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Role of Progranulin in Diagnosis of Early Onset Neonatal Sepsis

Khalid Mohamed Salah[1], Mohamed Abdelazem Abdelhamed Abdelrahman[1], Walaa Mohamed Samy[2], Sahbaa Fehr Mohamed[1]

1. Department of Pediatrics, 2. Department of Medical Biochemistry, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:** Mohamed Abdelazem Abdelhamed Abdelrahman,
Mobile: (+20) 01000200258, **E-mail:** dr.mabd10@gmail.com

ABSTRACT

Background: Neonatal sepsis is the medical term for a bloodstream infection in newborn infants younger than 28 days old. Procalcitonin (PCT) and C-reactive protein (CRP), are non-specific infection markers, which are being employed as biomarkers to help diagnose neonatal sepsis. This study aimed to correlate the progranulin (PGRN) serum level to early onset neonatal sepsis (EOS)**.**

Patients and methods: This cross-sectional study was carried out at tertiary referral Neonatology Unit, Pediatric Hospital and Medical Biochemistry Department, Zagazig University during the period from May 2021 to May 2022. Confirmation of neonatal sepsis was done by total blood count and, in cases of suspicion, blood culture. All neonates had their blood drawn for whole blood count, CRP, PCT, and PGRN testing prior to beginning treatment.

Results: The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the PGRN diagnostic test were 81.8%, 96.7%, 75.5%, and 95.5%, respectively. PCT has a sensitivity of 72.7%, specificity of 95.6%, PPV of 66.7%, NPV of 96.7%, and accuracy of 93.1% in terms of diagnostic accuracy. Sensitivity, specificity, PPV, NPV, and accuracy of CRP diagnostics were all above average.

Conclusion: PGRN may be used as biomarker for EOS diagnosis.

Keywords: Neonatal sepsis, Progranulin.

INTRODUCTION

Neonatal early-onset sepsis (EOS) is a term used to describe sepsis that strikes infants within 72 hours of delivery. EOS may be caused by organisms transmitted from the mother. Without antibiotic therapy, EOS can advance quickly, cause neurological problems, and result in infant mortality [(1)]**.**

The gold standard test for a conclusive diagnosis of EOS is a microbial culture, however it takes two to three days for the results to be available, which is too long for an early diagnosis. Empirically chosen broad-spectrum antibiotics are frequently used to treat high-risk neonates, although doing so could promote the emergence of resistance bacteria. Procalcitonin (PCT) and C-reactive protein (CRP), non-specific infection markers, have been employed more and more as biomarkers to aid in the diagnosis of EOS, but their predictive ability is still insufficient [(2)]**.**

Finding novel, potent, and precise indicators for the early detection of EOS is thus urgently needed.Progranulin (PGRN), is a protein with 593 amino acids. A wide range of cell types, including astrocytes and microglia, neurons, and endothelial cells involve PGRN. It has been related to immunology, infection, and inflammation. PGRN is raised in sepsis and mediates the recruitment of macrophages, which is crucial for host defense, according to studies, which is significant [(3)]. This study aimed to correlate the progranulin serum level to early onset neonatal sepsis.

PATIENTS AND METHODS

The patients were recruited in this cross-sectional study from tertiary referral Neonatology Unit, Pediatric Hospital and Medical Biochemistry Department, Zagazig University. We conducted the study on 102 infant, females and males were (50%) each, with mean±standard deviation of gestational age was 37.15±1.21 and it ranged between 34 and 40 week. They were diagnosed as neonates with early onset sepsis.

Inclusion criteria:

Neonates (full term and preterm) admitted with sepsis within the first 72 hours after birth, based on maternal risk factors (chorioamnionitis, premature membrane rupture lasting longer than 18 hours). Clinical signs such as tachycardia and respiratory distress/apnea (heart rate >190 beats/min), or bradycardia (heart rate <90 beats/min), abdominal distension, food intolerance, neurological symptoms (such as convulsions, irritability, or lethargy), cardiovascular impairment (such as pallor or peripheral cyanosis and mottled skin with capillary refill delayed >3s). Conventional laboratory tests (WBC < 5000/mm^3 or >20000/mm^3, I/T ratio >0.12, platelet count<100,000/μl). Positive blood culture results.

Exclusion criteria: patients with metabolic and genetic disorders, cerebral hemorrhage, established intrauterine viral infection, prior antibiotic use, absence of parental consent, and patients with congenital abnormalities.

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All selected newborns had full examination and history-taking. Evidence of neonatal sepsis was done by complete blood count and blood culture for suspected cases. Blood samples for CRP, PCT and PGRN were collected from each newborn at the same time before initiating therapy.

Methods:

At the time of the infant's admission to the NICU, a sample of venous blood was taken along with a sample for blood cultures, procalcitonin (PCT), C-reactive protein (CRP), platelet Count (PLT), white blood cell count (WBC) and immature by total ratio (I/T ratio) and progranulin (PGRN). The samples were centrifuged for 6 minutes at 3000 g, then serum was frozen in sterile vials at 18°C. When all samples were taken, all PGRN measurements were made. The enzyme-linked immunosorbent assay (ELISA) kit measured the concentration of human progranulin (PGRN) in samples using a double-antibody sandwich design. Using Human progranulin kits which made in china by Sunred producer. Neonates (fullterm and preterm) admitted with sepsis within the first 72 hours after birth, based on the following factors:

1. Maternal risk factors (prolonged rupture of membranes>18 h, chorioamnionitis).
2. Clinical symptoms including respiratory distress/apnea, tachycardia (heart rate>190 beats/min), or bradycardia (heart rate<90 beats/min), cardiovascular compromise (e.g., paleness or peripheral cyanosis and mottled skin with capillary refill delayed>3s), neurological signs (seizures, irritability, or lethargy), feeding intolerance, or abdominal distension.
3. Results of conventional laboratory tests (WBC < 5000/mm3 or>20000/mm3) I/T ratio>0.12, Platelet count<100,000/µl).
4. Positive results of blood culture.

According to the above considering factors all recruited patients were categorized into four groups:

- Proven sepsis: positive blood or cerebrospinal fluid cultures.(No.=11)
- Probable sepsis: negative cultures, ≥3 abnormal findings. (No.=38)
- Possible sepsis: negative cultures, two abnormal findings. (No.=32)
- Unlikely sepsis: negative cultures, single abnormal finding. (No.=21)

These groups were determined before the initiation of the study

Ethics approval: The caregivers of the patients were notified for different diagnostic and treatment options and a written informed consent was signed. Zagazig University's Institutional Review Board (IRB) gave the study their approval. The protocol for the study adhered to the Helsinki Declaration, the World Medical Association's rule of ethics for human subjects' research.

Statistical Analysis

With the help of the statistical program for research Statistical package for social science (version 18), the data were coded, inputted, and analyzed. The following were employed as descriptive statistics: mean, standard deviation, range, frequency, and percentage. In order to examine the relationship for categorical data, the Chi-Square test (X^2) was utilized. The ROC curve was also used. A P value of 0.05 was considered significant.

RESULTS

Table 1 shows the mean gestational age and the mean weight of the studied patients.

Table (1): Demographic data among studied cases

	Mean ± SD	Range
Gestational Age (weeks)	37.15± 1.21	34.0- 40.0
Weight (kg)	3.03± 0.55	1.90- 3.90

The number of cases was 102 infants. Females and males were (50%) each.

Table 2 shows the results of examining the studied cases.

Table (2): Examination among the studied cases

	Range	Mean ± SD
APGAR Score at 5 minutes	5.0- 9.0	7.03± 0.855
Head circumference	33.0- 36.0	34.66± 0.836
Respiratory Rate	60.0- 80.0	68.65± 4.65
Heart Rate	90.0- 200.0	141.96± 24.94

Table 3 shows that blood culture was positive among 10.8% of the studied cases.

Table (3): Blood culture among the studied cases

		No.	%
Blood Culture	**Negative**	91	89.2
	Positive	11	10.8

Table 4 shows that the studied groups' outcomes differed in a statistically significant way as 27.3% of proven sepsis cases died while 13.2% of probable sepsis cases died and there was no death between possible and unlikely sepsis groups.

Table (4): Comparison between the studied groups regarding outcome

			Proven sepsis	Probable sepsis	Possible sepsis	Unlikely sepsis	Chi^2	P. value
Outcome	Died	No.	3	5	0	0	11.741	0.008
		%	27.3%	13.2%	0%	0%		
	Improved	No.	8	33	32	21		
		%	72.7%	86.8%	100.0%	100.0%		

Table 5 shows that 53 cases were positive sepsis by PGRN.

Table (5): PGRN, PCT and CRP in diagnosis of sepsis

	Positive		Negative	
	Number	Percentage	Number	Percentage
Progranulin	53	51.96%	49	48.04%
Procalcitonin	39	38.24%	63	61.76%
CRP	27	26.47%	75	73.52%

Table 6 shows the diagnostic criteria of PGRN, PCT, and CRP. PGRN had the highest value of **s**ensitivity, specificity, PPV, NPV and accuracy.

Table (6): Diagnostic accuracy of PGRN, PCT and CRP in diagnosis of cases

	Cut off value	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy
Progranulin	50	0.827	81.8%	96.7%	75%	97.8%	95%
Procalcitonin	0.95	0.819	72.7%	95.6%	66.7%	96.7%	93.1%
CRP	36	0.785	63.6%	93.4%	53.8%	95.5%	90.2%

DISCUSSION

In the current study, there was a statistically significant difference between the proven sepsis and the non-proven sepsis regarding mean value of gestational age and weight. This agreed with **Belachew and Tewabe** [(4)] who claimed that one of the elements affecting neonatal sepsis was the newborn's birth weight. Neonatal sepsis was 1.42 times more likely to happen in infants under 2.5 kg than in those weighing 2.5 kg or more. This result is in line with studies of **Dong and Speer** [(5)]. Neonatal infections are more likely to occur in newborns with low birth weight because they are more likely to be premature, have undeveloped immune systems, are unable to feed, readily lose heat, and have poor glycogen stores [(6)]. This was in disagreement with **Elgendy *et al.*** [(7)] who discovered that gestational age and weight didn't differ significantly.

According to the current study, there was a statistically significant decline among proven sepsis than not proven sepsis regarding Apgar score. Similarly, **Gonzalez *et al.*** [(8)] found a significant statistical decrease in the first-minute and fifth-minute Apgar scores in the patient group compared to the control group.

The current study found that patients with proven sepsis had statistically higher mean CRP levels than those without. This agrees with **Ahmed and Salah**[(9)] and **Shalaby *et al.*** [(10)] who observed an increase in CRP in the sepsis patients. This concurs with the previous studies as well as **El-Shafie *et al.*** [(11)] and **Dursun *et al.*** [(12)]. Some studies have concluded that CRP is still a valuable and accurate acute-phase protein for sepsis diagnosis particularly in developing nations, despite presence of a number of novel markers of infection [(13)]. These outcomes were consistent with **Boraey *et al.*** [(14)], and **Hofer *et al.*** [(15)]. They noted that the most common laboratory test was CRP, as accurately diagnose newborn sepsis, was simple to measure, and was more reasonably priced. Particularly in settings with limited resources, the simple use of CRP as a marker for infant sepsis makes it easy to diagnose. In reaction to bacterial infections or other inflammatory disorders, serum levels of CRP may increase by 100–1000 times, and concentrations are proportional to the severity of illness [(16)]. However, **Naher *et al.*** [(17)] have reported that CRP elevations may be caused by physiologic rise after delivery or illness unrelated to

infections, hence they are not always diagnostic for sepsis.

In this study, the mean PCT value was statistically higher in proven sepsis group than other groups. This was consistent with the conclusions of **Mohsen *et al.*** [18], who discovered that procalcitonin (PCT) levels increased significantly among newborns with sepsis compared to controls. PCT concentrations recorded in septic neonates were 5–20 times higher than those found in healthy babies [19]. Within six hours of the start of an infection, higher PCT levels can be found; they peak between 18 and 24 hours later and continue to be elevated for up to 48 hours (the half-life of PCT in peripheral blood is around 24 hours) [20]. A multicenter randomized controlled trial, by **Stocker *et al.*** [21] conducted that PCT might be used to direct the use of antibiotics to infants who may have EOS. They discovered that stopping treatment based on PCT had no negative effects and dramatically cut the use of antibiotic therapy in a population where culture-proven infections are uncommon.

According to the results of the current study, the mean PGRN value was statistically greater in proven sepsis than in other groups. This agreed with **Yang *et al.*** [22] who assessed the value of PGRN, IL-33, IL-17a, IL-23, IL-6, TNF-α, IFN-γ, GM-CSF, and the conventional biomarkers PCT and CRP in a Chinese cohort of high-risk newborns for the early detection of EOS. According to their findings, the blood PGRN levels in EOS neonates but not non-EOS neonates increased over time, increasing PGRN's predictive power. Both adult and pediatric sepsis patients' PGRN levels are higher than those of the equivalent controls [3].

Our results were in line with a prior study that assessed the progranulin (PGRN) diagnostic value contrasted its effectiveness with that of other used markers in early-onset neonatal sepsis (EOS), such as procalcitonin (PCT) and C-reactive protein (CRP). According to that study, sepsis patients' levels of PGRN could significantly increase [23].

CONCLUSION

PGRN may be used as biomarker for the detection of EOS, and it's better to be used combined to CRP and PCT. Blood culture remain the gold standard tool to diagnose EOS.

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