Use of Mannose-Binding Lectin Gene Polymorphisms and the Serum MBL Level for the Early Detection of Neonatal Sepsis

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Abstract

Background Mannose-binding lectin (MBL) is a component of innate immunity and is particularly important in neonates, in whom adaptive immunity has not yet completely developed. MBL deficiency and *MBL2* gene polymorphisms are associated with an opsonization defect and have been associated with neonatal sepsis.

Aim The aim of our study was to assess serum MBL levels and genotype *MBL2* genes to determine whether they can serve as markers for predicting neonatal sepsis in neonatal intensive care units.

Patients and Methods A case-control study was conducted with 114 neonates classified into two groups: the septic group included 64 neonates (41 preterm and 23 full-term infants), and the non-septic control group included 50 neonates (29 preterm and 21 full-term infants). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to genotype *MBL2* gene exon 1 (rs1800450) and (rs1800451) SNPs. Enzyme-linked immunosorbent assay (ELISA) was used to measure MBL serum concentrations.

Results The polymorphic genotypes BB and AC at codons 54 and 57, respectively, showed higher frequencies than the wild-type genotype (AA) (14.1% versus 12.9% and 28.1% versus 19.4% respectively) in both groups, and this difference was greater in the septic group than in the non-septic group; however, the differences did not reach statistical significance. The B and C allele frequencies were also higher in the septic group than in the non-septic group, but the differences did not reach statistical significance ($p = 0.282$ and 0.394 , respectively). The serum levels of MBL were significantly lower in the septic group than in the non-septic group ($p = 0.028$).

Conclusion This study found no association between MBL levels or *MBL2* exon 1 genotypes or alleles and neonatal sepsis risk. Further studies with larger sample sizes are needed to determine the role of the *MBL2* gene as a risk factor and early predictor of neonatal sepsis.

Keywords

- mannose-binding lectin
- neonatal sepsis
- gene polymorphism

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Introduction

Neonatal sepsis is a common health problem worldwide, both among full-term and preterm infants.¹ Although great improvements have been made toward reducing the complications of sepsis in preterm infants, sepsis remains a major cause of significant mortality and morbidity among very low-birth-weight (<1,500 g) infants.²

A strong demand for further improvements necessitates the search for biomarkers for the early diagnosis of sepsis. Although the gold standard for the diagnosis of sepsis is the isolation of the organism from blood via blood culture, this process requires a long period of 24 to 48 hours and a large blood sample to provide optimal results due to the low level of bacteremia in infants.³ Additionally, early diagnosis is difficult because the clinical signs and symptoms of neonatal sepsis are usually nonspecific. Thus, the timely and accurate identification of sepsis is important for implementing the appropriate antibiotic treatment to reduce the risk of adverse outcomes.⁴

Mannose-binding lectin (MBL) is a soluble pattern-recognition molecule⁵ that is produced by the liver and is an acute-phase protein that activates the lectin pathway of the complement system and subsequent inflammatory mechanisms. The importance of MBL functions during the first months of life, when the efficacy of innate immunity is crucial, has led to the proposal that MBL should be included in biomarker panels for the early detection of neonatal infections.⁶

The MBL serum levels are genetically determined. The *MBL2* gene is one of the most extensively studied candidate genes reported to be associated with sepsis and infectious disease.⁷ The *MBL2* gene is located on chromosome 10q11.2 and contains five exons. Variations in the *MBL* gene change its peptide chain structure and influence the formation and stability of oligomeric MBL.^{8,9} Five widely studied *MBL2* gene polymorphisms were reasonably related to decreased MBL serum levels. These include three structural variants of exon 1, namely, codon 52 (rs5030737C/T, A/D), codon 54 (rs1800450G/A, termed A/B), and codon 57 (rs1800451G/A, termed A/C) (wild-type allele termed A and the three variant alleles [D, B, and C] collectively termed O), and two promoter variants at –550 (rs11003125G/C, termed H/L) and –221 (rs7096206G/C, termed Y/X).^{10–12}

The function of MBL is known to be influenced by common SNPs that are found in the promoter region and in exon 1, and these SNPs affect gene expression levels, the MBL protein structure, and MBL serum levels.¹³

Low MBL plasma levels, mainly due to genetic influences, have been largely associated with susceptibility to invasive infections and a poor outcome.¹⁰ On the other hand, the excessive activation of this protective system may be responsible for a detrimental unbalanced inflammatory and coagulation response, as observed in inflammatory diseases, transplant rejections, and diabetic nephropathy.¹⁴ Whether MBL can influence susceptibility to common pathogens and the development of severe infections has been investigated, but there is still no consensus on the clinical relevance of its deficiency or the indications for replacement therapies.¹⁵

The aim of this study was to determine whether the genetic polymorphisms in exon 1 of the *MBL2* gene (rs1800450 and rs1800451) and serum MBL levels could serve as markers for the early prediction of neonatal sepsis in neonatal intensive care units (NICUs).

Patients and Methods

This is a case-control study that was conducted from January 2016 to September 2018. The study included 114 neonates admitted to the NICU of Cairo University Children Hospital El Mounira. They were divided into two groups. Group 1, the patient group, included 64 neonates who met any of the following criteria for sepsis: (1) temperature instability (fever or hypothermia); (2) circulatory dysfunction (tachycardia, bradycardia, hypotension, or a delayed capillary refill time); (3) respiratory dysfunction (tachypnoea, grunting, cyanosis, apnoea, the need for supplemented oxygen, or the need for ventilation); (4) gastrointestinal dysfunction (feeding intolerance, abdominal distention, or bloody stool); or (5) neurological dysfunction (irritability, lethargy, hypotonia, or convulsion).¹⁶ The changes in hematological parameters were processed according to the Rodwell et al hematological scoring system (HSS) for the diagnosis of neonatal sepsis.¹⁷ The exclusion criteria were as follows: major congenital anomalies, hypoxic ischemic encephalopathy, or a suspected inborn error of metabolism. Group 2, the control group, included 50 neonates who had no clinical or laboratory signs of sepsis. The study was approved by the local ethics committee of the faculty of medicine–Cairo University, and informed consent was obtained from all parents.

Every neonate was subjected to (1) the acquisition of a full history involving the maternal history of premature rupture of membranes >18 hours, maternal history of fever, maternal history of antenatal steroid or antibiotic use, maternal history of pregnancy-related diseases (diabetes, preeclampsia), and neonatal history, which included the gestational age, birth weight, sex, Apgar score, and mode of delivery (vaginal or cesarean section); to (2) a full clinical examination including vital sign measurements (heart rate, respiratory rate, blood pressure, and temperature), a general examination and a systemic examination (cardiac, respiratory, abdominal, and neurological); and to (3) laboratory investigations, where venous blood samples were collected into sterile vacutainers, the serum was separated and stored at –20°C until the time of assessment, and EDTA blood for DNA extraction was stored at –20°C until extraction.

Laboratory Workup

(1) Serum MBL levels were measured using ELISA (NOVA ELISA Kit, Bioneovan Co., Ltd. Beijing, China) according to the manufacturer's instructions.

(2) Genotyping of the *MBL2* gene was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for codons 54 (rs1800450) and codon 57 (rs1800451) of exon 1. All laboratory workups were performed at the chemical pathology laboratories, Faculty of Medicine, Cairo University.

Mannose-Binding Lectin Genotyping

DNA was extracted from all EDTA blood samples using the salting out technique.¹⁸ Extracted DNA was then amplified at exon 1 codons 54 and 57 of the *MBL2* gene using polymerase chain reaction using the following primers: forward primer, 5'-GTA GGA CAG AGG GCA TGC TC-3' and reverse primer, 5'-CAG GCA GTT TCC TCT GGA AGG -3'. The following amplification protocol was used with a computerized Biometra thermocycler: initial denaturation at 95°C for 6 minutes, followed by 35 cycles of denaturation at 95°C for 40 seconds, annealing at 57.8°C for 40 seconds, and extension at 72°C for 40 seconds, with a final extension at 72°C for 10 minutes. The amplified 349-bp DNA band was then digested with BanI and MOBII restriction enzymes for codons 54 and 57, respectively. The wild-type *MBL2* allele for all codons was designated as the A allele, which was cut with the BanI enzyme into two bands (260 and 89 bp), while the variant B allele for codon 54 remained uncut (349 bp). For codon 57, the wild-type allele remained uncut (349 bp) by the MOBII enzyme, while the variant C allele was cut into two bands (270 and 79 bp). The digested products were detected in a 3.5% agarose gel containing ethidium bromide by performing electrophoresis on a gel electrophoresis apparatus and visualized by UV transillumination (Promega, United States).

The results of other routine laboratory investigations, including CBCs, CRPs, BUNs, creatinine measurements, liver function tests, urine analyses, urine cultures and sensitivity, and blood cultures, were collected from the patient records.

Statistical Analysis

Statistical analysis was done using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, United States). Quantitative data were expressed as mean $\pm$ standard deviation (SD) and compared using *t*-test when normally distributed, and as median and range using Mann-Whitney U test when not normally distributed. Allele frequency was calculated using

the allele counting method (each individual is represented by 2 alleles and allele frequency = number of mutated alleles/total number of alleles). Genotype frequencies fulfilled Hardy Weinberg equilibrium. The Chi-square test was used to compare allele and genotype frequency among different studied groups. The ROC curve was used to deduce a cutoff level of MBL. A *p*-value < 0.05 was considered significant.

Results

Among the 64 septic neonates, 41 (64.1%) were born before 37 weeks of gestational age (preterm) and among the non-septic neonates, 29 (58.0%) were preterm. Demographic and clinical data of the studied groups are summarized in ►Table 1.

Most of the neonates in the septic group presented with respiratory distress syndrome on admission (78.1%), while 9.3% had jaundice, 6.3% had seizures, and 6.3% were growing neonates (►Fig. 1). To establish feeding,

hematological scoring system (HSS) results of the studied groups are presented in ►Table 2. The hemoglobin and platelet counts were significantly lower in the septic group than in the non-septic group, but the total leukocyte count, immature neutrophils, immature neutrophil/total neutrophil ratio (I/T) and immature neutrophil/mature neutrophil (I/M) ratios were significantly higher in the septic group than in the non-septic group.

The biochemical parameters of the studied groups are presented in ►Table 3. Total bilirubin and albumin levels were higher in the non-septic group than in the septic group. The median CRP value in the septic group was 48 mg/dL.

Urea, creatinine, and AST levels were significantly higher in the septic group than in the non-septic group (*p* < 0.05), while there were no significant differences in the ALT and direct bilirubin levels (*p* = 0.68 and 0.077, respectively) between the groups.

Table 1 Demographic and clinical data of the studied groups

	Septic group n = 64	Non-septic group n = 50	<i>p</i> -Value
Gestational age (weeks)*	34.3 $\pm$ 3.5	34.0 $\pm$ 2.9	0.422
Head circumference (cm)*	32.9 $\pm$ 2.9	33.1 $\pm$ 2.9	0.65
Birth weight (gm)*	2202.5 (760.0–5200.0)	1500 (900–3470)	0.359
Length (cm)*	44.2 $\pm$ 3.8	45.1 $\pm$ 5.0	0.028
Age (days)**	1 (1–23)	2 (1–11)	0.032
Maturity			0.509
Preterm	41 (64.1%)	29(58.0%)	
Full term	23 (35.9%)	21 (42.0%)	
Sex			0.868
Male	31 (48.4%)	25(50.0%)	
Female	33 (51.6%)	25(50.0%)	
Turbid amniotic fluid			<0.001
Positive	32 (50.0%)	0 (0.0%)	
Negative	32 (50.0%)	31 (100.0%)	
Mode of delivery			0.082
NVD No	13 (20.3%)	18(36.0%)	

Note: Data are represented as * the mean $\pm$ standard deviation (SD) and ** the median (range) or number and percentage. A *p*-value < 0.05 is significant. Abbreviation: NVD: normal vaginal delivery.

Fig. 1 The clinical diagnosis of the studied groups on admission.

In the septic group, blood cultures were positive for 54 patients (84.4%) and negative for the remaining 10 (15.6%). In the septic group, *Klebsiella* showed the highest incidence (46.5%), and Amikin was the antibiotic with the highest sensitivity (25.6%) (►Fig. 2).

Results of Serum Mannose-Binding Lactose and MBL2 Genotyping

The serum levels of MBL were significantly lower in the septic group than in the non-septic group, ($p = 0.028$) (►Table 4). Using ROC curve, serum MBL levels ≤ 0.726 $\mu\text{g/mL}$ had a sensitivity of 53.1%, specificity of 74.0%, PPV of 72.3%, NPV of 55.2%, and accuracy of 62.3% for diagnosis of sepsis. Using

this cutoff value, MBL deficiency was significantly more frequent in septic patients (53.1%) compared with the non-septic group (26%) ($p = 0.004$) (►Fig. 3).

The MBL2 genotyping at codon 54 and codon 57 of exon 1 (►Table 4) showed no statistically significant differences between the septic and non-septic groups with respect to genotype frequency ($p = 0.102$ and 0.356 , respectively) or allele frequency ($p = 0.282$ and 0.394 , respectively). However, at codon 57, the frequencies of the polymorphic genotype (AC) and the polymorphic C allele were higher in the septic group than in the non-septic group, but the difference did not reach statistical significance ($p = 0.356$ and 0.394 , respectively).

Neither the serum MBL levels nor the MBL2 genotype was statistically significantly different when we divided the study groups into preterm and full-term infants, as shown in ►Table 5 (►Fig. 4).

Table 2 The hematological scoring system results of the studied groups

	Septic group n = 64	Non-septic group n = 50	p-Value
Hb (g/dL)	13.4 $\pm$ 3.3	15 $\pm$ 2.7	0.017*
TLC (cell/mm ³)	14.6 (3.3–66.0)	10.0 (5.1–21.0)	<0.001
Platelets (cell/mm ³)	134.0 (8.0–598.0)	241.0 (113–549.0)	0.001
Total neutrophils (%)	58.0 (18.0–88.0)	46.0 (22.0–77.0)	0.003
Immature neutrophils (%)	16.0 (0.0–46.0)	2.0 (0.0–11.0)	<0.001
I/T ratio	0.3 (0.0–0.8)	0.04 (0.0–0.19)	<0.001
I/M ratio	0.43 (0.0–7.6)	0.05 (0.0–0.83)	<0.001

Note: All data are represented as the median (range), except *hemoglobin levels are represented as the mean $\pm$ standard deviation.

Abbreviations: Hb: hemoglobin, TLC: total leucocytic count, I/T: immature neutrophil/total neutrophil ratio, I/M: immature neutrophil/mature neutrophil ratio, p-Value < 0.05 is significant.

Table 3 Biochemical parameters of the studied groups

Biochemical parameters	Septic group n = 64	Non-septic group n = 50	p-Value
Urea (mg/dL)	47.0 (12.0–107.0)	36.0 (14.0–68.0)	0.002
Creatinine (mg/dL)	0.8 (0.2–1.7)	0.65 (0.3–1.0)	0.009
AST (units/L)	47.0 (6.0–233.0)	34.0 (13.0–75.0)	0.009
ALT (units/L)	24.5 (6.0–219.0)	24.0 (5.0–67.0)	0.680
Albumin (gm/dL)	2.9 $\pm$ 0.7	3.5 $\pm$ 0.6	<0.001*
Total bilirubin (mg/dL)	7.85 (0.0–18.0)	11.1 (4.5–23.0)	0.004
Direct bilirubin (mg/dL)	0.7 (0.0–1.5)	0.89 (0.15–1.7)	0.077

Note: All data are represented as the median and range except *albumin is expressed as the mean $\pm$ standard deviation. A p-value < 0.05 is significant.

Fig. 2 Results of blood cultures in the septic group.

There were no significant associations between the mean serum MBL levels and *MBL2* genotypes and alleles when premature and full-term infants in the septic and non-septic groups were compared.

The correlations between MBL levels and clinical characteristics showed that only birth weight and head circumference were negatively correlated with serum MBL levels ($r = -0.248$ and -0.344 , respectively, and $p = 0.048$ and 0.005 , respectively) (►Fig. 5A, B, ►Table 6).

There were no associations between *MBL2* gene polymorphisms at codon 54 or 57 of exon 1 and MBL deficiency in the sepsis group.

Discussion

The importance of MBL function during the first few months of life, when the efficacy of innate immunity is crucial, has

Table 4 MBL serum levels, genotypes and alleles frequency (codons 54 & 57) among septic and non-septic groups

		Septic group n = 64	Non-septic group n = 50	p-Value
		No (%)	No %	
MBL levels (µg/mL)		0.69 (0.18–3.84)	0.82 (0.05–1.59)	0.028
Codon 54 Genotypes Alleles	AA	30 (46.9)	13 (26.0)	0.055
	AB	25 (39.1)	30 (60.0)	
	BB	9 (14.1)	7 (14.0)	
	A allele	66.4%	56.0%	0.108
	B allele	33.6%	44.0%	
Codon 57 Genotypes Alleles	AA	46 (71.9)	40 (80.0)	0.317
	AC	18 (28.1)	10 (20.0)	
	A allele	85.9%	90.0%	0.354
	C allele	14.1%	10.0%	

Abbreviation: MBL, mannose-binding lectin presented as median (range), $p < 0.05$ is considered significant

led some researchers to propose that MBL should be included in biomarker panels for the early detection of neonatal infections.⁶ The serum levels of MBL were significantly lower in the septic group than in the non-septic group, ($p = 0.028$) (►Table 4). Using ROC curve, serum MBL levels ≤ 0.726 µg/mL had a sensitivity of 53.1%, specificity of 74.0%, PPV of 72.3%, NPV of 55.2%, and accuracy of 62.3% for diagnosis of sepsis. Using this cutoff value, MBL deficiency was significantly more frequent in septic patients (53.1%) compared with the non-septic group (26%) ($p = 0.004$). Our results were in agreement with those of Frakking et al, Auriti et al, El-Shimi et al, Ozdemir et al, de Benedetti et al, Dzwonek et al, Wahab Mohamed et al, Xue et al, and who reported that neonates with neonatal sepsis had lower serum MBL levels than control neonates.^{19–25}

Frakking et al explained that MBL deficiency was associated with an opsonization defect and was associated with recurrent infections, especially in immunocompromised individuals. Neonates are considered to be immunocompromised because adaptive immunity has not yet developed, and immune defense depends on maternal antibodies and innate immunity. Therefore, neonates, especially in premature neonates admitted to the neonatal intensive care unit, are prone to developing infections that are sometimes life-threatening.¹⁹

The comparison of the MBL levels of the preterm and full-term infants in this study showed no significant difference ($p > 0.05$). This was in agreement with the reports by El-Shimi et al and Frakking et al, who found that the mean MBL serum level was insignificantly lower in preterm neonates than full-term neonates.^{19,21} The combination of prematurity and low MBL levels increased the risk of sepsis to 70%. This increase was because low MBL levels might be associated with a decreased capacity for the phagocytosis or opsonization of microorganisms.

The results of our study showed no association between *MBL2* gene polymorphisms at codon 54 or 57 of exon 1 and neonatal sepsis risk. The most common genotype in the septic group was AA (46.9%), followed by AB (39.1%) and BB (14.1%). These results were in agreement with the results by Klostergaard et al, who found that the A/B and A/C

Fig. 3 MBL deficiency ($\leq 0.726 \mu\text{g/mL}$) in the septic and non-septic groups. MBL, mannose-binding lactose.

polymorphisms had no significant association with sepsis risk.²⁶ Additionally, Huh et al reported that the A/B variant had no significant association with sepsis among Korean neonates with sepsis.²⁷ Koroglu et al reported similar results with regard to the common genotypes among the Turkish population, where genotype AA was the most common, followed by AB and BB.²⁸ In contrast to these findings, Ozkan et al demonstrated that the B allele of the A/B polymorphism was a risk factor for sepsis in Turkish patients.²⁹

The *MBL2* polymorphism at codon 54 (rs1800450) causes the Gly54Asp variation, which leads to the production of non-functional monomers that interfere with the formation of higher-order oligomers of the mannose-binding protein, leading to alterations in the protein's functional activity and circulating levels.^{30–32} Thus, the association of *MBL2* gene polymorphisms with the occurrence of sepsis is still controversial. In addition, gene polymorphisms are different between regions and ethnicities.

Table 5 MBL serum levels, genotypes, and allele frequencies among preterm and full-term infants in the septic and non-septic groups

		Septic group n = 64		Non-septic group n = 50		p-Value
		Preterm n = 41	Full term n = 23	Preterm n = 29	Full term n = 21	
MBL levels (ug/ml)		0.77 (0.20–3.84)	0.59 (0.18–1.23)	0.86 (0.05–1.30)	0.75 (0.49–1.59)	0.092
Codon 54						
Genotypes Alleles	AA	19 (46.3%)	11 (47.8%)	7 (24.1%)	6 (28.5%)	0.114
	AB	16 (39.1%)	9 (39.1%)	14 (48.3%)	14 (66.7%)	
	BB	6 (14.6%)	3 (13.1%)	8 (27.6%)	1 (4.8%)	
	A allele	65.9%	67.4%	48.3%	61.9%	0.135
	B allele	34.1%	32.6%	51.7%	38.1%	
Codon 57						
Genotypes Alleles	AA	30 (73.2%)	16 (69.6%)	20 (69.0%)	18 (85.7%)	0.550
	AC	11 (26.8%)	7 (30.4%)	9 (31.0%)	3 (14.3%)	
	A allele	86.6%	84.8%	84.5%	92.9%	0.618
	C allele	13.4%	15.2%	15.5%	7.1%	

Abbreviation: MBL, mannose-binding lectin, $p < 0.05$ is considered significant.

Fig. 4 Codon 57 (AA and AC) genotypes among septic and non-septic groups.

Fig. 5 Correlation of weight (A) and head circumference (B) with MBL level in the septic group. MBL, mannose-binding lactose.

The contribution of MBL deficiency to the pathophysiology of several respiratory diseases has been extensively analyzed. Lin et al demonstrated that MBL deficiency increased the risk of recurrent infective exacerbations in patients with chronic obstructive pulmonary disease and worsened the disease outcome.³³ MBL deficiency has been associated with respiratory disease progression in cystic fibrosis, as well as with bronchiectasis formation in common variable immunodeficiency. Chalmers et al suggested that MBL might be an important modifier of disease severity in non-cystic fibrosis bronchiectasis because patients with low MBL serum levels ($< 0.2 \mu\text{g/mL}$) displayed a higher frequency of chronic colonization with bacteria, more frequent severe exacerbations during follow up, and a worse quality of life.³⁴

Table 6 MBL deficiency in relation to genotypes of codons 54 and 57 in the septic group

	MBL Level ($\mu\text{g/mL}$)		p-Value
	MBL ≤ 0.726	MBL > 0.726	
	No. (%)	No. %	
Codon 54			
Wild AA ($n = 30$)	16 (53.3%)	14 (46.7%)	0.975
Mutant AB + BB ($n = 34$)	18 (52.9%)	16 (47.1%)	
Codon 57			
Wild AA ($n = 46$)	24 (52.2%)	22 (47.8%)	0.807
Mutant AC ($n = 18$)	10 (55.6%)	8 (44.4%)	

Conclusion

We found no association between *MBL2* gene polymorphisms and neonatal sepsis, but a higher frequency of polymorphic genotypes and alleles was observed in neonates with sepsis. Serum MBL levels did not differ between preterm and full-term neonates in the two groups. The serum levels of MBL were significantly lower in the septic group than in the non-septic group, ($p = 0.028$) (► **Table 4**). Using ROC curve, serum MBL levels ≤ 0.726 $\mu\text{g}/\text{mL}$ had a sensitivity of 53.1%, specificity of 74.0%, PPV of 72.3%, NPV of 55.2%, and accuracy of 62.3% for diagnosis of sepsis. Using this cutoff value, MBL deficiency was significantly more frequent in septic patients (53.1%) compared with the non-septic group (26%) ($p = 0.004$). Further investigations are needed with a large sample size to confirm that low serum MBL levels and the presence of B and C alleles of the *MBL2* exon 1 gene may be associated with neonatal sepsis among neonates.

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