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Original Article

Clinical feature and serum markers in patients with myasthenia gravis and connective tissue disorder

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Abstract: Background: The coexistence of myasthenia gravis (MG) and connective tissue disorder (CTD) disease is rarely reported and until now few published studies have been found on MG-CTD disease. In this study we aimed to investigate the clinical features of MG-CTD patients and explore the relationship between the expression level of several serum factors and clinical features of MG-CTD patients. Methods: 24 patients diagnosed with MG-CTD syndrome were collected in our study. Enzyme-linked immunosorbent assay (ELISA) was performed to detect the expression levels of serum factors including IL-17, IL-23, PTP, PTK, AChR-Ab, and MuSK-Ab. In addition, the difference of expression levels of serum factors in patients and control groups was analyzed by T test. Chi-squared test was used to analyze the relationship between serum factors and clinical features. Results: The results indicated that the expression of serum factors including IL-17, IL-23, PTP and AChR-Ab were significantly increased in MG-CTD patients compared to the normal control group ($P<0.05$). Moreover, the outcome showed that the serum factor were closely related with clinical features, as IL-23 positive expression affecting Thymectomy (P=0.006, OR=0.063, 95% CI=0.007-0.590), and AChR-Ab affecting CTD type (P=0.019, OR=0.111, 95% CI=0.016-0.789) and Thymectomy (P=0.003, OR=0.040, 95% CI=0.003-0.477). Conclusion: Several serum factors showed increased expression levels in MG-CTD patients and the factors might be potential useful serum markers for MG-CTD.

Keywords: Myasthenia gravis, connective tissue disorder, serum marker, IL-23, AChR-Ab

Introduction

Myasthenia gravis (MG) is an antibody-mediated autoimmune process at the neuromuscular junctions clinically characterized by weakness and fatigability of skeletal muscles [1] and is caused by autoantibodies against muscle nicotinic acetylcholine receptor (AChR). Connective tissue disorder disease (CTD) such as rheumatoid arthritis, scleroderma, as well as autoimmune diseases of both peripheral and central nervous system such as dermatomyositis and neuromyelitis optica have been reported to occur together with MG [2-5]. There is an increased incidence of other autoimmune disorders during the course of MG [6]. MG occurs with thymoma in 15% of patients [7]. Thyroid diseases have been observed in more than 5% of MG patients [8]. Although previous studies have reported the MG usually coexisted with various CTD including autoimmune diseases, the studies on the diagnostic and treatment strategies for MG and CTD disease were not too much.

Based on the current research, acetylcholine receptor (AChR) at neuromuscular junctions is the major autoantigen for MG [9]. There is a relationship between the antibody against AChR and disease in an individual patient [10, 11]. the autoantibody response is T cell dependent, with CD4+ T cells providing help for B cells to produce anti-AChR antibodies [12, 13]. Recently, a new T-helper subset, Th17, has been identified and found to have a role in several autoimmune diseases, such as multiple sclerosis and systemic lupus erythematosus. These cells secrete a variety of cytokines, including IL-17, IL-21 and IL-22, with proinflammatory function [11]. Bai et al. [14] have reported that IL-17 was a key to autoantibody responses in an experimental murine autoimmune myasthenia gravis model. The authors noted that IL-17 elicited higher antibody responses to the autoanti-

Table 1. Patient demographics and clinical characteristics

Parameters	No. (total n=24)
Female gender, n (%)	22 (91.7)
Age at MG onset (years), mean (range)	38.1 (7-64)
Age at CTD onset (years), mean (range)	41.2 (11-64)
CTD type (%)	
Systemic lupus erythematosus (SLE)	10 (41.7)
Systemic sclerosis (SSc)	14 (58.3)
ANA (%)	22 (91.7)
Clinical manifestations of MG, n (%)	
Dysarthria	6 (25)
Dysphagia	9 (37.5)
Dysphonia	8 (33.3)
Proximal weakness	6 (25)
Dyspnoea	3 (12.5)
Thymectomy, n (%)	
Performed	6 (25)
Not performed	18 (75)
Anti-AChR antibody, n (%)	16 (66.7)
EMG, n (%)	13 (54.2)

MG, Myasthaenia gravis; CTD, Connective tissue disorder; EMG, electromyography.

gen AChR. Besides, there are other serum factors and antibodies relating with MG, such as IL-23, MuSK-Ab [15-17]. However, the role of serum factors in MG-CTD disease was still not clear.

In this study, we attempted to investigate the clinical features of patients with MG and CTD disease, detect the expression levels of serum factors in patients and control groups and explore the relationship of serum factors with clinical features in MG-CTD patients.

Materials and methods

Patients and samples

In this study, 24 patients with MG-CTD were recruited from January 2012 to May 2014, at the Central Hospital of Wuhan. The diagnosis of MG was made on the basis of clinical findings and confirmed by single fiber electromyography (SFEMG). CTD was defined according to the American College of Rheumatology (ACR) classification. Besides 16 healthy participants (10 women, 6 men; mean age 38.5 years, range 13-62 years) served as normal controls (NC). Our study received prior approval by local Ethic Committee of Central Hospital of Wuhan and informed consent was obtained from each participators.

Blood samples were obtained by venipuncture and immediately centrifuged (3000 r/min) for 15 min. Serum samples were stored at -80°C until assay. All samples were measured in duplicate and analyzed simultaneously. Moreover, Repetitive nerve stimulation and chest computerized tomography and/or chest magnetic resonance imaging were documented for all patients.

Enzyme-linked immunosorbent assay (ELISA)

For the quantification of IL-23 and IL-17 in human serum samples, Human IL-23 and IL-17 Quantikine Elisa Kit (R&D Systems, Minneapolis, MN) were used following manufacturer's guidelines. The concentration of protein tyrosine phosphatas (PTP) and protein tyrosine kinase (PTK) were detected using ELIAS PTP kit and PTK kit (Sigma company, United Stated), respectively. What's more, the expression levels of AChR-Ab and MuSK-Ab was also analyzed by ELISA Kits (Product Code: E01A0203 and E01A0452, BG company, Shanghai, China). The detection sensitivity for AChR-Ab and MuSK-Ab was 0.1 ng/ml and the absorbance of VERSAmax used the automatic enzyme standard detector.

Statistical analysis

All statistical data were analyzed by SPSS version 18.0 software (SPSS Inc, IL, USA). Data were expressed as mean ± standard deviation (SD). T test was used to estimate differences of serum antibodies expression level between MG-CTD group and NC group. The association between expression level of serum antibodies and clinical features were estimated by Chi-squared test. $P<0.05$ was considered to be statistically significant.

Results

Clinical characteristic in MG-CTD patients

Based on the recorded clinical information of the patients we collected demographic variables, information on duration, manifestations, disease activity, and treatment of CTD, as well as basic descriptors for MG, including manifestations and chronology. A summary of the data was presented in **Table 1**.

Table 2. The expression level of serum factors in MG-CTD and control groups

Group	IL-17 (pg/ml)	IL-23 (pg/ml)	PTP (ng/ml)	PTK (ng/ml)	AChR-Ab (ng/ml)	Musk-Ab (ng/ml)
MG-CTD	5.84±1.40	212.29±29.92	82.33±7.86	48.37±9.47	0.77±0.11	0.37±0.06
NC	4.62±0.99	60.94±7.72	17.44±1.82	46.56±8.52	0.37±0.08	0.31±0.06
P value	0.004	0.000	0.000	0.541	0.000	0.066

Table 3. Relevance of serum factors with clinical features of MG-CTD patients

Serum factors		IL-23 (positive/negative)			AChR-Ab (positive/negative)		
		P value	OR	95% CI	*P* value	OR	95% CI
CTD type	SLE	0.151	0.250	0.035-1.775	0.019	0.111	0.016-0.789
	SSc						
Thymectomy	Performed	0.006	0.063	0.007-0.590	0.003	0.040	0.003-0.477
	Not performed						

Serum IL-17, IL-23, PTP, PTK, AChR-Ab and MuSK-Ab levels in patients and healthy controls

We detected serum concentrations of IL-17, IL-23 PTP, PTK, AChR-Ab and MuSK-Ab levels in patients with MG-CTD and the normal control group. The results indicated that the level of IL-17 IL-23, PTP and AChR-Ab was significantly higher in patients with MG-CTD than normal controls and the difference in the two groups was statistically significant ($P<0.05$), while the level of serum PTK and MuSK-Ab showed no significant difference in patients with MG-CTD and healthy controls ($P>0.05$, **Table 2**).

Relationship between serum factors and clinical features

The expression levels of serum factors were different in patients group and normal control group. Thus we attempted to explore the correlation of significant serum factors with clinical features. As shown in **Table 3**, IL-23 was associated with thymectomy ($P=0.006$, OR=0.063, 95% CI=0.007-0.590), moreover, AChR-Ab was closely related with CTD type ($P=0.019$, OR=0.111, 95% CI=0.016-0.789) and Thymectomy ($P=0.003$, OR=0.040, 95% CI=0.003-0.477). However, other factors showed no significant correlation with clinical features (all, $P>0.05$).

Discussion

MG is caused by pathogenic autoantibodies directed towards the skeletal muscle AChR. Other antibodies associated with MG include those against muscle-specific tyrosine kinase (MuSK) and striational proteins (titin and ryanodine receptor) [18, 19]. MuSK proteins are neuromuscular junction proteins present in mature muscle cells. Patients who test negative for anti-AChR antibodies typically have anti-MuSK antibodies [20]. Anti-titin is the most common anti-striational protein antibody present in MG patients. Anti-titin antibodies are associated with increased disease severity and are more often found in patients with thymoma and late onset MG [19, 20]. By contrast, in CTD, antibodies are directed towards components of the cell nucleus (ANA) and the pathogenic mechanism for these ANA antibodies is partly understood. Autoantibody production is the result of multiple immune cell abnormalities that involve B-cell, T-cell, and monocyte lineages.

MG and CTD have several common clinical characteristics, such as the onset age and sex distribution. Both of the diseases are manifested by cycles of exacerbation and remission, positive ANA, and thymus hyperplasia [21, 22]. According to the cases reviewed from the literature, either disease can occur after one has been diagnosed and no specific trigger for the second disorder has been described. This has been considered a precipitating factor for the development of CTD, where the loss of thymic hormones is thought to result in defects in T-suppressor cell function [23]. In humans, long-term thymectomized MG patients display mild T-cell lymphopaenia, which is associated with hypergammaglobulinaemia and evidence of B-cell hyperactivity. In addition, many of these patients have high titres of a variety of autoantibodies, including anti-dsDNA and anti-cardiolipin antibodies (aCL) [24]. However, no

significant clinical improvement or change in the circulating autoantibody titres was noted in MG patients with associated connective tissue diseases.

In our study, we found that the expression levels of serum factors including IL-17, IL-23, PTP, and AChR-Ab were higher in patients with MG-CTD than in normal controls. We also found that PTP and MuSK-Ab in patients with MG-CTD showed no significant difference compared with NC group. Moreover, we analyzed the relationships of serum factor levels with clinical features. We have arrived at the conclusion that IL-23 and AChR-Ab expression were closely correlated with clinical features. Integrated above all the evidence, we can conclude an important role for serum factors in the screening and diagnosis of patients with MG-CTD.

Previous studies have analyzed the role of autoantibodies and cytokines in patients with MG. For example, Uzawa A et al. have found an intensive cytokines and chemokines profile in the serum of anti-AChR antibody positive patients with MG and determined that the increased cytokine levels are linked to clinical MG parameters [1]. Zheng S et al. also reported that anti-inflammatory cytokine IL-22 might be protective for MG and might be a therapeutic target [9]. In addition, as a important type of CTD, SLE has been reported to significantly associated with MG, and useful treatment for the coexistence disease has been found [25-27]. Therefore, further studies should focus on the diagnosis and treatment of MG-CTD to save more and more patients.

In summary, our results show a significant increase in IL-17, IL-23, PTP and AChR-Ab levels in the serum of patients with MG and CTD. Close relationship between serum factors with clinical features of MG-CTD patients was also found. Moreover, the pathogenic and inflammatory mechanisms at neuromuscular junctions that are exerted by cytokines could be important in patients with MG and CTD. Serum antibody therapy could have the potential for treating MG-CTD. Further studies, including those of patients with seronegative MG, are required to confirm the details of the serum markers in patients.

Disclosure of conflict of interest

None.

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