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Role of high-mobility group box 1 in late onset neonatal sepsis

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Abstract

Neonatal sepsis is an important cause of morbidity and mortality. High mobility group box1 protein (HMGB1) is a cytokine that can mediate inflammation. The aim of this research was to investigate the role of HMGB1 in diagnosis and prognosis of late onset neonatal sepsis. This observational case-control study included 80 newborn infants ≥37 weeks of gestation. Newborn infants were assigned into two groups: the late-onset neonatal septic group included 40 infant cases, and the control group included 40 newborn infants. Clinical sepsis score, hematological sepsis score and serum level of C-reactive protein were assessed and blood culture performed. HMGB1 was measured by an enzyme-linked immunosorbent assay. There was a significant increase of HMGB1 in the late-onset neonatal septic group than the control group (p< 0. 001). The best cut off point of HMGB 1 to discriminate against the late-onset sepsis cases from the control newborn infants was > 68 ng/ml with a sensitivity of 97.5%, specificity of 95%, positive predictive value of 95.1% and negative predictive value of 97.4% with total accuracy of 0.99%. The values of HMGB1 were not affected by gestational age, birth weight, postnatal age or gender. There were no significant differences in mean values between survival and non-survival cases. The best cut off value to predict mortality in the late onset sepsis group was >167.8 ng/ml with a sensitivity of 60% and specificity of 54.29%. In conclusion, this study suggested that HMGB1 is a promising marker for diagnosis of late onset neonatal sepsis in full term infants, on the contrary HMGB1 could not predict mortality in neonatal septic patients.

Keywords: HMGB1, sepsis markers, Late onset neonatal sepsis, newborn infants, neonatal mortality.

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Introduction

Neonatal sepsis is an invasive infection, usually bacterial, occurring during the neonatal period. The symptoms and signs of neonatal sepsis are multiple and non-specific.[1] Neonatal sepsis is divided into two categories: early-onset and late-onset sepsis (LONS). Early onset occurs within the first 3 days, while LONS refers to the presentation of sepsis after 3 days.[2, 3] LONS may be caused by pathogens acquired at delivery, bottle feeding, or during hospital care. Although in most cases LONS onset is often inconspicuous, the clinical course may be alarmingly fulminant leading to septic shock and death within hours of onset.[4] Neonatal sepsis is one of the most important causes of neonatal morbidity and death. The diagnosis of neonatal sepsis depends on clinical findings, culture

results, complete blood count (CBC), differential leucocyte count, C-reactive protein (CRP), and sepsis criteria. Also, some new markers are suggested for early diagnosis and to predict prognosis.[5, 6,7]

High mobility group box protein 1 (HMGB1) is a single polypeptide chain of 215 amino acids in length and organized into two DNA binding regions termed A and B boxes and an acidic tail. It has many rules inside and outside the cell. Inside the cell, it binds DNA and regulates transcription, determines chromosomal architecture. While outside the cell, it acts as an alarming factor to activate the immune system and mediate physiological and pathological responses.[8] A growing number of studies revealed that HMGB1 is a cytokine that can mediate inflammation,[9, 10] It not only mediates inflammation response but also plays an important role in the immunosuppression of sepsis, however it is still considered as a conflicting molecule in sepsis. Further research on the association of HMGB1 and sepsis phenotypes is necessary to determine the utility of HMGB1 as a biomarker.[11] Nevertheless, the role of HMGB1 in the assessment of neonatal sepsis and prediction of mortality is not fully studied. The aim of this research was to investigate the role of HMGB1 in diagnosis and prognosis of late onset neonatal sepsis.

Patients and Methods

This observational case-control study included 80 newborn infants. It was conducted over a period of 12 months from April 2020 to March 2021. The LONS group included 40 full term infants whose clinical manifestations started 72 hours after birth and were confirmed by positive blood culture. We excluded preterm infants and newborn infants with inborn errors of metabolism. The control group included 40 full term infants with no clinical or laboratory evidence of sepsis.

The sepsis clinical diagnosis was based on the presence of at least two clinical symptoms such as temperature instability, cardiovascular and/or respiratory instability including bradycardia or tachycardia, hypotension, apnea, tachypnea or increased oxygen requirements, feeding intolerance, poor suckling, abdominal distension, irritability, lethargy, or hypotonia. In addition, at least two laboratory findings including complete blood count, positive CRP, thrombocytopenia, metabolic acidosis, and positive culture were also considered for diagnosis of sepsis. Abnormal radiological findings of pneumonia and necrotizing enterocolitis were also considered for diagnosis of sepsis. A hematological score (a score depends on total white blood cells count, total polymorphonuclear leukocytes count, immature to mature polymorphonuclear cell ratio and platelet count of > 5) was diagnosed as sepsis.

All neonates were subjected to full history taking, clinical examination, estimation of clinical sepsis score and laboratory investigations including complete blood count (CBC), blood culture, CRP and hematological sepsis score. Blood samples were collected at the time of diagnosis of sepsis. Measurement of HMGB1 was determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits (catalogue no. 326054329, Shino-Test Corporation, Japan), according to the manufacturer's instructions.

Statistical Analysis

Collected data were coded, revised, and statistically analyzed by the statistical package for social science (SPSS, IBM, Chicago) version 20. Discrete variables were expressed as counts and percentages. Continuous variables are stated as medians and interquartile ranges or means and standard deviation. Differences in categorical variables were calculated using the Chi-square test or Fisher exact test. The Student t-test or Mann-Whitney test were used for analysis of continuous variables. Spearman's rank coefficient test was used for correlation between HMGB1, and other studied variables. The ANCOVA test was used to study the effect of gestational age, birth weight, gender, and postnatal age on HMGB1 values. The receiver operating characteristic curve (ROC) was used to detect cut-off value and sensitivity and specificity. The statistical significance was defined as $p< 0.05$.

Results

Demographic data for patients and controls

The results showed that there is no difference between the septic and control groups in gender, length, and birth weight. Nevertheless, there was a statistically significant decrease in gestational age (p<0.004) and a statistically significant increase in postnatal age in the septic group (p <0.001)), (Table 1).

Table 1. Comparison between the late onset neonatal sepsis group and the control group according to demographic data.

	Control (n = 40)		LOS (n = 40)		*p* value
	No.	%	No.	%	
Gender					
Male	27	67.5	25	62.5	$^{\chi 2}$0.05
Female	13	32.5	15	37.5	
Birth weight (Mean ± SD)	3.47 ± 0.41		3.45 ± 0.32		^{t}0.05
Gestational age (wks.) Mean ± SD	38.20 ± 1.07		37.60 ± 0.67		^{t}0.004
Length (cm) (Mean ± SD)	50.20 ± 0.39		50.30 ± 0.79		^{t}0.05
Postnatal age (days) Mean ± SD	7.10 ± 2.19		12.40 ± 5.73		^{t}<0.001

SD: Standard deviation t: Student t-test χ^2: Chi-square test, LOS: Late onset septic. $p \leq 0.05$ is significant.

Laboratory investigations

There was a significant increase in the mean values of HMGB1 in the LONS group; the median IQR was 167.40 (112.69–313.15)ng/ml, which was higher than in the control group, 23.94 (14.75 – 27.85) ng/ml, and p<0.001.

Also, there was a statistically significant increase in HMGB1 median values in female infants, 295.00 (165.78-327.1) ng/ml, than male infants, 130.00 (110.6-305) ng/ml. However, there were no differences between the survival and non-survival newborn infants in LONS (Table 2).

Table 2. Comparison of the late onset neonatal sepsis group, control group, gender, and survival and non-survival according to change in HMGB1 maker.

	HMGB1 (ng/ml)/Median and IQR	^{U}p value
LONS	167.40 (112.69–313.15)	<0.001
Control	23.94 (14.75 – 27.85)	
Male	130.00 (110.6-305)	0.043
Female	295.00 (165.78-327.1)	
Survival	167.00 (113.1-311.2)	NS
Non-survival	168.86 (112.5-320.0)	

IQR: Inter quartile range U: Mann Whitney test. $p > 0.05$ is not significant (NS).

LONS: late-onset sepsis. HMGB1: High mobility group box1 protein.

The receiver operating characteristic curve (ROC) showed that the best cut-off value of HMGB1 to discriminate neonates with LONS from the control group was > 68 ng/ml with a sensitivity of 97.5%, specificity of 95%, positive predictive value (PPV) of 95.1%, negative

predictive value (NPV) of 97.4% with total accuracy of 0.99%. Nonetheless, the ROC showed that the best cut-off point of HMGB1 to predict mortality in the LONS group was >167.8 ng/ml with a sensitivity of 60%, 54.29% specificity, and a total accuracy of 0.54%, (Table 3).

Table 3. Agreement, sensitivity, and specificity for HMGB1 to discriminate patients from controls and to predict mortality in the LONS group.

HMGB1 (ng/ml)	AUC	*p* value	95% CI		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
LONS and control groups	0.997	<0.001	0.990	1.0	>68	97.50	95.0	95.1	97.4
Survival and non-survival	0.537	NS	0.252	0.823	>167.8	60.0	54.29	15.8	90.5

AUC: Area Under a Curve CI: Confidence Intervals NPV: Negative predictive value PPV: Positive predictive value. $p > 0.05$ is not significant (NS).

The ANCOVA test showed that HMGB1 mean values were not affected by gestational age, birth weight, postnatal age, and gender (Table 4).

There was a statistically significant increase in total white blood cells, total neutrophils, immature neutrophils, and immature neutrophil/total neutrophil ratio in the LONS group than the control group ($p< 0.001$). Thrombocytopenia was reported in 72.5% of the LONS group. The hematological sepsis score and clinical sepsis score were significantly higher among the LONS group than the control group ($p<0.001$).

Table 4. Effect of sepsis, Gestational age, postnatal age, Birth weight, and Gender on HMGB1.

	Control F	Control p	LONS F	LONS P
Groups	60.817	<0.001*	-	-
Gestational age (weeks)	0.001	NS	0.021	NS
Postnatal age (hours)	1.523	NS	0.653	NS
Birth weight (kg)	0.482	NS	0.062	NS
Gender	2.263	NS	2.254	NS

ANCOVA test, F : (value of Ancova) LONS = Late onset neonatal sepsis $p > 0.05$ is not significant (NS).

Discussion

A growing number of in vivo and in vitro investigations revealed that HMGB1 plays a pivotal role in the processes of inflammatory response and immunosuppression of sepsis.[12] In the current study we focus on further understanding on the role of HMGB1-associated pathogenesis in neonatal sepsis, as it may assist in early diagnosis and development of treatment strategies.

In this study there were higher levels of HMGB1 in the LONS group in comparison to the control group ($p<0.001$). This result is comparable with that reported by Zhuo and Liao, 2019, who found that the relative expression of HMGB1 mRNA in peripheral blood mononuclear cells of neonates in the sepsis group was significantly higher than that in the local infection group and the control group.[13] HMGB1 plays an important role in the pathogenesis of neonatal sepsis by activating the toll-like receptor 4 (TLR4)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway and inducing the secretion of inflammatory factors including interleukin (IL)-8.[13] Also in adult patients, the study by Yoo et al., 2021, demonstrated a

statistically significant trend of increase in median values of HMGB1 between control, sepsis and septic shock groups.[11] On the contrary, in children HMGB1 it seems to be of minor importance.[14]

HMGB1 acts as a pro-inflammatory molecule during the early stages of sepsis. However, the extracellular HMGB1 could also induce immune tolerance and immunosuppression when released by other somatic cells. In contrast, intracellular HMGB1 can induce protective autophagy and contribute to cell survival.[15, 16] By delivering lipopolysaccharide and promoting endocytosis, HMGB1 activates the noncanonical inflammasome pathway and induces pyroptosis.[17] Pyroptotic macrophage death may accelerate undesirable immune hyperactivity and immunosuppression, which is a potential mechanism associated with late mortality from sepsis.[18] These contradictory results indicate that the functions of HMGB1 in sepsis are complex and different.[12]

This study revealed that at cut off value of > 68 ng/ml, HMGB1 had a sensitivity of 97.5% and specificity of 95% to diagnose late-onset neonatal sepsis with a positive predictive value of 95.1% and negative predictive value of 97.4%. These data show the ability of HMGB1 to diagnose sepsis and to exclude cases with no sepsis with high sensitivity and specificity. Also, the HMGB1 value was not affected by the gestational age, birth weight, postnatal age, and gender whether in the control or late-onset sepsis group. Also, we found no significant differences between the types of bacteria detected in the blood culture.

In the current study, there was no difference in HMGB1 between survival and non-survival cases. Furthermore, our study showed that the best cut off point of HMGB1 to predict mortality in the LONS sepsis group was >167.8 ng/ml with a sensitivity of 60%, specificity of 54.29%, PPV of 15%, and NPV of 90.5%. This indicates high negative predictive value for mortality, but moderate sensitivity and specificity with low positive predictive values. The study by Zhou et al., 2018, revealed that platelet-derived HMGB1 facilitated neutrophil activation and reactive oxygen species generation, which were critical for the ability of neutrophil to promote bacterial clearance.[19] On the contrary, the study by Karlsson et al., 2008, demonstrated that the increase in Delta HMGB1 was associated with mortality.[20] These data imply that persistent, non-contained HMGB1 release may be more revealing of worse prognosis than a self-limiting peak and that targeting the HMGB1 pathway may have therapeutic potential in the late stages of sepsis.[21] One study of mice suffering from sepsis showed that reducing the release of HMGB1 can effectively improve their survival.[22] HMGB1 promotes sepsis-induced organ dysfunction through suppressing neutrophil ability to clear bacteria and so enhancing persistent inflammation. HMGB1 decreased the capacity of neutrophil to kill bacteria through mediating neutrophil nicotinamide adenine dinucleotide phosphate oxidase dysfunction in patients surviving septic shock.[23] These contradictory results would indicate the need for more studies of the functions of HMGB1 and its role in mortality.

In conclusion, the values of HMGB1 were not influenced by gender, birth weight, gestational age and postnatal age. The study findings indicated that HMGB1 may be used in diagnosis of late onset neonatal sepsis with high sensitivity and specificity.

Author Contributions

HHZ, data collection and training and writing original draft. SE, conception and design of the study, interpretation of data and final approval of the version to be submitted. IME, data Laboratory investigation, results analysis and approval of the final version

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of

Medicine for Girls, Al-Azhar University (approval 202003188, dated 11/3/2020).

Informed consent

An informed consent was obtained from the parents or caregivers of each neonate before enrollment in the study.

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