

# Maternal Serum Mannose-Binding Lectin Levels and Serum Parameters in Severe Preeclampsia

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## Abstract

**Objective:** We have aimed to compare the serum mannose-binding lectin (MBL) levels in severe preeclampsia with those in uncomplicated pregnancies and healthy women and to investigate the correlation with serum biochemical markers.

**Materials and Methods:** This study includes 27 patients with severe preeclampsia (Group 1); 27, with uncomplicated pregnancy (Group 2); and 25 healthy women of reproductive-age (Group 3). We have made complete blood counts and measured the prothrombin time, activated partial thromboplastin time, the international normalized ratio; and the serum levels of urea, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and MBL.

**Results:** The mean serum MBL level was significantly higher ( $p \leq 0.05$ ) in Group 1 than in Groups 2 and 3, while the MBL levels did not differ significantly between Groups 2 and 3 ( $p > 0.05$ ). High MBL level was not correlated with age, blood pressure, complete blood count, haemostatic parameters, and liver and renal functions.

**Discussion:** Serum MBL level increases significantly in severe preeclampsia, but is not correlated with the degree of the severity.

**Keywords:** mannose-binding lectin, preeclampsia

## Özet

### Ağır Preeklampsi Hastalarında Serum Mannoz-Bağlayıcı Lektin ve Serum Biyokimyası

**Amaç:** Ağır preeklampsi hastaları, komplikasyonsuz gebeler ve üreme çağında gebe olmayan sağlıklı kadınlarda serum mannoz-bağlayıcı lektin (MBL) düzeylerinin karşılaştırılması ve serum biyokimyası ile korelasyonunun araştırılması amaçlandı.

**Materyal ve Metot:** Çalışma; 27 ağır preeklampsi hastasını (Grup 1), 27 komplikasyonsuz gebeyi (Grup 2) ve 25 üreme çağında gebe olmayan sağlıklı kadını (Grup 3) içeriyordu. Olgularda tam kan sayımı, protrombin zamanı, aktive parsiyel tromboplastin zamanı, uluslararası normalizasyon oranı ve serum üre, kreatinin, ürik asit, aspartat aminotransferaz, alanin aminotransferaz, laktat dehidrogenaz, MBL düzeyleri ölçüldü.

**Sonuçlar:** Ortalama serum MBL düzeyi Grup 2 ve 3 arasında anlamlı farklılık göstermezken ( $p > 0.05$ ), Grup 1 düzeyi Grup 2 ve 3'ten anlamlı olarak daha yüksekti ( $p < 0.05$ ). MBL düzeyi yaş, arteriyel kan basıncı, tam kan sayımı, hemostatik parametreler, karaciğer ve renal fonksiyon testleriyle korele değildi.

**Tartışma:** Serum MBL düzeyi, ağır preeklampsi grubunda anlamlı olarak artar, ancak hastalığın şiddeti ile korele değildir.

**Anahtar sözcükler:** mannoz-bağlayıcı lektin, preeklampsi

## Introduction

Preeclampsia is observed in 5% of the deliveries and is characterized by hypertension and proteinuria. Although it is clinically evident after the 20<sup>th</sup> week of gestation, the

underlying immunopathological process begins early in the 1<sup>st</sup> trimester (1,2). Excessive maternal inflammatory response to fetal allograft is implicated in the destruction of the fetomaternal interface. Because mannose-binding lectin (MBL) is an important immune modulator of the innate immune system, its level in the serum may increase and reflect the severity of preeclampsia (3).

Few studies in the literature have evaluated the serum MBL levels or its genotyping in pregnancy-related complications (4-7). If an association exists between the serum MBL level

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and preeclampsia, it is obvious that assessing the serum MBL level could be helpful in the management of those patients. In this study, we have compared the serum MBL levels in severe preeclampsia with those seen in uncomplicated pregnancies and healthy women and investigated the correlation with serum biochemical markers.

## Materials and Methods

The following subjects were included in this study: 27 severe preeclampsia patients (Group 1), 27 pregnant women without any complications (Group 2), and 25 healthy women of reproductive-age (Group 3). All of the pregnant women had completed more than 28 weeks of gestation. Women with type 1, type 2, or gestational diabetes mellitus; rheumatological diseases; chronic renal disease; local or systemic infections; premature labor; premature rupture of membranes; and multiple gestations were excluded from the study.

The participating women provided informed consent, and the study was deemed to be in accordance with Helsinki declaration II and approved by the ethical committee of Uludağ University Medical Faculty.

Severe preeclampsia was defined as blood pressure greater than 160/110 mmHg, as measured on at least 2 occasions 6 hours apart and proteinuria greater than 2 (+), as assessed using a dipstick (>300 mg/dl) on 2 occasions.

We centrifuged 5 ml venous blood from each patient at 3000 rpm, and the serum thus obtained was separated to determine urea, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) levels. Complete blood counts and urinalysis of each patient were performed. The serum samples for MBL determination were stored at  $-20^{\circ}\text{C}$  until the end of the study.

Serum MBL was measured using enzyme-linked immunosorbent assay (ELISA) (KIT 030, Antibodyshop, Grusbakken 8, DK-2820 Gentofte, Denmark). ELISA was performed in microwells coated with mannan from *Saccharomyces cerevisiae*. The serum specimens were diluted in a calcium-containing buffer. Aliquots of calibrators and diluted serum samples were incubated in mannan-precoated microwells at room temperature for 1 hour. Functionally active MBL present in the solutions bound to the mannan-coated wells via its carbohydrate-binding domains. Unbound material was removed by washing. Biotinylated monoclonal detection antibodies were added to each test well and incubated at room temperature for 1 hour. The detection antibody attached to the bound MBL oligomers via the carbohydrate-binding domains that were not occupied during binding to the mannan coat. Unbound detection antibody was removed by washing. Horseradish peroxidase (HRP)-conjugated streptavidin was added to each test well and

allowed to form a complex with the bound biotinylated antibody at room temperature for 1 hour. The unbound conjugate was removed by washing. A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) was added to each test well and incubated in a dark environment for 15 minutes. The bound HRP-streptavidin reacted with the substrate to generate a colored product. The enzymatic reaction was stopped chemically, and the color intensity was read at 450 nm in an ELISA reader. The color intensity (absorbance) indicated the concentration of the functionally active MBL originally added to each well. The results for the calibrators were used to construct a calibration curve from which the concentrations of functionally active MBL in the test specimens were read.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) v13.0. The distribution patterns of the groups were analyzed using the Shapiro-Wilk test. T-test was used in the comparison of 2 groups; and one-way analysis of variance, in comparison of more than 2 groups with normal distribution. Mann-Whitney U test was used in the comparison of 2 groups; and Kruskal-Wallis test, in the comparison of more than 2 groups without normal distribution. The categorized data were analyzed using Pearson  $\chi^2$  test and Fisher's exact test. Pearson's correlation coefficient was calculated, and correlation analysis was made to determine any association between the variables. Statistical significance was defined as  $p \leq 0.05$ .

## Results

The mean ( $\pm$ SD) ages, systolic and diastolic blood pressures, serum MBL levels and complete blood counts of the groups are seen in Table 1. Prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) tests were only performed in severe preeclampsia patients (Table 1).

The mean ( $\pm$ SD) serum AST, ALT, urea, creatinine, uric acid and LDH levels and their significance levels are shown in Table 2. Although there were some significant differences between the mean serum ALT and creatinine levels of the groups, both were within the physiological limits in all groups. Esbach levels were only measured in severe preeclampsia patients (Table 2).

The mean serum MBL level in Group 1 was significantly ( $p < 0.05$ ) higher than those in Groups 2 and 3, while the difference between the levels in the latter 2 groups was not significant ( $p > 0.05$ ) (Table 1). However, serum MBL levels did not show any significant correlation with the age, blood pressures, complete blood counts, haemostatic parameters and serum liver and renal function tests in all groups ( $p > 0.05$ ).

## Discussion

Struggle for life begins with fecundation. The fetus has paternal alloantigens and should induce immune tolerance to

**Table 1.** Age, blood pressure, serum mannose-binding lectin levels, complete blood counts and serum haemostatic marker levels of the groups (Mean ±SD)

	Group 1 (n=27)	Group 2 (n=27)	Group 3 (n=25)
Age (years)	30.3±1.2 <sup>a,b</sup>	29.5±1.0 <sup>a,c</sup>	29.1±1.0 <sup>b,c</sup>
Systolic blood pressure (mmHg)	168.5±2.5 <sup>d,e</sup>	114±1.6 <sup>d,a</sup>	112.1±1.2 <sup>e,a</sup>
Diastolic blood pressure (mmHg)	113.3±1.3 <sup>d,e</sup>	74.2±1.5 <sup>d,a</sup>	69.8±1.3 <sup>e,a</sup>
Mannose-binding lectin (ng/ml)	3112.6±128.4 <sup>f,g</sup>	2457.3±221.7 <sup>f,a</sup>	2052.8±248.9 <sup>g,a</sup>
Hemoglobin (g/dl)	13.3±0.2 <sup>f,g</sup>	12.4±0.2 <sup>f,a</sup>	11.9±0.1 <sup>g,a</sup>
Leukocyte count (cell/μl)	11 247.8±520.6 <sup>a,d</sup>	10 896.5±525.8 <sup>a,e</sup>	7160.3±392.7 <sup>d,e</sup>
Platelet count (cellx10 <sup>3</sup> /μl)	208.2±18.8 <sup>a,d</sup>	217.3±11.3 <sup>a,e</sup>	307.2±14.3 <sup>d,e</sup>
Prothrombin time (sec)	10.80±0.85	-	-
Activated partial thromboplastin time (sec)	25.04±3.24	-	-
International normalized ratio	0.94±0.09	-	-

p>0.05: a, b, c; p≤0.001: d, e; p≤0.05: f, g.

**Table 2.** Serum AST, ALT, urea, creatinine, uric acid, LDH and Esbach levels of the groups (Mean ±SD)

	Group 1 (n=27)	Group 2 (n=27)	Group 3 (n=25)
AST (U/L)	51.3±8.2 <sup>d,e</sup>	22.6±1.5 <sup>d,a</sup>	22.4±1.6 <sup>e,a</sup>
ALT (U/L)	41.9±8.3 <sup>f,a</sup>	19.3±1.8 <sup>f,b</sup>	23.9±1.4 <sup>a,b</sup>
Urea (mg/dl)	30.3±1.8 <sup>d,f</sup>	17.5±1.1 <sup>d,a</sup>	22.5±1.4 <sup>f,a</sup>
Creatinine (mg/dl)	0.85±0.49 <sup>a,d</sup>	0.6±0.02 <sup>d,e</sup>	0.85±0.02 <sup>a,e</sup>
Uric acid (mg/dl)	6.5±0.2 <sup>d,e</sup>	3.7±0.1 <sup>d,h</sup>	2.8±0.1 <sup>e,h</sup>
LDH (U/dl)	384.4±16.6 <sup>d,e</sup>	261.4±8.6 <sup>d,a</sup>	223.6±13.3 <sup>e,a</sup>
Esbach	3.14±1.20	-	-

p>0.05: a, b, c; p≤0.001: d, e, h; p≤0.05: f, g.

be implanted in the endometrium. The environment becomes more challenging as trophoblasts invade the maternal spiral arteries to construct the fetomaternal interface. The chorionic villi extend into the newly formed sinuses to establish fetomaternal transport of the nutrients (1,8). However, such an exposure of paternal alloantigens to the maternal circulation stimulates the immune system, and the complement system becomes activated. Complement components are deposited in the placenta physiologically, but in preeclampsia, an inflammatory response is induced and exceeds the physiological limits (9-12).

Mannose-binding lectin plays an important role in the modulation of the innate immune system. It binds the carbohydrate moieties of microorganisms and activates the complement system via the lectin pathway (13,14). It also contributes to the modulation of a complement-independent pathway, removal of immune debris, promotion of apoptosis, and removal of apoptotic cells (13,15,16). Several authors have suggested MBL deficiency in the immunopathogenesis of recurrent miscarriages and have proposed that insufficient maternal inflammatory response would prevent trophoblastic invasion and remodeling of spiral arteries (6,7,17,18). However, until recently, the normal levels in pregnancy were not clearly defined. In 2006, van de Geijn et al. observed a 140% increase in serum MBL levels during pregnancy (19). They assumed that the MBL levels would decline to the

decline to the normals at 6 weeks postpartum and could be considered as the baseline value. Patients were their own controls based on this assumption which could have been a cause of bias in that study. As expected, the mean systolic and diastolic blood pressures in our study were significantly higher in the severe preeclampsia group than in the other groups. Because there were only few reports on serum MBL levels in uncomplicated gestation and severe preeclampsia, we had to measure these levels in the 3 groups of patients. Though it was not significant, we found serum MBL level increased during pregnancy compared to the levels in healthy reproductive-age group.

Sziller et al. evaluated the gene polymorphism in MBL codon 54 in pregnancy, which is associated with low MBL production (4). They hypothesized that this polymorphism protected against preeclampsia; in contrast, van de Geijn et al. reported no association between the MBL genotypes and preeclampsia (5). In our study, we evaluated the serum MBL levels instead of genotypes, because gene expressions can vary. We found the mean serum MBL level significantly increased in severe preeclampsia group compared to other 2 groups, probably indicating the underlying excessive oxidative stress and inflammation.

The degree of involvement of MBL in the immunopathogenesis of preeclampsia and whether it is

involved in local or systemic inflammation remains unknown. Detection of MBL on the endothelium of spiral arteries can be considered as a proof of placental inflammation (20). In order to reveal its role in systemic inflammation, we have performed complete blood counts and assessed the parameters of haemostasis and of liver and renal functions in severe preeclampsia.

The increased hemoglobin level observed in the severe preeclampsia group was considered as a common finding caused by hemoconcentration. The higher mean leukocyte count and the lower mean platelet count in Groups 1 and 2 were consistent with physiological changes during pregnancy (21).

The serum urea and creatinine levels decrease in uncomplicated pregnancies due to increased glomerular filtration rate. However, in preeclampsia, the development of glomerular endotheliosis decreases the glomerular filtration rate; hence, the serum levels of urea, uric acid, and creatinine increase. Sibai et al. have shown that the serum uric acid level correlated with the severity of preeclampsia (22). The serum levels of urea, uric acid, and creatinine in our study were consistent with the findings reported in the literature. Although Group 2 subjects demonstrated significantly higher mean serum uric acid levels than Group 3 subjects, the levels in both groups were within physiological limits.

The increased mean levels of serum AST, ALT, and LDH were attributed to the oxidative changes reported to occur in preeclampsia (23-29). Earlier studies have demonstrated that increased trophoblast destruction at the maternal-fetal interface and endothelial cell injury can alter eicosanoid metabolism, thereby resulting in high levels of circulating lipid peroxide levels and oxidative stress, with excessive consumption of antioxidants. As the inflammatory reaction at the maternal-fetal interface increases, the activity of the complement system is believed to promote the removal of immune complexes and apoptotic cells (16). In such a situation, the levels of circulating markers of innate immunity, particularly that of MBL, is expected to increase, and reflect the severity of the ongoing immunological process. However; we were not able to find any significant correlation between the serum MBL levels and age, blood pressures, complete blood counts, haemostatic parameters, and liver and renal functions in the severe preeclampsia group.

High levels of MBL in uncomplicated pregnancy may be physiological because placenta formation with trophoblastic invasion of spiral arteries should be accompanied by inflammation to some extent. It is a physiological wound, with healing and remodeling lasting throughout the whole gestation. MBL deficiency may slow down the inflammatory reaction by decreasing the rate of complement activation, which is necessary for trophoblast invasion and attachment of the fetus to the uterus, and

hence may result in miscarriage. However, increase in MBL levels beyond physiological limits may increase the activity of the complement system, which can destroy the maternal-fetal interface and induce preeclampsia.

In conclusion, we have found significantly increased serum MBL levels in severe preeclampsia, which was not correlated with age, blood pressure, complete blood count, haemostatic parameters, and liver and renal functions. MBL might play a role in the immunopathogenesis of severe preeclampsia but it does not appear to be correlated with the severity of preeclampsia. Lack of any correlation between the serum MBL level and other parameters might be due to the small sizes of the experimental groups and a correlation would be overt in studies with larger groups. Because the data on serum levels and genotypes of MBL are conflicting and insufficient in the literature, further studies are required on MBL levels in complicated pregnancies.

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