Mannose-binding lectin gene polymorphism and its effect on short term outcomes in preterm infants

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Abstract

Objective: Mannose-binding lectin, which belongs to the collectin family, is an acute-phase reactant that activates the complement system. This study aimed to investigate the effect of MBL2 gene polymorphism on short-term outcomes in preterm infants.

Method: Infants of <37 gestational weeks who were admitted to the neonatal intensive care unit during a two-year period were enrolled in this prospective study. The neonates were categorized into two groups according to their MBL2 genotypes. Normal MBL2 genotype was defined as MBL2 wild-type (AA genotype), whereas mutant MBL2 genotype was defined as MBL2 variant-type (AO/00 genotype). The relationship between MBL2 genotype and short-term morbidity and mortality was evaluated.

Results: During the two-year study period, 116 preterm infants were enrolled in this study. In MBL2 variant-type, mannose-binding lectin levels were significantly lower and incidences of mannose-binding lectin deficiency (MBL level <700 ng/mL) were higher (p <0.001). In this group, the prevalence of respiratory distress syndrome and mortality was significantly higher (p <0.001, p >0.03 respectively). In the MBL2 wild-type group, the prevalence of necrotizing enterocolitis (NEC) was higher (p=0.01). Logistic regression analyses revealed that MBL2 variant-type had a significant effect on respiratory distress syndrome development (odds ratio, 5.1; 95% confidence interval, 2.2–11.9; p <0.001).

Conclusions: MBL2 variant-type and mannose-binding lectin deficiency are important risk factors for respiratory distress syndrome development in preterm infants. Additionally, there is an association between MBL2 wild-type and NEC. Further studies on this subject are needed.

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Keywords

Mannose-binding lectin; Preterm; Respiratory distress syndrome

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Introduction

Mannose-binding lectin (MBL) is an acute-phase reactant that activates the complement system. It belongs to the collectin family of proteins, which includes lung surfactant protein A (SP-A) and SP-D. MBL plays a key role in first-line immune responses as a component of innate immunity. Because adaptive immunity is underdeveloped in preterm infants, innate immunity gains higher importance. The MBL2 gene is located on the long arm of chromosome 10, and mutant MBL2 alleles occur as a result of three single-point mutations in this gene (B, C, and D). Although functional MBL levels are low in heterozygous polymorphisms, MBL levels in homozygous polymorphisms are so low that they may not even be determinable. MBL activates the complement system by binding to mannos or sugar motifs, which are found in many microorganisms, and plays an important role in innate immunity and inflammation. In newborns, an increase in sepsis frequency is observed when MBL levels are low.

The mortality and morbidity rates in preterm infants are higher than those in term infants. As gestational week and birth weight decrease, the risk of complications increases. In preterm infants, complications are observed in the early (neonatal period) and late periods (after discharge). Although the survival rate of most preterm infants has improved because of advances in medical care, the incidence of short-term complications remains relatively stable. Short-term complications increase the risk of long-term sequelae.

In recent years, many studies have been conducted on the importance of MBL during the neonatal period, and most of these are associated with sepsis. In sepsis, proinflammatory and anti-inflammatory cytokine ratio is vital in terms of defense against infectious agents. The imbalance in this ratio is manifested by increased morbidity and mortality during the neonatal period. Increased cytokine levels have a significant role in the pathophysiology of morbidities such as respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BPD), and retinopathy of prematurity (ROP). This prospective study aimed to investigate the association of MBL2 polymorphism with short-term outcomes in preterm infants.

Materials and methods

All preterm infants of <37 gestational weeks who were admitted to the neonatal intensive care unit (NICU) of the Uludag University Medical School during a two-year period were enrolled in this prospective study. The neonates were categorized into two groups according to their MBL2
Neonatal ROP, IVH immediately ples levels of deficiency.

After these changes, blood cultures were analyzed using the fully automated BACTEC method in a BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany). LOS was determined by the time at which sepsis occurred between 4 and 30 days after birth.13 IVH was evaluated by cranial ultrasound examinations, which were performed by the same pediatric radiologist and diagnosed using the Papile classification system.14 NEC was diagnosed according to clinical and radiographic findings and classified according to modified Bell’s criteria.15 BPD was classified into three groups in terms of BPD severity depending on the duration and level of supplemental oxygen and mechanical ventilatory support at 36 weeks postmenstrual age.16 ROP was classified according to the International Classification of Retinopathy of Prematurity.17

The MBL levels and gene polymorphisms were assessed within 3 h in most infants and within 24 h after birth in all infants. The blood samples for the measurement of MBL levels were collected in a test tube and these blood samples were centrifuged within thirty minutes after obtaining. After the centrifugation process, serum of the samples were immediately stored at −80 °C until analysis.

Blood samples were analyzed using enzyme-linked immunosorbent assay. PCR and restriction fragment length polymorphism were used for MBL2 genotyping. Serum MBL levels were measured using an immunoassay kit (Oligomer ELISA kit; Antibody Shop, Copenhagen, Denmark) according to the manufacturer instructions. The lowest detectable MBL concentration was 10 ng/mL. For the definition of the functional MBL deficiency, this study used two different cut-off values of MBL concentration. An MBL level < 700 ng/mL was determined as deficiency and <150 ng/mL as severe deficiency.14,15 DNA was extracted from the blood samples using a commercially available kit (Puregene, Gentra, MN, United States), and MBL2 genotyping was performed using these samples. DNA samples were maintained at −20 °C until use. All genotypes were detected using PCR and restriction enzyme digestion. Exon 1 of MBL2 was amplified by PCR. The primer sequences were 5'-GGA GCA AGG GCA TGCTC-3' and 5'-CAG GGA TCC TCT GGA AGG-3'. In all, 349 bp PCR product was digested with Banl for exon 54 and codon 54, and 57, respectively. The normal allele (allele A) is cut into two fragments with Banl (lanes 2 and 5), 89 and 260 bp. The variant allele (allele O) remains uncut (lanes 1 and 6). Both uncut and digested fragments are seen in AO heterozygote (lanes 3 and 4). L: 100 bp DNA ladder.

Figure 1 DNA fragments on agarose gel electrophoresis after restriction enzyme digestion of exon 1 of the mannose-binding lectin (MBL2) gene codon 54. In all, 349 bp PCR product was digested with Banl for exon 54 and codon 54, and 57, respectively. The normal allele (allele A) is cut into two fragments with Banl (lanes 2 and 5), 89 and 260 bp. The variant allele (allele O) remains uncut (lanes 1 and 6). Both uncut and digested fragments are seen in AO heterozygote (lanes 3 and 4). L: 100 bp DNA ladder.

and 5'-CAG GCA TCC TCT GGA AGG-3'. In all, a 349-bp PCR product was digested with Banl and Mbol for exon 54 and codon 57, respectively. The normal allele (allele A) was cut into two fragments with Banl, 260 and 89 bp. The variant allele B (rs1800450) and allele D (rs5030737) remained uncut. Mbol cleaved the variant allele C (rs1800451) into 270 and 79 bp fragments. The fragments were visualized using electrophoresis on 2% agarose gel. At electrophoresis, the dual band at the restriction site was defined as a heterozygous mutation, whereas the single band was defined as a homozygous mutation. As stated, the normal structural MBL2 allele was named A, whereas alleles B, C and D (mutation in codons 54, 57 and 52) were named O. A representative gel electrophoresis of the MBL2 exon 1 codon 54 polymorphisms is shown in Fig. 1.

This study was approved by the Ethics Committee of Uludag University Medical School and conformed to the standards set by the Declaration of Helsinki (15.01.2013-1/20). All parents provided informed consents prior to the inclusion of their children in the study.

Statistical analysis

Statistical analysis was performed using SPSS v. 20.0 software (SPSS Inc., Chicago, IL, United States). The results are presented as median (interquartile range) for the variables showing non-Gaussian distribution and mean ± standard deviation for data showing normal distribution. Student’s
Mannose-binding lectin and outcomes in preterms

Table 1  Neonatal and maternal characteristics of the study population.

|                                      | MBL2 wild-type | MBL2 variant -type | p     |
|--------------------------------------|----------------|-------------------|-------|
|                                      | (n = 69)       | (n = 47)          |       |
| GA at birth, median in weeks (range) | 30 (29–33)     | 31 (29–33)        | 0.6a  |
| Birth weight, g (mean ± SD)          | 1539 ± 574     | 1459 ± 556        | 0.5b  |
| Sex, n (%)                           |                |                   |       |
| Male                                 | 40 (58)        | 29 (62)           |       |
| Female                               | 29 (42)        | 18 (38)           | 0.7c  |
| SGA, n (%)                           | 13 (19)        | 14 (30)           | 0.1c  |
| Cesarean delivery, n (%)             | 58 (84)        | 36 (77)           | 0.3c  |
| Apgar score, median (range)          |                |                   |       |
| Minute 1                             | 7 (5–8)        | 6 (4–7)           | 0.1a  |
| Minute 5                             | 8 (7–9)        | 8 (7–9)           | 0.1a  |
| Antenatal steroid, n (%)             |                |                   |       |
| None                                 | 33 (48)        | 30. (64)          |       |
| Single course                        | 18 (26)        | 9. (19)           |       |
| Repeat course                        | 18 (26)        | 8. (17)           |       |
| Maternal preeclampsia, n (%)         | 17 (25)        | 15 (32)           | 0.4c  |
| Maternal Infection, n (%)            | 3 (4)          | 4 (9)             | 0.4c  |
| PPROM, n (%)                         | 13 (19)        | 6 (13)            | 0.4c  |
| Chorioamnionitis, n (%)              | 4 (6)          | 2 (4)             | 0.7c  |
| MBL levels, (ng/mL) median (range)   | 993 (257–1812) | 10 (10–473)       | <0.001a |
| MBL deficiency, (MBL level <700ng/mL, n (%)) | 30 (44) | 45 (96) | <0.001c |

Values with significance are presented in bold.

MBL2, mannose-binding lectin; GA, gestational age; SGA, small for gestational age; PPROM, preterm premature rupture of membranes.

a Mann–Whitney U test.
b Student’s t-test.
c Chi-squared test.

t-test was used for group comparisons of normal distributions, and the Mann–Whitney U test was used for group comparisons of non-normal distributions. The chi-squared test and Fisher’s exact test were used for the comparison of categorical variables. Logistic regression analysis was performed to investigate the effect of MBL2 genotype on RDS. The analysis included factors that were demonstrated in the literature to have an effect on RDS: gestational age, birth weight, sex, antenatal steroid administration, and MBL2 genotype were included in the analysis. A p-value of <0.05 was considered statistically significant.

Results

Overall, 131 preterm infants were included in this study. Ten were excluded because of blood sample insufficiency, four because of major congenital abnormalities, and one because of major surgery. In the final analysis, a total of 116 preterm infants were included: 69 with MBL2 wild-type (AA genotype) and 47 with MBL2 variant-type (AO/OO genotype). Overall, the rate of MBL2 variant-type in preterm infants was 41%. MBL levels were significantly lower and MBL deficiency and severe deficiency were higher in MBL2 variant-type than in MBL2 wild-type (p < 0.001). Table 1 shows the demographic features of the study population. Codon 57 and 52 polymorphisms were not detected in any of the 116 preterm infants during the genetic evaluation. MBL2 codon 54 genotype and allele frequencies were 59% for MBL2 wild-type (AA genotype) and 41% for MBL2 variant-type (AO and OO genotype).

Evaluation of short-term morbidity based on MBL2 genotype revealed that RDS and mortality rates were significantly higher in the MBL2 variant-type group (p < 0.001, p = 0.03; respectively). NEC was found to be more prevalent in the MBL2 wild-type group (p = 0.01). There was no difference between the MBL2 wild-type and variant-type groups in terms of IVH, BPD, ROP, and LOS (Table 2). Consideration of short-term morbidity based on MBL levels revealed that RDS was significantly higher in both the MBL deficient and severely deficient groups (p < 0.001). NEC was found to be more common with normal levels of MBL (p = 0.002). There was no significant difference in infants with or without MBL deficiency with respect to IVH, BPD, ROP, LOS, and mortality (Table 3). Further, because univariate analyses revealed that RDS development was more common in the MBL2 variant-type, the effect of gestational age, birth weight, gender, antenatal steroid use, and MBL2 genetics, which are factors that may affect RDS development, was investigated by logistic regression analysis. MBL2 variant-type was found to be an independent factor for the development of RDS (OR: 5.1, 95% CI: 2.2–11.9, p < 0.001).

Discussion

It was observed that MBL levels were lower in preterm infants with MBL2 variant-type than in those with MBL2 wild-type. RDS was significantly more common in the MBL2
variant-type group and also in the MBL deficient group. Additionally, the mortality rates were higher in preterm infants with MBL2 variant-type. In the study model, MBL2 variant-type was a significant independent factor for RDS after adjusting for the effects of other factors. Besides, the prevalence of NEC was higher in the MBL2 wild-type group and with normal MBL levels. It is believed that these findings will contribute toward accumulating evidence on the effect of MBL in preterm morbidities.

The collectin family and MBL play an important role in the primary immune elimination of invasive microorganisms in the innate immune response as well as in the regulation of ongoing immune responses against microbial invasion. Studies have reported an association between MBL deficiency or variant genotype as well as infection and pulmonary pathologies. Pulmonary function impairment has been reported in patients with MBL deficiency or variant type as well as infection and pulmonary pathologies. Pulmonary function impairment has been reported in patients with MBL deficiency or variant type as well as infection and pulmonary pathologies. In some studies, similar to the results obtained in the present study, it has been shown that MBL deficiency or variant type cause respiratory morbidity independent of infection. There is high sequence homology between MBL and SP-A and SP-D. The genes encoding these proteins are located on the long arm of chromosome 10 and belong to a similar lineage. SP-A and SP-D are involved in the removal of many pathogens in the lungs, and although SP-A is particularly known for its immune functions, RDS is associated with decreased SP-A levels. Mutant MBL2 genetics are associated with insufficient surfactant protein-A production, which may facilitate the development of RDS. Similarly, the present study also found a significant increase in RDS prevalence and mortality in patients with mutant MBL2 genetics. Early selective surfactant therapy in RDS has been reported to reduce pulmonary injury and mortality. It is believed that during the evaluation of MBL2 genotype at the time of delivery in preterm infants with high RDS risk and ≥32 gestational weeks and in borderline cases with an indication for surfactant, the early administration of surfactants to patients with mutant MBL2 genetics will reduce mortality and pulmonary morbidities.

In recent years, there has been an increased interest in the association between MBL and inflammatory morbidities. It has been reported that MBL activates the lectin pathway of complement, resulting in ischemia-perfusion damage. In patients with MBL2 wild-type, higher MBL levels have been reported and associated with NEC, resulting in reperfusion injury after intestinal ischemia. In agreement with these findings, in the present study the prevalence of NEC was higher in preterm infants with MBL2 wild-type and normal MBL levels. However, some studies have reported that there is no association between MBL2 genotype and NEC. In the present work, the development of NEC may have been relatively more common because of significantly higher mortality rates in patients with MBL2 wild-type. Because there

### Table 2  Frequency of early neonatal outcomes according to mannose binding lectin genotypes.

| Outcome               | MBL2 wild-type(n=69) | MBL2 variant-type(n=47) | p*  |
|-----------------------|-----------------------|-------------------------|-----|
| RDS, n (%)            | 21 (30)               | 31 (66)                 | <0.001 |
| IVH, (Papille grade 3-4), n (%) | 3 (4)                 | 2 (4)                   | 0.9  |
| NEC, (>grade 1), n (%) | 9 (13)                | 0 (0)                   | 0.01 |
| BPD, (grade 2-3), n (%) | 16 (23)              | 6 (13)                  | 0.2  |
| ROP, (>stage 2), n (%) | 10 (15)               | 3 (6)                   | 0.2  |
| LOS, n (%)            | 18 (26)               | 16 (34)                 | 0.4  |
| Mortality, n (%)      | 6 (9)                 | 11 (23)                 | 0.03 |

a Chi-squared test.
Values with significance are presented in bold.
MBL2, mannose-binding lectin; RDS, respiratory distress syndrome; LOS, late onset sepsis; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity.

### Table 3  Mannose binding lectin levels in relation to early neonatal outcomes.

| MBL deficiency | Normal MBL>700 ng/mL(n=41) | p*  |
|----------------|----------------------------|-----|
| <150 ng/mL(n=36) | 28 (72)                  | 1 (3) | <0.001 |
| 150–700 ng/mL(n=36) | 23 (64)                  | 2 (5)  | 0.7  |

a Chi-squared test.
Values with significance are presented in bold.
MBL, mannose-binding lectin; RDS, respiratory distress syndrome; LOS, late onset sepsis; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity.

MBL2, mannose-binding lectin; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; BPD, bronchopulmonary dysplasia; ROP, retinopathy of prematurity.
are debatable opinions in the literature on MBL2 genotype and NEC development, additional studies are needed to clarify this issue.

In this study, in agreement with previous data, no correlation was found between MBL2 genotype and MBL levels with inflammation-associated pathologies, BPD, IVH, and ROP. The evaluation of the association between MBL2 genotype and morbidity was the common aspect of these studies. It would be misleading to evaluate the association of MBL2 genotype and MBL levels obtained at the time of delivery with morbidities alone. Because MBL levels increase as the gestational week increases in the MBL2 wild-type group, the evaluation of morbidity development with MBL levels obtained at different postnatal weeks may provide more accurate results to demonstrate the association between lectin pathway and inflammatory morbidities. There is a clear need for extensive studies to investigate the association between MBL and inflammatory morbidities in premature infants.

Although the association of MBL2 genotype with culture-proven sepsis has not been reported in the literature, the association between MBL2 genotype and early clinical sepsis has been reported. In contrast, the association between MBL deficiency and sepsis has been reported in many studies. In the present study, no association was found between MBL2 genotype and MBL levels with LOS. The authors believe that inadequate immune response to infection is observed because low MBL levels in the early postnatal weeks in preterm infants even if the MBL2 genotype is wild-type. Therefore, future studies should evaluate MBL values at the time of sepsis together with genotype.

This study had some limitations. Morbidity was evaluated based on only MBL2 genotype and with the levels of MBL within 24 h after birth because the MBL levels of the infants were not reassessed during the subsequent postnatal days. Additionally, the results obtained with a limited number of cases may not reflect the overall results. The strength of this study was that it evaluated the association of MBL2 genotype with MBL level and morbidities in preterm infants and simultaneously examined the MBL level in the first 24 h of life.

In conclusion, the presence of MBL2 variant-type and low MBL levels are important risk factors for RDS development in preterm infants. Additionally, there is an association between MBL2 wild-type and NEC. Considering the importance of showing that MBL2 variant-type is an independent predictor of RDS, further prospective randomized studies on this topic are clearly required.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Speletas M, Gounaris A, Sevdali E, Kompoti M, Konstantinidi K, Sokou R, et al. MBL2 genotypes and their associations with MBL levels and NICU morbidity in a cohort of Greek neonates. J Immunol Res. 2015;2015:478412.
2. Dzwonek AB, Neth OW, Thiebaut R, Gulczynska E, Chilton M, Hellwig T, et al. The role of mannose-binding lectin in susceptibility to infection in preterm neonates. Pediatr Res. 2008;63:5–680.
3. Ozkan H, Koksal N, Cetinkaya M, Kilic S, Celebi S, Oral B, et al. Serum mannose-binding lectin (MBL) gene polymorphism and low MBL levels are associated with neonatal sepsis and pneumonia. J Perinatol. 2012;32:7–210.
4. van der Zewt WC, Catsburg A, van Elburg RM, Savelkoul PH, Vandenbroucke-Grauls CM. Mannose-binding lectin (MBL) genotype in relation to risk of nosocomial infection in pre-term neonates in the neonatal intensive care unit. Clin Microbiol Infect. 2008;14:5–130.
5. Koroglu OA, Onay H, Erdemir G, Yalaz M, Cakmak B, Aksu M, et al. Mannose-binding lectin gene polymorphism and early neonatal outcome in preterm infants. Neonatology. 2010;98:12–305.
6. Xue J, Liu AH, Zhao B, Si M, Li YQ. Low levels of mannose-binding lectin at admission increase the risk of adverse neurological outcome in preterm infants: a 1-year follow-up study. J Matern Fetal Neonatal Med. 2016;29:9–1425.
7. Auriti C, Prencipe G, Inglese R, Azzari C, Ronchetti MP, Tozzi A, et al. Role of mannose-binding lectin in nosocomial sepsis critically ill neonates. Hum Immunol. 2010;71:8–1084.
8. Eichenwald EC, Stark AR. Management and outcomes of very low birth weight. N Engl J Med. 2008;358:11–700.
9. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ. 2012;345:e7976.
10. Prencipe G, Azzari C, Moriondo M, Devito R, Inglese R, Pezzullo M, et al. Association between mannose-binding lectin gene polymorphisms and necrotizing enterocolitis in preterm infants. J Pediatr Gastroenterol Nutr. 2012;55:5–160.
11. Walti H, Couchard M, Relier JP. Neonatal diagnosis of respiratory distress syndrome. Eur Respir J Suppl. 1989;3:6–22s.
12. Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, et al. European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants-2010 update. Neonatology. 2010;97:17–402.
13. Stoll BJ, Hansen N, Farianoff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110:91–285.
14. Papile LA, Burstein J, Burstein R, Kocher H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92:34–529.
15. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am. 1986;33:179–201.
16. Cakmak BC, Calkavur S, Ozkany F, Koroglu OA, Onay H, Ilirili G, et al. Association between bronchopulmonary dysplasia and MBL2 and IL1-RN polymorphisms. Pediatr Int. 2012;54:8–863.
17. International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol. 2005;123:991–9.
18. Luo J, Xu F, Lu GJ, Lin HC, Feng ZC. Low mannose-binding lectin (MBL) levels and MBL genetic polymorphisms associated with the risk of neonatal sepsis: an updated meta-analysis. Early Hum Dev. 2014;90:64–557.
19. Lin CL, Shu LK, Lin JC, Liu CY, Chian CF, Lee CN, et al. Mannose-binding lectin gene polymorphism contributes to recurrence of infective exacerbation in patients with COPD. Chest. 2011;139:43–51.
20. Chalmers JD, Fleming GB, Hill AT, Kilpatrick DC. Impact of mannose-binding lectin insufficiency on the course of cystic fibrosis: a review and meta-analysis. Glycobiology. 2011;21:82–271.
21. Gong MN, Zhou W, Williams PL, Thompson BT, Pothier L, Christiani DC. Polymorphisms in the mannose binding lectin-2 gene and acute respiratory distress syndrome. Crit Care Med. 2007;35:48-56.

22. Seaton BA, Crouch EC, McCormack FX, Head JF, Hartshorn KL, Mendelsohn R. Review: structural determinants of pattern recognition by lung collectins. Innate Immun. 2010;16:50-143.

23. Chang HY, Li F, Li FS, Zheng CZ, Lei YZ, Wang J. Genetic polymorphisms of SP-A, SP-B, and SP-D and risk of respiratory distress syndrome in preterm neonates. Med Sci Monit. 2016;22:100-5091.

24. Bahadue FL, Soll R. Early versus delayed selective surfactant treatment for neonatal respiratory distress syndrome. Cochrane Database Syst Rev. 2012;11:CD001456.

25. Hartz A, Pagel J, Humberg A, Preuss M, Schreiter L, Rupp J, et al. The association of mannose-binding lectin 2 polymorphisms with outcome in very low birth weight infants. PLoS One. 2017;12:e0178032.