Maternal serum mannose-binding lectin in severe preeclampsia

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Summary

Purpose: We aimed to investigate the level of serum mannose-binding lectin (MBL) in severe preeclamptic patients, women with uncomplicated pregnancies, and healthy reproductive-age females and its impact on gestational age at delivery and birth weight. *Methods:* Serum MBL levels were measured in 27 severe preeclampsia patients (Group 1), 27 patients with uncomplicated pregnancies (Group 2), and 25 healthy reproductive-age women (Group 3). *Results:* The mean serum MBL was significantly higher ($p \leq 0.05$) in Group 1 than in Groups 2 and 3, while the levels in Groups 2 and 3 did not significantly differ. The mean gestational age at delivery and mean birth weight were significantly lower in Group 1. In Group 1, serum MBL was negatively correlated ($p \leq 0.05$) with the gestational age at delivery and birth weight. *Conclusion:* Serum MBL increased in preeclampsia and was negatively correlated with the gestational age at birth and birth weight, indicating an underlying immunopathogenesis.

Key words: Mannose-binding lectin, Preeclampsia.

Introduction

In approximately 5% of cases, labor is complicated by preeclampsia – a disease that is unique to pregnancy and associated with maternal and fetal morbidity and mortality [1]. Preeclampsia is commonly manifested as hypertension and proteinuria in the second half of pregnancy [2]. Although its pathophysiology remains obscure, it is considered an inappropriate maternal immunological response against the fetal allograft [3, 4]. Traces of excessive maternal inflammation that cause defective trophoblast invasion and placentation can be seen both in the tissue and circulation [5-8]. Mannose-binding lectin (MBL) is one of the factors involved in the innate immune system [9]. MBL plays an important role against microorganisms by binding their carbohydrate moieties and activating the complement system via the lectin pathway [10, 11]. It is also a strong modulator of the complement-independent pathway and apoptosis [12].

Only a few studies evaluating the serum level of MBL and its genetic expression in some pregnancy-related complications have been reported in the literature [13-16]. Considering the proposed immunopathogenesis of preeclampsia and the importance of MBL in innate immunity, we investigated serum MBL levels in severe preeclamptic patients and compared them to the levels in patients with uncomplicated pregnancies and healthy reproductive-age females.

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Materials and Methods

Twenty seven severe preeclampsia patients (Group 1), 27 pregnant women without any complications (Group 2), and 25 healthy reproductive-age women (Group 3) were included in the study. All 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.

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 Uludag University Medical Faculty.

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

We centrifuged 5 ml venous blood from each patient at 3000 rpm, and the separated serum for MBL determination was stored at –20°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 mannose from Saccharomyces cerevisiae. The serum specimens were diluted in a calcium-containing buffer. Aliquots of calibrators and diluted serum samples were incubated in mannose-precoated microwells at room temperature for one hour. Functionally active MBL present in the solutions bound to the mannose-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 one hour. The detection antibody attached to the bound MBL oligomers via the carbohydrate-binding domains that were not occupied during binding to the mannose 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 one 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. The t-test was used in the comparison of two groups; and one-way analysis of variance, in comparison of more than two groups with normal distribution. The Mann-Whitney U test was used in the comparison of two groups, and the Kruskal-Wallis test in the comparison of more than two groups without normal distribution. The categorized data were analyzed using Pearson chi-square test and Fisher's exact test. Pearson's correlation coefficient was calculated, and correlation analysis was performed to determine any association between the variables. Statistical significance was defined at $p \leq 0.05$.

Table 1. — *Age, gravida, abortion, blood pressure and mannose-binding lectin levels of the groups (mean ± SD).*

	Group 1 (n = 27)	Group 2 (n = 27)	Group 3 (n = 25)
Age (years)	30.3 ± 1.2	29.5 ± 1.0	29.1 ± 1.0
Gravida	2.1 ± 0.4	2.3 ± 0.3	0.6 ± 0.2
Abortion	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.3
Systolic Blood Pressure (mmHg)	168.5 ± 2.5	114 ± 1.6	112.1 ± 1.2
Diastolic Blood Pressure (mmHg)	113.3 ± 1.3	74.2 ± 1.5	69.8 ± 1.3
MBL (ng/ml)	3112.6 ± 128.4	2457.3 ± 221.7	2052.8 ± 248.9

Table 2. — *Gestational ages at the time of serum sampling and at birth, routes of delivery, and gender of the newborns (mean ± SD).*

		Group 1 (n = 27)	Group 2 (n = 27)
Gestational age at serum sampling (days)		232.3 ± 5.1	223.5 ± 7.2
Gestational age at birth (das)		234.4 ± 4.4	273.6 ± 1.8
Birth weight (grams)		1695.7 ± 119.4	3370.3 ± 86.9
Route of delivery	Vaginal	7 (25.9%)	14 (53.8%)
	Cesarean section	20 (74.1%)	12 (46.2%)
Gender of newborn	Female	16 (59.3%)	15 (55.6%)
	Male	11 (40.7%)	12 (44.4%)

Results

As seen in Table 1, the demographic characteristics of the groups were comparable ($p > 0.05$) with regard to the mean ages and the number of abortions. The mean gravida was significantly ($p \leq 0.001$) lower in Group 3 than in Groups 1 and 2, while those in Groups 1 and 2 did not significantly differ ($p > 0.05$). As expected, the mean systolic and diastolic blood pressures in Group 1 were significantly ($p \leq 0.001$) higher than those in Groups 2 and 3, while the blood pressures in Groups 2 and 3 did not significantly differ ($p > 0.05$). The mean (± SD) Esbach value in the severe preeclampsia group was 3.1 ± 0.2 g/day. The mean serum MBL level in Group 1 was significantly higher ($p < 0.05$) than those in Groups 2 and 3, while the difference between the levels in the latter two groups was not significant ($p > 0.05$).

As seen in Table 2, the mean gestational ages at the time of serum sampling in Groups 1 and 2 were comparable ($p > 0.05$); in contrast, the mean gestational age at delivery and the mean birth weight were significantly ($p \leq 0.001$) lower in Group 1. The rate of cesarean section was significantly higher in Group 1 as opposed to that in Group 2 ($p < 0.05$), but the ratios of the gender of the newborns in these groups were comparable ($p > 0.05$) (Table 2). Acute fetal distress was the most frequent (78%) indication for cesarean section in the severe-preeclampsia group.

Serum MBL level was negatively correlated with the gestational age at delivery ($r = –0.57$, $p \leq 0.01$) and the birth weight ($r = –0.52$, $p \leq 0.01$) in Group 1. No correlation with age, gravida, proteinuria, systolic and diastolic blood pressures was seen in the groups ($p > 0.05$).

Discussion

Pregnancy is a unique situation in which a fetus with paternal alloantigens can survive in a uterus that is equipped with the maternal immune system. Trophoblasts invade the maternal spiral arteries and construct the feto-maternal interface by extending chorionic villi into sinuses formed by remodeled spiral arteries. The villi coated by syncytiotrophoblasts float freely within the maternal circulation for exchange transport of nutrients [1, 5]. This intrepid presentation of paternal antigens to the maternal immune system activates the complement system, and complement components physiologically accumulate in the placenta [17, 18]. This immune process is restrained by the development of immune tolerance, but the deposits of complement-activation products increase in a redundant manner in pregnancies complicated by preeclampsia [19, 20].

MBL is an important modulator of the innate immune system, and it participates in the activation of the complement system via the lectin pathway, in the modulation of inflammation, promotion of apoptosis, and removal of immune complexes and apoptotic cells [10, 21, 22]. MBL was detected on the endothelium of the spiral arteries [23]. Kilpatrick *et al.* [24] reported that MBL deficiency might be associated with recurrent miscarriages (RM). They found low levels of MBL in 16% of female partners and 14% of male partners among couples with RM compared to < 5% of controls. In 1999, Christiansen *et al.* confirmed the findings of this study [15]. They reported a highly significant ($p < 0.01$) correlation between the magnitude of exposure (frequency of MBL deficiency) and the severity of the disease (number of miscarriages)

and proposed that MBL deficiency in women and RM might be causally related. However, although the frequency of MBL deficiency was higher in both Danish and Scottish women with RM than in controls, the difference was only significant when the two groups were combined. In 1999, Kilpatrick *et al.* tested 218 females and 179 male partners among couples with RM and 376 blood donors as controls [16]. In this study, they suggested that only MBL values ≤ 0.1 mg/ml should be considered clinically significant risk factors for spontaneous abortion. In 2002, Kruse *et al.* detected a significantly increased prevalence of low MBL levels in women with RM, irrespective of the cutoff level for low MBL, whether 50 or 100 ng/ml [25]. They hypothesized that abnormalities in the classic complement pathway might result in impaired immune-complex elimination and that since MBL activates the complement pathway, its deficiency might predispose to RM. In our study, we did not aim to elucidate the association between serum MBL levels and abortion rates. However, the mean numbers of abortions in the groups were comparable, and no correlation was seen between the serum MBL levels and the number of miscarriages ($p > 0.05$).

In 2006, van de Geijn *et al.*, found that during an uncomplicated pregnancy, the serum MBL concentration increased to 140% compared to the baseline, which was defined as the concentration at six months postpartum [26]. This increase was present in the first trimester and did not significantly increase as the pregnancy advanced; on the contrary, it declined at six weeks postpartum. However, our findings were not in agreement with those of Geijn *et al.* Although the mean serum MBL level in women with uncomplicated pregnancies tended to be higher than that in healthy reproductive-age women, this difference did not reach statistical significance ($p > 0.05$). The study by Van de Geijn *et al.* was unique; however, a major bias in their study could be that the same patients served as the controls after delivery [26]. Further, they did not measure the patients' pregestational serum MBL levels. Instead, they compared the gestational levels with the postpartum levels assuming that the patients would completely recover by the 6th postpartum month and that these values could be accepted as the nongestational baseline values.

Few studies reported in the literature have investigated the MBL status in preeclampsia. MBL can be studied directly by measuring the actual serum level or indirectly by determining the relevant genotypes. Sziller *et al.* studied the MBL codon 54 gene polymorphism, which is compatible with low MBL production, and concluded that carriage of this polymorphism protected against preeclampsia [13]. They hypothesized that an MBL-mediated event might be involved in the pathogenesis of preeclampsia. In contrast to the findings of Sziller *et al.*, Van de Geijn *et al.* observed no association between the MBL genotypes and preeclampsia [14]. In our study, we measured the serum levels of MBL, but did not investigate the genotypes because they show varying expressions. In contrast to Sziller and Van de Geijn, we detected significantly higher mean serum MBL levels in the severe preeclamptic patients compared to the other two groups, maybe as a reflection of increased oxidative stress and inflammation ($p < 0.05$). Because there is not so much literature about serum MBL levels in uncomplicated gestation and preeclampsia, we measured serum levels in all three groups of patients. We found some increase in serum MBL levels during pregnancy but that did not reach significancy when compared to the healthy reproductive-age group; hence the significant increase in the severe preeclampsia group was attributed to the toxemia itself.

Deficient maternal MBL concentration has also been described as a risk factor for preterm birth and reduced birth weight [27]. Kruse *et al.* reported that the median birth weight tended to be lower among women with MBL levels of 100 ng/ml than among women with normal MBL levels; however, the difference was not significant [25]. Furthermore, when they excluded the preterm births, they observed that the birth weights of the term infants born to women with MBL levels ≤ 100 ng/ml were significantly lower as compared to those of infants born to women with normal MBL levels. On the contrary, in a recently published report, van de Giejn *et al.*, investigated whether MBL polymorphisms play a role in preterm birth [28]. Serum MBL levels are in close association with single nucleotide polymorphisms in the structural gene. Individuals with the AA wildtype genotype have the highest MBL serum concentrations, and individuals with the OO genotype, which represent the variant alleles B, C and D, the lowest [29]. Van de Geijn *et al.*, have found that the high MBL production genotype group A was associated with shorter gestational age at delivery (274 days) compared to the intermediate MBL production genotype group B (283 days) and the low MBL production group genotype C (284 days). High MBL genotype group A constituted 86% of the preterm births, however there was no significant difference in gestational ages among the groups when the analysis was restricted to the women that gave birth at term. In our study, the mean gestational age at delivery and the mean birth weight were significantly ($p \leq 0.001$) lower in the severe-preeclampsia group compared to that in the uncomplicated-pregnancy group. The finding that severe preeclampsia is associated with increased rate of preterm deliveries was not surprising [30]. The important finding, in our study, was the presence of negative correlations between the serum MBL level and gestational age at delivery ($r = –0.57$, $p \leq 0.01$) and birth weight ($r = –0.52$, $p \leq 0.01$) in the severe-preeclampsia group, which may indicate an underlying immune pathology.

Some authors have reported that decreased MBL levels are harmful to pregnant women, while others have reported contradictory findings; perhaps, the truth lies somewhere inbetween [14, 25]. High levels of MBL in normal pregnancy may indicate a physiological role acquired during evolution. Placentation is a complex process involving trophoblast invasion and spiral artery remodeling. It is a physiologic wound, and healing and

remodeling are prolonged, probably lasting throughout gestation. During this process, new tissue formation and remodeling occur repeatedly. MBL may play a dual role during the entire process. Its deficiency may decrease inflammatory reaction by decreasing the rate of complement activation, which is necessary for trophoblast invasion and attachment of the fetus to the uterus, and may result in miscarriage. Defects in the removal of inflammatory debris may contribute to the immunopathogenesis. However, increased MBL levels beyond physiological limits may increase the activity of the complement system that can destroy the maternal-fetal interface and induce preeclampsia.

In conclusion, we found that the serum MBL level was increased in preeclampsia, and that it negatively correlated with the gestational age at birth and the birth weight, indicating an underlying immunopathogenesis. However, it should be remembered that the serum MBL level is influenced by any inflammatory event such as trauma and infection. Because the knowledge regarding the association between MBL and preeclampsia reported in the literature is incomplete, further studies involving larger groups of patients are required.

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