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Research Article

Neonatal Sepsis After Birth: Microbiological and Immunomodulatory Aspects

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Abstract

A clinical phenomenon known as neonatal sepsis is characterized by infection-related signs and symptoms in the first month of life, either with or without bacteremia. The main objective of the present study was to study the role of Toll-like receptors as a diagnostic marker for neonatal bacterial sepsis and its possibilities for differentiation between viral and bacterial neonatal infections. To gather information from patients, a cross-sectional study design was used. Study sample included 50 neonates who were categorized as a low-risk group and a high risk-group for sepsis. Both blood cultures and toll like receptors were investigated. Results showed that 18% of blood culture were positive, and the most prevalent bacterium was Group B *Streptococcus*. Comparing high-risk group with low-risk group for toll like receptors, CRP, and WBC showed that TLRs, CRP were higher in high-risk group compared with low-risk group ($p < 0.05$), except the levels of WBC that did not show significant variations ($p = 0.072$). Together, the study's findings showed how crucial it is to use toll-like receptors for diagnostic purposes.

Keywords: *Neonatal Sepsis; Blood Culture; Group B Streptococcus; Toll Like Receptor; Diagnostic Marker*

Introduction

Neonatal sepsis is a medical condition that manifests as infection-related symptoms in the first month of life, either with or without bacteremia. It covers a variety of infant systemic diseases, including pneumonia, arthritis, osteomyelitis, septicemia, meningitis and urinary tract infections [1]. The most frequent cause of newborn mortality, sepsis accounts for 30 to 50 percent of all neonatal deaths in underdeveloped nations. Up to 20% of newborns are thought to suffer sepsis, while only 1% of them pass away from sepsis-related causes. With early detection, appropriate antibiotic medication, and vigorous supportive care, sepsis-related death can be generally avoided [2].

A class of transmembrane receptors called toll-like receptors (TLRs) is crucial for the host's defense against microbes. Human immune-related cells like monocytes, neutrophils, macrophages, dendritic cells, T cells, B cells and NK cells primarily express TLRs [3]. They start the production of cytokines, chemokines and certain adhesion molecules, which all contribute to the inflammatory response [4]. There are currently about eleven human TLRs known, and each one is involved in a certain intracellular signaling pathway. TLRs 1, 2, 4, 5 and 6 are typical for bacterial products, TLRs 3, 7 and 8 are characteristic for viral infection and TLR-9 is associated with bacterial and viral inflammatory response [5].

Sepsis demonstrated that TLR-2 and TLR-4 expression on monocytes in septic patients is higher than in healthy persons, indicating that toll-like receptors play a critical role in the modulation of systemic responses to pathogens during sepsis [6]. More than 2 million of

newborns and infants under the age of 6 months die each year worldwide due to infection, despite advances in medicine and technology, particularly in developing countries approximately one-third of neonatal death is because of severe infections [7].

The unique features of neonatal immune system, although affords some protection against infectious diseases in a number of infants, contributes to the impaired response to a range of pathogens, resulting in their enhanced susceptibility to severe disease. This burden of infection highlights early-life susceptibility, and the urgent need for a better understanding of the immune mechanisms that contribute to the neonatal susceptibility [8].

Neonatal sepsis is a clinical syndrome that includes signs and symptoms of infection in the first month of life, either with or without bacteremia. This includes a variety of infant systemic illnesses include septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. Obvious infections like conjunctivitis and oral thrush are typically excluded from the definition of newborn sepsis [9].

Study Objectives

The main objective of the present study was to study the role of Toll-like receptors as a diagnostic marker for neonatal bacterial sepsis and its possibilities for differentiation between viral and bacterial neonatal infections.

Methods and Subjects

Study design and setting

A cross-sectional study was conducted to collect data from participants within the same time frame. The study was conducted in NICU of Al-Azhar Assiut University hospital from January 2016 to December 2017.

Study sample

A total of 50 neonates, 25 of them at high-risk of sepsis that met the inclusion criteria, and 25 neonates with low-risk sepsis.

Inclusion criteria

The following criteria were applied: term neonates, normal birth weight neonates, neonates with risk factors of sepsis such as membrane rupture for more than 18 hours, maternal fever more than 38ºC and fetal heart rate settled > 160 x / min.

Study procedure

Blood culture

All blood cultures were carried out for at least 72 hours. By applying enhanced bacteriological techniques (BACTEC) and BACT/ALERT blood culture systems, bacterial growth was discovered within 12 - 24 hours. With these cutting-edge methods, bacteria might be found in concentrations as low as 1 - 2 colony-forming units (cfu) per milliliter.

Human toll like receptor 2 (TLR2) ELISA kit measurement

ELISA kit using Sandwich-ELISA was followed. This kit's Micro Elisa strip plate has been pre-coated with a TLR2-specific antibody. The appropriate Micro Elisa strip plate wells were filled with standards or samples, which were then mixed with the designated antibody.

Each Micro Elisa strip plate well was then filled with a Horseradish Peroxidase (HRP)-conjugated antibody that was specific for TLR2. Free parts were removed by washing. Each well receives a dose of the TMB substrate solution. Only the wells containing TLR2 and HRP-conjugated TLR2 antibodies had blue appearances before turning yellow after the stop solution was added. At a wavelength of 450 nm, the optical density (OD) was measured spectrophotometrically. The relationship between the OD value and TLR2 concentration is linear.

Human toll like receptor 8 and 9 (TLR8, 9) quantification using an ELISA kit

The same procedure used in measuring TLR2 was followed for measuring TLR8 and TLR9.

Results

Socio-demographic characteristics, history data and birth weight among studied groups (High and low risk neonates

As shown in table 1, the general characteristics of study sample among high-risk group and low risk group did not significantly vary for all variables ($p > 0.05$ for all), except for the variable "mode of delivery" in which elective C.S was more among high-risk group (80%) compared with low-risk group (60%). Both emergent and vaginal tube were prevalent among low-risk group. The differences in delivery mode were statistically significant ($p = 0.045$).

	High risk group (n = 25)		Low risk group (n = 25)		
	n	%	n	%	P-value
Age at entry in the study (Mean ± SD)					
Age (days)		2 ± 2	3 ± 2		0.052
Sex distribution					
Females	10	40.0%	18	72.0	1.000
Males	15	60.0%	7	28.0	
Residence					
Rural	13	52.0%	15	60.0%	0.792
Urban	12	48.0%	10	40.0%	
Mode of delivery					
Elective C.S	20	80.0%	15	60.0%	*
Emergent C.S	3	12.0%	6	24.0%	0.045
Vaginal delivery	2	8.0%	4	16.0%	
Maternal medications					
Corticosteroid	1	4.0%	1	4.0%	0.914
Insulin	3	12.0%	3	12.0%	
Anti-hypertensive	6	24.0%	5	20.0%	
Antibiotics	4	16.0%	4	16.0%	
Gestational age (GA)					
Gestational age by Ballard score Mean ± SD	38.15 ± 2.19		38.8 ± 2.12		0.815
Mean ± SD Birth weight, (kg)					
Birth weight (kg)	3.42 ± 0.48		3.21 ± 0.51		0.170
Apgar score					
Apgar score (New Ballard score) Mean ± SD					0.052
1st minute	7 ± 2		8 ± 1		
5th minute	9 ± 1		10 ± 0		

Table 1: *Socio-demographic characteristics, history data and birth weight among studied groups (High and low risk neonates).*

Blood culture results

As it can be seen from table 2, the prevalence of positive cultures was 18%. The most frequent isolated bacteria were 20% group B *streptococcus*, 8% *E. coli*, 4% *Staphylococcus* and 4% *Klebsiella* species.

	n = 50	%
Blood culture		
Positive	9	18
Negative	41	82
Isolated organism from high-risk group		
	n = 25	**%**
Group B *streptococci* (GBS)	5	20
E. coli	2	8
Staph. aureus	1	4
Klebsiella	1	4

Table 2: *Results of blood culture.*

The relationships between study groups in relation to TLRs and CRP and WBC

As indicated in table 3, the differences in means for TLR2, LR8, TLR9 and CRP were statistically significant ($p < 0.05$) and patients who were classified in high-risk group had higher levels than their counterparts in low-risk group. On the other hand, the difference of WBCs levels was not statistically significant ($p = 0.072$).

	High risk group (n = 25)	Low risk group (n = 25)	P value
	Median (IQR)	Median (IQR)	
TLR2	1245 (531.5 - 2197.5)	573 (504.5 - 1500)	0.049*
TLR9	2394 (1355.5 - 4658)	1333 (829 - 1581.5)	0.001**
TLR8	2341 (484.5 - 6984.5)	1271 (804.5 - 1925)	0.001**
CRP	27.2 (6.8 - 120.5)	3.8 (2.75 - 5.25)	0.001**
WBCs	13.6 (8.4 - 15.85)	9.8 (7.05 - 13.6)	0.072

Table 3: *Comparison between studied groups as regard Toll-like receptors (2, 8 and 9), CRP and WBCs.*

**Statistically significant difference ($p < 0.05$), **Highly statistically significant difference ($p < 0.01$), IQR: Interquartile Range.*

The relation of toll like receptors (2, 8 and 9) to CRP and blood culture results in high-risk neonates

As indicated in table 4, there were significant differences ($p < 0.05$) in the levels of TLR2, TLR9, CRP and WBCs in groups with patients who were positive for CRP and blood culture compared with patients in groups with positive CRP and negative for blood culture. The difference for TLR8 was not statistically significant ($p = 0.65$).

Variables	CRP +ve and blood culture +ve (n = 9)	CRP +ve and blood culture -ve (n = 16)	
	Median (IQR)	Median (IQR)	P value
TLR 2 pg /ml	1380 (1218-2197.5)	809 (531.5 - 2016)	0.01*
TLR 9 pg /ml	2683 (1798.5-4658)	1562 (1355.5-3929)	0.001**
TLR 8 pg /ml	2594 (1986-6984.5)	2613 (484.5-5672)	0.65
CRP mg / dl	68 (49-120)	18.5 (6.8 - 63.6)	0.01*
WBCs (10^3/mm^3)	14.95 (11.3-15.85)	9.2 (8.4 - 10.2)	0.01*

Table 4: *Relation of toll like receptors (2, 9 and 8) to CRP and blood culture results in high-risk neonates.*

Discussion

A clinical state of bacteremia with signs and symptoms of infection in the first four weeks of life is known as neonatal sepsis. Pathogenic bacteria that enter the bloodstream can produce septicemia, an intense infection with little localization, pneumonia, or meningitis when they become predominately localized to the lungs or meninges [10]. Infants, particularly preterm infants, suffer high mortality and morbidity as a result of neonatal sepsis [11]. Despite significant advancements in newborn intensive care medicine, little is known about the etiology of neonatal sepsis, which quickly progresses from infection to a systemic inflammatory response. Without a shadow of a doubt, sepsis is a clinical symptom of an unbalanced immune response to invading infections [12]. However, multiple clinical studies using immuno-modulatory methods in newborns with known or suspected illnesses did not increase survival [13]. The discovery of a diversified system of pattern recognition receptors (PRR) based on *in vitro* research or data obtained in the murine system led to the development of several novel theories surrounding the etiology of sepsis [14]. As a result, animals with genetic TLR4 deficiencies or mutations have impaired LPS signaling and are more resistant to endotoxin shock [15], whereas animals lacking TLR2 and MyD88 are more vulnerable to *Staphylococcus aureus* infections [16].

A study using a mouse model of experimental poly-microbial sepsis provides the strongest support yet for the pathophysiological relevance of TLR in infectious illnesses. After sepsis was created, TLR2 and TLR4 gene expression and protein levels rose in the liver and lungs. These changes were highly linked with sepsis-induced fatality [17]. TLR 2 is important in the recognition of live *Staphylococcus epidermis*, a nosocomial pathogen that causes catheter-associated bacteremia in the immune-compromised host. Following i.v. *Staphylococcal epidermis*, TLR 2-deficient mice have decreased cytokine and chemokine production and impaired clearance of bacteremia [18]. On intracellular vesicles, there are TLRs 3, 7, 8, and 9. (endosomes, lysosomes). TLR 3 recognizes double-stranded RNA. TLR 5 binds bacterial flagellum. TLRs 7 and 8 both recognize single-stranded RNA. TLR 9 recognizes CpG-rich hypo-methylated DNA [19]. Experimental endotoxemia in adults revealed up-regulation of TLR2 on monocytes but no changes on neutrophils following LPS infusion, whereas TLR4 was highly down-regulated on neutrophils but not significantly regulated in monocytes in the first *in vivo* investigation in humans with respect to TLR expression [20]. The aim of the study was to study the role of Toll-like receptors as a diagnostic marker for neonatal bacterial sepsis and its possibilities for differentiation between viral and bacterial neonatal infections. In the study there were no statistically significant differences between studied groups (high and low risk groups) as regard gestational age (P = 0.815), birth weight (P = 0.170), age at entry in the study (P = 0.052), residence (P = 0.792) and maternal medications (P = 0.914). In our study sex distribution among high-risk group revealed male predominance (60%) but not statistically significant. Sex distribution among low-risk group revealed female predominance (78%), These results were found to be in agreement with results of studies done by Satar and Özlü [21] and Shrestha., *et al*. [22] who reported that male infants had a higher incidence of neonatal sepsis than female infants. Also, our results were found to be in agreement with the results of Yadav., *et al*. [23] and Eschborn and Weitkamp [24] who found that males represented 65% and 62% of their septic patients respectively.

The cause may be related to mutations in X-linked immune-regulatory genes that are involved in thymus function or immunoglobulin production. Male infants had a higher incidence of neonatal sepsis than female infants. However, Trotman [25] found that female gender was associated with poor outcome in neonates with bacterial sepsis. Therefore, there was variation in the impact of gender on sepsis between trials.

In the study done by Adatara., *et al*. [26], it was shown that cesarean delivery was associated with greater risk of neonatal sepsis, this may be due to risk factors of sepsis rather than the CS itself which done under complete septic conditions.

Conclusion

An interplay has been found between different members of Toll like receptors (TLRs) and the patho-genetic network that lead to neonatal sepsis (TLR 2,9 and 8) as evidenced by their high levels in high risk neonates compared to low risk one. TLRs might play a vital role in differentiating bacterial from viral infections and this can be indirectly concluded by differences between those neonates with +ve and others with -ve blood cultures. TLRs could be used as a tool for detecting early onset sepsis and this could be supported by their rise as early as the 1st day in high-risk neonates. Diagnosis of neonatal sepsis was not only depending on raised levels of TLRs but must be supported by other markers such as CRP, leukocytosis, I/T ratio and +ve cultures. Group B *Streptococci, E. coli, Klebsiella* and *Staph. aureus* were revealed to be the most prevalent isolated bacteria in our investigation.

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