Procalcitonin as a Diagnostic Marker of Sepsis in Neonates

Muhammad Jameel Ashraf[1], Syed Kashif Abbas[2], Atika Sher[3]

Abstract

Objective: To determine the diagnostic accuracy of serum procalcitonin levels (PCT) by taking blood culture as gold standard.

Methods: This was a cross-sectional study conducted in neonatal intensive care unit (NICU) of Liaquat National Hospital, Karachi over a period of six months from June 2017 to December 2017. A total of 141 babies were enrolled in the study that had evidence of neonatal sepsis. Non-probability consecutive sampling was the technique used in our study for sample collection. Later on, after completion of data of required sample, a data base was developed on SPSS for windows version 22.0 for data analysis. Mean, median and standard deviation were calculated for continuous while frequency along with percentages was drawn for qualitative variables. Pearson Chi-square test (χ^2) was applied on all qualitative variables by taking p-value <0.05 as significant. Finally, the diagnostic accuracy of serum procalcitonin levels in neonatal sepsis was calculated in terms of sensitivity, specificity, positive predictive values and negative predictive values using blood culture as gold standard.

Results: Of 141 neonates, 68 were males and 73 were females which constitutes 48.2% and 51.8%, respectively. The mean weight of neonates was 2.49 ± 0.55 kg, while mean height and FOC (frontal-occipital circumference) were found to be 46.22 ± 3.17 cm and 33.29 ± 1.41 cm, respectively. The sensitivity of PCT in this study was found to be 70.90% while the specificity was 77.90%. Furthermore, positive predictive value (PPV) and negative predictive value (NPV) accounted for 67.24% and 80.72%, respectively.

Conclusion: Although blood culture is the gold standard method to diagnose neonatal sepsis in neonates, however PCT has also shown good accuracy with quick results in diagnosing neonatal sepsis in this study. The early use of PCT in neonatal infection may definitely help us limiting number of neonates putting on antibiotics due to suspected risk of infection.

Key Words: Neonatal sepsis, procalcitonin, diagnosis, sensitivity, specificity.

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Introduction

Sepsis is the leading cause of death in children. Sepsis is caused by infection in blood stream, and thus, defined as a systemic inflammatory response syndrome (SIRS)[1]. The pathogenesis of sepsis depends upon various inflammatory mediators, such as coagulation, adaptive and innate immune response and intermediary metabolism products. These factors altogether interrelate and provide guidance to this abnormal response[2].

Neonatal sepsis is defined as an invasive bacterial infection that occurs in newborn which occurs in the first 4 weeks of life[3]. Basically, there are two types of sepsis in neonates; early-onset neonatal sepsis (EONS), that occurs ≤7 days of birth and late-onset neonatal sepsis (LONS) that develops beyond 7 days of birth[4]. According to the World Health Organization (WHO), the numbers of children born each year is about 130 million, in which about

[1-3] Department of Pediatrics,
Liaquat National Hospital, Karachi

Correspondence: Dr. Muhammad Jameel Ashraf
Department of Paediatrics, Liaquat National Hospital
Email: sj_ashraf@hotmail.com
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4 million babies die each year due to neonatal sepsis[5].

The gold standard for diagnosing sepsis is positive blood culture or culture from any other sterile body fluid, for instance cerebrospinal fluid (CSF) or urine culture results[6]. In the developed countries, the reported incidence of culture proven sepsis fluctuates between 1-8 cases/1000 live births[7]. Study conducted by Lutfullah et al. reported the overall incidence of early onset neonatal sepsis is about 31.3 cases/1000 live births[8]. Moreover, local studies showed the incidence of neonatal sepsis in Pakistan ranges from 14-63/1000 live births[9].

Although blood culture is the gold standard for the diagnosis of sepsis, it takes about 48-72 hours to report the results[10]. On the other hand, serum procalcitonin levels (PCT), an acute-phase reactant and indicator of sepsis in neonates, provide real-time availability of results[11]. PCT is a diagnostic biomarker for sepsis in individuals with SIRS. Several studies have shown the efficacy of PCT in determining neonatal sepsis. Moreover, it is a precursor of calcitonin, a 116 amino acid protein which is secreted by the C cells of thyroid gland in a normal situation[12]. However, these levels may increase in conditions that are septicemia, pneumonia, meningitis and urinary tract infection[13]. Since serum levels of PCT increase physiologically in the first few days of life, its efficacy as a biomarker in early onset sepsis is limited[14].

Recently, a meta-analysis was done that enrolled 1959 neonates. Results showed that the pooled sensitivity of PCT was found 81% and a specificity of 79%, respectively[15].

The aim of conducting this study to determine the diagnostic accuracy of serum procalcitonin levels (PCT) in neonatal sepsis by taking blood culture as gold standard, thus by getting the sensitivity and specificity of PCT, we would be able to get accurate and real time results in neonatal sepsis. That ultimately would improve the neonatal outcome by early identification and prompt management.

In our study, the value of PCT that considered positive was >2 ng/mL. Moreover, this test was analysed with the help of immunoluminometric assay method (Liaison-BRAHMS PCT).

Diagnostic accuracy was defined as the ability of serum procalcitonin level to correctly diagnose neonatal sepsis taking blood culture as gold standard. True positive was considered as a positive concentration of serum procalcitonin levels with positive blood culture reports. True negative was negative concentration of serum procalcitonin levels with negative blood culture reports. False positive meant that positive concentration of serum procalcitonin levels with negative blood culture reports. False negative was considered as a negative concentration of serum procalcitonin levels with positive blood culture reports. Sensitivity= TP/TP+FN*100, specificity= TN/TN+FP*100, positive predictive value= TP/TP+FP*100, negative predictive value= TN/TN+FN*100. (TP= True Positive, TN= True Negative, FP= False Positive, FN= False Negative).

Subjects and Methods

This was a cross-sectional study conducted in neonatal intensive care unit (NICU) of Liaquat National Hospital, Karachi over a period of six months from June 2017 to December 2017. Ethical committee approval (ERC) was obtained before conducting the study. A total of 141 babies, both indoor and outdoor, were enrolled in the study that had evidence of neonatal sepsis. For the objective of the study, sample size has been calculated by taking confidence level (1-α) as 95% with desired precision (d) of 8% for sensitivity and 6% for specificity, sensitivity of 92.8% while specificity of 75% with approximate population estimation of 44% taken from the parent study[16,17]. By putting all the values the sample size calculated was 141.

Neonatal sepsis in our study was defined on following clinical manifestations that included respiratory distress (respiratory rate more than 70/min on two separate occasion in a day), inconsolable cry, lethargy, convulsion (episodes of seizures lasting more than 10 seconds in a single day) and feeding intolerance. While exclusion criteria included all neonates with history of prior administra-

tion of antibiotic therapy, any evidence of perinatal asphyxia, aspiration syndromes, congenital anomalies and laboratory finding suggestive of inborn error of metabolism. Collection of data was performed on a predesigned pro forma. It included patient admission number, sex, age, history, physical examination and laboratory tests. Written informed consent was obtained from parents of study participant. Non-probability consecutive sampling was the technique used in our study for sample collection. Under aseptic condition blood samples were taken for blood culture and for serum PCT levels. It was made sure by assigned head nurse to send these samples to pathology department in controlled and aseptic environment. Later, on daily basis, blood culture reports were followed for 7 days. Moreover, reports with positive results were again reconfirmed.

For collecting samples, standard procedure was adapted for both PCT and blood culture. Serum PCT was analysed with the help of immune luminometric assay method (Liaison-BRAHMS PCT). In order to draw sample for PCT, sterilisation of the skin was done. Approximately 1-2 mL of blood (with no anti-coagulant) was taken in a bottle tube. Later that tube was centrifuged for 5 minutes; this causes the serum to separate using a suitable pipette. Afterwards, 5L of sample was dropped on control beside each drop of latex. Finally, rotate the slide was rotated in machine for few minutes, the results was displayed on the computer screen. The value of PCT that considered positive was >2 ng/mL.

For blood culture, sample of blood was taken under strictly hygienic condition that is according to the standard guideline in order to avoid contamination. Proper scrubbing was done prior to sample collection, staff that was involved in the procedure wore sterile glove. Sample collection was done by taking blood from a vein after proper sterilisation of the skin thoroughly with sterile spirit swab. Approximately, 1-3 mL of blood was taken and put in a blood culture bottle (BD BACTEC Peds plus/F culture vials). Later under BACTEC machine, culture media bottle was kept at 37ºC that automatically detected growth of organisms if present. Upon detection of organism, the machine gives bleep. In that case, the culture media would be then taken out of the machine and put on a culture media plates. Culture media plates included blood, chocolate and Mecconci as a media. Finally, if there was any evidence of significant growth found then that growth was subjected to sensitivity.

Later on after completion of data of required sample, a data base was developed on SPSS for windows version 22.0 for data analysis. Mean, median and standard deviation were calculated for continuous variables such as weight, height and frontal-occipital circumference (FOC). Frequency along with percentages were drawn for qualitative variables like gender, gestational age, blood culture reports (positive/negative), procalcitonin levels (significant/insignificant) and outcome; while Pearson Chi-square test (χ^2) of all qualitative variables were applied by taking p-value <0.05 as significant. All data with significant p-values were then analysed. Finally the diagnostic accuracy of serum procalcitonin levels in neonatal sepsis was calculated in terms of sensitivity, specificity, positive predictive values and negative predictive values using blood culture as gold standard.

Results

There were total 141 neonates who were admitted with complain of neonatal sepsis during the tenure. Out of them, 68 were males and 73 were females which constitute 48.2% and 51.8%, respectively (Table 1). The mean weight of neonates was 2.49 ± 0.55 kg while mean height and FOC was found 46.22 ± 3.17 cm and 33.29 ± 1.41 cm, respectively.

Of total admitted neonates, 57 babies were indoor and remaining 84 were admitted from outdoor that comprises of 40.4% and 59.6%, respectively. The numbers of preterm admissions were slightly high as compared to term admissions. Of total number of admissions, 51.1% were preterm neonates while 48.9% were term neonates. Laboratory results of new born babies showed 58 with positive PCT, whereas 83 with negative PCT values constituting 41.1% and 58.9%, respectively. While 55 neonates presented with positive blood culture and

86 presented with negative blood culture reports. Moreover, 19 indoor babies had positive blood culture where as, on the other hand, 36 outdoor babies had positive blood culture. The most common organism that was found in blood culture was Acinetobacter while least common organism found was E. coli accounting 34.5% and 16.4%, respectively. Other organisms included Burkholderiacepacia that was found in 21.8% and Klebsiella was found in 27.3% cases. Moreover, 26 term neonates and 32 preterm showed positive PCT results blood culture was found positive in 24 term neonates and 31 preterm neonates, respectively. Likewise, high numbers of outdoor neonates were seen with significant serum PCT levels as compared to indoor neonates. Of total 58 neonates with significant serum PCT levels, 36 outdoor had significant serum PCT level while only 22 were shown to have significant PCT levels overall. The most common organism in term babies were Acinetobacter where as Burkholderia Cepacia and Klebsiella were found to be the leading cause of infection in preterm babies (Table 2). Moreover, Acinetobacter is also the leading cause of neonatal sepsis in indoor babies while Klebsiella is the most common organism that affected outdoor babies. Even with discrepancy in the numbers of admission between indoor and outdoor babies, 38.59% indoor babies had significant serum PCT levels as compared to outdoor babies that constitute about 42.85% that had significant serum PCT levels. Similarly, 33.33% indoor admission were found to have positive blood culture reports while 42.85% outdoor admission were seen with positive blood culture reports, respectively.

The sensitivity of PCT in our study was found to be 70.90%, while the specificity was 77.90%. Furthermore, positive predictive value (PPV) and negative predictive value (NPV) accounted for 67.24% and 80.72%, respectively as determined by Pearson chi-square test (χ^2) = 33.01, p-value= 0.000. The outcome of our study showed that out of 141 neonates, 116 got discharged home safely, 4 left against medical advice (LAMA), 6 got discharged on request (DOR) and 15 babies expired, which constitutes 82.3%, 2.8%, 4.3%, and 10.6%, respectively. While out of 15 expired babies, 9 were preterm and 6 were term babies. Moreover, female babies account for high number of expiries compared to male babies. A total of 9 female were expired compared to male that was recorded 6 in number. With respect to indoor/outdoor babies, total 9 outdoor babies were expired while 6 babies that were admitted indoor were expired. The length of stay of neonates in nursery in our study was 4.83 ± 1.67 days.

Table 1. Frequency of age, admission status, gestational age, procalcitonin levels, blood culture and organism's distribution in neonatal sepsis at a tertiary care hospital

n= 141	N	Percentage (%)
Gender		
Male	68	48.2%
Female	73	51.8%
Admission Status		
Indoor	57	40.4%
Outdoor	84	59.6%
Gestational Age		
Term	69	48.9%
Preterm	72	51.1%
Procalcitonin Levels		
Positive	58	41.1%
Negative	83	58.9%
Blood Culture		
Positive	55	39%
Negative	86	61%
Organisms		
Acinetobacter	19	34.5%
E. coli	9	16.4%
Burkholderia Cepacia	12	21.8%

Discussion

This study determines the diagnostic accuracy of serum procalcitonin levels in neonatal sepsis. Diagnosis of neonatal sepsis remains challenging because of nonspecific signs and symptoms or vague clinical presentation. In our study, female babies developed sepsis more than male babies compared to study conducted by Sidra et al. which showed male babies are more prone to develop sepsis as compared to female babies[18]. This could be due to the reason that the numbers of female admitted in our study might be higher that the number of male admissions.

Moreover, preterm babies developed more infection as compared to term babies in our study. Similar results were found in other published stud-

ies[19]. This might be due to the fact that preterm has predisposition of neonatal sepsis. Due to early delivery and weak immune response, preterm babies are more prone to develop sepsis. Furthermore, the most important indicator of evaluating neonatal health status in hospital is length of stay. It was found slightly higher in preterm babies compared to term babies. This is again due to fact that preterm babies usually presents with multiple issue such as respiratory distress syndrome (RDS), haematological variation due to stress and feeding and sucking issues that make them stay longer in nursery compared to term babies. With respect to laboratory investigations, serum PCT and blood culture has also shown more positive results and determines more chances of developing neonatal sepsis in preterm babies. The main reason of this is due to maternal infections such as chorioamnionitis and leaking that cause the baby to deliver early as well as chances of getting more infection due to maternal infections. Another alarming parameter is the birth weight, majority of babies in our study was found to have low birth weight, which is again one of the major contributing factors that increase the likelihood of developing neonatal sepsis. Another striking finding in our study is the high numbers of outdoor babies that were admitted with diagnosis of neonatal sepsis. Most outdoor babies were presented with positive blood culture as compared to indoor babies. Same results were observed with serum PCT levels. The proportion of significant serum PCT levels were found higher in outdoor babies. Although it has been observed in our study that the proportion of significant serum PCT levels and positive blood cultures is high in outdoor babies as compared to indoor babies, however there is also a discrepancy among the number of admissions in indoor versus outdoor babies. For this reason, indoor and outdoor babies were individually evaluated with respect to the number of admissions. Nonetheless, the results came out to be the same. Even with individual evaluation, there was an upward trend observed in indoor babies with significant serum PCT levels as well as positive blood culture reports compared to indoor babies that had less patients with these significant findings. Finally, the sensitivity of PCT in our study was found to be 70.90%, while the specificity was found 77.90%. Compared to study conducted by Seth Kwabena Amponsah et al. that showed sensitivity and specificity of PCT was 87.5% and 63 %, respectively[19]. The mild variation in these results might be due to difference in the tools used in assessing the laboratory results as well as nursing care and set up. The majority babies with true positive results are preterm and outdoor admissions. So this clearly emphasises that prematurity is one of the leading cause of sepsis in neonates. On the other hand, the increase proportion of neonatal sepsis was documented in those neonates who were admitted from outdoor. This might be due to the fact that maternal infection during pregnancy and their antenatal care are the major contributing factors that may increase or decrease the risk of neonatal sepsis. So providing healthy antenatal care during pregnancy is the key factor that helps us mitigating the severity of neonatal infections and thus improving the outcome of babies admitted in the NICU. In summary, the assessment is going on to determine the validity of PCT in the diagnosis of neonatal sepsis. However, so far it has been observed by meta-analysis that PCT is a very useful tool in detecting earlyneonatal sepsis and it has shown a beneficial role in diagnosis, prognosis and response to treatment in patients with neonatal sepsis.

The main limitation of our study is the cost of serum PCT levels, that is the main hindrance of restricted sample size in that range. We believe that increase in the numbers of sample could provide us better and more reliable conclusions about the importance of determination of procalcitonin levels. Thus, improve the neonatal outcome by early identification and prompt treatment.

Conclusion

Although blood culture is the gold standard method to diagnose neonatal sepsis in neonates, however PCT has also shown good accuracy with quick results in diagnosing neonatal sepsis in our study. The early use of PCT in neonatal infection may definitely help us limiting the number of neonates putting on antibiotics due to suspected risk of infection. Moreover, those neonates who have been

on antibiotics due to sepsis, the value of PCT also help us determine the estimated length of stay of babies in nursery. That ultimately would provide release of both mental as well financial stress of parents. Furthermore, early identification of neonatal sepsis by means of PCT compared to blood culture reports could assist us in reducing neonatal morbidity and mortality and improve the neonatal outcome by initiating immediate treatment and neonatal care.

Conflict of Interest

This is to state that all authors have certified that they have NO affiliations with or involvement in any organisation or entity with any financial interest or nonfinancial interest in the subject matter or materials discussed in this manuscript.

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