Long-term Efficacy of Modified Guilu Erxian Jiao Formula in the Treatment of Patients with Myasthenia Gravis: A Retrospective Cohort Study

Clinical Study Protocol

1 Study Subjects

A retrospective analysis was performed on the medical records of patients with myasthenia gravis (MG) who attended the First Affiliated Hospital of Henan University of Chinese Medicine and Henan Academy of Medical and Pharmaceutical Sciences from January 2020 to January 2024. The final on-site or telephone follow-up for the outcomes of the enrolled cases was completed by July 31, 2024.

2 Diagnostic Criteria

2.1 Western Medicine Diagnostic Criteria

The criteria were formulated in accordance with the *Guidelines for the Diagnosis and Treatment of Myasthenia Gravis in China (2020 Edition)* issued by the Chinese Society of Neuroimmunology, Chinese Society for Immunology. A clinical diagnosis of MG can be made if the patient presents with typical clinical manifestations of MG, supported by any one of the neostigmine test, neuroelectrophysiological assessment, or serum specific antibody detection, and other diseases are excluded (Table 1).

Table 1 Western Medicine Diagnostic Criteria for Myasthenia Gravis

Item	Description
Clinical Manifestations	Any skeletal muscle throughout the body can be affected, presenting with fluctuating weakness and fatigability. Symptoms follow a pattern of "worse in the evening and better in the morning", aggravated after activity and relieved after rest. The extraocular muscles are the most commonly affected, presenting with symmetric or asymmetric ptosis and/or binocular diplopia, which are the most common initial symptoms of MG.
Pharmacological Findings	Positive neostigmine test.
Neuroelectrophysiology	A decrement of more than 10% in wave amplitude with low-frequency stimulation on repetitive nerve stimulation (RNS) examination.
Antibodies	Acetylcholine receptor (AChR) antibodies can be detected in the blood of most patients with generalized MG. A small number of patients with AChR antibody-negative MG can be tested positive for muscle-specific kinase (MuSK) antibodies, low-density lipoprotein receptor-related protein 4 (LRP4) antibodies, or titin antibodies.

2.2 Western Medicine Classification Criteria

The criteria were formulated in accordance with the Myasthenia Gravis Foundation of America (MGFA) Clinical Classification (2000) (Table 2).

Table 2 MGFA Clinical Classification Criteria

Classification	Symptom Manifestation
Type I	Any ocular muscle weakness, may be accompanied by weak eyelid closure, with normal muscle strength in other muscle groups.
Type II	Mild weakness in muscle groups other than the ocular muscles, may be accompanied by ocular muscle weakness.
Type IIa	Mainly affecting limb muscles and/or trunk muscles, with or without lesser degree of bulbar muscle involvement.
Type IIb	Mainly affecting bulbar muscles and/or respiratory muscles, with or without lesser degree of limb and/or trunk muscle involvement.
Type III	Moderate weakness in other muscle groups regardless of the degree of ocular muscle weakness.
Type IIIa	Mainly affecting limb muscles and/or trunk muscles, with or without lesser degree of bulbar muscle involvement.
Type IIIb	Mainly affecting bulbar muscles and/or respiratory muscles, with or without lesser degree of limb and/or trunk muscle involvement.
Type IV	Severe weakness in other muscle groups regardless of the degree of ocular muscle weakness.
Type IVa	Mainly affecting limb muscles and/or trunk muscles, with or without lesser degree of bulbar muscle involvement.
Type IVb	Mainly affecting bulbar muscles and/or respiratory muscles, with or without lesser degree of limb and/or trunk muscle involvement.
Type V	Endotracheal intubation with or without mechanical ventilation (excluding routine postoperative use). Cases receiving only nasogastric feeding without endotracheal intubation are classified as Type IVb.

3 Case Selection Criteria

3.1 Inclusion Criteria

(1) Meeting the diagnostic criteria of the Chinese Medical Association, with MGFA classification Type I-IV;(2) Meeting the diagnostic criteria of the China Association of Chinese Medicine, with TCM syndrome differentiation of spleen and kidney deficiency;(3) No age or gender restrictions;(4) Received conventional western medicine treatment for ≥1 month (pyridostigmine bromide tablets combined with prednisone acetate tablets);(5) Complete medical records.

Only patients who met all the above criteria were included in this study.

3.2 Exclusion Criteria

(1) Patients with concurrent other severe systemic diseases, severe impairment of heart, liver, kidney or other organ functions, or history of psychiatric disorders;(2) Patients with incomplete medical records.

Patients who met any of the above criteria were excluded.

4 Grouping Method

All enrolled patients received conventional western medicine treatment, and were grouped as follows:(1) Treatment Group: Patients who took the modified Guilu Erxian Jiao formula for a cumulative duration of ≥1 month per year(2) Control Group: Patients who only received conventional western medicine treatment

The conventional western medicine regimen consisted of pyridostigmine bromide tablets combined with prednisone acetate tablets. The modified Guilu Erxian Jiao formula was composed of Colla Carapacis et Plastri Testudinis 10g, Colla Cornus Cervi 10g, Ginseng Radix et Rhizoma 10g, Lycii Fructus 10g, and Astragali Radix 30g, with the dosage halved for minor patients.

5 Data Collection

5.1 Baseline Information

General information including name, gender, age, body mass index (BMI); medical history information including age at onset, disease duration, initial affected muscle groups, involved muscle

groups; auxiliary examination results including serum autoantibody levels, thymus status, neostigmine test, RNS and other information were extracted from the patients' medical records and filled in the relevant sections of the Case Report Form (CRF). On-site or telephone follow-up was conducted on the outcome events of the patients, including the occurrence of relapse, time and cause of relapse, and medication tapering status, to further supplement and improve the CRF.

5.2 Primary Outcome Measure

(1) Relapse RateRelapse was defined as recurrence of MG symptoms or a significant increase in MG medications after the patient achieved Minimal Manifestation Status (MMS) or better. The overall relapse rate and relapse rates at different time points (within 6 months, 6-12 months, 12-24 months, 24-36 months, and 36-48 months after treatment) were calculated.

5.3 Secondary Outcome Measures

(1) Time to First Dose Reduction of Conventional Western MedicineTime to first dose reduction was defined as the interval between the date of the first dose reduction and the initial consultation, measured in months. The time to first dose reduction of pyridostigmine bromide tablets and prednisone acetate tablets was calculated for both groups respectively.

(2) TCM Syndrome Score ScaleThe TCM syndrome score was formulated with reference to the *Guiding Principles for Clinical Research of New Traditional Chinese Medicines* issued by the Ministry of Health of the People's Republic of China in 2002 (Appendix 1). The total TCM syndrome score and scores of each individual symptom were calculated.

(3) Myasthenia Gravis-Activities of Daily Living (MG-ADL) ProfileThe core function of the MG-ADL is to quantitatively analyze the correlation between the patient's clinical manifestations and daily functional status to reflect the severity of the disease, which includes 8 items (Appendix 2).

(4) Incidence of Myasthenic CrisisMyasthenic crisis was defined as rapid deterioration of MG condition requiring immediate airway opening and assisted ventilation, or MGFA classification Type V.

(5) MGFA Clinical ClassificationThe Myasthenia Gravis Foundation of America (MGFA) Clinical Classification (2000) was adopted, including Type I, Type IIa, Type IIb, Type IIIa, Type IIIb, Type IVa, Type IVb, and Type V.

(6) Hamilton Anxiety Rating Scale (HAMA)The HAMA is a clinical scale used for the diagnosis and severity grading of anxiety states (Appendix 3).

(7) MGFA Post-Intervention Status (MGFA-PIS)The MGFA-PIS was developed by the Myasthenia Gravis Foundation of America (Table 3). A favorable outcome was defined as achieving any of Complete Stable Remission (CSR), Pharmacologic Remission (PR), or Minimal Manifestation Status (MMS).

Table 3 MGFA Post-Intervention Status (MGFA-PIS)

Grade	Description of Post-Intervention Status
Complete Stable Remission (CSR)	No symptoms or signs of myasthenia for at least 1 year, during which no MG medications were received; no evidence of muscle weakness found on examination by a professional neurologist, with minimal weak eyelid closure allowed.
Pharmacologic Remission (PR)	The criteria are the same as CSR, except that the above state is achieved through medication (excluding cholinesterase inhibitors).
Minimal Manifestation Status (MMS)	No functional limitation caused by myasthenia, with certain muscle weakness detected on examination by a professional neuromyopathy physician.
Improved (I)	Significant reduction in clinical symptoms of myasthenia or significant decrease in the dosage of MG treatment medications compared with baseline.

Grade	Description of Post-Intervention Status
Unchanged (U)	No significant change in clinical symptoms and dosage of MG treatment medications compared with baseline.
Worse (W)	Significant aggravation of clinical symptoms of myasthenia or significant increase in the dosage of MG treatment medications compared with baseline.
Exacerbation (E)	New clinical symptoms appear after CSR, PR or MMS has been achieved.
Died Of MG (D)	Death from MG or complications of MG treatment, or death within 30 days after thymectomy.

6 Autoantibody Detection

Acetylcholine receptor antibody (AChR-Ab) was detected using an enzyme-linked immunosorbent assay (ELISA) kit from RSR Ltd. (UK), and titin antibody (Titin-Ab) was detected using an ELISA kit from DLD Diagnostika GmbH (Germany). The cut-off values for positive AChR-Ab and Titin-Ab were 0.45 nmol/L and 1, respectively.

7 Statistical Analysis

Medical records and follow-up data were entered into a database using Microsoft Excel software, followed by double review and verification. Incorrect information was rechecked against the original medical records, and the verified data were imported into SPSS 26.0 software for statistical analysis.

Measurement data conforming to normal distribution were expressed as mean ± standard deviation ($\bar{x}$ ± SD) and analyzed by independent samples t-test; those not conforming to normal distribution were expressed as median and interquartile range [M(P25, P75)] and analyzed by rank sum test. Enumeration data were expressed as number of cases (n) and rate (%), and analyzed by chi-square (χ^2) test. A P-value < 0.05 was considered statistically significant.

Kaplan-Meier (K-M) survival analysis was used to plot the cumulative relapse probability risk curve, and the Log-Rank test was performed for hypothesis testing. Cox multivariate regression analysis was conducted to identify statistically significant variables associated with relapse, and to screen out relevant protective and risk factors for MG relapse.

Appendix 1: TCM Syndrome Score Scale

Table A1 TCM Syndrome Score Scale

Symptom	Mild (1 point)	Moderate (2 points)	Severe (3 points)	Score
Sallow Complexion	Pale yellow without dirt, with luster	Slightly dull and sallow, with faint luster	Dull sallow complexion with dirt, no luster	
Shortness of Breath and Reluctance to Speak	Speaks little only when asked repeatedly	Does not speak unless asked	Unwilling to speak at all	
Chest Distress	Mild chest distress	Obvious chest distress with frequent deep sighs	Feeling of chest oppression as if stuffed	
Epigastric Distension	Epigastric distension after meals, resolves spontaneously within 30 minutes	Epigastric distension after meals, resolves spontaneously within 2 hours	Persistent epigastric distension and abdominal bloating	
Reduced Food Intake	Slight decrease in food intake	Decreased food intake	Markedly reduced food intake	
Loose Stools	Unformed stool	Loose stools 2-3 times a day	Watery loose stools more than 4 times a day	
Soreness and Weakness of Loin and Knees	Intermittent soreness and weakness of the waist and knees	Dull aching pain, requiring frequent postural changes	Severe lumbago as if broken, persistent and unrelieved, only relieved by medication	

Symptom	Mild (1 point)	Moderate (2 points)	Severe (3 points)	Score
Cold Intolerance and Cold Limbs	Subjective feeling of cold	Frequent cold intolerance or cold hands and feet in winter, requiring extra clothing	Body temperature often fails to reach normal level, cold hands and feet in both winter and summer	
Frequent Nocturia	Nocturia 2 times	Nocturia 3 times	Nocturia 4 times or more	
Total Score	-	-	-	

Note: 0 points for absence of the above symptoms; 1 point for mild symptoms; 2 points for moderate symptoms; 3 points for severe symptoms.

Appendix 2: Myasthenia Gravis-Activities of Daily Living (MG-ADL) Profile

Table A2 Myasthenia Gravis-Activities of Daily Living (MG-ADL) Profile

No.	Item	0 points	1 point	2 points	3 points	Score
1	Speech	Normal	Intermittent hyponasality or nasal twang	Continuous hyponasality or nasal twang, but intelligible	Speech is unintelligible	
2	Chewing	Normal	Fatigue when eating hard food	Fatigue when eating soft food	Nasogastric tube feeding with liquid diet	
3	Swallowing	Normal	Occasional choking	Frequent choking leading to dietary changes	Nasogastric tube feeding with liquid diet	
4	Breathing	Normal	Shortness of breath after exertion	Shortness of breath at rest	Mechanical ventilation support	
5	Impaired ability to brush teeth or comb hair	None	Strenuous, but can be completed without rest	Requires rest midway	Complete loss of these abilities	
6	Impaired ability to stand up from a chair	None	Mild, sometimes requires hand support	Moderate, often requires hand support	Severe, requires assistance from others	
7	Diplopia	None	Occasional, not daily	Daily, but not persistent	Persistent	
8	Ptosis	None	Occasional, not daily	Daily, but not persistent	Persistent	
-	**Total MG-ADL Score (0-24)**	-	-	-	-	

Appendix 3: Hamilton Anxiety Rating Scale (HAMA)

Table A3 Hamilton Anxiety Rating Scale (HAMA)

Symptom	Score (0-4 points)
Anxiety: Worry, preoccupation, feeling that the worst is going to happen, irritability.	0 1 2 3 4
Tension: Feelings of tension, fatigability, inability to relax, emotional reactions, easy crying, tremors, feelings of restlessness.	0 1 2 3 4
Fears: Fear of the dark, strangers, being alone, animals, travel by car or bus, crowds.	0 1 2 3 4
Insomnia: Difficulty falling asleep, frequent awakening, light sleep, excessive dreaming, night terrors, fatigue on waking.	0 1 2 3 4
Cognitive Function: Or memory and attention disorders. Inability to concentrate, poor memory.	0 1 2 3 4
Depressed Mood: Loss of interest, lack of pleasure in previous hobbies, depression, early morning awakening, diurnal variation (worse in the morning and lighter in the evening).	0 1 2 3 4
Musculoskeletal System: Muscle soreness, inflexibility of movement, muscle	0 1 2 3 4

Symptom	Score (0-4 points)
twitching, limb twitching, chattering of teeth, trembling of voice.	
Sensory System: Blurred vision, chills and hot flushes, feelings of weakness, tingling all over the body.	0 1 2 3 4
Cardiovascular System: Tachycardia, palpitations, chest pain, throbbing of blood vessels, feeling of fainting, missed heart beats.	0 1 2 3 4
Respiratory System: Chest tightness, feeling of suffocation, sighing, difficulty breathing.	0 1 2 3 4
Gastrointestinal Tract: Difficulty swallowing, belching, indigestion (postprandial abdominal pain, burning sensation in the stomach, abdominal distension, nausea, fullness in the stomach), borborygmus, diarrhea, weight loss, constipation.	0 1 2 3 4
Genitourinary System: Frequent urination, urgent urination, amenorrhea, frigidity, premature ejaculation, erectile dysfunction, impotence.	0 1 2 3 4
Autonomic Nervous System: Dry mouth, flushing, pallor, easy sweating, goose bumps, tension headache, piloerection.	0 1 2 3 4
Behavior at Interview: (1) General manifestations: Tension, inability to relax, fidgeting, finger biting, clenched fists, fidgeting with handkerchief, facial muscle twitching, constant foot tapping, hand tremors, frowning, stiff facial expression, sighing respiration, pallor; (2) Physiological manifestations: Swallowing, hiccups, rapid heart rate at rest, tachypnea (more than 20 breaths per minute), hyperactive tendon reflexes, tremors, dilated pupils, eyelid twitching, excessive sweating, exophthalmos.	0 1 2 3 4

Note: A score ≥29 indicates severe anxiety; a score of 21 indicates marked anxiety; a score ≥14 indicates definite anxiety; a score >7 indicates possible anxiety; a score <7 indicates no anxiety.