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Original article

Mannose-binding lectin deficiency in preterm neonates

Background: *Mannose-binding lectin (MBL) is a collagenous protein that plays a role in innate immunity. MBL deficiency is associated with an opsonization defect and has been associated with recurrent infections, especially in immunocompromised individuals. Neonates are considered to be immunocompromised because adaptive immunity has not yet been developed.*
Objective: *This study was done to evaluate the levels of MBL in premature neonates and to determine the relation between MBL deficiency and development of sepsis.*
Methods: *This case- control study was conducted on 64 neonates classified into 2 groups; 39 preterm neonates with gestational age (G.A) <36 weeks and 25 healthy full term neonates. Measurement of mannose-binding lectin (MBL) serum level was done on the first day of life using ELISA technique.*
Results: *Mean MBL plasma level was found to be lower in preterm than full term neonates, yet this difference did not reach statistical significance. There was a negative correlation albeit an insignificant one, between MBL level and GA. The deficient group (those with MBL level ≤0.7μg/ml) had higher incidence of sepsis, albeit an insignificant one, than the non deficient group. A highly significant positive correlation was demonstrated between MBL plasma level in neonatal and umbilical cord blood samples.*
Conclusion: *Premature neonates have low MBL serum levels which could be measured in either their venous or umbilical cord blood efficiently. Further studies are needed to investigate the relationship between MBL deficiency and neonatal sepsis and whether measuring MBL levels might be used to identify which neonates are prone to infections.*

Keywords: Mannose binding lectin, neonates, preterm, sepsis.

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INTRODUCTION

Mannose-binding lectin (MBL), also called mannose binding protein (MBP), is a calcium-dependent serum protein that plays a role in the innate immune response by binding to carbohydrates on the surface of a wide range of pathogens (viruses, bacteria, fungi, protozoa) where it can activate the complement system or act directly as an opsonin[1].

Circulating MBL concentrations and functional activity are correlated with common genetic variants in the MBL2 gene. Three single nucleotide polymorphisms (SNPs) in codons 52, 54 and 57 (D, B and C variants, respectively) of exon-1 lead to reduced functional plasma MBL concentrations[2] .

MBL deficiency arising from mutations and promoter polymorphisms in the MBL2 has been associated with increased risk, severity, and frequency of infections and autoimmunity[3] .

Neonates are prone to develop infections which are sometimes life-threatening, especially in premature patients admitted to the neonatal intensive care unit[4].

Neonatal MBL concentration increases during the first weeks after birth, both in premature and term neonates[5]. Low MBL concentrations have been related to lower gestational age[6]. Therefore, low MBL concentrations may not only be explained by MBL2 gene mutations, but also by prematurity[7] .

So, we aimed to evaluate the levels of MBL in premature neonates and to determine the relation between MBL deficiency and development of sepsis.

METHODS

Study design

This prospective case control study was conducted on 64 neonates, as a stratified non random sample, enrolled from neonatal intensive care units of Obstetric and Gynecology Hospital, Ain Shams University and El-Matareya Teaching Hospital during the period from June 2008 to November

2008. An informed consent was taken from the parents before their enrollment in the study.
Neonates were divided into 2 groups:

The study group: It included 39 preterm neonates, 32 males and 7 females with gestational ages less than 36 weeks and they were classified into two groups, septic group and non septic group according to sepsis score[8]. The control group included 25 healthy full term neonates, 11 males and 14 females appropriate for gestational age (FT-AGA).

Exclusion criteria: Neonates were excluded from the study if they had:
- History of maternal fever and chorioamnionitis.
- Congenital malformation.
- Chromosomal abnormalities.
- Premature rupture of membranes (PROM)>18 hours.

Careful history was taken including maternal, obstetric, and perinatal history. In addition, full clinical examination, birth weight measurement, and gestational age assessment were done.

Laboratory investigations:

Blood samples were withdrawn from all neonates by venipuncture within the first 24 hours of admission, and cord blood samples were withdrawn from 17 of them in the delivery room (10 samples from preterm neonates and 7 from full term neonates).

1. Two mL venous blood/ cord blood were collected in a plain tube and were allowed to clot for 30 min to estimate MBL serum level by ELISA technique (Sanquin Reagents, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands). Samples were then centrifuged at 3000 rpm for 10 min. The supernatant was separated in aliquots and stored at -20°C until time of assay.

MBL serum level ≤0.7 µg/ml was considered deficient; an optimal cut-off plasma level for MBL deficiency was 0.7 µg/ml[7].

2. Three mL venous blood were collected 3 days after birth, 1 week after birth and when indicated and divided as follows:

a) Two mL were collected on K-EDTA tube for CBC analysis and were immediately analyzed on Coulter Counter Gen (Coulter Electronics Corporation Hielach, Florida, USA). Differential leucocytic count was done on Leishman stained peripheral blood smear.

b) One mL was collected and was immediately analyzed for CRP with titer analysis by latex agglutination test. (Kit provided by Teco diagnosis, 1268 N. Lakeview Ave Anaheim, CA 92807 USA).

Statistical Methods

Data were analyzed using Statistical Package for Special Science (SPSS) software computer program version 13. Quantitative data were described using mean ± standard deviation, median (interquartile range); qualitative data were described in the form of numbers and percentages. Student t-test of two independent samples was used for comparison of normally distributed quantitative variables while Mann-Whitney test was used for non- parametric data. Chi-square test was used for comparison of qualitative variables. Wilcoxon signed rank test was used to compare follow up with initial values. Correlation between continuous variables was performed using Spearman correlation coefficient (r). The relative contribution of some variables to the risk of MBL deficiency was expressed as the OR with a 95% confidence interval (CI).

RESULTS

The demographic and clinical characteristics of studied neonates are listed in table (1). The study group included 39 preterm neonates with mean gestational age 32.97±2.12 weeks and mean birth weight 1.89±0.54 kg. The control group included 25 fullterm neonates with mean gestational age 39.04±1.27 weeks and mean birth weight 3.2±0.4 kg. There was a highly significant difference in gestational age and birth weight between both groups and a significant difference in gender**.**

A highly significant decrease in Apgar scores at 1 and 5 minutes was found in preterm group, and no significant difference between both groups in mode of delivery and number of neonates whose mothers had PET**.**

Mean MBL serum level was found to be insignificantly lower in preterm than full term neonates (p=0.058). (Table 2).

A negative correlation albeit an insignificant one, was detected between MBL plasma level of neonates of the preterm group and their gestational ages (r = - 0.116, p>0.05), while there was a highly significant negative correlation between their MBL serum level and their birth weights (r = - 0.447, p<0.01). An insignificant positive correlation was detected between MBL serum level of preterm and fullterm neonates together with their gestational ages (r = 0.149, p>0.05) and birth weights (r = 0.035, p>0.05).

Fifteen patients of our preterm group developed sepsis after the first week of life. On comparing the septic and nonseptic groups, there was no significant difference as regards the MBL serum level. (Table 3). However 50 % of preterms with

MBL levels ≤0.7µg/ml (deficient in MBL) developed sepsis in the face of 26.3 % only in the non deficient group (those with MBL levels >0.7µg/ml), although the difference was not significant (p>0.05). (Figure 1).

A highly significant positive correlation was demonstrated between MBL serum level in neonatal and umbilical cord blood samples. (Figure 2).

On estimating the different risk factors which may affect the MBL serum level, gestational age (<37 weeks), birth weight (<1500 gm), preterm neonates with RDS, gender, mothers with preeclampsia, and mode of delivery, all were found to have insignificant effects. (Table4).

Table 1. Demographic data of study (preterm) and control (full term) groups.

Variable	Preterm n=39	Fullterm n=25	P value
Gestational Age (Wks) (mean ± SD)	28 – 36 32.97 ± 2.12	37 – 42 39.04 ± 1.27	0.000**
Birth Weight (kg) (mean ± SD)	1.0 – 3.0 1.89 ± 0.54	2.7 – 4.0 3.2 ± 0.4	0.000**
Male / Female	32 / 7	11 / 14	0.002*
SVD / LSCS	16 / 23	12 / 13	0.58
APGAR at 1 min at 5 min	3 – 6 5.13±0.69 6 – 9 8.33±0.73	5 – 7 6.04±0.45 8 – 10 9.08±0.4	0.000** 0.000**
PET (%)	7 (17.9%)	2 (8%)	0.26
No RDS RDS grade II III IV	9 (23%) 13 (33.33%) 15 (38.4%) 2 (5.1%)	No RD	

SVD: Spontaneous Vaginal Delivery, LSCS: Lower Segment Caesarean Section, PET: Preeclamptic toxemia, RDS: Respiratory Distress Syndrome, ** Highly Significant, * Significant

Table 2. Comparison of MBL serum level between preterm and full term neonates.

MBL level in neonatal blood sample (µg/ml)	Preterm n=39	Fullterm n=25	P value
Range	0.1 – 2.8	0.2 – 6.5	0.058
Mean ± SD	1.06 ± 0.79	2.12 ± 2	

MBL: Mannose binding lectin

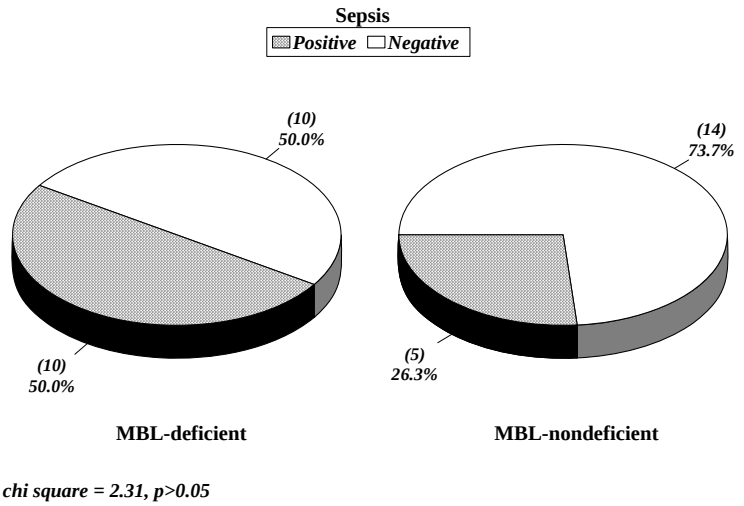


Figure 1. Comparison between MBL deficient and MBL non deficient preterm neonates as regards development of sepsis**.**

Table 3. Comparison between septic and non septic groups of preterm neonates as regards MBL serum level.

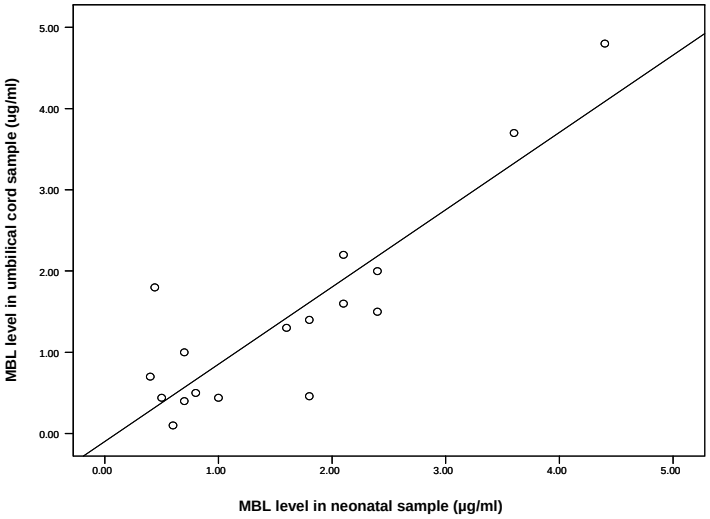
Preterm group	n	MBL level (µg/ml)		P value
Non septic group	24	**Range**	0.1 - 2.4	0.33 (P>0.05)
		Mean ± SD	1.146 ± 0.79	
Septic group	15	**Range**	0.1 - 2.8	
		Mean ± SD	0.933 ± 0.82	

MBL: Mannose binding lectin

Table 4. Association of different factors with risk of MBL deficiency

Variable	Odds ratio	95% CI of odds ratio	
		Lower	upper
GA	2.237	0.784	6.386
BW	0.278	0.067	1.147
RDS	1.250	0.280	5.585
PET	0.333	0.075	1.475
Sex	1.413	0.487	4.100
Delivery	0.723	0.266	1.969

GA: gestational age, BW: birth weight, RDS: respiratory distress syndrome, PET: preeclamptic toxaemia



r =0.88, p<0.01

Figure 2. Correlation between MBL serum level in neonatal and umbilical cord samples.

DISCUSSION

In neonates, low MBL levels are associated not only with variant MBL2 genotype, but also with low gestational age. Detection of MBL deficiency at birth should be based on actual MBL plasma levels rather than on MBL2 genotype[7]. This was concluded by van der Zwet et al.[9], they demonstrated that there was no relationship between MBL genotype and the risk of nosocomial sepsis or pneumonia, even after correction for birth-weight, because of an insufficient correlation between genotype and the concentration of functional MBL.

In the present study, the first day MBL level was found to be lower in preterm group (mean MBL=1.06 µg/mL) when compared to the full term group (mean MBL=2.12 µg/mL). Yet this difference was not statistically significant. This could be explained by the fact that only 10 of our preterm patients were less than 32 weeks, and some studies as the study done by de Benedetti et al.[10] demonstrated that MBL levels were related to gestational age. Neonates with GA>32 weeks had MBL levels significantly higher than those with GA<32 weeks. So, they concluded that low MBL levels in neonates may also be secondary to a maturational defect; possibly involving the liver secretory capacity i.e. prematurity is associated with insufficient MBL production by the liver.

Our results were in agreement with those of Frakking et al.[7], who demonstrated no statistical significant difference between preterm and full term groups regarding MBL levels.

Also, we have found that MBL levels in preterm neonates alone or together with fullterm neonates were not significantly correlated with gestational age. Other researchers found this relationship significant[7,11]. The finding in this study of a significant negative correlation of MBL with birth weight in the preterm group was contradictory with another study which demonstrated that MBL level was not affected by birth weight suggesting that small for gestational age preterm neonates have similar MBL levels to appropriate for gestational age preterm neonates[6].

Various factors were tested to determine which was more influential on MBL level and it was found that gestational age, birth weight, RDS, PET, gender, and mode of delivery were all non-significant. It is to be noted that in our study an optimal cut off serum level for MBL deficiency was 0.7 µg/mL, while in the studies of Lau et al[6] and Ozdemir et al.[12], the cut off value was 0.4 µg/mL.

Analysis of our results revealed that 50 % of the MBL deficient preterm neonates and 26.3 % of the MBL non deficient preterm neonates developed sepsis, yet this difference was not statistically significant. Some studies like ours did not find an increased sepsis risk in neonates with low MBL levels[11]. Also on comparing the septic and nonseptic groups, the lower mean MBL level of the septic group was not statistically significant. This could be explained on the basis that the number of the neonates in the study group was small. A larger scale study would give a better insight on the relation of MBL to sepsis. Furthermore development of sepsis is multifactorial in which many deficiencies besides that of MBL come into play.

In a study done to outline the role of MBL in determining the susceptibility of preterm neonates to severe sepsis, cord blood MBL was measured in 44 preterm neonates and was found to be significantly lower in neonates who developed sepsis than those who did not develop sepsis[12].

In another study, the plasma levels of MBL were determined at birth in 88 neonatal intensive care patients (71 premature). Thirty-five neonates (40%) had low, i.e. ≤0.7 µg/ml, MBL plasma levels at birth. The authors concluded that MBL deficiency increases the susceptibility to infections in premature and term NICU-patients. The presence of low (≤0.7 µg/ml) MBL plasma levels at birth appeared to be associated with early onset sepsis (EOS). In addition, neonates with low MBL plasma levels appeared to have an increased risk of severe infections during the first month of life compared to neonates with normal plasma levels[13].

There is a report of an increased risk of nosocomial sepsis with low MBL levels at birth in a cohort of 206 neonates admitted to NICU. The authors suggested that MBL is protective against the development of neonatal sepsis[10,14]. In a study done to investigate the association between serum MBL levels and development of sepsis in neonates, the authors demonstrated that high serum MBL levels protect from sepsis independent of gestational age or birth weight[10].

The combination of prematurity and low MBL levels increases the risk of sepsis to 70%[14]. This is because low MBL levels might be associated with decreased capacity of phagocytosis or opsonization of microorganisms[13]. Binding of MBL to *Staph. aureus* was found to be markedly impaired at concentrations of <0.6 µg/ml[15].

In the current study, 17 paired first day neonatal and umbilical cord samples showed highly significant correlation in their MBL level (r = 0.88). A similar correlation was reported by Frakking et al.[7] (r = 0.95). Therefore, umbilical cord blood can be used to detect MBL deficiency as umbilical cord sampling is easier and less invasive than venous puncture in neonates[7].

In conclusion, premature neonates tend to have low MBL serum concentrations which could be measured in either their venous or umbilical cord blood efficiently. A higher percentage of preterm neonates deficient in MBL develop sepsis.

Further studies are needed to investigate the relationship between MBL deficiency and neonatal sepsis and whether measuring MBL levels might be used to identify which neonates are prone to infections.

REFERENCES

1. **Koch A, Melbye M, Sorensen P, Homoe P, Madsen HO, Molbak K et al.** Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. JAMA 2001; 285 (10): 1316-21.
2. **Madsen HO, Garred P, Kurtzhals JA, Lamm LU, Ryder LP, Thiel S, et al.** A new frequent allele is the missing link in the structural polymorphism of the human mannan-binding protein. Immunogenetics 1994; 40 (1): 37–44.

3. **Larsen F, Madsen HO, Sim RB, Koch C, Garred P**. Disease-associated mutations in human mannose-binding lectin compromise oligomerization and activity of the final protein. J Biol Chem 2004; 279 (20): 21302-11.

4. **Baltimore RS.** Neonatal sepsis: epidemiology and management. Paediatr Drugs.2003; 5 (11): 723–40.

5. **Aittoniemi J, Miettinen A, Laippala P, Isolauri E, Viikari J, Ruuska T, et al.** Age-dependent variation in the serum concentration of mannan-binding protein. Acta Paediatr.1996; 85 (8):906–9.

6. **Lau YL, Chan SY, Turner MW, Fong J, Karlberg J.** Mannose-binding protein in preterm infants: developmental profile and clinical significance. Clin Exp Immunol. 1995; 102 (3): 649–54.

7. **Frakking FN, Brouwer N, Zweers D, Merkus MP, Kuijpers TW, Offringa M et al.** High prevalence of mannose-binding lectin (MBL) deficiency in premature neonates. Clin Exp Immunol. 2006; 145 (1): 5-12.

8. **Griffin MP, Lake DE, O'Shea TM, Moorman JR.** Heart rate characteristics and clinical signs in neonatal sepsis. Pediatr Res 2007; 61 (2): 222-7.

9. **van der Zwet WC, Catsburg A, van Elburg RM, Savelkoul PH, Vandenbrouke-Grauls CM.** Mannose-binding lectin (MBL) genotype in relation to risk of nosocomial infection in preterm neonates in the neonatal intensive care unit. Clin Microbiol Infect. 2008; 14 (2):130-5.

10. **de Benedetti F, Auriti C, D'Urbano LE, Ronchetti MP, Ravà L, Tozzi A, et al.** Low serum levels of mannose binding lectin are a risk factor for neonatal sepsis. Pediatr Res.2007; 61 (3):325–8.

11. **Hilgendorff A, Schmidt R, Bohnert A, Merz C, Bein G, Gortner L.** Host defence lectins in preterm neonates. Acta Paediatr 2005; 94 (6): 794–9.

12. **Ozdemir O, Dinleyici EC, Tekin N, Colak O, Aksit MA.** Low mannose-binding lectin levels in susceptibility to neonatal sepsis in preterm neonates with fetal inflammatory response syndrome. J Matern Fetal Neonatal Med. 2010. (Epub ahead in print).

13. **Frakking FN, Brouwer N, van Eijkelenburg NK, Merkus MP, Kuijpers TW, Offringa M, et al.** Low mannose-binding lectin (MBL) levels in neonates with pneumonia and sepsis. Clin Exp Immunol. 2007; 150 (2): 255-62.

14. **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 (6): 680-5

15. **Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW.** Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun. 2000; 68 (2):688–93.