

The relationship between serum iron levels and AChR-Ab and IL-6 in patients with myasthenia gravis

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Abstract. – OBJECTIVE: To investigate the correlation between serum iron (SI) levels and acetylcholine receptor antibodies (AChR-Ab) and interleukin 6 (IL-6) in patients with myasthenia gravis (MG).

PATIENTS AND METHODS: A total of 76 patients with myasthenia gravis (MG group) between July 2015 to March 2018, and another 50 healthy subjects during the same period were selected for this study. SI levels in the peripheral blood serum of all participants were measured using the colorimetric method (a SI level of $< 8.95 \mu\text{mol/L}$ was considered the standard for SI deficiency). Enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA) were used to detect the expression and levels of IL-6 and AChR-Ab in the peripheral blood of all participants. The presence and levels of IL-6 and AChR-Ab in the serum of MG patients and normal healthy subjects were compared. The levels of IL-6 and AChR-Ab in MG patients with normal SI levels and those with SI deficiency were analyzed.

RESULTS: The SI deficiency rate, AChR-Ab positivity rate, AChR-Ab levels, and IL-6 levels in the MG group were significantly higher than those in the control group (73.68% vs. 26.00%, 81.58% vs. 0.00%, $1.05 \pm 0.40 \text{ nmol/L}$ vs. $0.21 \pm 0.09 \text{ nmol/L}$, and $183.54 \pm 35.26 \text{ ng/mL}$ vs. $121.43 \pm 28.45 \text{ ng/mL}$, respectively; all p -values were < 0.01). In MG patients, the levels of AChR-Ab and IL-6 in the SI deficiency group were significantly higher than those in the normal SI group ($1.15 \pm 0.34 \text{ nmol/L}$ vs. $0.81 \pm 0.45 \text{ nmol/L}$ and $193.12 \pm 31.70 \text{ ng/mL}$ vs. $156.74 \pm 31.19 \text{ ng/mL}$, respectively; all p -values were < 0.01). The correlation analysis showed that SI levels were negatively correlated with AChR-Ab and IL-6 levels in MG patients ($r = -0.776$, $r = -0.663$, both p -values were < 0.01).

CONCLUSIONS: Iron deficiency in MG patients and SI levels are negatively correlated with AChR-Ab and IL-6 levels in MG patients.

Key Words:

Myasthenia gravis, Serum iron, AChR-Ab, IL-6.

Introduction

Myasthenia gravis (MG) is a neuromuscular junction disease characterized by isolated or systemic skeletal muscle weakness and fatigue whose symptoms are aggravated by activity and alleviated by rest¹. Currently, the prevalence of MG is 77-150 per 1 million people with a ratio of males to females of approximately 2:3². The pathogenesis of MG has not been completely clarified, but MG is an autoimmune disease mediated by B lymphocytes. A variety of cytokines and antibodies produced by B cells play an important role in the pathogenesis of MG, among which the most common are acetylcholine receptor antibodies (AChR-Ab) and interleukin 6 (IL-6). Iron is a trace element required by the human body for the synthesis of hemoglobin. Recent studies^{3,4} have shown that patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), are more likely to have decreased serum iron (SI) levels. SI levels in MG patients and their relationship with AChR-Ab and IL-6 levels have not been previously reported. Therefore, this study intends to explore the role of SI in the pathogenesis of MG by analyzing changes in SI levels and their relationship with AChR-Ab and IL-6 levels in MG patients. Further studies elucidating the pathogenesis of MG are required.

Patients and Methods

Patients

The MG group consisted of 76 patients admitted from July 2015 to March 2018, including 31 males and 45 females, aged 22-62 years with an average age of (31.86 ± 4.32) years. A healthy control group of 50 healthy subjects was selected, in-

cluding 20 males and 30 females, aged 23-61 years with an average age of 32.12 ± 4.28 years. The two groups were matched for age and sex. All subjects or their families signed informed consent.

The inclusion criteria included 1) meeting the expert consensus on the diagnosis and treatment of myasthenia gravis⁵, 2) complete clinical data, 3) no history of cerebral infarction or cerebral hemorrhage as confirmed by computed tomography (CT) or magnetic resonance imaging (MRI) examinations, 4) clear mind, able to match the researcher, 5) informed consent signed by the patient or their family, and 6) no history of iron supplementation. The exclusion criteria included 7) patients with severe heart, liver, kidney, or lung dysfunction, 8) patients with severe system diseases, 9) history of malignant tumors, and 10) inability to cooperate.

Methods

Serum samples were collected in the morning. Approximately 5 mLs of peripheral venous blood were taken from each participant and allowed to coagulate for 15 minutes at room temperature, followed by centrifugation at 3,000 R/minute for 10 minutes. After centrifugation, the serum was collected and stored at -80°C in an ultra-low temperature refrigerator for future detection of SI, IL-6, and AChR-Ab levels. SI was detected using a Hitachi 7600 automatic biochemical instrument for colorimetric detection purchased from Roche Diagnostics Co., LTD (Basel, Switzerland). According to relevant standards, a SI level of < 8.95 $\mu\text{mol/L}$ was considered the standard for a SI deficiency⁶. IL-6 was detected using an enzyme-linked immunosorbent assay kit that was purchased from Wuhan Jinkairui Bioengineering Co., LTD. Expression levels and the presence of AChR-Ab were detected by radioimmunoassay according to the kit instructions. The kit was purchased from the RSR Company in Milton Keynes, UK, and a serum AChR-Ab concentration of > 0.5 nmol/L was considered positive for AChR-Ab expression.

According to the SI levels, the two groups were divided into SI deficiency and SI control groups. The rates and levels of IL-6 and ACh-Ab in patients with SI deficiencies and normal SI levels were compared, and the correlation between SI levels and serum IL-6 and ACh-Ab levels was analyzed.

Statistical Analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Measurement data

were expressed as mean \pm standard deviation, and comparisons between groups were performed using *t*-tests. Enumeration data were expressed as percentages and compared by χ^2 tests. Spearman's correlation analysis was used to perform the correlation analysis. A *p*-value < 0.05 was considered statistically significant.

Results

Levels of SI, AChR-Ab, and IL-6 Compared Between the Two Groups

There were 56 cases of SI deficiency (SI deficiency group) and 20 cases with normal levels (SI normal group) in the MG group. The SI deficiency rate in the MG group was significantly higher than that of the healthy control group ($p < 0.01$). The positive rate and expression levels of AChR-Ab and IL-6 in MG patients were significantly higher than those in the control group ($p < 0.01$) (Table I).

Comparison of AChR-Ab and IL-6 Levels in Peripheral Blood of Patients with Different SI Levels Within the MG Group

Patients in the MG group were divided into a SI deficiency group (< 8.95 $\mu\text{mol/L}$, $n = 56$) and a normal SI level group (≥ 8.95 $\mu\text{mol/L}$, $n = 20$) according to the diagnostic criteria of SI deficiency. When comparing the levels of AChR-Ab and IL-6 of the two groups, the results showed that the levels of AChR-Ab and IL-6 in the SI deficiency group were significantly higher than those in the normal SI group (1.15 ± 0.34 nmol/L vs. 0.81 ± 0.45 nmol/L , 193.12 ± 31.70 ng/mL vs. 156.74 ± 31.19 ng/mL , all *p*-values were < 0.01) (Table II).

Correlation Analysis of SI Levels with AChR-Ab and IL-6 Levels in the MG Group

SI levels were negatively correlated with AChR-Ab and IL-6 levels in MG patients ($r = -0.776$, $r = -0.663$, respectively, both *p*-values were < 0.01). Data are presented in Figures 1 and 2.

Discussion

MG is an autoimmune disease. In MG the body produces an abnormal immune response against its acetylcholine receptor, which destroys the body's self-tolerance and leads to a massive loss of acetylcholine receptors on the postsynaptic

Table I. Comparison of the levels of SI, AChR-Ab, and IL-6 between the two groups.

Group	n	SI lack rate (n, %)	AChR-Ab positive rate (n, %)	AChR-Ab (nmol/L)	IL-6 (ng/mL)
MG group	76	56 (73.68)	62 (81.58)	1.05 ± 0.40	183.54 ± 35.26
Healthy control group	50	13 (26.00)	0 (0.00)	0.21 ± 0.09	121.43 ± 28.45
χ^2/t		27.68	80.30	14.58	10.41
<i>p</i>		< 0.01	< 0.01	< 0.01	< 0.01

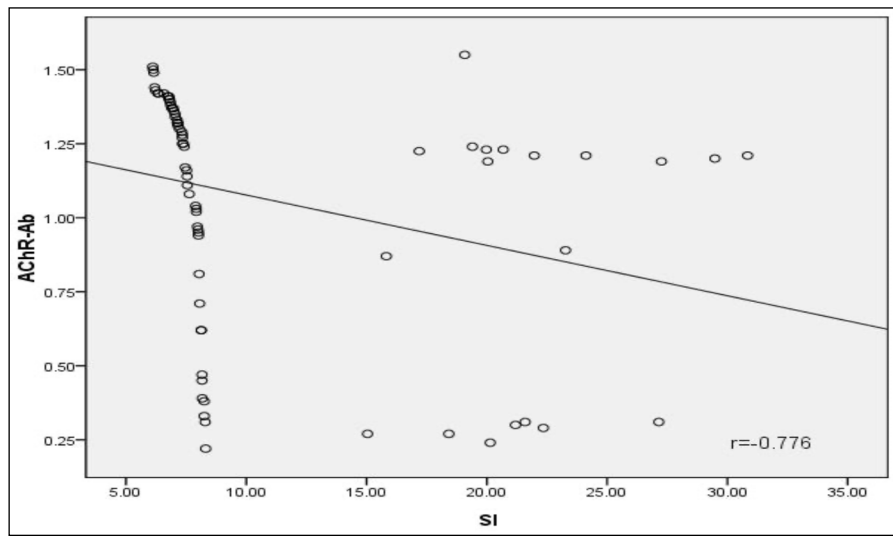


Figure 1. Correlation analysis of SI levels with AChR-Ab in the MG group.

membrane of the motor endplate, which leads to dysfunction of neuromuscular transmission⁷. Humoral immunity and cellular immunity mediate the autoimmune responses in MG, and AChR-Ab and IL-6 play an important role in these responses. Studies⁸ have shown that AChR-Ab can be detected in the peripheral blood in more than 50% of ocular muscle MG patients and 85% of systemic MG patients. IL-6 is a cytokine secreted by Th2 cells, which can activate quiescent T cells and further induce the terminal differentiation of activated T cells, lead to an increase in AChR-

Ab secretion, and participates in the pathogenesis of MG⁹⁻¹¹. The level of IL-6 in the serum of MG patients has been correlated with the severity and treatment efficacy of MG to a certain extent¹². In our study, the levels of AChR-Ab and IL-6 in the serum of MG patients were significantly higher than those of healthy controls, which was due to the immune dysfunction of MG patients and the activation of T and B cells, resulting in the production of AChR-Ab and IL-6 in large quantities. This also supports that AChR-AB and IL-6 are closely related to the pathogenesis of MG¹³.

Table II. Comparison of AchR-Ab and IL-6 levels in the peripheral blood of patients with different SI levels within the MG group.

Group	n	AChR-Ab (nmol/L)	IL-6 (ng/mL)
SI deficiency group	56	1.15 ± 0.34	193.12 ± 3170
SI normal group	20	0.81 ± 0.45	156.74 ± 31.19
<i>t</i>		3.51	4.42
<i>p</i>		0.0007	< 0.01

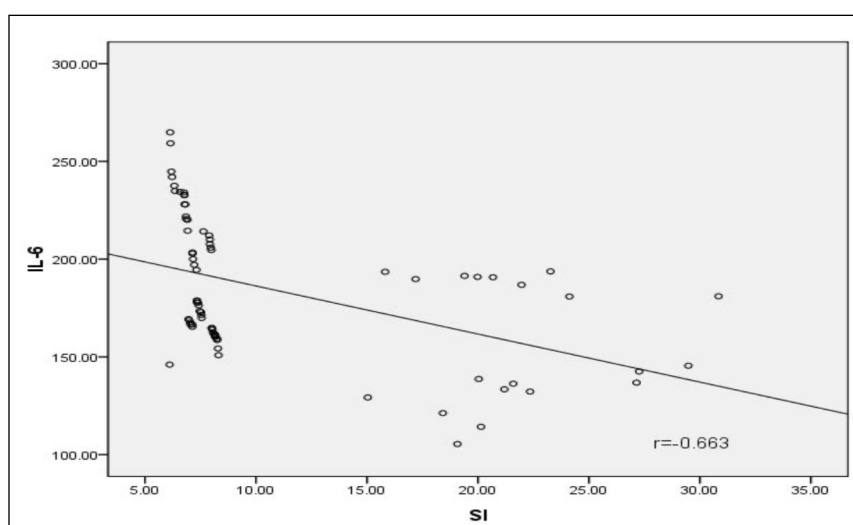


Figure 2. Correlation analysis of SI levels with IL-6 in the MG group.

In our study, the SI levels of MG patients were significantly lower than that of healthy controls. MG patients often experience masticatory weakness, dysphagia, and other conditions. The amount of food intake in such patients was significantly reduced leading to a deficiency of iron in the body¹⁴. At the same time, MG patients can have anxiety, depression, and other negative emotions due to the long-term burden of the disease leading to a decreased appetite and reduced intake of trace elements¹⁵. In addition, MG patients often develop lung infections and required glucocorticoid and immune agent treatments which can result in iron absorption and utilization disorders¹⁶.

Recent literature has shown that patients with SLE, rheumatoid arthritis (RA), and other immune diseases are more likely to have a SI deficiency. In our study, the rate of SI deficiency in MG patients was significantly higher than that of healthy controls. At the same time, SI levels in MG patients were negatively correlated with serum AChR-Ab and IL-6 levels, suggesting that SI plays an immunomodulatory role in antibody or cytokine production. The possible mechanisms of this role are speculated as follows: 1) iron plays a role in regulating immune function and a long-term iron deficiency may cause a decline in immune regulatory functions and immune surveillance functions. This can lead to the production of autoantibodies, activation of complement, and the induction of autoimmune diseases¹⁷. 2) Iron is an indispensable trace element for microbial growth

and reproduction. Patients with iron deficiency are more likely to have infections leading to the production of more inflammatory cytokines, such as IL-6, IL-4, and interferon- γ (IFN- γ). These inflammatory cytokines can aggravate MG through the immune pathways described above¹⁸. On the contrary, the exacerbation of MG can further lead to deficiencies of SI due to eating complications and hormone use which can lead to a vicious cycle.

Conclusions

The results of our study showed that SI levels were decreased in MG patients, and SI levels were negatively correlated with AChR-Ab and IL-6. However, this study was only a single-center clinical study with a small sample size and no basic molecular research was conducted on the relationship between MG and SI levels. Therefore, the relationship between the incidence of MG and SI deficiency is not completely clear, which is the focus of future research studies by our group.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Availability of Data and Materials

All data, models, and code generated or used during the study appear in the submitted article.

Ethics Approval

This study was approved by People's Hospital of Deyang City Ethics Committee.

Informed Consent

All subjects or their families signed informed consent.

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