± 0.387</td>
</tr>
</tbody>
</table>

Notes: a $P > 0.05$, compared with the control group (Pre-treat); $P < 0.05$, compared with the control group; $P < 0.05$, compared with the same group before treatment.

<table>
<thead>
<tr>
<th>Item</th>
<th>Pre-treat</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial group (n = 30)</td>
<td>Control group (n = 30)</td>
<td></td>
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</tr>
</tbody>
</table>

Notes: $P > 0.05$, compared with the same group before treatment.

Figure 1. Comparison of the level of serum AchRAb among the two groups before and after treatment ($^aP < 0.05$, $^bP < 0.05$).

Table 3. Comparison of Evaluation of QMG Scoring among The Two Groups ($\bar{x} \pm s$).

<table>
<thead>
<tr>
<th>Item</th>
<th>Pre-treat</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial group (n = 30)</td>
<td>Control group (n = 30)</td>
<td></td>
</tr>
<tr>
<td>AChRAb positive</td>
<td>10.80 ± 4.92a</td>
<td>12.10 ± 5.70</td>
<td>10.86 ± 4.98</td>
</tr>
<tr>
<td>AChRAb levels</td>
<td>9.70 ± 3.82bc</td>
<td>6.19 ± 3.57cd</td>
<td>9.41 ± 11.18</td>
</tr>
</tbody>
</table>

Notes: $P > 0.05$, compared with the control group (Pre-treat); $P < 0.05$ (3 months), $P < 0.01$ (6 months), compared with the control group; $P < 0.05$ (3 months and 6 months) compared with the same group before treatment.

Figure 2. Comparison of clinical efficacy among the groups before and after treatment (case). After 3 months of treatment, $^aP > 0.05$(improvement rate), $^bP < 0.05$(Total effective rate), compared with the control group; After 6 months of treatment, $^aP < 0.05$(improvement rate), $^bP < 0.05$, compared with the same group before treatment.
increased synaptic space, eventually led to the incidence of MG. It is reported\(^\text{16-19}\) that AChRAb can be detected in the serum of more than 85% of generalized myasthenia gravis patients, AChRAb levels and the severity of the clinical manifestations of MG was associated. Although AChRAb were not detected in the serum of about 15% of patients, and these patients were referred to the antibody-negative MG (Seronegative MG, SNMG)\(^\text{20}\), the SNMG patients’ clinical manifestations in line with MG antibodies patients seemed basically similar, were also sensitive to immunosuppressive therapy, and also copied the MG model after the SNMG patient’s serum or IgG to be injected to mice\(^\text{21}\). In addition to AChRAb mediated therefore, there was may be other antibodies or molecules involved in the MG pathogenesis. However, the changes of serum AchRAb levels can still be detected in patients with MG in order to guide clinical diagnosis and treatment. At present, nonspecific immunity inhibitory is still mainly adopted in the clinical treatment of MG patients, and wherein the cholinesterase inhibitors therapy on patients is still used as the preferred drug which has affected on the development in the treatment of MG\(^\text{22,23}\). Furthermore, long-term use of steroid drugs caused changes in their image, such as having a moon-like face, being fat and having hair loss, which also affects the patient’s self-esteem and psychological aspects, and also often lead to high medical costs\(^\text{15}\). At present there is still a lack of effective prevention and therapeutic measures on MG patients\(^\text{24,25}\). As we all know that traditional herbal medicines have been used for thousands of years and are beneficial in prevention and treatment of many diseases, including MG. Greater attention is being given to such medicines due to their varied biological actions and low toxicity.

According to the theory of TCM, the MG belongs to “TCM Flaccidity” category. The main physiological functions of the TCM kidneys are: storing essence; dominating growth, development and reproduction; regulating water metabolism and receiving Qi. The kidneys also take charge of the bone and manufacture marrow. Therefore, it is considered as the “foundation of prenatal life”. If the kidney essences insufficiency or primary Qi deficiency will affect growth and development as well as muscle function directly, which lead to the disease appearing eventually; “The TCM spleen is the foundation of postnatal life”, it is also viewed as the source for the production and transformation of Qi and blood, the TCM spleen dominate muscles, limbs, if this function of the spleen and stomach is deficient, will cannot nourishing muscles and bones, and the limbs will become flaccid and disuse eventually. Our previously related studies\(^\text{26,27}\) had also shown that TCM pathogenesis of MG were spleen-kidney deficiency type, which was probably the most common type in clinical research. Under this premise, we developed the basic prescription called “Jianpi Bushen Granules” for the treatment of the MG patients with spleen-kidney deficiency type. Huangqi (Radix Astragali) included in the prescription can regulate immune with two-way function, and to reduce serum AchRAb level by enhancing the inhibitory activity of T lymphocytes, Gouqi (Lycium chinense) are play a regulatory role by increasing the number and activity of T lymphocytes, and Danggui (Radix Angelicae Sinensis) can potent enhance the non-specific immune function but suppress humoral immune\(^\text{28,29}\), and so on.

In this study, on the Western conventional treatment’s foundation, we plused Chinese herbal compound JBG with invigorating spleen-kidney role to treat MG, in accordance with the principles of evidence-based medicine, using randomized, controlled method to confirmed that the use of Chinese herbal compound combined with Western medicine can significantly improve the total effective rate of the trial group after treatment (50%, 3 months, 86.67%, 6 months), there was significant difference \(P < 0.05\) compared with the control group (23.33%, 3 months, 40.6% 6 months). We also found that the patients’ serum AchRAb levers in the trial group were significantly lower than that of the control group after treatment, there was significant difference between the two groups \(P < 0.05\), with statistical significance. Moreover, the patients’ serum AchRAb levers in the two groups were significantly lower than that of the same group before treatment, there was also significant difference \(P < 0.05\). Although the positive rate of patients’ serum AchRAb was no significant difference between the two groups before and after treatment, of that were more significant reduced compared with the same group before treatment. We speculated that the reason might be because of the relatively small samples, and the relatively short duration of treatment. According to the composition of prescription principles in TCM Formulae, we known that JBG was mainly suitable for the treatment of ocular and systemic myasthenia gravis (equivalent to the TCM of Flaccid patients with spleen-kidney deficiency type), which consisted of Huangqi (Radix Astragali), Gouqi (Lycium chinense), Taizishen (Radix Pseudostellariae), and so on, has invigorating the kidney-spleen as well as elevating Yang Qi functions. Based on the classical theory of TCM, we analysed that Huangqi (Radix Astragali) and Gouqi (Lycium chinense) can reinforcing kidney-spleen and elevating Yang Qi as the principal drugs of this decoction, while compatible effects of the minister drug Heshouwu (Radix Polygoni Multiflori) and Shanzhuyu (Fructus Corni) can nourishing the liver-kidney, Taizishen (Radix Pseudostellariae) can replenishing qi to invigorate the spleen; and compatible effects of the assistant drug Danggui (Radix Angelicae Sinensis) can promoting circulation of Qi and blood. This organic inner joint of the decoction can significantly increase the clinical effectiveness via tonifying the spleen-kidney, to enhancing the nutrition of muscles and bones, and ultimately achieve the purpose for treatment on MG. In addition, our previous studies\(^\text{5-8,28,30-32}\) have also shown that JBG can reduce the level of IFN-1 and it’s mRNA and then decrease AChRAb level in blood and reduce severity of myasthenia, can promote the growth of new axon in NMJ, increase the number of synaptic vesicles and reduce the synaptic injury in model rat of trial autoimmune MG(EAMG), and can effectively regulate the levels of some EAMG-related differential proteins expressive. In this study, we found that the use of the JBG combined with Western Medicine therapy on Patients with MG can obtain better clinical effects via effectively regulated the serum AchRAb levels of patients,
which also verified the existence of a correlation between the level of serum level of AchRAb and clinical symptoms of patients in MG\cite{33}, and there was no any adverse reaction appeared in the course of the study. However, this study still can not fully explained that the integrative treatment on MG with a long-term advantage, and also does not provide enough evidence based medicine for the study on MG due to the time and sample size and other factors. Then, exactly which part of the JBG does play a key role in the treatment for MG? Or which channels of JBG does adjust the body’s immunological environment and then to display the combined effect of it? Obviously, these should be urgently concerned issue of future studies. Therefore, it is necessary to carry out more in-depth multidisciplinary study on the the fundamental mechanisms of JBG therapy on MG using the more cutting-edged scientific and technical means, such as DNA technology, molecular biology techniques, and so on to furtherly clarify pathogenesis of MG, and provide more effective and well treatment for MG.

Further evaluation on long-term and more in-depth study of JBG will be carried out by continuous follow-up studies.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**REFERENCES**