DIAGNOSTIC VALUE OF MANNOSE-BINDING LECTIN SERUM LEVEL IN NEONATAL SEPSIS

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ABSTRACT

Background: Sepsis is one of the most important diseases in newborns and is responsible for 45% of late deaths in the neonatal intensive care units (NICUs).

Objective: The aim of the study was to evaluate mannose-binding lectin (MBL) serum level as adiagnostic value of neonatal sepsis.

Subjects and method: This case–control study was conducted on68 neonates admitted to the neonatal ICU. The 68 neonates were divided into two groups: the patient group and the control group. The patient group included34 cases with neonatal sepsis and the control group included 34 healthy neonates. Serum levels of MBL were measured by immunoassay. The results were tabulated and analyzed with SPSS.

Results: Serum MBL levels were significantly lower in the neonates with sepsis than in the control group (27.05 ± 8.43 vs. 73.4 ± 20.4 ng/ml; P < 0.001). The lowest MBL levels were detected in those infants with septic shock. MBL had high sensitivity (97.1%) and specifi city (100%) as well as positive (100%) and negative (94.4%) predictive values to detect sepsis.

Conclusion and key messages:MBL serum level could be considered a sensitive and specific marker for prediction of neonatal sepsis. Neonates with significant decrease in MBL are at increased risk for developing sepsis and septic shock.

Keywords: mannose-binding lectin, newborn infants, sepsis, sepsis.

INTRODUCTION

Neonatal sepsis, sepsis neonatorum, and neonatal septicemia are terms that have been used to describe the systemic response to infection in newborn infants. There is little agreement on the proper use of the terms i.e. whether their use should be restricted to bacterial infections, positive blood cultures, or severity of illness [1].

Early detection and management of neonates with doubtful sepsis are important to prevent dangerous and life-threatening complications. Compared with the obvious and useful therapeutic choices, the diagnosis of suspected neonatal sepsis is challenging. In premature infants, the diagnosis of sepsis is more complicated, because of the nonspecific clinical manifeststions and the deficiency of accurate diagnostic tests [2].

Neonatal sepsis may be categorized as early or late onset. Eighty-five percent of newborns with early-onset infection present within 24 hours, 5% present at 24-48 hours, and a smaller percentage of patients present within 48-72 hours. Onset is most rapid in premature neonates[3].

Mannose-binding lectin (MBL) is a plasma protein that plays an important role in the innate immune defence. It is antibody independent which comes into play within

the first 12 hours from microorganisms' contact MBL activates the lectin pathway of the
complement system by binding to various microorganisms. This leads to opsonization and enhanced phagocytosis[4]. MBL is an example of a pattern-recognition receptor present as a free protein in blood plasma. MBL binds to bacterial surfaces that display a particular arrangement of carbohydrate sugar residues, mannose or fructose Once MBL recognizes a pathogen, its lectin domain will bind to mannose, or other carbohyrate sugar residues on the pathogen surface, and activate the complement via the MB-lectin pathway .The complement system or "complements", helps the ability of antibodies and phagocytic cells to clear pathogens from an organism. It is part of the immune system called the innate immune system that is not adaptable and does not change over the course of an individual's lifetime. However, it can be recruited and brought into action by the adaptive immune system [5].

PATIENTS AND METHODS

We conducted this case–control study on a patient group of 34 cases with neonatal sepsis and a control group of 34 healthy neonates at the NICU. The study was approved by the research ethics committee. Both preterm and full-term newborn infants Gestational age > 30 weeks, Birth weight > 1.000 Kg , maternal fever or PROM>18, Lethargy and poor suckling were enrolled in the study. Informed parental consent was obtained for all babies in the study. Definitive sepsis was diagnosed if the patients had the combination of a positive blood culture and clinical or laboratory evidence of sepsis. Clinical markers of sepsis included poor circulation (pallor, hypotension, and tachycardia or bradycardia), increased oxygen requirements or ventilation parameters, temperature instability, lethargy or irritability, abdominal distension, and poor suckling. Laboratory markers included abnormal leukocyte count, increased I/T (immature to total neutrophil) ratio, low platelet count, and raised C-reactive protein. Infants with neonatal asphyxia, metabolic disease, congenital malformations, or neonatal major surgical problems were excluded.

All cases underwent the following:

- All neonates were subjected to full clinical history taking of newborns stressing on prenatal(premature labor,maternal peripartum fever or infection,premature rupture of membrane, amniotic fluid problems, maternal bleeding,history of maternal disease causing any neoantal distress. Socioeconomic status of his or her family and maternal age); Natal history(cord around neck, resuscitation at birth, invasive procedure, obstructed labor, mode of delivery, sex ,gestational age); Postnatal history(Age, sex, weight,Signs of respiratory distress,Feeding problems, Manifestations of neoantal sepsis as irritability, decreased activity jaundice, pallor, mottling, rashes, poor suckling, loss of interest in feeding, vomting, diarrhea, abdominal distension and convulsions)
- Thorough clinical examination: Generalexamination (apgar score,estimation of gestational age, level of consciousness, color, vital signs ,respiratory rate ,heart rate & temperature measurements),Regional examination(head , neck, limbs ,back and genitalia),systemicexamination (neurology ,cardiac, chest and abdomen),neurological examination (neoantal reflexes, tone and level of consciousness),cardiovascular examination(respiratory rate, signs of respiratory distress and apnea)abdominal examination(abdominal distension, hepatomegaly and splenomegaly, ascitis, dilated veins and rash).
- Laboratory investigations: This included complete blood count, differential white blood cell count, platelet count, C-reactive protein, blood culture they were performed using(Bact/ALERT 3D 160 for identification of the microorganism,vitek2 for antibiotic susceptibility testing (ast) and treatment given) and serum MBL.

Measurement of serum mannose-binding lectin level: Sample collection:Four milliliters of venous blood were collected on EDTA; 2 ml was left clotted to be separated for CRP. Other 2 ml was used for assuring Mannose binding lectin. The samples were submitted immediately to centrifugation for 15 minutes. The plasma was obtained and stored at -20°C, for assessing Mannose binding lectin.Determination of plasma Mannose binding lectin levels by ELISA technique[6].Plasma Mannose binding lectin level was assessed by an ELISA methodology using Mannose binding lectin (human) kit, supplied by Chongqing Biospes Co.,Ltd.

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS version 16.Continuous Quantitative variables e.g. age were expressed as the mean ± SD & median (range), and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Continuous data were checked for normality by using Shapiro Walk test. Independent samples Student's t-test was used to compare two groups of normally distributed data.Mann-whitney test was used to compare two groups of not-normally distributed data.Categorical data were compared using Chi-square test (χ^2 test).All tests were two sided. p-value < 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically insignificant (NS). Validity of the screening test (MBL) was assessed in the terms of sensitivity, specificity, predictive value positive, predictive value negative and accuracy.

RESULTS

The clinical characteristics of the study groups are summarized and illustrated in Table 1. There was highly statistical significant difference between the studied groups as regarding respiratory rates. But there were non-significant difference among them in pulse rate and in temperature.

Our results showed that there was highly statistical significant difference between both groups as regarding hemoglobin, PT, TLC and IT ratio.It was noticed that all babies in sepsis group had lower levels in all lab characteristics in comparison to the control group .However IT ratio was higher in sepsis than in control group .There was highly statistical significant difference between the studied groups as regarding different types of organisms and blood culture which was positive in 76.5 % of sepsis group. There was highly statistical significant difference between both groups as regarding CRP and MBL levels,as shown in table 2.

Table 3 shown that there was significant positive correlation between MBL level and Hb, PT and TLC and non-significant correlation between MBL and gestational age and birth weight.There was negative correlation between MBL level and CRP, pulse and respiratory rates which was significant only in CRP.

Table 4 shows that MBL had high sensitivity (94.1%)specifi city (100%), and positive (100%) and negative (94.4%) predictive values to detect sepsis (Fig. 1). CRP had sensitivity (75%)specifi city (65%), and positive (68.4%) and negative (73.3%) predictive values to detect sepsis

Table 1 Vital signs of the studied groups:

Vital signs	sepsis group(n=34)	control group(n=34)	t-test	p-value
Pulse:				
Mean±SD	136.7±15.6	133.7±9.09	0.967	0.337(NS)
Median(range)	139.5(89-170)	136(112-150)		
Respiratory Rate:				
Mean±SD	56.1±10.9	45.5±4.83	5.099	<0.001(HS)
Median(range)	55.5(30-75)	45(38-56)		
Temperature:				
Mean±SD	37.1±0.48	36.9±0.25	0.998	0.332(NS)
Median(range)	37(36.1-38.5)	37(36.5-37.4)		

S: significant difference (p<0.05). NS: non-significant difference (p>0.05).
*:Mann-whitney test.

Table 2 Laboratory findings of the studied groups:

Laboratory findings	sepsis group(n=34)	Control group(n=34)	t-test	p value
Hemoglobin				
Mean ±SD	11.9±1.56	15±1.14	-9.256	<0.001(HS)
Median (range)	14(9.4-23)	14.8(11.4-17.5)		
Platelet count				
Mean ±SD	130.1±77.1	240.4±71.8	-4.987	<0.001(HS)
Median (range)	117.5(38-298)	229(136-420)		
WBC				
Mean±SD	8.28±3.05	14.4±3.41	-7.728	<0.001(HS)
Median(range)	9.5(3.2-33)	12(6.8-18)		
IT ratio				
Mean±SD	0.27±0.08	0.16±0.02	-5.658	<0.001(HS)
Median(range)	0.29(0.06-0.39)	0.16(0.12-0.20)		
CRP				
Mean±SD	49.2±35.5	3.77±0.90	7.464	<0.001(HS)
Median(range)	48(6-96)	3.6(2.4-5.9)		
MBL				
Mean±SD	27.05±8.43	73.4±20.4	- 12.24	<0.001(HS)
Median(range)	23.5(15-43)	67.5(46-100)		
Blood culture [n(%)]				
+	26(76.5)	0(34)		
-	8(23.5)	0(100)		

CRP, C-reactive protein; MBL, mannose-binding lectin; WBC, white blood cell, aThe values are calculated using the Mann–Whitney U-test, bThe value is calculated using the independent-sample Student's t-test, cThe value is calculated using the $\chi 2$-test, $P < 0.05$, significant.

Table 3 The correlation between MBL level and different parameters among the studied group:

Variable	MBL level	
	r	p
CRP:	**-0.677**	**<0.001****
Hemoglobin:	**0.622**	**<0.001****
PT:	**0.592**	**<0.001****
TLC:	**0.588**	**<0.001****
Pulse:	-0.127	0.304
Respiratory rate:	-0.460	<0.001**
Gestational age:	0.094	0.444
Birth weight:	0.148	0.228

Table4 C-reactive protein and mannose-binding lectin as markers for neonatal sepsis

Cutoff point	AUC	Sens.	Spec.	PVP	PVN	Accuracy(CI)	P value
MBL≤ 42	0.918	94.1%	100%	100%	94.4%	97.1%	<0.001
CRP≥5.95	0.782	75%	65%	68.4%	73.3%	70.6%	<0.001

AUC, area under the curve; CI, confi dence interval; CRP, C-reactive protein; MBL, mannose-binding lectin; PVP Predictive value positive; PVN Predictive value negative; SN, sensitivity; SP, specifi city; l, ‡P < 0.001 (HS), P < 0.001 (HS).

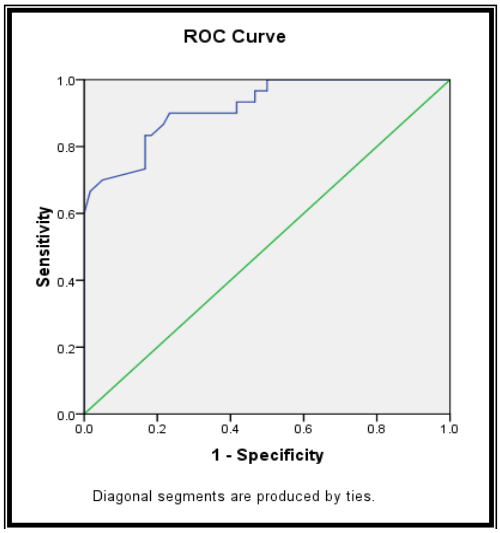

Figure (1): Receiver Operator Curve of MBL level as an indicator for sepsis.

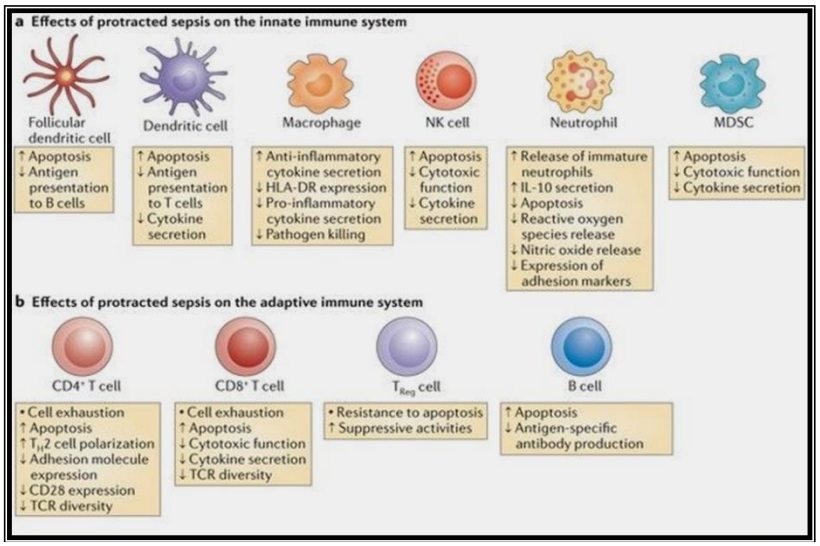

Figure (2): a | Sepsis has diverse and important effects on all cellular elements that comprise the innate immune system. Sepsis rapidly triggers extensive apoptosis in follicular dendritic cells, dendritic cells, immature macrophages, natural killer (NK) cells and myeloid-derived suppressor cells (MDSCs). Conversely, sepsis delays neutrophil apoptosis a result that is thought to be secondary to the mechanisms of neutrophil activation. After initial mobilization and activation of neutrophils, subsequent neutrophils that are released from the bone marrow have lower bactericidal functions and decreased cytokine production. Recent data show that a subset of neutrophils release large amounts of the immunosuppressive cytokine interleukin-10 (IL-10). Decreased HLA-DR expression on antigen-presenting cells, including monocytes, macrophages and dendritic cells is a hallmark of sepsis, which may impair the optimal presentation of microbial antigens to T cells.

b | Sepsis causes a marked loss of CD4+ and CD8+ T cells, as well as B cells. Regulatory T (TReg) cells are more resistant to sepsis-induced apoptosis and, consequently, there is an increased percentage of TReg cells in the circulation of patients with sepsis relative to the other lymphocyte subsets. This contributes to the formation of a more immunosuppressive phenotype. Surviving CD4+ and CD8+ T cells either shift from a pro-inflammatory T helper 1 (TH1) cell phenotype to an anti-inflammatory TH2 cell phenotype or develop an 'exhaustive' phenotype that is characterized by increased programmed cell death 1 expression and reduced cytokine secretion. CD4+ T cells have decreased expression of CD28 and reduced T cell receptor (TCR) diversity, which are both likely to contribute to the impaired [7].

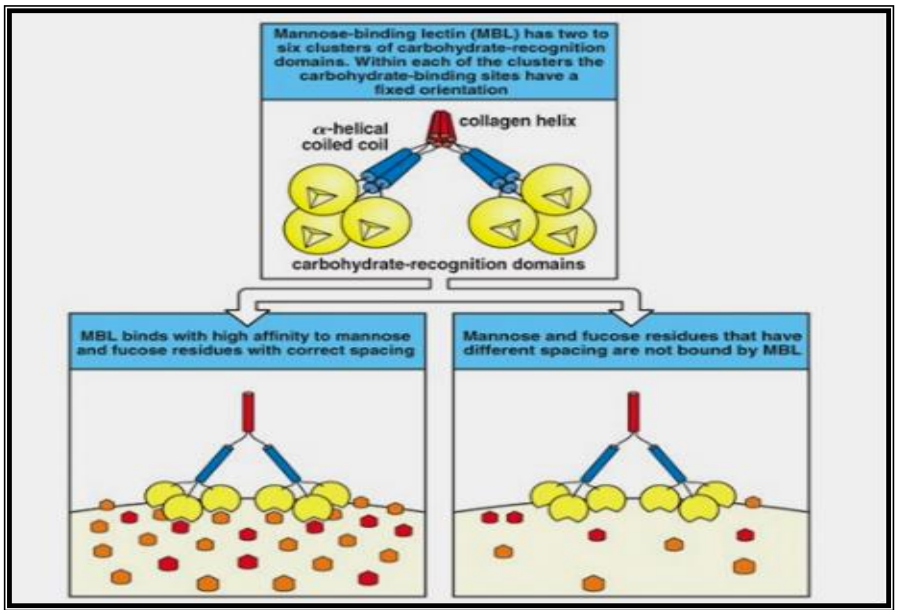

Figure (3): MBL recognizes bacterial surfaces by their particular spacing of carbohydrate residues.[5]

DISCUSSION

Sepsis is a common and lethal syndrome [8]. Constitute a large proportion of the critically ill population and, although outcomes have improved,mortality remains higher than 25–30%, and even 40–50% when shock is present. In our study, there was no statistically significant difference between septic and control groups in age and sex except gestational age and MBL [9]. **Auriti et al., (2010)** investigated the association of Mannose-Binding Lectin (MBL) serum levels with Nosocomial Sepsis, their changes overtime during infection, their relation with pathogens, with the MBL_2 genotype and their relationship with mortality. Low MBL levels on admission increased the risk of infection, independently from gestational age and invasive procedures. The median peak MBL level during infection was higher than the median level on admission and was correlated with it[10]. **Özkan et al., (2012)** determined the serum Mannose-Binding Lectin (MBL) levels and the frequency of MBL gene polymorphisms in infants with neonatal sepsis. Serum MBL levels were significantly lower in infants with sepsis compared with the control group .Additionally, in the study group, the mean serum MBL levels were found to be significantly lower in the premature infants compared with the term infants [11].

DE Benedetti et al., (2014) determined that low Serum levels of Mannose Binding Lectin are a risk factor for neonatal sepsis by measured serum MBL levels on admission by (ELISA).Serum MBL levels on admission were significantly lower in infants with sepsis; interquartile range ,particularly in those with confirmed sepsis compared with infants without sepsis, and infants with CoNS-positive blood culture. Results suggest that the simple measurement of serum MBL levels on admission may help to identify neonates at high risk of developing sepsis in the NICU [12].

CONCULSION

Mannose Binding Lectin is a novel marker that may be used for diagnosis of neonatal sepsis. MBL could be used in the diagnosis of neonatal sepsis with more efficacy of that of CRP. The study revealed that neonates tend to have low MBL serum levels. MBL serum level could be considered as sensitive and specific marker for prediction of neonatal sepsis.

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