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A role for mannose-binding lectin, a component of the innate immune system in preeclampsia

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Abstract

Problem—Mannose-binding lectin (MBL) is a pattern-recognition receptor that activates complement and modulates inflammation. Homozygosity for the most common allele of the *MBL2* gene associated with high MBL serum concentrations is more prevalent in patients with preeclampsia. The objective of this study was to determine maternal plasma MBL concentrations in normal pregnant women and patients with pre-eclampsia.

Method of study—This cross-sectional study included normal pregnant women (n=187) and patients with preeclampsia (n=99). Maternal plasma MBL concentrations were determined by ELISA.

Results—Women with preeclampsia had higher median maternal plasma MBL concentration than normal pregnant women. MBL concentration distribution curves were three-modal, the subintervals in normal pregnancy were low (<143.7), intermediate (143.7–1898.9) and high (>1898.9ng/ml). The proportion of normal pregnant women was larger in the low subinterval, while the proportion of patients with preeclampsia was larger in the high subinterval (p=0.02). Normal pregnant women in the high subinterval had a larger rate of placental underperfusion than those in the low and intermediate subintervals (P = 0.02).

Conclusions—Median maternal plasma MBL concentration is elevated in patients with preeclampsia and a larger proportion of these patients is in the high subinterval than normal pregnant women, suggesting that this innate immune system component is involved in the mechanisms of disease in preeclampsia.

Keywords

innate immune system; MBL genotypes; pattern-recognition receptor; single nucleotide polymorphism; maternal systemic inflammation

INTRODUCTION

During normal pregnancy, the maternal immune system undergoes specific adaptations (e.g. white blood cell count elevation,¹ complement activation^{2,3}) in order to maintain host defense against infections and to tolerate a semi-allograft.^{3–14} In contrast to the mild intravascular systemic inflammation observed in normal pregnancies, preeclampsia has been proposed to be characterized by highly exaggerated intravascular inflammation^{3,4,14–21} and activation of the innate immune system.^{22–25}

Mannose-binding lectin (MBL) is a pattern-recognition receptor of the innate immune defense that is produced by the liver.^{26–28} MBL has the ability to bind to certain carbohydrate ligands on microorganisms and altered-self structures, and activate the lectin pathway of the complement pathway through MBL-associated serine proteases (MASPs).^{26–29} MBL can also promote complement-independent opsonophagocytosis, apoptosis, and modulate inflammatory processes.²⁷

The *MBL2* gene contains single-nucleotide polymorphisms (SNPs) in its promoter and coding regions, and serum MBL concentrations have large inter-individual differences depending on these genotypes.^{30–32} Non-pregnant individuals with the most common alleles have high serum MBL concentrations (>1000 ng/ml), whereas individuals with variant alleles have intermediate (100–1000 ng/ml) or low/deficient (<100 ng/ml) concentrations.^{28,33} MBL deficiency has been reported to increase the susceptibility to microbial infections.^{27,34} In fact, maternal *MBL2* gene codon 54 polymorphism that results in low serum MBL concentrations has been associated with higher susceptibility to histological chorioamnionitis³⁵ and preterm birth at <29 weeks of gestation.³⁶

In contrast, MBL has an active pro-inflammatory role in chronic diseases and high serum MBL concentrations predispose to increased hypoxia/reperfusion injury and microvascular complications in diabetes.^{37–47} Of importance, a recent study reported that homozygosity for the common allele that is associated with high serum MBL concentrations was more frequent in women with preeclampsia and HELLP syndrome than in normal pregnant women, suggesting that an MBL-mediated event might be involved in the pathogenesis of this syndrome.⁴⁸

The aims of this study were: 1) to determine the maternal plasma MBL concentrations in normal pregnant women and in patients with preeclampsia; 2) to compare the distribution of women with high, intermediate and low MBL concentrations; and 3) to correlate maternal plasma MBL concentrations with histopathological findings in the placenta.

MATERIAL AND METHODS

Study design and population

This cross-sectional study included pregnant women with preeclampsia (n=99) and normal pregnancies (n=187). Patients with multiple pregnancies, stillbirth, and fetal congenital or chromosomal abnormalities were excluded. Samples and data were retrieved from the bank of biological samples and clinical databases of the Perinatology Research Branch. All women provided written informed consent prior to the collection of samples. The utilization of samples for research purposes was approved by the Institutional Review Boards of both Wayne State University and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NIH/DHHS). Many of the samples used in this study had previously been utilized to study the biology of inflammation and angiogenesis in normal pregnant women and women with preeclampsia.

Definitions

Women were considered to have a normal pregnancy if they met the following criteria: 1) no medical, surgical, or obstetrical complications and 2) delivery of a normal term (≥ 37 weeks) infant whose birthweight was between the 10th and 90th percentiles for gestational age.⁴⁹ Preeclampsia was defined as hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, measured at two different time points 4 hours to 1 week apart) developing after 20 weeks of gestation and proteinuria (≥ 300 mg in a 24 hour urine collection, or two random urine specimens obtained 4 hours to 1 week apart containing $\geq 1+$ protein by dipstick or one dipstick measurement with $\geq 2+$ protein).^{50–52} Severe preeclampsia was defined as systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg and/or proteinuria greater than 5 g in a 24 hour collection or $>3+$ on one dipstick measurement.⁵³ Severe preeclampsia was also diagnosed in the presence of oliguria, pulmonary edema, seizures, thrombocytopenia, abnormal liver enzymes associated with persistent epigastric or right upper-quadrant pain, or persistent and severe central nervous system symptoms).⁵³ Small for gestational age (SGA) neonate was defined as birthweight below the 10th percentile for gestational age at birth.⁴⁹

MBL oligomer immunoassay

Concentrations of oligomerized MBL in maternal plasma were determined by the use of specific and sensitive enzyme-linked immunoassays purchased from Antibody-Shop A/S (Gentofte, Denmark). Maternal plasma samples and standards were incubated in duplicate wells of the microtiter plates pre-coated with monoclonal antibodies specific for the carbohydrate recognition domain (CRD) of MBL. During this incubation MBL present in maternal plasma or standards bound to the antibody-coated wells through its CRDs. After repeated washing and aspiration to remove all unbound substances, a biotinylated monoclonal detection antibody specific for oligomerized MBL was added to each well of the assay plates. Following incubation, unbound detection antibody was removed by repeated washing, and the assay plates were incubated with horse-radish peroxidase-conjugated streptavidin solution and allowed to form complexes with the bound biotinylated antibody. Unbound conjugate was removed by repeated washing, and a chromogenic peroxidase substrate containing tetramethyl-benzidine was added to each well of the assay plates. Color developed in proportion to the amount of MBL bound in the initial step. The color development was stopped with the addition of an acid solution, and the color intensity was read using a programmable spectrophotometer (Ceres 900 Micro-plate Workstation, Bio-Tek Instruments, Winooski, VT, USA). The concentrations of oligomerized MBL in maternal plasma samples were determined by interpolation from individual standard curves composed of human MBL. The calculated inter- and intra-assay coefficients of variation for MBL oligomer immunoassays in our laboratory were 5.5% and 5.7%, respectively. The lower limit of detection (sensitivity) was calculated to be 0.11 ng/ml.

Placental histopathological examinations

Placental tissue samples were taken by systematic random sampling, and subsequently fixed in 10% neutral buffered formalin overnight, and embedded in paraffin. Five μ m-thick paraffin sections were stained with haematoxylin and eosin, and examined using bright-field light microscopy. Histopathological examinations were performed by three pathologists who were blinded to the clinical information based on the diagnostic criteria previously described.⁵⁴

Statistical analysis

The Kolmogorov–Smirnov test was used to determine whether the data was normally distributed. The Spearman's rho test was employed to determine the relationship between

maternal plasma MBL concentrations and gestational age at blood draw. Comparisons between groups were performed using Chi-Square test for categorical and Mann-Whitney test for continuous variables.

To characterize the distribution of maternal plasma MBL concentrations in the study groups, MBL concentration values were $\log_{10}$ transformed to improve normality of the data distribution. Data mass densities were estimated using the “*density*” method⁵⁵ implemented in the R statistical software.⁵⁶ These resulting mass density curves are similar to typical histograms, except that the distributions are continuous and smooth, and the areas under the curves are 1; the density values represent the probability of observing the concentration given at the X axis. The cut-offs for the MBL concentration subintervals were empirically determined by using the concentration values corresponding to the two minimums on the normal pregnancy distribution curve. The number of patients and their placental histopathological findings were compared between the two groups in all three concentration subintervals. Multiple logistic regression analysis was performed to reveal the association between maternal age, nulliparity, smoking, placental histopathological findings, maternal plasma MBL concentrations, and preeclampsia.

The R statistical software (www.r-project.org) and SPSS version 12.0 (SPSS Inc., Chicago, IL) were used for the analyses. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Demographic data

Table I displays the demographic and clinical characteristics of the study groups. The median birthweight and gestational age at delivery were significantly higher and the proportion of nulliparous women was significantly lower in normal pregnant women than in patients with preeclampsia.

Maternal plasma MBL concentrations in the study groups

1) Maternal plasma MBL concentrations did not change with advancing gestational age in normal pregnancy ($R^2=0.0008$, $P=0.7$). 2) Patients with preeclampsia had a significantly higher median maternal plasma MBL concentration [1128 ng/ml (range: 13.6–11870)] than normal pregnant women [886.3 ng/ml (range: 6.1–7805.1), $p<0.01$] (Figure 1). 3) Women with severe preeclampsia ($n=88$) had a higher median maternal plasma MBL concentration [1294.9 ng/ml (range: 13.6–11870.0)] than women with mild preeclampsia ($n=11$) [525.7 ng/ml (range: 15.8–4684.5)], although this difference was not statistically significant ($p=0.08$).

Characteristics of the MBL concentration distributions in the study groups

1) The $\log_{10}$ MBL concentrations showed a common three-modal distribution with three typical subintervals in both study groups (Figure 2). 2) The normal pregnant cut-offs between the concentration subintervals were empirically determined by using the concentration values corresponding to the two minimums (143.7 ng/ml and 1898.9 ng/ml) on the normal pregnancy distribution curve (Figure 2). 3) The proportion of patients in the low, intermediate and high concentration subintervals (0–143.7; 143.7–1898.9; and >1898.9 ng/ml) were significantly different between the two study groups. A smaller proportion of women with preeclampsia had low plasma MBL concentrations and a larger proportion of these patients had high MBL concentrations than women in the normal pregnancy group (11.1 % vs. 24.1 % and 42.4 % vs. 31.5 %, respectively) (Table II).

Correlation of placental histopathological findings with maternal plasma MBL concentrations

Placental histopathological findings consistent with maternal underperfusion (composite of remote and recent villous infarcts, increased syncytial knots, villous agglutination, increased intervillous fibrin, distal villous hypoplasia, persistent muscularization of basal plate arteries, mural hypertrophy of decidual arterioles, and acute atherosclerosis of basal plate arteries/decidual arterioles) were more frequent in normal pregnant women with high MBL concentrations (>1898.9 ng/ml) than in those with low and intermediate ($0-1898.9$ ng/ml) concentrations ($p=0.02$) (Table III). Similarly, placental histopathological findings consistent with maternal underperfusion were more frequent in patients with preeclampsia than in normal pregnant women in all concentration subintervals (low: $p=0.009$, intermediate: $p<0.001$ and high: $p=0.02$) (Table IV). Multiple logistic regression analysis revealed that maternal underperfusion and nulliparity had strong positive associations, maternal plasma MBL concentration had a weak but significant association, while smoking and maternal age had no association with preeclampsia (Table V).

DISCUSSION

Principal findings of this study

1) Women with preeclampsia had a significantly higher median maternal plasma MBL concentration than normal pregnant women; 2) a larger proportion of women with preeclampsia had high MBL concentrations than women in the normal pregnancy group; 3) the rate of placental histopathological findings consistent with maternal underperfusion was greater in normal pregnant women with high MBL concentrations than in those with low and intermediate MBL concentrations; 4) placental underperfusion was more frequent in patients with preeclampsia than in normal pregnant women in all concentration subintervals; and 5) multiple logistic regression analysis revealed that nulliparity, maternal plasma MBL concentrations and maternal underperfusion were independently associated with preeclampsia.

Structural and functional characteristics of MBL

Mannose binding lectin is an innate pattern recognition receptor and acute phase reactant secreted into the circulation by the liver⁵⁷⁻⁵⁹ that is involved in the first-line of host-defense against microorganisms.^{27:28:59:60} MBL is comprised of 25 kDa subunits, which form collagenous triple helices that are further oligomerized to 150-450 kDa bouquet-like higher-order structures.^{28:59:61-63} These higher oligomeric forms interact with carbohydrate arrays through several CRDs, thus, with increased avidity and strengthened binding.⁶¹⁻⁶³

MBL binds to a wide variety of ligands on microbial (e.g. bacterial, fungal, protozoan, viral and yeast) and on altered-self (e.g. upon ischemia-reperfusion) structures.^{27:28:59} Ligand binding results in the activation of MBL associated serine proteases (MASPs).^{27-29:59:62:64-66} Active MASPs specifically cleave complement factors in an antibody and C1-independent manner, which leads to the activation of the complement cascade.^{27:34:65:67:68} MBL can also promote opsonophagocytosis in the initial phase of the immune response to pathogens,^{27:67:68} phagocytotic uptake of apoptotic cells,^{27:68} modulation of the inflammatory response,^{27:68-72} and the cleavage of coagulation factor XIII and fibrinogen.^{27:68:73}

The *MBL2* gene promoter contains heat-shock consensus sequences and glucocorticoid-responsive elements that may be responsible for increased serum MBL concentrations upon acute phase reactions.^{58:74:75} On the other hand, certain SNPs in the *MBL2* gene promoter

are associated with decreased gene expression and low or deficient serum MBL concentrations.^{27:30:59:76:77} Single nucleotide polymorphisms in exon 1 at codons 52 (Arg→Cys), 54 (Gly→Asp) and 57 (Gly→Glu), referred to as variants D, B and C, respectively, lead to the synthesis of structurally impaired, functionally deficient MBL that have low plasma half life.^{27:28:30:59:61-63:76:78:79}

Approximately half of the population is homozygous for the wild-type allele (variant A) and has high serum MBL concentrations (>1000 ng/ml).^{28:32} Heterozygotes for variant B, C and D alleles have intermediate serum MBL concentrations (100–1000 ng/ml), while approximately 10% of the population is homozygote or compound heterozygote for the variant alleles, and by definition, MBL deficient (<100 ng/ml).^{28:32:34:59} Serum MBL concentrations further vary in each coding genotype depending on the promoter polymorphisms closely linked to the coding SNPs.^{27:30:32:59:76:77}

MBL deficiency

MBL deficiency is one of the most common human immune deficiencies, as MBL variant B occurs in 22–28% of Eurasian populations, variant C in 50–60% of sub-Saharan African populations, and variant D in 14% of European populations.^{27:30:31:76} It has been suggested that variant alleles and low MBL concentrations might confer protection for the hosts,^{27:28:48:59:63:78:80:81} and that MBL polymorphisms may have been maintained in advantage for heterozygous individuals by heterosis in analogy with sickle cell trait.^{80:81} According to the two main hypotheses, positive selective forces might have acted on MBL variant alleles, because 1) MBL promotes the uptake of intracellular parasites (e.g. Mycobacteria, Leishmania) and MBL deficiency was suggested to be protective against these infections;^{30:30:31:63:76:80-82} and 2) MBL leads to complement activation, subsequent release of inflammatory mediators and tissue damage,^{81:82} and low serum MBL concentrations may be beneficial for reducing these harmful consequences.

Pro-inflammatory role of MBL

The lectin pathway has an important role in initiating the activation of the complement after hypoxia–reoxygenation in human endothelial cells³⁷ and in rat myocardium.³⁸ The inhibition of the lectin pathway in a rat myocardial reperfusion model,³⁸ as well as MBL deficiency in knockout mice protected against reperfusion injuries of the gut, kidney and heart.⁴¹⁻⁴³ MBL is also involved in solid-organ transplantation-related ischemia/reperfusion injuries, as MBL depositions were observed in injured kidney transplants,³⁹ and a high pre-transplant serum MBL concentration turned to be independent risk factor for death-censored graft loss and was associated with more severe form of rejection leading to decreased renal allograft survival.⁴⁴ Serum MBL concentrations were also significantly higher in new-onset type 1 diabetes patients than in healthy controls; thus, MBL was hypothesized to be involved in the pathogenesis of the disease.^{63:82:83} High-MBL producing common alleles and high MBL serum concentrations were also implicated in diabetic microvascular complications, nephropathy and retinopathy.^{40:45-47:83}

MBL in normal pregnancy

This is the first study to determine maternal plasma MBL concentrations in a population of pregnant women that is mainly of African-American origin. Herein, plasma MBL concentrations outlined a three-modal distribution curve in both groups of pregnant women either with normal pregnancy or with preeclampsia. These findings are consistent with previous reports that described three MBL concentration ranges for non-pregnant individuals, which were closely associated with their genotypes.^{28:30:31:47:59} As genotyping was not available in this study, we used a statistical method to define empirically the normal pregnancy cut-offs between the concentration subintervals in our study

population. These concentration cut-off values (143.7 and 1898.9 ng/ml) were higher than the previously published cut-offs (100 ng/ml and 1000 ng/ml) for Caucasian non-pregnant individuals.²⁸ This can either be the result of the difference between: 1) the study populations; 2) the measurable concentrations of MBL in serum and in plasma; 3) the methods of the calculations; and 4) the pregnant and non-pregnant state.

Indeed, a recent longitudinal study reported increased maternal serum MBL concentrations throughout pregnancy.⁸⁴ Maternal serum MBL concentrations increased before 12 weeks of gestation up to 140% of the non-pregnant basal concentration measured 6 months post-partum, and were consistently higher during pregnancy.⁸⁴ This is in accord with our findings that maternal plasma MBL concentrations did not change significantly as a function of gestational age in the second half of the pregnancy. Importantly, the same study⁸⁴ revealed that parallel with the changes in serum MBL concentrations, similar findings were also observed in the serum MBL–MASP complex activity (172% increase in pregnancy) and MBL pathway activity (164% increase in pregnancy), with a very strong correlation between those three variables at all time points ($R>0.93$, $p<0.01$).⁸⁴

Another study³ has demonstrated that normal pregnancy is associated with the activation of the complement system as determined by increased maternal plasma C3a, C4a, and C5a concentrations,³ which did not change along gestation.³ Since the plasma concentration of C3a had only a weak positive correlation with C4a and C5a concentrations, it was hypothesized that not all C3a was generated after C4, and that other than the classical pathway of the complement might be involved in its activation.³

The results of these studies suggest that increased complement activation through the lectin pathway is part of the mild systemic inflammation observed in normal pregnancies, it may have essential functions in protecting the mother against infections, and this phenomenon may also reflect an additional mechanism in the shift from the adaptive to innate immunity during normal pregnancy.

MBL in pregnancy complications

Maternal gene polymorphism in codon 54 (variant B) that results in low MBL serum concentrations has been associated with higher susceptibility for preterm birth at <29 weeks of gestation,³⁶ and was also found to be associated with a higher frequency of histological chorioamnionitis in cases of preterm birth at <35 weeks of gestation.³⁵ Codon 52 (variant D) polymorphism was over-represented in preterm infants born before 36 weeks of gestation than in infants born at term.⁸⁵ Several studies have also revealed that low serum and plasma MBL concentrations were associated with increased risk for unexplained recurrent miscarriages.^{86–89} Thus, high maternal serum MBL concentrations were suggested to be beneficial for pregnancy outcome.⁸⁴

MBL in preeclampsia

This study has demonstrated that women with preeclampsia had a higher median maternal plasma MBL concentration than normal pregnant women, and high maternal plasma MBL concentrations were independently associated with preeclampsia. In addition, a larger proportion of women with preeclampsia fell into the high MBL concentration subinterval than normal pregnant women, as well as a larger proportion of normal pregnant women had deficient plasma MBL concentrations than women with preeclampsia.

The association between MBL genotypes and the risk for developing preeclampsia is under debate. Sziller et al.⁴⁸ presented that homozygosity for the common A allele in codon 54, known to be associated with high serum MBL concentrations, was more frequent in women with preeclampsia (OR: 2.5; 95% CI: 1.02–6.6) and HELLP syndrome (OR: 2.1; 95% CI:

1.08–4.14) than in normal pregnant women. Moreover, the carriage of codon 54 polymorphism (variant B allele), responsible for low serum MBL concentrations had a protective effect against the development of preeclampsia and HELLP syndrome.⁴⁸ In contrast, van de Geijn et al. did not find association between three structural and two promoter polymorphisms in the *MBL* gene and preeclampsia.⁹⁰ However, these authors reported that low MBL production genotypes might be considered as disease modifiers among women with severe preeclampsia and eclampsia.⁹⁰ Both of these studies included only Caucasian women.

Measuring plasma MBL concentrations, Wang et al using an own developed immunoassay did not find significant difference between patients with preeclampsia and those with normal pregnancy.²⁵ The inconsistency between the two studies could either be the consequence of the differences in the ethnicity of the study populations, severity of the disease, or the immunoassays used or the sample size. Of note, in spite of the different ethnicities, the proportion of primiparous patients and the association between MBL expressors and preeclampsia in our study concurs with those of Sziller et al.⁴⁸ Moreover, Kilpatrick et al. found a higher median serum MBL concentration among primiparous women with preeclampsia and their husbands than in healthy blood donors,⁹¹ further validating the results of Sziller et al. and ours.

The question of how high maternal blood MBL concentrations may confer a risk for preeclampsia has not been answered. It has been suggested that maternal complement activation by MBL may contribute to the destruction of paternal antigen expressing trophoblasts at the maternal–fetal interfaces.^{23;48;92;93} Indeed, previous studies revealed increased complement split product deposition in the villous stroma^{22;23;93} and at the trophoblast basal membrane^{22;93} in preeclampsia. Concerning this hypothesis,^{48;93} the excessive damage of the invading trophoblasts would increase the possibility of insufficient trophoblast invasion of spiral arteries, subsequent placental hypoxia and the development of symptoms characteristics of preeclampsia.^{48;93} Accordingly, maternal carriage of the *MBL2* gene variant B allele, responsible for decreased serum MBL concentrations, was suggested to result in a decreased capacity for complement-dependant trophoblast lysis.⁴⁸

Previous studies on patients with preeclampsia found increased complement split products in the maternal circulation,⁹⁴ enhanced placental deposition of complement components in the walls of uterine spiral arteries,^{95;96} fetal stem vessels,^{22;23;93} as well as in kidney glomerular capillary walls.⁹⁷ Although complement component depositions were also found in normal term and preterm placentas^{92;98} and in uteroplacental arteries in normal pregnancies,⁹⁹ these were consistently milder than in placentas taken from patients with preeclampsia. These results may suggest a similar role for MBL in these earlier published pathophysiological changes in preeclampsia as in the microvascular complications in diabetes, in hypoxia/reperfusion injury or in kidney transplant rejection.^{37–47}

The study reported herein revealed that placental histopathological findings consistent with maternal underperfusion were more frequent in normal pregnant women with high MBL concentrations, which may propose that high circulating MBL concentrations augment some initial changes in placental maternal microvasculature. As histopathological findings consistent with maternal underperfusion were more frequent in placentas of patients with preeclampsia than in normal pregnant women, independent from MBL concentration subintervals, high circulating MBL concentrations do not seem to be closely related to the development of more frequent and severe microvascular damage detected in preeclampsia.

Although the exact pathophysiological role of MBL in preeclampsia still has to be resolved, it is tempting to speculate that the higher concentrations of MBL and complement split

products in the maternal circulation predominantly reflect exaggerated intravascular inflammation^{3,4,14–21} and the activation of the innate immune system^{22–25} in preeclampsia.

Limitations of this study

The *MBL2* gene polymorphisms were not examined in this study; thus, our observations were solely based on the analysis of maternal plasma MBL concentrations. Placental histopathological findings were accessible for 129 women with normal pregnancy and 98 with preeclampsia.

Conclusions

The median maternal plasma MBL concentration is higher in women with preeclampsia than in normal pregnant women, and the distribution of patients in the MBL concentration subintervals is different between the study groups, with a higher proportion of patients with preeclampsia in the high concentration subinterval. These results suggest that this component of the innate immune system is involved in the mechanisms of disease in preeclampsia.

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Figure 1. Maternal plasma MBL concentrations in the study groups

Women with preeclampsia had significantly higher maternal plasma MBL concentrations [median: 1128 ng/ml (range: 13.6–11870.0)] than normal pregnant women [median: 886.3 ng/ml (range: 6.1–7805.1), $p < 0.01$].

Figure 2. Data mass densities and ranges of maternal plasma MBL concentrations in the study groups. (A) The log₁₀ MBL concentrations showed a common three-modal distribution with three typical subintervals in both groups. The area under both density curves is 1; the density values represent the probability of observing the concentration given at the X axis. **(A–B)** The cut-offs between the concentration subintervals were empirically determined by using the concentration values (143.7 ng/ml and 1898.9 ng/ml) corresponding to the two local minimums on the normal pregnancy group distribution curve. **(B)** Concentration values are represented in ng/ml.

Table I

Demographic and clinical characteristics of the study population

	Normal pregnancy (n=187)	Preeclampsia (n=99)	p value
Maternal age (years) ¹	25 [21–29]	23 [20–29]	NS
Nulliparity ²	50 (26.7)	59 (59.6)	<0.001
African-American origin ²	151 (80.7)	80 (80.8)	NS
Smoking ^{2, 3}	38 (22.1)	13 (13.5)	NS
Gestational age at delivery (weeks) ¹	39.6 [38.9–40.4]	36.0 [32.3–38.6]	<0.001
Birth-weight (g) ¹	3345 [3100–3600]	2200 [1560–2800]	<0.001
Cesarean section ²	76 (40.6)	52 (52.5)	NS

Values are presented as median [range] or number (percent).

Comparisons were performed among all groups with Chi-Square test² and Mann-Whitney tests.¹

³Normal pregnancy (n=172); preeclampsia (n=96)

NS: Statistically not significant.

Table II

Number of patients in the MBL concentration subintervals

Concentration subinterval (ng/ml)	Normal pregnancy (n=187)	Preeclampsia (n=99)
0–143.7	45 (24.1)	11 (11.1)
143.7–1898.9	83 (44.4)	46 (46.5)
>1898.9	59 (31.5)	42 (42.4)

Values are represented as numbers (percentages).

The proportion of patients in the concentration subintervals in the two study groups was significantly different (Pearson Chi-Square test: $p=0.02$).

Table III

Placental Lesions According to MBL Concentration Subintervals in Normal Pregnant Women

Concentration subinterval (ng/ml)	Maternal underperfusion (n=17)	No maternal underperfusion (n=112)
0-1898.9	8 (47.1)	84 (75)
>1898.9	9 (52.9)	28 (25)

Values are represented as numbers (percentages).

The proportion of patients in the concentration subintervals was significantly different (Pearson Chi-Square test: $p=0.02$).

Table IV

Placental Lesions According to Study Groups and MBL Concentration Subintervals

Concentration subinterval (ng/ml)	Normal pregnancy (n=129)	Preeclampsia (n=98)	p value
0–143.7	2 (1.1)	5 (5.1)	0.009
143.7–1898.9	6 (3.2)	29 (29.3)	< 0.0001
>1898.9	9 (4.8)	21 (21.2)	0.020

Values are represented as numbers (percentages).

Table V

Multiple logistic regression analysis of the association of maternal age, nulliparity, smoking, plasma MBL concentrations, placental maternal underperfusion and preeclampsia

Factor	OR [95% CI]	p value
Maternal age	1.025 [0.962–1.092]	NS
Nulliparity	5.626 [2.620–12.081]	< 0.001
Smoking	0.485 [0.205–1.148]	NS
Plasma MBL concentrations	1.00017 [1.00001–1.0003]	0.037
Maternal underperfusion	6.334 [3.07–13.07]	< 0.001

NS: Statistically not significant.