

ORIGINAL ARTICLE

Altered Serum Cytokines Profile in Patients with Myasthenia Gravis

¹Rasha M. Elnagar, ²Nouran E. Samra, ²Safaa M. El-Ageery*, ³Nawal S. Gouda, ⁴Enas Gouda, ⁵Amal M. Hammad, ⁶Mazin M. Eladaroussy, ⁷Aya Zhran, ⁸Rania Talaat

¹Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt; Basic Medical Sciences Department, College of Medicine, Al-Maarefa University, P.O.Box71666, Riyadh 11597, Saudi Arabia

²Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Egypt

³Microbiology Department, Faculty of Medicine, Northern Border University, Saudi Arabia

⁴Clinical Pathology Department, Hematology Unit, Faculty of Medicine, Mansoura University, Egypt

⁵Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Al-Azhar University, Damietta, Egypt

⁶MBBCH, Faculty of Medicine, Mansoura University, Egypt

⁷Neurology Department, Faculty of Medicine, Mansoura University, Egypt

⁸Medical Microbiology and Immunology Department, Faculty of Medicine, Helwan University, Egypt

ABSTRACT

Key words:
Serum cytokines,
myasthenia gravis

***Corresponding Author:**
Safaa M. EL-Ageery, Medical
Microbiology and Immunology
Department, Faculty of
Medicine, Mansoura University
Tel: +201090888263
safageery@gmail.com

Background: One of the most common neuromuscular junction disorders is autoimmune myasthenia gravis (MG). Cytokines profile is expected to be of most important in MG pathogenesis. **Objectives:** In this research we aimed to analyze serum cytokines profiles in acetylcholine receptor (AChR) antibody positive MG patients and determine the link between these cytokines and clinical parameters of MG. **Methodology:** Forty AChR antibody positive MG patients and 40 healthy participants (HC) were included in this study. Serum cytokines including IL-2, IL-4, IL-6, IL-15, IL-17 A, IFN- γ , were measured by enzyme linked immunosorbent assay (ELISA). The correlations between serum cytokines levels and clinical profile were analyzed. **Results:** Serum levels of IL-6, IL-15, IL-17 were significantly higher in MG patients than in HC. Serum levels of IL-4 were significantly lower in MG patients than in HC. According to MG Foundation of America (MGFA) classification, generalized MG group (G-MG) had higher levels of IL-6 and IL-17 than HC, without statistical significant variation among oculomotor MG (O-MG) and HC. We also found that early onset MG (EOMG) had significantly higher IL-2 levels compared to late onset MG (LOMG). We also found significant higher IL-17 levels in thymoma associated MG patients (TMAG) than in MG with normal thymus group. Serum levels of IL-6 and IL 17 were positively correlated with AChR antibodies concentration and quantitative MG (QMG) scores in all MG patients. **Conclusion:** we can conclude that the pathogenic inflammatory effects exerted by cytokines at neuromuscular junctions are important in MG patients.

INTRODUCTION

One of the most common neuromuscular junction disorders is autoimmune myasthenia gravis¹. MG shows different clinical manifestations, ranging from ocular motility troubles, generalized or focal fatigable muscle weakness up to neuromuscular respiratory failure². In general, auto-antibodies targeting post synaptic neuromuscular junction are the main cause of MG. According to the type of auto-antibodies, MG is classified as acetylcholine receptor antibody positive (AChR Ab MG), low density lipoprotein receptor related protein 4 antibody positive (Lrp4 Ab MG), muscle specific kinase antibody positive (MuSK Ab MG) and triple seronegative MG (t-SNMG)³. AChR antibodies are

produced by T cell dependent and B cell mediated autoimmune mechanism, resulting in complement system activation with subsequent postsynaptic muscle membrane inflammation. Moreover, AChR Ab MG represents about 80% of MG patients⁴.

Cytokines control immune response in inflammatory disorders. Naive CD4⁺ T cell can differentiate into T helper types such as Th1, Th2, Th9, Th17, Th22 or regulatory T cells (Treg) as a result of different cytokines actions⁵. Based on the particular cytokines released by the T cell subsets, various inflammatory responses can be promoted. Therefore, cytokines profile is expected to be of most important in MG pathogenesis. Furthermore, it was observed that Th1 and Th17 could get worse pathogenesis of experimental autoimmune myasthenia gravis (EAMG), in MG animal models, while, Treg could

improve EAMG pathogenesis⁶. So in our research, we evaluated intensive serum cytokines profiles in AChR Ab MG patients and determined the link between these cytokines and clinical parameters of MG.

METHODOLOGY

Study design

Forty AChR Ab MG patients and 40 healthy participants (HC group) of matching age and sex were included in this study. The patients data were collected regarding age, sex, disease duration, AChR antibody titer and E-L-T classification (EOMG with age at onset ≤ 49 years, LOMG with age at onset ≥ 50 years, and TAMG)⁷. Other data including MGFA clinical classification⁸ and QMG scores⁸ were also collected at the time of serum sampling.

Exclusion criteria were: MG associated with malignant tumors (except thymoma), acute cerebrovascular illness, and acute infections within the previous 4 weeks.

AChR antibodies assay

AChR antibodies concentration was detected via ELISA using a human AChR antibodies ELISA Kit (R&D, USA) in accordance with manufacturer instructions. Optical density values could be taken at 450 nm. The range of test was 20–500 pmol/l, and concentration values above 20 were considered positive.

Cytokine measurement

Blood sampling was taken after all night fasting and were frozen at -80°C . Serum levels of cytokines including IL-2, IL-4, IL-6, IL-15, IL-17 A, IFN- γ , were measured by ELISA, using reagent kits of R&D Systems Inc, MN, USA, in accordance with the manufacturer protocols.

Correlation between serum cytokines and clinical MG profiles

The correlations between serum cytokines level and clinical profile were analyzed, such as AChR Ab titer, MGFA classification (ocular or generalized type), QMG scores and the presence of thymoma in all MG patients.

Statistical Analysis

All data were expressed as mean $\pm$ SD, median (minimum - maximum), or count and percentage. Data normality was checked via Shapiro-Wilk test. Comparisons between two groups were analyzed by Mann-Whitney U test and Chi square. Correlation between studied parameters was performed by Spearman rank correlation coefficient. P value < 0.05 was considered significant.

RESULTS

Clinical profile of MG patients

The clinical characters of MG patients at serum sampling time were as follows: mean age $\pm$ SD (range): 50.4 ± 8.67 (29–68) years, female ratio: 22/40 (55%); mean disease duration, 11.9 ± 20.3 (0–82) months, mean AChR Ab titer, 139.13 ± 114.93 (20–490) pmol/L, E-L-T classification, EOMG = 11, LOMG = 29, TAMG = 13, median MGFA classification: 2 (1–5), mean QMG score, 9.5 ± 5.9 (1–25) points.

Serum cytokines profile

Serum levels of IL-6, IL 15, IL 17 were significantly higher in MG patients than in HC group ($p < 0.001$ for IL-6 and IL-15 and $p = 0.007$ for IL-17). Serum levels of IL 2 and IFN- γ were insignificantly higher in MG patients than in HC group. Serum levels of IL-4 were significantly lower in MG patients than in HC group ($p < 0.001$) (table 1, figure 1).

Changes in serum cytokines levels according to MGFA clinical classification

According to MGFA classification, G-MG showed higher IL-6 and IL-17 levels than HC ($p < 0.001$ and $p = 0.014$ respectively), without statistical significant variation among O-MG and HC. Serum levels of IL-15 levels were significantly higher in either O-MG or G-MG patients than in HC group ($p < 0.001$ for each). Serum levels of IL-4 were significantly lower in either O-MG or G-MG patients compared with HC group ($p < 0.001$ for each). There were no statistical significant differences detected in the other cytokines levels in MG patients (either G-MG or O-MG) compared to healthy group (table 2, figure 2).

Changes in serum cytokines levels based on E-L-T classification

Based on E-L-T classification, we found that EOMG had significantly higher IL-2 levels compared with LOMG ($p < 0.001$). No statistical significant difference in the other cytokines levels were detected between EOMG and LOMG (Table 3, figure 3). We also found significant higher IL-17 levels in TAMG patients than in MG with normal thymus group ($p < 0.001$) (table 4, figure 4).

Correlations between serum cytokines with QMG scores and with the concentration of AChR antibodies

Serum levels of IL-6 and IL 17 were positively correlated with the concentration of AChR antibodies ($r = 0.313$, $p = 0.049$ and $r = 0.372$, $p = 0.018$, respectively) and QMG scores ($r = 0.382$, $p = 0.015$ and $r = 0.812$, $p < 0.001$, respectively) in all MG patients (Fig.5).

Table 1: Serum cytokines profile in the MG and HC groups

Cytokine (pg/mL)		HC (n = 40)	All MG (n = 40)	P value
IL2	mean±SD	12.52 ± 1.16	14.64 ± 8.17	0.644
	Median (min – max)	12.4 (10.5 - 15.1)	13.35 (2.4 - 34.1)	
IL4	mean±SD	3.9 ± 0.6	2.22 ± 0.6	<0.001*
	Median (min – max)	4.09 (2.12 - 4.22)	2.04 (1.86 - 4)	
IL6	mean±SD	11.18 ± 4.07	27.95 ± 10.18	<0.001*
	Median (min – max)	10.96 (4.12 - 21.92)	27.4 (10.3 - 54.8)	
IL15	mean±SD	3.52 ± 1.1	7.36 ± 2.87	<0.001*
	Median (min – max)	3.55 (1.7 - 6.1)	7.05 (2.6 - 14)	
IL17A	mean±SD	12.87 ± 5.12	15.88 ± 6.83	0.007*
	Median (min – max)	11.83 (2.43 - 24.50)	16.6 (2.38 - 26.8)	
IFN-γ	mean±SD	103.72 ± 25.92	106.69 ± 28.93	0.627
	Median (min – max)	98.2 (68.9 - 189.1)	103.3 (63.5 - 189.1)	

$p^* < 0.05$ is considered significant. Data are expressed as mean±SD, median (minimum-maximum).

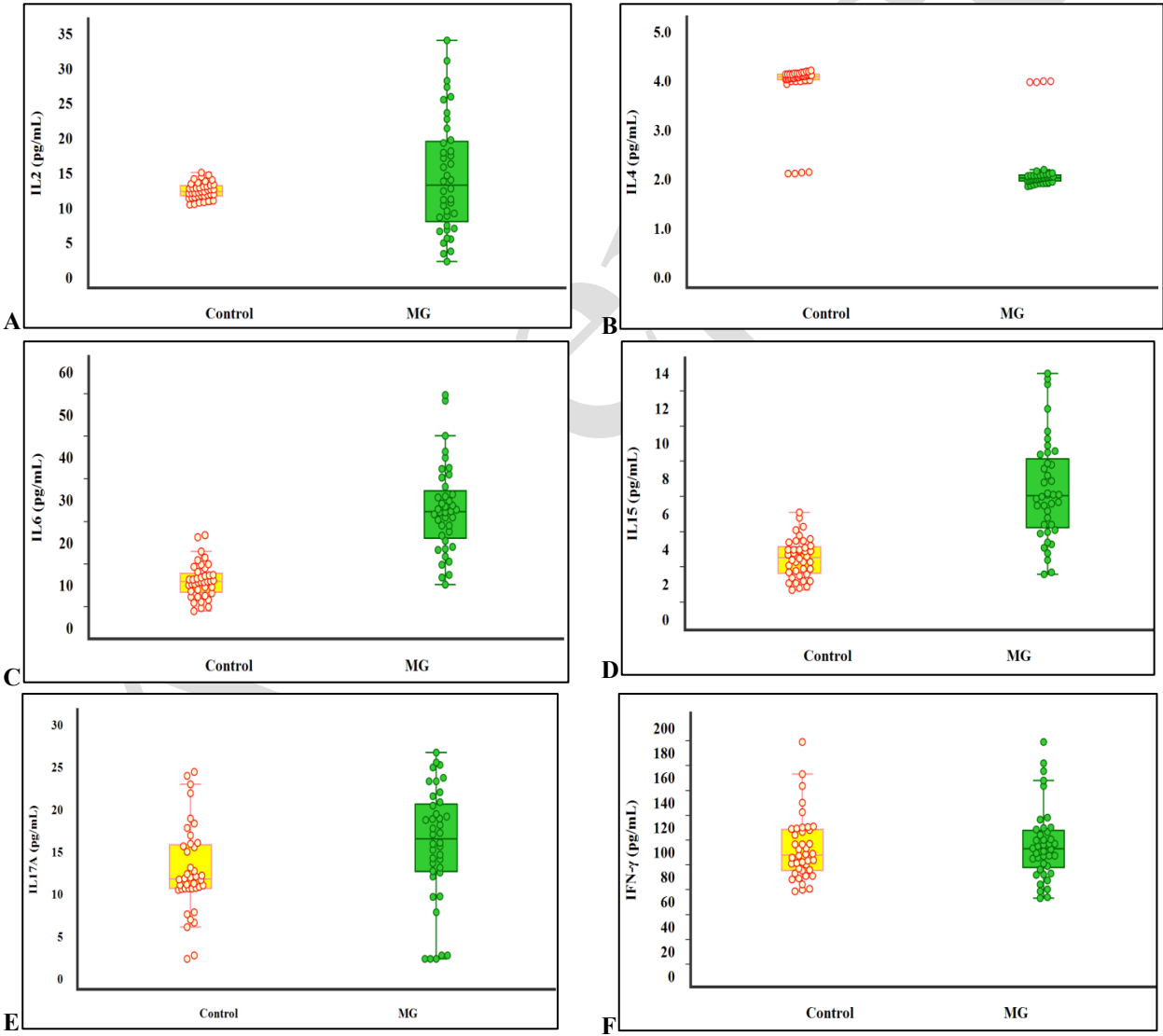


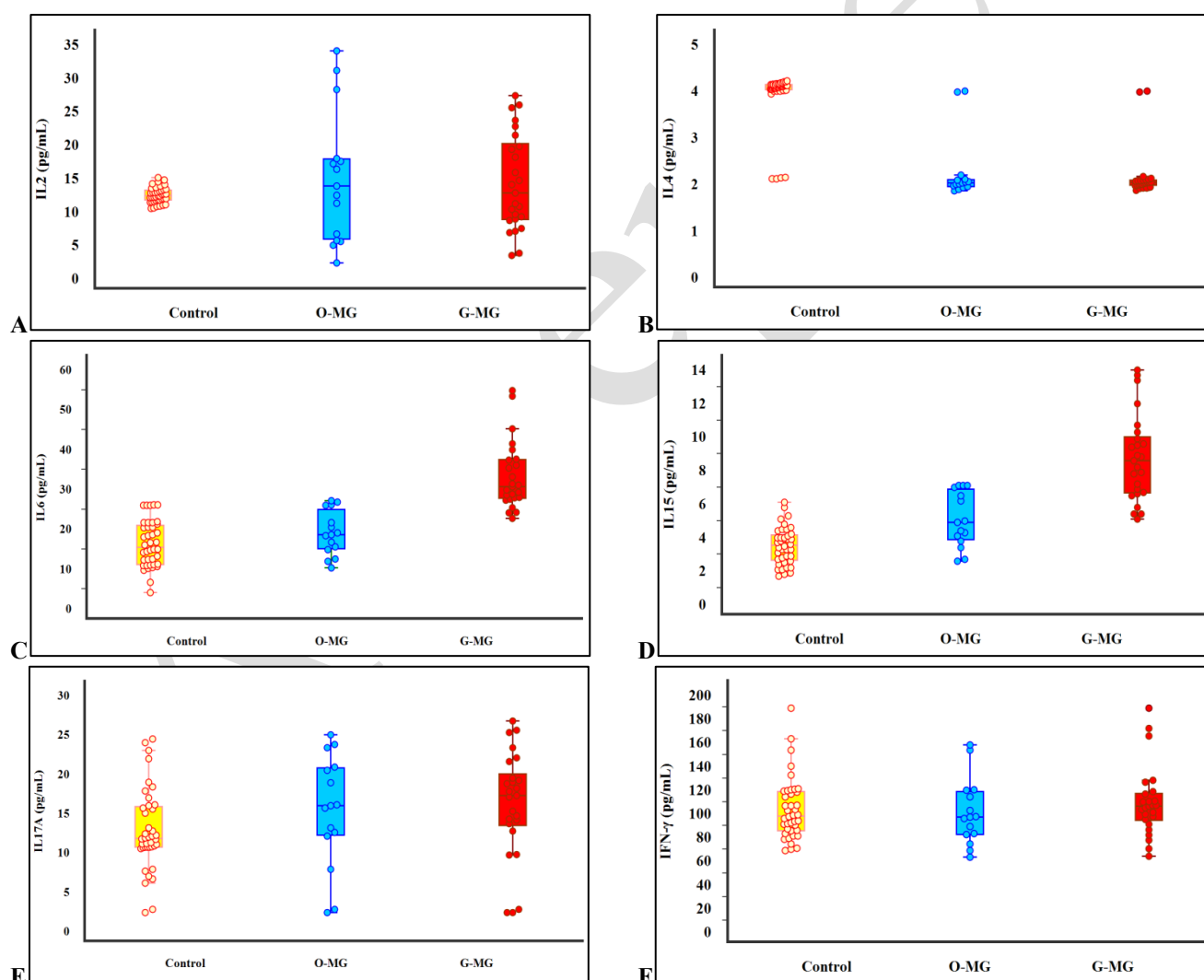
Fig. 1: Serum cytokines profile in the MG and HC groups. (A) IL2, (B) IL4, (C) IL6, (D) IL15, (E) IL17A, and (F) IFN γ .

Serum levels of IL-6, IL 15 ($p < 0.001$ for each), IL 17 ($p = 0.007$) were significantly higher in MG patients than in HC group ($p < 0.001$). Serum levels of IL-4 were significantly lower in MG patients than in HC group ($p < 0.001$).

Table 2: Changes in serum cytokines levels according to the MGFA clinical classification

Cytokine (pg/mL)		HC (n = 40)	O-MG (n = 15)	G-MG (n = 25)	P1 value	P2 value
IL2	mean±SD	12.52 ± 1.16	15.06 ± 9.82	14.38 ± 7.22	0.433	0.930
	Median (min – max)	12.4 (10.5 - 15.1)	13.9 (2.4-34.1)	12.8 (3.5 - 27.4)		
IL4	mean±SD	3.9 ± 0.6	2.28 ± 0.7	2.18 ± 0.55	<0.001*	<0.001*
	Median (min – max)	4.09 (2.12 - 4.22)	2.03 (1.86-4)	2.04 (1.87 - 4)		
IL6	mean±SD	16.36 ± 5.74	19.1 ± 5.59	33.26 ± 8.47	0.121	<0.001*
	Median (min – max)	15.52 (4.12 - 26.10)	18.6(10.3-27.2)	30.7 (22.7 - 54.8)		
IL15	mean±SD	3.52 ± 1.1	5.08 ± 1.64	8.73 ± 2.58	0.002*	<0.001*
	Median (min – max)	3.55 (1.7 - 6.1)	4.9 (2.6-7.1)	8.6 (5.1 - 14)		
IL17A	mean±SD	12.87 ± 5.12	15.42 ± 7.08	16.15 ± 6.82	0.067	0.014*
	Median (min – max)	11.83 (2.43 – 24.50)	16.0 (2.43 -25)	17.3 (2.38 - 26.8)		
IFN-γ	mean±SD	103.72 ± 25.92	101.54 ± 28	109.78 ± 29.59	0.698	0.338
	Median (min – max)	98.2 (68.9 - 189.1)	97.4 (63.5-158.3)	106.4 (64.2 - 189.1)		

P1: comparison between O-MG and HC, P2: comparison between G-MG and HC, $p^* < 0.05$ is considered significant. Data are expressed as mean±SD, median (minimum-maximum).

**Fig. 2:** Serum cytokines levels according to the MGFA clinical classification. (A) IL2, (B) IL4, (C) IL6, (D) IL15, (E) IL17A, and (F) IFN γ .

G-MG showed higher IL-6 ($p < 0.001$) and IL-17 ($p = 0.014$) levels than HC, without statistical significant variation among O-MG and HC.

Table 3: Changes in the serum cytokines levels in EOMG and LOMG groups

Cytokine (pg/mL)		EOMG (n=11)	LOMG (n =29)	P value
IL2	mean±SD	21.29 ± 5.88	7.99 ± 3.05	<0.001*
	Median (min – max)	19.6 (13.9 - 34.1)	8.1 (2.4 - 12.8)	
IL4	mean±SD	2.32 ± 0.73	2.11 ± 0.45	0.461
	Median (min – max)	2.04 (1.91 - 4)	2.04 (1.86 - 3.98)	
IL6	mean±SD	26.2 ± 8.93	29.7 ± 11.25	0.495
	Median (min – max)	26.65 (10.3 - 41.5)	27.95 (15.6 - 54.8)	
IL15	mean±SD	6.96 ± 2.65	7.77 ± 3.09	0.659
	Median (min – max)	7 (2.6 - 12)	7.05 (4.1 - 14)	
IL17A	mean±SD	15.96 ± 6.7	15.79 ± 7.14	0.799
	Median (min – max)	15.85 (2.38 - 25.6)	18.3 (2.4 - 26.8)	
IFN-γ	mean±SD	109.58 ± 24.16	103.8 ± 33.41	0.174
	Median (min – max)	106.85 (64.2 - 172.2)	97.5 (63.5 - 189.1)	

$p^* < 0.05$ is considered significant. Data are expressed as mean± SD, median (minimum-maximum).

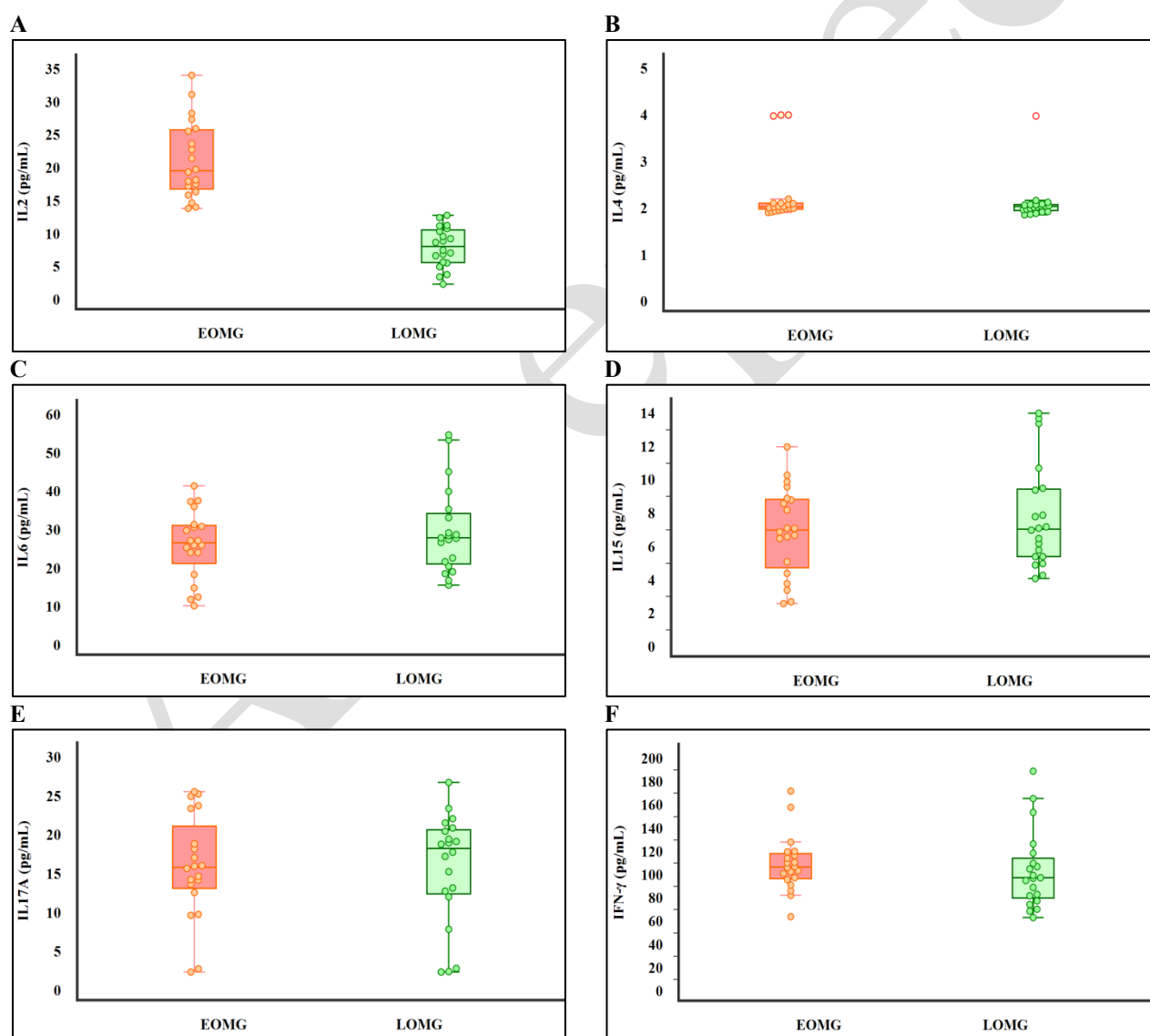


Fig. 3: Serum cytokines levels in EOMG and LOMG subgroups. (A) IL2, (B) IL4, (C) IL6, (D) IL15, (E) IL17A, and (F) IFN γ . EOMG had significantly higher serum IL-2 levels compared with LOMG ($p < 0.001$).

Table 4: Changes in the serum cytokines levels in MG with normal thymus and MG with abnormal thymus

Cytokine (pg/mL)		MG with normal thymus (n = 27)	MG with abnormal thymus (n = 13)	P value
IL2	mean±SD	14.83 ± 7.96	14.23 ± 8.91	0.732
	Median (min – max)	12.8 (3.5 - 34.1)	16.4 (2.4 - 31.2)	
IL4	mean±SD	2.18 ± 0.53	2.3 ± 0.75	0.493
	Median (min – max)	2.04 (1.87 - 4)	2 (1.86 - 4)	
IL6	mean±SD	27.51 ± 9.1	28.86 ± 12.48	0.820
	Median (min – max)	28 (12 - 53.4)	26.1 (10.3 - 54.8)	
IL15	mean±SD	7.1 ± 2.56	7.9 ± 3.49	0.648
	Median (min – max)	6.9 (2.7 - 13.7)	7.1 (2.6 - 14)	
IL17A	mean±SD	13.62 ± 6.16	20.56 ± 5.87	<0.001*
	Median (min – max)	15.3 (2.38 - 22.1)	23.4 (7.9 - 26.8)	
IFN-γ	mean±SD	111.14 ± 31.87	97.43 ± 19.53	0.376
	Median (min – max)	105 (64.2 - 189.1)	99.5 (63.5 - 120.4)	

$p^* < 0.05$ is considered significant. Data are expressed as mean± SD, median (minimum-maximum).

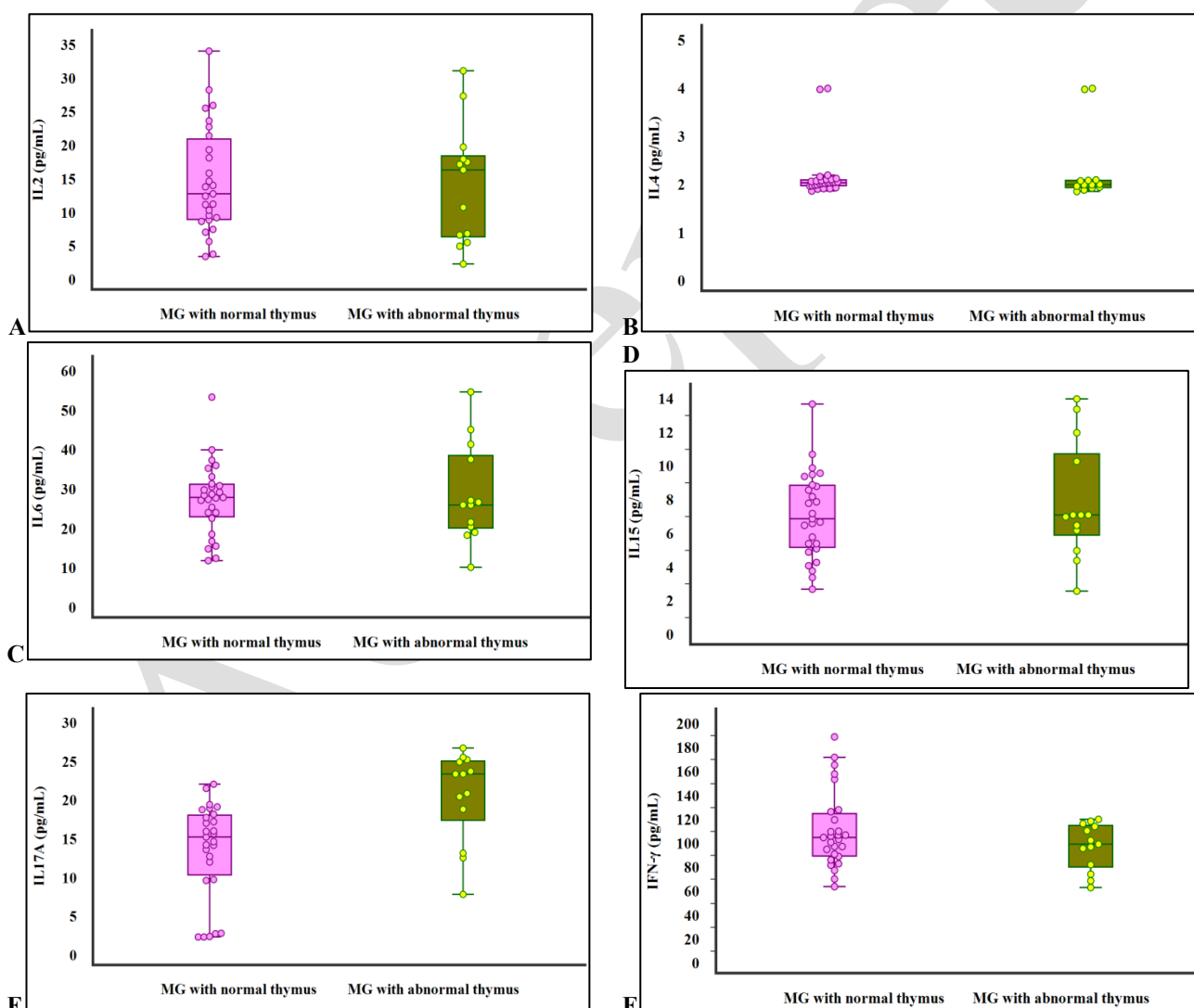


Fig. 4: Serum cytokines levels in MG with normal thymus and MG with abnormal thymus. (A) IL2, (B) IL4, (C) IL6, (D) IL15, (E) IL17A, and (F) IFN γ .

Serum IL-17 was significantly elevated in TAMG patients than in MG with normal thymus group ($p < 0.001$).

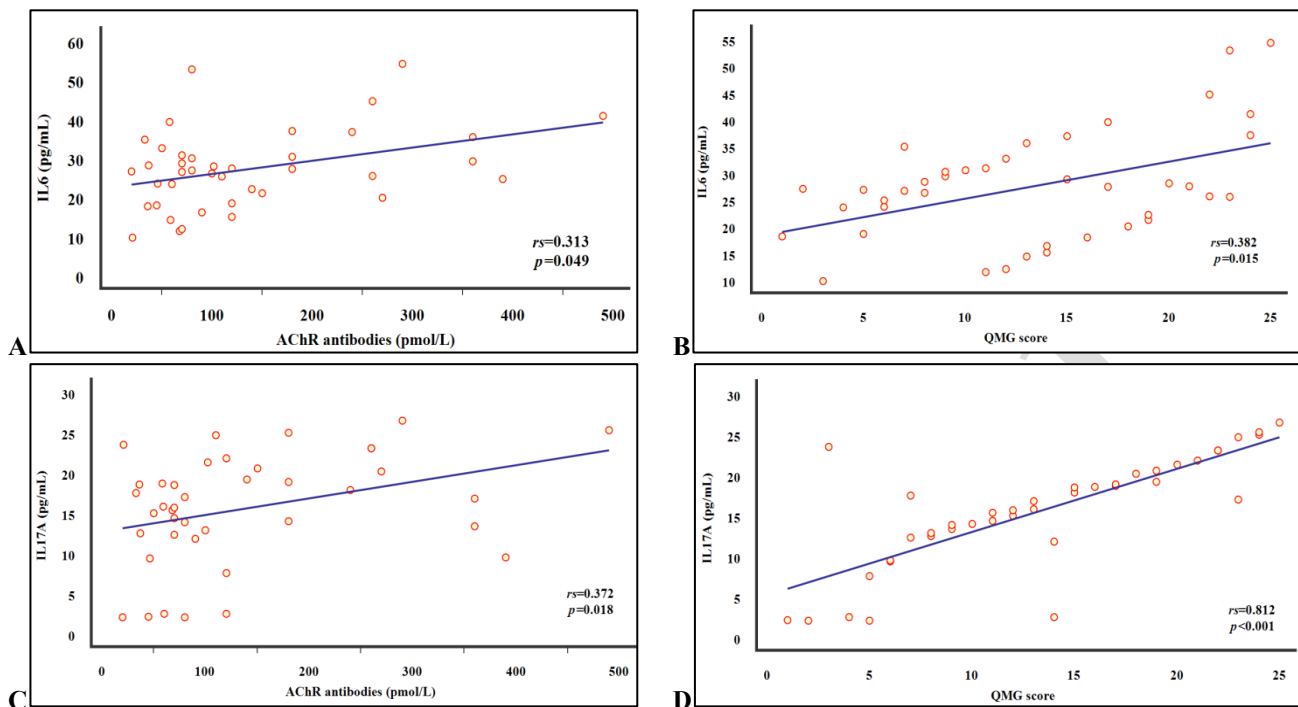


Fig. 5: Correlations between serum IL6 and IL17A cytokine levels with QMG scores and with the concentration of AChR antibodies

(A) IL-6 was positively correlated with the concentration of AChR antibodies, (B) IL-6 was positively correlated with QMG scores, (C) IL-17 was positively correlated with the concentration of AChR antibodies, (D) IL-17 was positively correlated with QMG scores.

DISCUSSION

Myasthenia gravis is antibody mediated, T cell dependent autoimmune disorder. Long term antibody response requires particular T cell subsets mainly CD4⁺ T cell. Cytokines released from CD4⁺ T cells control production of antibody by B cells⁹. In our study we aimed to analyze an intensive serum cytokines profile in AChR Ab MG patients and determined the link between these cytokines and clinical parameters of MG.

In our study, we selected our patients with positive AChR antibody as an immunological marker for MG diagnosis. In general, MG is caused by auto-antibodies against MuSK, LRP4 or AChR in the postsynaptic membrane of neuromuscular junction. Antibody targeting AChR in postsynaptic endplate of neuromuscular junction is the most frequent antibody category in MG patients³. AChR antibody has been used as a diagnostic biomarker after being described and associated with pathophysiology of MG in the 1970s. AChR antibodies are detected in 80-85 % of G-MG patients and 50-70 % of O-MG patients, with 97-99 % specificity¹⁰. Additionally, AChR antibodies are not only considered as a diagnostic biomarker in MG, but also predict acceptable responses to thymectomy in non TAMG patients¹¹. This is because AChR antibodies are always present in TAMG patients¹².

The cytokines deregulation is linked to pathogenesis of various autoimmune disorders. Cytokines imbalance has been reported in MG with variations between studies. These variations could be reduced by means of cellular estimation of cytokines⁹. IL-6 is formed via different immunological cells, like B cell, T cell, macrophage and dendritic cell¹³. However, since IL-6 increase was an inclusive outcome of several immunological cells, the pathological role of different immune cells cannot be specified in MG². Besides, IL-6 is released by the muscle cell in AChR Ab dependent mode. IL-6 is able to stimulate plasma cell proliferation and antibody secretion. Also, it promotes T cell propagation, encourages cytotoxic T cell activation, and provokes Th17 and Tfh cells differentiation, which are associated with MG pathogenesis¹⁵. In this study, serum IL-6 levels were significantly higher in MG participants than in HC. We also found that the G-MG group showed significantly elevated IL-6 than HC, without statistical significant difference between O-MG and HC groups. Our study also showed that IL-6 level was positively correlated with QMG score and AChR antibodies concentration in all MG patients. Most studies were similar to our findings about IL6^{2,13}. It was reported that IL6 was correlated with MG severity, signifying that IL-6 causes exacerbation of MG pathogenesis¹⁵. Conversely, higher serum IL-6 level was not established in AChR Ab MG patients, in other studies^{16,17}. This discrepancy reflects the importance of

methodological difference. Furthermore, IL-6 is a pleiotropic cytokine, playing both pro- and anti-inflammatory actions. This does not make IL-6 a definite MG diagnostic biomarker¹⁴. Jonsson and coauthors¹⁸ support the effectiveness of IL-6 inhibitor (tocilizumab) for AChR Ab MG treatment. Perhaps, IL-6 level could be considered a pharmacodynamics biomarker, as it could measure the outcome of therapeutic interference, but more researches are required to found standard values and confirm the results¹⁸.

IL15 is secreted by activated monocytes, dendritic cells, epithelial cells and fibroblasts. Unlike IL-2, IL-15 is not produced by activated T cell. IL-15 is a pleiotropic proinflammatory cytokine with multiple biological effects, and it is expressed in numerous inflammatory diseases¹⁹. IL-15 can activate T cell, macrophage and neutrophil; and is vital for dendritic cell action in some model systems²⁰. In this study, serum IL-15 levels were significantly elevated in MG patients than in HC, and its levels were significantly higher in the G-MG or O-MG group than HC group. Similar result was reported by Uzawa et al¹⁶ who reported that serum IL-15 levels were significantly higher in MG patients than those in healthy participants. Actually, increased levels of IL-15 in both serum and inflamed tissue have been detected in multiple autoimmune disorders that may be linked with their pathogenesis¹⁹. IL-15 neutralization was found to have a valuable effect in psoriasis models. This information holds promise that IL-15 pathway antagonism has a therapeutic effect in inflammatory autoimmune disorders²¹. Concerning MG, preceding researches have reported that IL-15 has the principal role in pathogenesis of EAMG^{22,23}. Production of cytokines by muscle cells has been considered an important mechanism that may affect EAMG. It was reported that muscle cells can release IL-15 as a result of IL-4 stimulation either in vitro or in vivo (EAMG induction)²². Myocytes in rats with EAMG, which were provoked by either active immunization or by AChR antibodies passive transfer, showed increased IL-15 production. This result proposed that muscle cells result in inflammation through production of IL-15 and thus deteriorate EAMG clinical course. That's why, IL-15 may be implicated in MG pathogenesis²³.

Th17 cells are detected at inflammatory site in multiple human inflammatory disorders and are associated with several autoimmune disorders pathogenesis such as multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease²⁴. IL-17A is the characteristic cytokine of Th17 cell which is a proinflammatory cytokine that can up-regulate matrix metalloproteases and several chemokines, resulting in neutrophils chemotaxis toward inflammatory site²⁵. Th17 cells contribute to an EAMG model in IL-12 / IL-23 knockout mice²⁶. Th17 cells and IL-17 display an essential role in harmonizing related auto-reactive T cells and B cells, resulting in auto-antibodies production with

consequent progress to EAMG²⁷. In our study, serum levels of IL-17 were significantly higher in MG patients compared with HC group, and its serum levels were positively correlated with concentration of AChR antibodies and QMG scores in all MG patients. Also, we found that G-MG group showed significantly elevated IL-17 than HC, without statistical significant difference between O-MG and HC groups. In another study, it was confirmed that serum IL-17 was significantly elevated in G-MG compared with O- MG or HC, and serum IL-17 was correlated with AChR Ab titres²⁸. Moreover, IL-17 level was found to expect progression from ocular to generalized MG within the first 6 months after diagnosis²⁹. However, IL-17 prognostic capability was not reported in another cohort of MG patients¹⁶.

We also found significant higher serum IL-17 levels in TAMG patients than in MG with normal thymus group. A previous research reported that that both serum IL-17 and expression of IL-17 was significantly higher in MG cases with thymoma than healthy group. But there were no significant differences between MG patients with normal thymus or with thymic hyperplasia and HC groups. Additionally, there was decreased population of Th17 cells (%) after thymectomy in 10 MG patients with thymoma, without reaching the statistical significance¹⁵. Increased IL-17 in this condition may encourage T cells auto-reactivity and B cells, and subsequently stimulate formation of auto-antibodies and make worse the disease. Therefore, the observation of increased IL-17 in MG patients may suggest a thymoma specific phenomenon. As a whole, these findings signify that IL-17 is strongly linked to thymoma induced immune injury¹⁵.

IL-4 is a multifunctional pleiotropic cytokine which is released mainly by activated T cells, eosinophils, basophils and mast cells. IL-4 is critical for regulation of cell proliferation, apoptosis and numerous genes in Th2 lymphocytes expression³⁰. In our research, serum levels of IL-4 were significantly lower in MG patients than in the HC group. An earlier study found that IL-4 did not have a pathogenic effect in EAMG and might have a protective role in EAMG. IL-4 has a regulatory mechanisms through prevention of development of chronic AChR Ab mediated autoimmune reaction³¹. It was reported that MG manifestations may develop from disturbance of protective mechanism by down-regulation of IL-4 in MG patients¹⁶.

IL-2 is mostly produced via CD4+ T cell, and also can be synthesized via B cell, NK cell and monocyte. IL-2 is a T cell growth factor; increases NK cell cytotoxicity and stimulates immunoglobulin production by B cell. Moreover, IL-2 is a T-cell stimulating cytokine which stimulates effector T cells proliferation and differentiation and supports immunological polarization of Th1³². In our study, serum IL 2 levels were elevated in MG patients than in HC without statistically significant variations. Regarding E-L-T classification, we found that EOMG had significantly higher IL-2 levels compared

with LOMG. Similar findings were obtained by Wei et al² who reported a reduction in serum IL-2 levels in LOMG compared to that in EOMG. Although IL-2 has an important role in enhancement of immune system, only higher serum IL-2 was detected in MG cases, without consideration of IL-2 change in EOMG and LOMG³³. Another study reported that IL-2 in LOMG was lower compared to EOMG patients. The researchers explained this by the presence of co-morbidities like diabetes or hypertension associated with LOMG that also become more with age. Diabetic cases with LOMG showed lower IL-2 levels opposed to non diabetics³⁴. It was found that in diabetic non obese mice, lower IL-2 expression has been linked to higher possibility of MG development³⁵. Additionally, IL-2 can regulate inflammation through prevention of IL-6 associated signaling proceedings and differentiation of Th-17. IL-2 levels were found to be reduced in both type 1 and type 2 diabetes mellitus than in healthy controls. IL-2 with low dose could be used in treating type 1 diabetes^{36,37}. It was theorized that aging decreases the immune competence with subsequent decrease in IL-2 secretion which plays a significant role in LOMG pathogenesis, and it may be also a risk factor of LOMG associated with DM².

IFN- γ is mostly produced by NK and CD8+ T cells, which has a wide antiviral effect and immunoregulatory activity in adaptive and innate immunity, tumor immunity and autoimmune diseases. Th17 cells can differentiate into interferon- secreting cells, known as Th1/17 cells, a subtype that encourages autoimmunity³⁸. Our study revealed that serum levels of IFN- γ were higher in MG patients than in HC without statistically significant difference. Similar result was reported by a previous study². A cross sectional research detected that Th1/17 cells number was elevated in peripheral blood of AChR Ab MG cases compared to HC group. Moreover, Th1/17 cells are found in an elevated quantity in thymus gland of AChR Ab MG cases, with a positive correlation between Th1/17 cells number and severity of MG based upon QMG³⁹. Th1/17 numbers showed also an earlier turn down in responders compared with non-responders following treatment of AChR Ab MG patients. This indicates that Th1/17 cell levels can be considered as an early pharmacodynamic biomarker of an acceptable response to therapy. In addition, immunosuppressive treatment decreases IFN- γ levels in AChR Ab MG patients. So, serum IFN- γ can also be considered as a pharmacodynamic biomarker, instead of a diagnostic biomarker⁴⁰. Actually, the difference in cytokines measurement or the difference in numbers or profiles of MG patients displays a role in discrepancy among different studies.

In this study, some restrictions should to be taken in consideration. Firstly, the most important limitation is that our research only calculated serum level of cytokines, without cellular evaluation of cytokines, and so could not show what cells release the altered

cytokines. Furthermore, serum cytokines levels were not essentially a mirror to these levels at the neuromuscular junctions. Second, enrollment of MG patients has another limitation, because our patients had somewhat low disease severity, mostly classified as MGFA I & II. Additionally, because of inadequate sample size, comparison of several groups, for example AChR antibodies and AChR associated with MuSK or LRP4 antibodies, could not be done.

From this study it can be concluded that the pathogenic inflammatory effects exerted by cytokines at neuromuscular junctions are important in MG patients. Anti cytokines treatment possibly have the ability in MG treatment. Further researches including seronegative MG patients, should be done to confirm more details about cytokine profiles in MG patients. The improvement of novel therapy for MG requires more understanding of cytokine signaling pathways.

Ethical approval

The study was accepted by Institutional Review Board of the Faculty of Medicine, Mansoura University; code number: R.25.08.3322.

Conflicts of interest

- The authors declare that they have no financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Gilhus NE. Myasthenia gravis. *N Engl J Med*. 2016; 375(26): 2570–81.
2. Wei SL, Yang CL, Si WY, Dong J, Zhao XL, Zhang P, et al. Altered serum levels of cytokines in patients with myasthenia gravis. *Heliyon*. 2023; 10(1): e23745.
3. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis - autoantibody characteristics and their implications for therapy. *Nat Rev Neurol*. 2016; 12(5): 259–68.
4. Martinez Salazar A, Mokhtari S, Peguero E, Jaffer M. The role of complement in the pathogenesis and treatment of myasthenia gravis. *Cells*. 2025; 14(10): 739.
5. Jutel M, Akdis CA. T-cell subset regulation in atopy. *Curr Allergy Asthma Rep*. 2011; 11: 139–45.
6. Yang H, Zhang Y, Wu M, Li J, Zhou W, Li G, et al. Suppression of ongoing experimental autoimmune myasthenia gravis by transfer of RelB-silenced bone

- marrow dendritic cells is associated with a change from a T helper Th17/Th1 to a Th2 and FoxP3+ regulatory T-cell profile. *Inflamm Res*. 2010; 59: 197–205.
7. Murai H, Masuda M, Utsugisawa K, Nagane Y, Suzuki S, Imai T, et al. Clinical features and treatment status of adult myasthenia gravis in Japan. *Clin Exp Neuroimmunol*. 2014; 5: 84–91.
8. Jaretski A 3rd, Barohn R J, Ernstoff R M, Kaminski H J, Keesey J C, Penn A S, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. *Neurology*. 2000; 55: 16–23.
9. Çebi M, Durmus H, Aysal F, Özkan B, Gül GE, Çakar A, et al. CD4⁺ T cells of myasthenia gravis patients are characterized by increased IL-21, IL-4, and IL-17A productions and higher presence of PD-1 and ICOS. *Front Immunol*. 2020; 11: 809.
10. Peeler CE, Lott LBD, Nagia L, Lemos J, Eggenberger ER, Cornblath WT. Clinical utility of acetylcholine receptor antibody testing in ocular myasthenia gravis. *JAMA Neurol*. 2015; 72(10): 1170–4.
11. Gronseth GS, Barohn R, Narayanaswami P. Practice advisory: thymectomy for myasthenia gravis (practice parameter update): report of the guideline development, dissemination, and implementation subcommittee of the american academy of neurology. *Neurology*. 2020; 94(16): 705–9.
12. Andrés M. De León. Biomarkers in autoimmune myasthenia gravis: A narrative review. *NeuroMarkers*. 2025; 2(3): 100094.
13. Uzawa A, Akamine H, Kojima Y, Ozawa Y, Yasuda M, Onishi Y, et al. High levels of serum interleukin-6 are associated with disease activity in myasthenia gravis. *J Neuroimmunol*. 2021; 358: 577634.
14. Maurer M, Bougoin S, Feferman T, Frenkian M, Bismuth J, Mouly V, et al. IL-6 and Akt are involved in muscular pathogenesis in myasthenia gravis. *Acta Neuropathol Commun*. 2015; 3:1.
15. Wang Z, Wang W, Chen Y, Wei D. T helper type 17 cells expand in patients with myasthenia-associated thymoma. *Scand J Immunol*. 2012; 76(1): 54–61.
16. Uzawa A, Kawaguchi N, Himuro K, Kanai T, Kuwabara S. Serum cytokine and chemokine profiles in patients with myasthenia gravis. *Clin Exp Immunol*. 2014; 176(2): 232–7.
17. Yilmaz V, Oflazer P, Aysal F, Durmus H, Poulas K, Yentur SP, et al. Differential cytokine changes in patients with myasthenia gravis with antibodies against AChR and MuSK. *PLoS ONE*. 2015; 10: e0123546.
18. Jonsson DI, Pirskanen R, Piehl F. Beneficial effect of tocilizumab in myasthenia gravis refractory to rituximab. *Neuromuscul Disord*. 2017; 27(6): 565–8.
19. McInnes IB, Gracie JA. Interleukin-15: a new cytokine target for the treatment of inflammatory diseases. *Curr Opin Pharmacol*. 2004; 4: 392–7.
20. Rückert R, Brandt K, Bulanova E, Mirghomizadeh F, Paus R, Bulfone-Paus S. Dendritic cell-derived IL-15 controls the induction of CD8 T cell immune responses. *Eur J Immunol*. 2003; 33: 3493–503.
21. Villadsen LS, Schuurman J, Beurskens F, Dam TN, Dagnaes-Hansen F, Skov L, et al. Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. *J Clin Invest*. 2003; 112:1571–80.
22. Shandley S, Martinez S, Krolick K. IL-4 receptor as a bridge between the immune system and muscle in experimental myasthenia gravis I: up-regulation of muscle IL-15 by IL-4. *Clin Immunol*. 2009; 132: 246–56.
23. Stegall T, Krolick KA. Myocytes respond to both interleukin-4 and interferon-gamma: cytokine responsiveness with the potential to influence the severity and course of experimental myasthenia gravis. *Clin Immunol*. 2000; 94:133–9.
24. Pène J, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, et al. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol*. 2008; 180: 7423–30.
25. Alrashidi HE, EL-Ageery SM, Fawzy IF, Bahy-Eldeen A, Mahmoud R, Abdelhafez MS. The role of T helper 17 cells in *Helicobacter pylori* associated chronic gastritis. *Egyptian Journal of Medical Microbiology*; 2021; 30 (3): 119–126.
26. Wang W, Milani M, Ostlie N, Okita D, Agarwal RK, Caspi RR, et al. C57BL/6 mice genetically deficient in IL-12/IL-23 and IFN-gamma are susceptible to experimental autoimmune myasthenia gravis, suggesting a pathogenic role of non-Th1 cells. *J Immunol*. 2007; 178: 7072–80.
27. Mu L, Sun B, Kong Q, Wang J, Wang G, Zhang S, et al. Disequilibrium of T helper type 1, 2 and 17 cells and regulatory T cells during the development of experimental autoimmune myasthenia gravis. *Immunology* 2009; 128 (Suppl 1): e826–36.
28. Roche JC, Capablo JL, Larrad L, Gervas-Arruga J, Ara JR, Sánchez A, et al. Increased serum interleukin-17 levels in patients with myasthenia gravis. *Muscle Nerve*. 2011; 44: 278–80.

29. Xie Y, Li H feng, Jiang B, Li Y, Kaminski HJ, Kusner LL. Elevated plasma interleukin- 17A in a subgroup of myasthenia gravis patients. *Cytokine*. 2016; 78: 44–6.
30. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol*. 1999; 17: 701–38.
31. Ostlie N, Milani M, Wang W, Okita D, Conti-Fine BM. Absence of IL-4 facilitates the development of chronic autoimmune myasthenia gravis in C57BL/6 mice. *J Immunol* 2003; 170: 604–12.
32. Liao W, J.-X. Lin, W.J. Leonard, IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr Opin Immunol*. 2011; 23(5): 598-604.
33. Hu Y, Wang J, Rao J, Xu X, Cheng Y, Yan L, et al., Comparison of peripheral blood B cell subset ratios and B cell-related cytokine levels between ocular and generalized myasthenia gravis. *Int. Immunopharm*. 2020; 80: 106130.
34. Klimiec-Moskal E, Quirke M, Leite MI. Comorbidities in older patients with myasthenia gravis - comparison between early- and late-onset disease. *Acta Neurol Scand*. 2022; 145(3): 371-4.
35. Hulme MA, Wasserfall CH, Atkinson MA, Brusko TM. Central role for interleukin-2 in type 1 diabetes. *Diabetes*. 2012; 61(1): 14-22.
36. Suri S, Mitra P, Abhilasha A, Saxena I, Garg MK, Bohra GK, et al. Role of interleukin-2 and interleukin-18 in newly diagnosed type 2 diabetes mellitus. *J. Basic Clin Physiol Pharmacol*. 2021; 33(2): 185-90.
37. Khalil RG, Abdel-Moneim A, Yousef AI, Abdel-Rahman H, Zanaty MI, El-Sayed A. Association of interleukin-2, interleukin-21 and interleukin-23 with hyperlipidemia in pediatric type 1 diabetes. *Mol Biol Rep*. 2021; 48(7): 5421-33.
38. Stockinger B, Omenetti S. The dichotomous nature of T helper 17 cells. *Nat Rev Immunol*. 2017; 17(9): 535–44.
39. Ma Q, Ran H, Li Y, Lu Y, Liu X, Huang H, et al. Circulating Th1/17 cells serve as a biomarker of disease severity and a target for early intervention in AChR-MG patients. *Clin Immunol*. 2020; 218: 108492.
40. Li Y, Chen P, Huang X, Huang H, Ma Q, Lin Z, et al. Pathogenic Th17 cells are a potential therapeutic target for tacrolimus in AChR-myasthenia gravis patients. *J Neuroimmunol*. 2024; 396: 578464.