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ORIGINAL ARTICLE

Plasma Gelsolin Level as a diagnostic Biomarker in Early Onset Neonatal Sepsis at Zagazig University Hospitals

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ABSTRACT

Background: Neonatal sepsis is one of the major causes of neonatal morbidity and mortality and is often difficult to distinguish from many other conditions. Thus, investigating new markers for early and prompt diagnosis of sepsis in neonates is a major concern. The purpose of this study was to investigate the role of plasma gelsolin level as a potential marker in the diagnosis of early neonatal sepsis.

Methods: This case control study was carried out in the Intensive Neonatal Care Unit (NICU), Microbiology and Immunology Department, Zagazig University children hospital during the period from March 2018 to February 2019. 46 neonates were included in the study. There were divided randomly into two groups. Group 1 included 23 septic neonate group and group 2 included 23 apparently healthy sex and age-matched neonates served as a control group. All patients were evaluated clinically and investigated for routine laboratory workup and sepsis markers in addition to plasma gelsolin level using ELISA technique.

Results: The most important risk factors for sepsis were premature rupture of membranes (PROM), which was found in 30.4% and maternal fever in 26.1% in septic group. The mean value of plasma gelsolin was found significantly lower among cases in comparison to the control group.

Conclusions: Plasma gelsolin could be used as a reliable diagnostic marker of early neonatal sepsis.

Keywords: A diagnostic biomarker, plasma Gelsolin, neonatal sepsis.

INTRODUCTION

Neonatal sepsis is a systemic infection occurring in infants at ≤28 days of life and is an important cause of morbidity and mortality of newborns. Early-onset neonatal sepsis (EOS) has been variably defined based on the age at onset, with bacteremia or bacterial meningitis occurring at ≤72 h in infants hospitalized in the neonatal intensive care unit (NICU), versus <7 days in term infants. In preterm infants, EOS is most consistently defined as occurring in the first 3 days of life and is caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery. Late-onset sepsis (LOS) is sepsis occurring after 72 h in NICU infants and 7 days of life in term infants, has been variably defined as occurring up to the age of <90 or 120 days, and may be caused by vertically or horizontally acquired pathogens. Early-onset neonatal infections of viral or fungal etiology may also occur at <7 days of life and must be distinguished from bacterial sepsis. [1]

The incidence of culture-proven EOS in the United States is estimated to be 0.77 to 1 per 1,000 live births. The incidence and mortality are higher when very low birth weight (VLBW) infants are considered exclusively; for infants with a body weight of <1,000 g, the incidences are estimated to be 26 per 1,000 and 8 per 1,000 live births in premature infants with a birth weight of between 1,000 and 1,500 g. [2]Organisms causing EOS are typically colonizers of the maternal genitourinary tract, leading to contamination of the amniotic fluid, placenta, cervix, or vaginal canal. The pathogen may ascend when the amniotic membranes rupture or prior to the onset of labor, causing an intra-amniotic infection. Thus, the infant may acquire the pathogen either in utero or intra-partum. Risk factors for EOS include both maternal and infant factors. Maternal risks, such as

dietary intake of contaminated foods, can arise before labor and delivery, with *Listeria monocytogenes* contamination of refrigerated foods such as deli meats being the most important example. [3]As the signs and symptoms of neonatal sepsis are nonspecific, early diagnosis and prompt treatment remains a challenge. There has been a myriad of studies on various diagnostic markers like hematological indices, acute phase reactants, C-reactive protein, procalcitonin, cytokines, and cell surface markers among others. Nonetheless, further research is needed to identify a biomarker with high diagnostic accuracy and validity. Some of the newer markers have shown promising results thereby potentially aiding in early detection of neonates with sepsis. In order to decrease the widespread, prolonged use of unnecessary antibiotics and improve the outcome of the infants with sepsis, reliable identification of sepsis at an earlier stage is paramount. [4]

Thus, new biomarkers are still needed for diagnosis. Plasma Gelsolin (pGSN) is an actin-binding plasma protein. Furthermore, extracellular gelsolin binds lipopolysaccharide and lipoteichoic acid, which are major virulence factors of gram-negative and gram-positive bacteria. The result of this binding is the inhibition of gelsolin's F-actin depolymerizing activity. Thus, gelsolin inhibits the release of IL-8 from human neutrophils subjected to lipoteichoic acid, lipopolysaccharide and heat-inactivated bacteria treatment [5].

The aim of this study was to explore the role of pGSN level as a potential marker in the diagnosis of early neonatal sepsis.

METHODS

This case control study was conducted in NICU, Zagazig University Children Hospital, Microbiology and Immunology Department, Faculty of Medicine in collaboration with Molecular Biology Unit, Zagazig Scientific and Medical Research Center, during the period from March 2018 to February 2019. 46 neonates were included in the study. They were divided into 2 groups according the presence or absence of sepsis. Group 1 included 23 septic neonate group and group 2 included 23 apparently healthy sex- and age-matched served as a control group.

Inclusion criteria: For case group, all full term neonates in the first 72 hours after birth whom were admitted to Zagazig NICU with culture proven onset sepsis. Exclusion criteria: Parents refusing to share in the study or patients with multiple congenital anomalies.All participants in the study had subjected to complete history taking regarding gestational disease, drug intake, infection , maternal age, antenatal hemorrhage , exposure to X ray , mode of delivery, gestation, birth weight and detailed clinical examination includig estimation of gestational age, head, neck, back, and genitalia examination .Laboratory tests: complete blood count (CBC), quantitative assessment of the level of C-reactive protein (CRP), kidney function tests, blood culture and pGSN level by ELISA as indicated in manufacturer instuctions.Measuring the gelsolin level by ELISA: All reagents and samples were brought to room temperature (18-25°C) before use. All standards and samples were run in duplicate. 100µl of each standard and sample were added into appropriate wells and incubated covered for 1 hour, at room temperature. All wells were washed 4 times with 1x wash solution and discarded washing was done by filling each well with wash buffer (300µl) using a multichannel pipette. Complete removal of liquid at each step is essential to good performance after the last wash, Buffer was removed by aspirating or decanting. The plate was inverted and blot against clean paper towels. 100µl of 1x prepared biotinylated detection antibody to each well were added and incubated for 15 minutes at room temperature with gentle shaking. The solution was discarded. 100µl of prepared HRP-Streptavidin solution to each well were added. Incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded. 100µl of ELISA Colorimetric TMB Reagent (Item H) to each well were added. Incubated for 30 minutes at room temperature in the dark with gentle shaking. 50µl of Stop Solution (Item I) to each well was added. The plate was read at 450 nm immediately.

Ethical Clearance: Written informed consent to participate in the study was obtained from the patient's parents. Approval for the study was obtained from the Department of Pediatrics and Microbiology and Immunology, University Hospitals of Zagazig, following the approval of the Institutional Review Board (IRB). The research was carried out in compliance with the World Medical Association's Code of Ethics (Helsinki Decleration) of human-related studies.

STATISTICAL ANALYSIS

Data were collected, tabulated and analyzed by SPSS 20 software. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance; difference and association of qualitative variable by Chi square test (X^2). Differences between quantitative independent groups by t test. Kruskal-Wallis test was used instead of a one-way ANOVA to find out if two or more medians are different. Ranks of the data points are used for the calculations, rather than the data points themselves and Mann-Whitney test

was used to compare differences between two independent groups when dependent variables are either ordinal or continuous.

RESULTS

Table (1) showed the initial laboratory data of studied groups including CBC and CRP, electrolytes, liver function, kidney function, and bleeding profile. Table (2) showed that there was a statistically significant difference between cases and control group regarding pGSN. The mean value of pGSN was lower among cases than control group. Table (3) showed that there was a statistically significant negative correlations between pGSN and CRP. There was a statistically significant positive correlations between pGSN and platelet. There were no statistically significant correlations between pGSN and other numerical data. Table (4) showed that the mean value of pGSN was lower among positive severe sepsis than less severe sepsis.

Table (1): Distributions of the studied cases regarding laboratory data.

		Range	Mean + SD
CBC	WBC($x10^3/mm^3$)	8.20 - 24.20	**14.97 ± 3.96**
	Hb(gm/dl)	8 - 15	**10.79 ± 2.226**
	Platelet($x10^3/mm^3$)	80 - 220	**155.78 ± 46.56**
CRP		28 - 171	**86.739 ± 39.85**
Electrolytes	Sodium (mEq/L)	133 - 148	**140 ± 4.16**
	Potassium (mmol/L)	3.10 - 5.30	**3.89 ± 0.568**
	Magnesium (mEq/L)	1.50 - 2.40	**1.81 ± 0.247**
Liver function	ALT (U/L)	8.50 - 15	**11.208 ± 1.87**
	AST (U/L)	22 - 70.20	**40.62 ± 14.75**
Kidney functions	Creatnine (mg/dL)	0.70 - 1.30	**0.965 ± 0.186**
	Urea (mg/dL)	11.20 - 25	**18.226 ± 3.56**
PT (seconds)		10 - 15	**12.65 ± 1.49**
PTT (seconds)		**40 - 54**	**46.04 ± 4.279**

Table (2): Distributions of the studied cases regarding Plasma gelsolin.

		Range	Mean + SD	P. value
Plasma gelsolin (µg/mL)	**Cases**	69 – 123	97.79 ± 19.39	**0.02**
	Controls	**77.80 - 768.40**	**198.48 ± 198.73**	

Table (3): Correlation between Plasma gelsolin and Laboratory data of the studied groups.

Correlation	Pearson's correlation	
	r	p
Age * Plasma gelsolin	0.009	**0.969**
Weight * Plasma gelsolin	0.358	**0.093**
WBC * Plasma gelsolin	- 0.073	**0.740**
Hb * Plasma gelsolin	0.082	**0.709**
Platelet * Plasma gelsolin	+ 0.427	**0.042**
CRP * Plasma gelsolin	- 0.599	**0.003**
Sodium * Plasma gelsolin	-0.235	**0.280**
Potassium * Plasma gelsolin	-0.096	**0.662**
Magnesium * Plasma gelsolin	-0.008	**0.972**
ALT * Plasma gelsolin	0.268	**0.217**
AST * Plasma gelsolin	-0.060	**0.785**
Creatnine * Plasma gelsolin	0.001	**0.995**
Urea * Plasma gelsolin	-0.255	**0.239**
PT * Plasma gelsolin	0.077	**0.728**
PTT * Plasma gelsolin	**0.094**	**0.670**

Table (4): Comparison between severe sepsis and plasma gelsolin in cases group regarding plasma gelsolin.

		Sepsis severe N = (5)	Sepsis N=(18)	t. test	P. value
Plasma gelsolin (µg/ml)	**Range**	69 - 120	103.30 - 123	2.78	**0.011**
	Mean ± SD	**95.76 ± .37**	**115.76 ± .74**		

t = Student's t-test

DISCUSSION

Neonatal sepsis is an inflammation of the blood caused mainly by bacteria that develops in a child 28 days later. Neonatal sepsis is known as early-release or late-release. Early sepsis occurs in the first 3 days of life, while late sepsis occurs typically in 4-28 days of life. Group B Streptococcus (GBS), Escherichia coli, Coagulase-negative Staphylococcus, Haemophilus influenza, and Listeria monocytogenes are the pathogens that cause neonatal sepsis [6]. Because of the immune system of the baby, neonatal sepsis has distinct clinical symptoms relative to sepsis in children or adults, indicating a special immune response to infection in the infant [7]. The diagnosis of neonatal sepsis often presents a highly false negative rate and a delay in obtaining blood culture results due to the non-specific clinical symptom. Biomarkers are therefore urgently needed to distinguish the sepsis accurately at an earlier stage [8]. In reducing sepsis-induced mortality, it is known that early diagnosis and timely management of sepsis are crucial. This indicates that the early differentiation between sepsis and non-infectious systemic inflammatory response syndrome (SIRS) affects the outcome significantly [9].Gelsolin is a multifunctional, calcium-dependent actin regulatory protein that circulates in healthy human plasma. Gelsolin is mainly involved in the rapid extreme extraction and transfer of actin filaments from dead cells into the blood stream. In addition, gelsolin binds in the body to a variety of pro-inflammatory and bioactive molecules, including lysophosphatidic acid,1-phosphate sphingosine, fibronectin, and platelet-activating factor. Gelsolin also acts as a mediator of many physiological functions, including wound healing, neurological development, progression of cancer, and angiogenesis [10]. More recently, reduced levels of gelsolin were correlated with the incidence and prognosis of many diseases, including broncho-pulmonary dysplasia (BPD), hyperoxic lung injury, sepsis, and intracranial hemorrhage [11]. Throughout this study, the mean value of pGSN (µg/ml) was lower among positive severe sepsis than less severe sepsis. This agrees with Halis et al., [12] who designed the study in sepsis premature infants to investigate changes in pGSN levels and re-evaluate this change after treatment and its mortality relationship. Whether or not pGSN levels can be a new biomarker for neonatal sepsis was also investigated. The mean pGSN level in the sepsis group at the time of diagnosis was 33.98±1.44 µg / mL, which was significantly lower than the control group level (60.05±1.3 µg / mL, $p<0.001$). This study showed that, mean value of pGSN (µg/ml) was lower among positive severe sepsis than less severe sepsis. Kose et al., [13] revealed that pGSN admission levels are also lower in neonatal infants and adult sepsis patients than in non-septic critically ill ICU patients, including those diagnosed with SIRS. It can be inferred from the above findings that the rate of pGSN can be used as a possible indicator of sepsis incidence and sepsis-associated mortality, the same conclusion was reached by Kose et al., [13] who found that the concentration of pGSN in survivors of sepsis was higher than in non-survivors. This study showed that pGSN and CRP had statistically significant negative correlations. This was in agreement with Osborn et al. [14] who revealed a significant negative correlation between pGSN and CRP. Another predictor of sepsis is the decrease of platelets, which was found in the study (155.78 ± 46.56) in cases in compassion with controls. This was in agreement with the study of Hornik et al., [15] who found a significant decrease in platelets level. A positive correlation was found between pGSN and platelets level in the study.

CONCLUSION

pGSN could be used as a reliable diagnostic marker of early neonatal sepsis

RECOMMENDATIONS

A larger group of patients should be studied for a longer period to confirm the effects observed in this study.

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