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Original Article

Diagnosis of Neonatal Septicemia; Thinking Beyond Blood Culture

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Abstract

Background: Neonatal sepsis (NS) is a major cause of neonatal admissions to hospital and mortality. This study was conducted to evaluate different methods for rapid and reliable diagnosis of NS with an aim to rationalize the use of antibiotics and stay in Neonatal Intensive Care Unit.
Objectives: To compare the different available methods for rapid and reliable diagnosis of neonatal sepsis.
Study design, settings and duration: This comparative study was conducted in Emergency Department of National Institute of Child Health (NICH) Karachi for the period of six months.
Subjects and Methods: Neonates who were brought to the emergency department of National Institute of Child Health with suspicion of NS were clinically examined. Those, supposed to be septic were included as study population and informed written consent was taken from their parents. After this, 3 ml blood was drawn for complete blood count (CBC), blood culture, C-reactive protein (CRP), polymerase chain reaction (PCR) and neutrophilic ratio. PCR and CRP results were compared with blood culture for specificity, sensitivity, positive and negative predictive values.
Results: Out of 129 neonates, 23 (17.82%) were positive for bacterial DNA by PCR method while 42 (32.55%) were positive for infection when tested for C reactive protein marker of the blood. Whereas, only 15 (11.62%) were positive by blood culture. Sensitivity and specificity of PCR were 60% and 88% while positive and negative predictive values were 39% and 94% respectively. Sensitivity and specificity of C reactive protein were 60% and 71% while positive and negative predictive values were 21% and 93% respectively.
Conclusion: Our study revealed high specificity and positive predictive values of PCR suggesting that PCR is more reliable, rapid and specific adjunct of blood culture for diagnosis of NS.
Key words: Neonatal sepsis, blood culture, PCR, CRP.

Introduction

Neonatal sepsis (NS) is a major cause of neonatal admissions to hospital and neonatal mortality in both developed and developing countries.[1] In India, 30 out of 1000 births are victims of NS and 19% of all neonatal deaths are contributed by it.[2] On comparison with the developed countries, NS is three times more prevalent in developing countries including Pakistan.[3]

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Authors Contribution

AR has done the conceptualization of project and literature search. AR, MK and SFH did the data collection. AAM did the statistical analysis. Drafting, revision and writing of the manuscript were done by AR, MK, SFH and MAM.

A comparative study from Israel, has established a significant correlation between blood culture and polymerase chain reaction (PCR) for isolation and identification of various kinds of blood borne bacteria. According to that study, positive and negative predictive values of PCR when compared with blood culture were 100% and 98% respectively.[4,5] Moreover, a study conducted in Germany showed that quantitative PCR can detect *Escherichia coli* in blood stream much more earlier than other conventional methods.[6]

Interestingly, a study from China has reported high sensitivity and specificity of real time PCR in contrast to those of blood culture; hence, claimed RT-PCR to be more reliable, appropriate and quick technique to isolate the bacteriological cause of NS.[7] In another study, panel of experts proved that blood culture is not only less sensitive but it also delays the results for 2-3 days compared to PCR which offers increased sensitivity and rapid diagnosis[1]. Similarly, a comparative study conducted in Norway stressed upon the need of introduction of PCR in early diagnosis of sepsis.[8] Along with above mentioned findings, an observational study conducted at University of Pittsburgh USA, claimed that

besides rapid diagnosis and high negative predictive value, PCR decreases the use of antibiotics and stay in neonatal intensive care unit.[9]

Although, blood culture is still gold standard for isolation and confirmation of the etiological agent of the NS,[10] but its reliability is questioned when it gives false negative results in cases where either the amount of sample or bacterial load is low.[11,12] Rapid identification for underlying cause of NS is most important for a newborn admitted in NICU with a suspicion to be suffering from NS, Therefore addition of newer, quick and authentic methods can result into better management of NS along with reduction in use of broad spectrum antibiotics, cost of treatment and an ultimate reduction in mortality as well.[9] With all these advantages, polymerase chain reactions (PCR) can be easily performed to evaluate the different kinds of microorganisms like bacteria, yeasts, viruses and protozoa.[13]

Subjects and Methods

Ethical clearance was taken from Institutional Ethical Review Board (IERB) of NICH.

It was a comparative study of six months duration from February 2016 to August 2016 and purposive sampling technique was used for recruitment of subjects. A total of 150 neonates who were brought to the emergency department of National Institute of Child Health (NICH) with suspected sepsis and had not taken antibiotics before visiting this facility were included as study population. After taking written consent from parent/guardian, a pre coded questionnaire was used to obtain information regarding clinical history, demography and lab parameters. About 3 ml blood was drawn for CBC, CRP and real-time 16S rDNA PCR analysis.

It was done by using QIA (Qiagen) amp (amplification) DNA Mini-kit. 20 µl Of Qiagen proteinase k (20 mg/ml) was added to 200 µl of serum sample. Then 200 µl of buffer Al was added, Vortexed for 15 seconds then incubated at 56°C for 10 minutes. After incubation, 200 µl of 100% ethanol was added, centrifuged for 20 sec and transferred the filter into Qiagen Amplification spin column and centrifuged at 6000g or 8000RPM for 1 minute. Then transferred concentrate into new collection tube, added 500 µl buffer aw1 centrifuged at 8000 RPM for 1 minute and replaced the collection tube. Added buffer aw-2 500 µl, and centrifuged at maximum speed for 3 minutes. Discarded and replaced filtrate tube. Added 200 µl elusion buffer AE. Kept at room temperature for 1 minute then centrifuged at 8000 RPM for 1 minute.

Elute obtained from extraction step was loaded onto the Qiagen amplification DNA mini kit Column. Amplification reaction volume was 25µl including 12.5 µl master mix,5 µl elute,1.25 µl forward primer,1.25 µl reverse primer and 5 µl RNAs free water. Primer sequences were as follows: Forward Primers, 5'AAC-TGG AGG-AAG-GTG GGG AT3'Reverse primer 5'AGG AGG TGA TCC AAC CGC A-3.

1-2 ml blood collected for culture was inoculated directly into a single, pediatric sample-sized resin-containing blood culture bottle (Peds Plus; Becton Dickinson), and was loaded into the BACTEC 9240 automated blood culture instrument within 1 hour from the time of receipt in the laboratory. A few drops of sample was taken out by sterile syringe from positively detected bottle loaded into the BACTEC instrument and were inoculated on Blood and Macconkey agar culture plates. Purified colonies were Gram stained and identified by different biochemical reactions.

Complete blood count was done using sysmex-Kx-21 Hematology Analyzer (Sysmex Corporation, Kobe Japan). Slides were stained with leishman stain and examined for morphology and differential count.

Serum level of CRP was measured using a biochemistry auto analyzer Beckman coulter AU-480 USA.

Results

Out of 150 enrolled patients, 58 (87%) were male, and 63(42%) were female. Around 50 (37%) were born at home and 95 (63%) in the hospital; including 121 (81%) normal while 29 (19%) cesarean section births/deliveries. Mean age was 6.6 days ranging from half hour of birth to 28 days; mean weight was 2.73 Kgs with the range of 1.1 to 4.5 kgs (Table-1).

Table1: demographic profile of the Neonates.

Variable	*No. of subject (n=150)*			*%*
Gender				
Male	87			58.0
Female	63			42.0
Place of delivery				
Home	55			36.7
Hospital	95			63.3
Mode of delivery				
Normal vaginal delivery	121			80.7
Cesarean section	29			19.3
	Mean	±	*S.D*	*(Range)*
Age in days	6.6	±	8.05	0.04 - 28
Weight (kgs)	2.73	±	0.62	1.1 -4.5

Risk factors in mothers including febrile illness 21%, foul smelling meconium 35%, rupture of membrane 18%, prolonged labor 7%, prenatal asphyxia 10% and more than two risk factors were found among 9% mothers. Risk factors among neonates included history of resuscitation 39%, home delivery 33%, low birth weight 24% and prematurity 4%. Signs and symptoms included respiratory distress 57%, temperature instability 29%, abdominal distention 8%, apnea 6% and hypotonia were observed in 4% of the neonates (Table-2).

Mean hemoglobin was 15.03 gmdl in blood culture positive and 15.18 gm/dl in blood culture negative cases, red blood cells(RBCs) were 4.75×106 cells/l in blood culture positive while 4.86 ×106 cells/l in blood culture negative cases. White blood cells (WBCs) were 13.34×106 cells/l in blood culture positive cases and 13.65×106 cells/l in blood culture negative cases while platelets were 171.8×103 cells/l in blood culture positive cases and 234.1×103 cells/l in blood culture negative cases respectively (Figure-1).

Table 2: Clinical history and presentations of mothers and neonates. (n=150)

Variable	*No. of Subject*	*%*
Risk factors in mother		
Febrile illness	31	20.67
Foul smelling meconium	53	35.33
Rupture of membrane	27	18
Prolonged labour	10	6.67
Prenatal asphyxia	15	10
More than one risk factors	14	9.33
Risk factors in Baby		
Low birth weight	36	24
Prematurity	6	4
Home delivery	50	33.33
History of resuscitation	58	38.67
Sign and symptoms in baby		
Respiratory distress	86	57.33
Cyanosis	8	5.33
Temperature instability	29	19.33
Apnea	9	6
Hypotonia	6	4
Abdominal distention	12	8

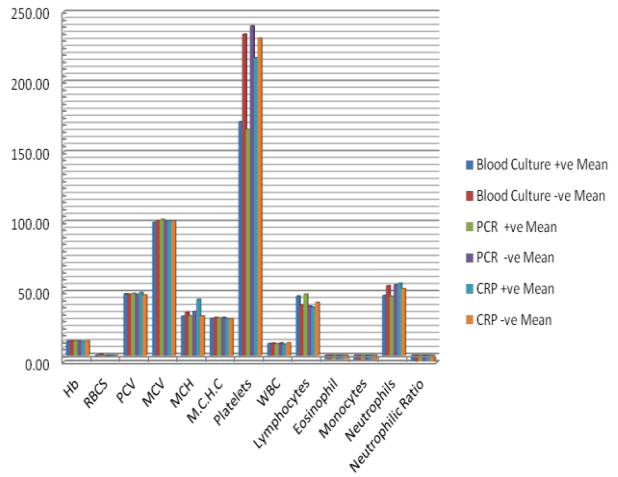

Figure 1: Complete blood count.

Blood and serum quantities of 21 samples were not sufficient; hence their results were not matching the criteria of calculation of sensitivity and specificity. Therefore, calculations were done on 129 samples. Twenty three out of 129 neonates (17.82%) were positive for bacterial DNA by PCR method, while 42 out of 129 (32.55%) were positive for infection when tested by C reactive Protein marker of the blood. Whereas, 15 out of 129 (11.62%) were positive by blood culture. Besides, equally high sensitivity and negative predictive values of PCR and CRP, specificity and positive predictive values of PCR are greater than those of CRP (Figure-2).

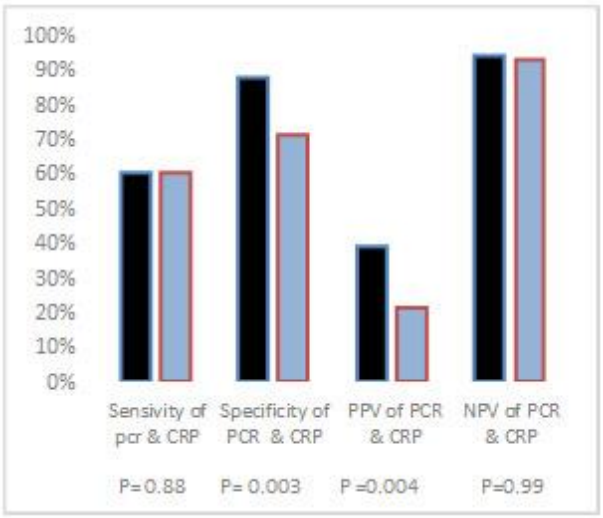

Figure 2: Comparison of C reactive protein and PCR results with blood culture.

Discussion

Neonatal sepsis contributes a major chunk of neonatal admissions to hospital and neonatal mortality. Therefore, to achieve better prognostic results, it is essential to diagnose it early. Owing to nonspecific signs and symptoms, clinical presentation of NS is unclear.[14,15] Therefore, it results into over investigation, excessive use of antibiotics, and development of drug resistance. Hence, it upsets the resources and health of concerned population.

Direct detection of bacterial DNA from the blood by PCR is an effective adjunct to blood culture for the diagnosis of NS. In this context, Reier-Nilsen *et. al.* revealed that PCR is 66.7% and 87.5% sensitive and specific in contrast to blood culture.[8] These findings are similar to our findings (60% and 88%). Chan *et. al.* further evaluated the utility of PCR for gram specific detection of blood stream infection and found PCR to be 86.4% and 99.0% sensitive and specific respectively towards gram negative bacteria. It was also proved to be a good detector of Gram positive bacteria with the sensitivity and specificity values of 73.7% and 98.5% respectively.[16] Interventions and modifications in molecular tests may increase the sensitivity and specificity even up to 100% but confirmation still needs culture.[17-19]

Another tool for rapid diagnosis of NS is CRP but it shows large variability in sensitivity ranging from 23% to 86%. While specificity varied from 65% to 97%.[20-24] In our study, sensitivity and specificity of CRP

remained to be 60% and 71% respectively. Reier-Nilsen in his study, established that CRP has a significant role in the diagnosis of NS.[8] Despite variability of results, CRP is still being used because it is cheaper and easy test to perform.

Our study, further demonstrates that in NS various hematological factors including WBC (White blood cells), neutrophils and platelets were in normal ranges and it has also been replicated in other studies.[25-27] Therefore, CBC may not be helpful in diagnosing NS. Despite delay in reporting, blood culture remains the gold standard investigation to diagnose sepsis because it gives confirmation and drug susceptibility patterns of etiological agents of NS.[28-30] Our findings suggest that besides, equally high sensitivity and negative predictive values of PCR and CRP, specificity and positive predictive values of PCR are greater than those of CRP. Further, modifications and enhancements of molecular assays may reinforce the utility of these valuable and rapid diagnostic tools in the context of NS.

Although blood culture is the gold standard diagnostic test for sepsis but high sensitivity and negative predictive values of PCR and CRP are pinpointing towards the use of these rapid tests as adjunct with blood culture. While on comparison, specificity and positive predictive values of PCR and CRP showed a remarkable difference. PCR can be used for early and more precise diagnosis of NS.

It may reduce the burden of patients in NICU (neonatal intensive care unit) and minimize the use of antibiotics.

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Conflict of interest: None declared.

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