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The relationship between COVID-19 and the complement system: mannose-binding lectin

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Abstract. – OBJECTIVE: Mannose-binding lectin (MBL) is one of the important parts of the complement system. In our study, we aimed to determine serum MBL levels and their relationship with intensive care hospitalization.

PATIENTS AND METHODS: Ninety COVID-19-positive patients from outpatient clinics and clinics were included in this study. The patients were evaluated in three groups as mild, moderate, and severe groups. Each group consisted of 30 patients. A venous blood sample was taken once from each patient. Serum MBL, C-reactive protein (CRP), lactate dehydrogenase (LDH), fibrinogen, D-dimer, and ferritin levels were measured.

RESULTS: The mean serum MBL levels of all patients were 695.46±324.42 ng/mL. One-way ANOVA test resulted in significant differences in serum CRP, LDH, fibrinogen, D-dimer, ferritin, and MBL levels between groups ($p<0.05$ for all comparisons). Post-hoc Tukey analysis showed significant differences in serum MBL levels between mild and severe groups and moderate and severe groups.

CONCLUSIONS: MBL may be used as a prognostic biomarker in COVID-19 patients. Further studies are needed to determine MBL in treatment strategies.

Key Words:
Complement system, COVID-19, Health, Mannose binding lectin.

Introduction

COVID-19 infection caused by SARS-CoV-2 was first seen in Wuhan, a city in China, leading to a global epidemic. The clinical and pathological features of acute infection may present with a wide spectrum of diseases ranging from asymptomatic infection to mild self-limiting symptoms and acute respiratory failure requiring intensive care[1]. Although the most common clinical findings are fever, cough, and fatigue, endothelial damage may occur in many vital organs due to excessive production of proinflammatory cytokines, especially in some patient groups. Proinflammatory cytokines synthesized by T lymphocytes and macrophages are held responsible for the damage. This phenomenon, called "cytokine storm", can result in multiple organ failure and mortality[2].

Acute respiratory distress syndrome (ARDS) is the primary cause of death in COVID-19[3] patients the primary reason for intensive care hospitalization[4]. Endothelial damage and activation play a major role in the exudative phase of ARDS because it may increase the likelihood of thrombosis as well as contribute to increased inflammation. In addition, as a result of endothelial damage, the lectin pathway of complement on the endothelial cell surface may be activated[5].

It has been suggested that the plasma-based complement system, which is an important part of innate immunity, is involved in the pathogenesis of COVID-19 by increasing inflammation and tissue damage. In addition, studies[6] have shown that it affects the promhrombotic system. Mannose-binding lectin (MBL), also known as mannose-binding protein (MBP), the relationship which we investigated with COVID-19 in our study, initiates the lectin pathway of complement activation[7]. MBL is a serum protein produced in the liver in the innate immune system[8]. This pattern recognition molecule that activates the lectin pathway of the complement system by binding to carbohydrates such as mannose on damaged host cells[9,10].

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MBL forms molecular complexes with MBL-associated serine proteases. The most important of these are known as MBL-associated serine protease-1 and MBL-associated serine protease-2 (MASPs-1, -2)[9,10]. MASPs-1 and MASPs-2 are activated when associated with MBL and bind to specific carbohydrate structures on microbial and cell surfaces. This leads to the cleavage of complement factors, C4 and C2, followed by the activation of the C3 and co-complement pathway and formation of C4b2b convertase[11]. Studies[10,12] have shown that MASP-1 has thrombin-like activity and can degrade factor XIII (FXIII). In addition, MASP-2 can also degrade prothrombin to thrombin. MASPs have an important place in the coagulation system and play an important role in the stabilization as well as activating the coagulation system[10].

There are studies[13] suggesting that the complement system is activated by hypoxic cells and tissues. COVID-19 may cause hypoxia leading to intensive care hospitalization in patients. Activation of the complement system may worsen the clinical course of the disease by increasing inflammation and endothelial damage. In addition, studies[14] have shown that the MBL pathway is associated with mortality.

In our study, we tried to determine the relationship between the level of MBL, which is one of the important parts of the complement system, in the serum of COVID-19 patients and the severity of the disease. We aimed to evaluate the relationship between MBL levels and intensive care hospitalization.

Patients and Methods

COVID-19-positive patients (who had at least one positive COVID-19 RT-PCR result within 7 days), ages between 18-75, male and female gender, who were admitted to COVID-19 outpatient clinics and clinics in Erzurum City Hospital were included in this study. Patients with known systemic diseases, pregnant patients, and lactating patients were not included in the study. The local Clinical Research Ethics Committee approved the study. All patients or their legal representatives were enlightened about the study and informed consent was taken.

Demographical data (age, gender) and chest X-ray results were obtained from the electronic automation system of the hospital. Subgroups were formed according to the clinical characteristics of the patients at hospital admission. Patients were evaluated in three groups. The first group was named the "mild group", including patients treated in the outpatient clinics and had no abnormalities in chest X-ray graphs. The second group was named the "moderate group", whose patients were treated in the clinic, out of intensive care conditions. The third group was named the "severe group", which included patients treated in the intensive care unit. Each group consisted of 30 patients.

Venous blood samples were obtained from all patients once at the time of admission to the hospital. Serum was obtained and aliquoted into two portions. First portions of each serum sample were used to measure C-reactive protein (CRP), lactate dehydrogenase (LDH), fibrinogen, D-dimer, and ferritin levels. The second portion from each aliquot was stored in a freezer at -20°C until the analysis day of MBL. Beckman Coulter AU5800 analyzer (Brea, CA, USA) was used to measure serum CRP, LDH, and ferritin levels. STA R Max 3 (Stago, Asnières-sur-Seine, France) hemostasis and coagulation analyzer was used to measure serum fibrinogen levels. Serum MBL levels were measured by the ELISA method in an ELISA microplate reader, using a commercial ELISA kit (BT Lab, Catalog No: E0335Hu, Lot no: 202111018, Shanghai, China).

Statistical Analysis

Data were analyzed by using SPSS version 25.0; (IBM Corp., Armonk, NY, USA) package program. The level of statistical significance was set at $p<0.05$. The normality of data was determined by Kolmogorov-Smirnov test. Data were presented as mean±standard deviation. A one-way ANOVA test was used to evaluate the differences between groups and a post-hoc Tukey test was performed to determine the pairwise differences between groups.

Results

Three groups were in similar characteristics in terms of age. There were no cases of death, intubation, and mechanical ventilation in the mild and moderate groups. Table I shows the demographic and clinical characteristics of the patients.

Biochemical values of patients and comparisons between groups are presented in Table II. The one-way ANOVA test showed that there were significant differences in serum CRP, LDH, fibrinogen, D-dimer, ferritin, and MBL levels between groups ($p<0.05$ for all comparisons). The mean serum MBL levels of all patients were 695.46±324.42 ng/mL. The post-hoc

Table I. Demographic and clinical characteristics of the patients.

	All (n=90)	Mild (n=30)	Moderate (n=30)	Severe (n=30)
Age (years)	56.2±13.2	47.1±14.2	57.2±10.1	64.4±16.1
Gender, M/F (%)	55/45	57/43	54/46	53/47
Death, n (%)	8 (8.8%)	0 (0 %)	0 (0 %)	8 (26.6%)
Intubation, n (%)	14 (15.5%)	0 (0 %)	2 (6.6 %)	12 (40%)
Mech. Vent. n (%)	29 (32.2%)	0 (0 %)	3 (10 %)	26 (86.6%)

Age is presented as mean±standard deviation, M/F: male/female, Mech. Vent. N.: Mechanical Ventilation Need.

Table II. Biochemical values of patients and comparisons between groups.

	Patients All (n=90)	Mild (n=30)	Moderate (n=30)	Severe (n=30)
CRP (mg/L)	42.55±45.33*	14.21±a	49.61±42.27	52.64±50.32[b]
LDH (U/L)	393.6±232.6*	235.6±72.3[a]	390.1±171.33[c]	551.7±257.12[b]
Fibrinogen (mg/dL)	400.6±144.12*	362.1±112.6[a]	471.9±157.1[c]	369.56±229.9
D-Dimer (ng/mL)	1,925.6±3,296.7*	325.50±276.41	1,988.15±2,032.18	3,461.92±7,550.5[b]
Ferritin (ng/ml)	686.4±634.27*	142.22±198.31[a]	776.87±725.3	1,126.24±601.40[b]
MBL (ng/mL)	466.30±188.84*	496.81±210.38	519.80±188.69[c]	382.30±135.47[b]

Results are expressed as mean ± standard deviation; *: $p<0.05$ for One-Way ANOVA test; [a,b,c]: One-way ANOVA Tukey post-hoc test p-values. [a]Significant difference between mild and moderate patients; [b]Significant difference between mild and severe patients; [c]Significant difference between moderate and severe patients. C-reactive protein (CRP), lactate dehydrogenase (LDH).

Tukey analysis showed that there were significant differences in terms of serum MBL levels between mild and severe groups and moderate and severe groups. (Figure 1).

Pearson's correlation analysis showed that there was a moderate positive correlation between fibrinogen and CRP levels (r=0.663; $p<0.01$). There were no significant correlations between pairwise comparisons of the other biochemical analytes.

Discussion

In this study, we determined the relationship between MBL levels and the severity of the disease in 3 groups of COVID-19 patients. Our results showed that there was no significant difference in MBL levels between mild and moderate patient groups. However, we observed that the MBL level was statistically significantly lower

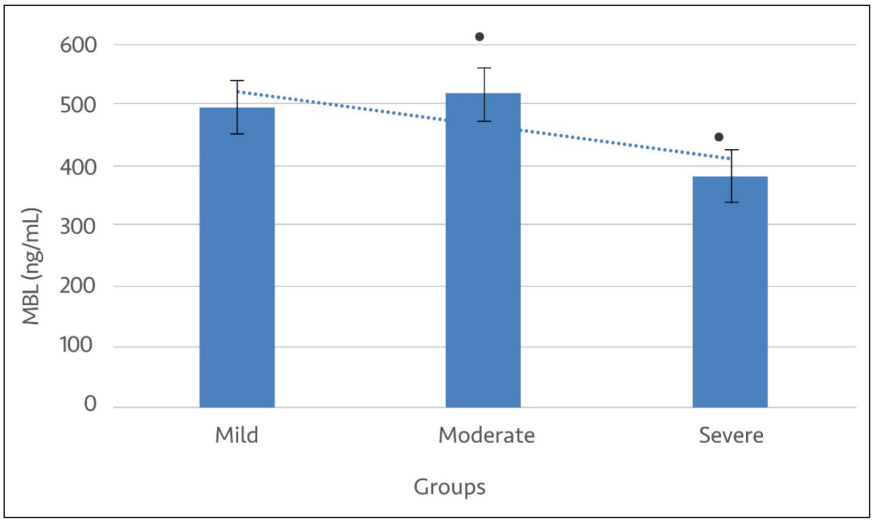

Figure 1. MBL values of groups. •: Significant difference between mild and severe patients and significant difference between moderate and severe patients (post hoc Tukey test p-value is lower than 0.05 for both comparisons).

in the severe patient group compared to the other groups. We found that LDH, CRP, D-Dimer, and ferritin levels, which were found to be important in the prognosis of COVID-19 in previous studies[15], were significantly higher in the severe group compared to mild and moderate groups. MBL level was statistically significantly lower in the severe patient group compared to the other groups.

COVID-19 is a multisystemic infection and the clinical status of patients ranges between mild and severe, including intensive care and even death[16]. It is thought that the main cause of disease severity and death is excessive inflammatory response[17]. Recent studies[18] have shown that the activation of the complement system initiates this dysregulated inflammatory response in COVID-19. In addition, studies[19] have reported elevated MBL levels in some critically ill COVID-19 patients.

In recent years, it has become increasingly clear that the complement system plays an important role in immune homeostasis, even in healthy individuals. Therefore, complement deficiencies are not only associated with infectious diseases but also with autoimmune and inflammatory diseases. Among the complement deficiencies identified in humans, the complement factor MBL deficiency has the highest frequency[20]. In a study[14] examining the relationship between the activation of the MBL pathway and mortality in COVID-19, it was demonstrated that patients with low MBL levels had more severe clinical status and the mortality rate was significantly high, in line with our study. It was concluded that complement activation was associated with the severity of the disease. In genetic studies[14] performed on COVID-19 patients, the incidence of MBL expression deficiency was found to be higher than in the control group. In the studies[21,22] carried out; the serum MBL level was found to be low in people with recurrent Clostridium difficile infections and recurrent sinusitis. In addition, MBL levels were found to be significantly low in patients with tuberculosis, which is an infectious disease that may lead to sepsis and intensive care admissions, and studies[23,24] have shown that this deficiency is associated with mortality. Considering these studies, it can be thought that infections develop more frequently in patients with MBL deficiency.

It has been shown[14] that MBL, which is one of the basic parts of the innate immunity, decreases due to the increase and dysregulation of the inflammatory response of COVID-19, and this is thought to be due to the overactivation of the complement system. The mortality rate was found[14] to be higher in patients who developed or had MBL deficiency. In addition, in a study[25], it was revealed that the survival rate of patients with high serum MBL levels who were hospitalized in the intensive care unit due to serious infection was higher.

In our study, only patients diagnosed with COVID-19 were evaluated, and MBL level could be used as a candidate biomarker in predicting the severity of other infectious diseases. In addition, by extending the follow-up period of the patients, more insight can be gained into whether the MBL level will be an indicator of mortality. More comprehensive studies can be done by increasing the number of patients.

Conclusions

Patients with low MBL levels present a severe course of COVID-19 and a higher rate of intensive care hospitalization. Agents that inhibit the activation of the complement system can prevent the irregular inflammatory response and prevent mortality caused by hyperinflammation. More comprehensive and detailed studies are needed to define MBL as a prognostic biomarker of COVID-19.

Conflict of Interests

The authors declare that there is no conflict of interest.

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Ethics Approval

The Institutional Review Board at Health Science University Faculty of Medicine approved this study (BEAH KAEK 2022/04-11), which was conducted in compliance with the 2013 version of the 1975 Helsinki Declaration.

Informed Consent

All patients or their legal representatives were enlightened about the study and informed consent was taken.

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