Polymerase Chain Reaction in Rapid Diagnosis of Neonatal Sepsis

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In a prospective study a total of hundred neonates who fulfilled the American College of Obstetrics and Gynecology's (ACOG) criteria for probable sepsis admitted to NICU of tertiary care armed forces hospital were investigated for evidence of sepsis. The investigation protocol included sepsis screen, blood culture and 1 mL of venous blood for molecular analysis by polymerase chain reaction (PCR) for bacterial DNA component encoding 16 s RNA in all cases. 100 newborns with probable sepsis were studied to evaluate the molecular diagnosis of sepsis using PCR amplification of 16 S RNA in newborns with risk factors for sepsis or those who have clinical evidence of sepsis. We compared the results of PCR with blood culture and other markers of sepsis screen (total leucocyte count (TLC), absolute neutrophil count (ANC), immature/total neutrophil count ratio (I/T ratio), peripheral blood smear, micro-ESR and C-reactive protein (CRP). Controls consisted of 30 normal healthy newborns with no overt evidence of sepsis. Sepsis screen was positive in 24 (24%) of cases in study group with sensitivity and specificity of 100% and 83.5% respectively. Blood culture was positive in 09(9%) with sensitivity of 69.2% and specificity of 100%. PCR was positive in 13(13%) of cases (9% are both blood culture and sepsis screen positive and 4% are positive by sepsis screen); the sensitivity of PCR was 100% and specificity was 95.6%. Blood culture is the most reliable method for diagnosis of neonatal sepsis. Polymerase chain reaction is useful and superior to blood culture for early diagnosis of sepsis in neonates.

Key words: *Bacteria, Neonatal sepsis, Pathogen testing, Polymerase chain reaction.*

CLINICAL diagnosis of sepsis in new born infants is not easy because symptoms and signs are non-specific. There is no laboratory test with 100% specificity and sensitivity, search has continued for a reliable test. Blood culture has been the gold standard for confirmation of diagnosis but the results of the test are available only after 48-72 hours. The neonates with "risk factors" for neonatal sepsis are thus treated with broad-spectrum antibiotics and require prolonged hospitalization.

The alterations of laboratory tests are corroborative parameters to diagnose neonatal sepsis. The sensitivity of each laboratory test is far from 100%(1-5). The measurements of IL-1 receptor antagonist, IL-6, IL-8, are still confined to the research laboratories(6,7). The advent of polymerase chain reaction has made it possible to have a 100% specific and sensitive method for diagnosis of bacterial sepsis in a short time(8,9).

DNA sequence present in all bacteria such as portions of the DNA encoding the 16-S-ribosomal-RNA has been used to define an organism as a bacterium (9). Those sequences are amplified with PCR, using an automated method allowing a rapid diagnosis. By using Multiplex-PCR simultaneous amplification of more than one genetic locus using more than one primer pair can be used to differentiate various etiological agents responsible for

sepsis based on the molecular weight of the fragments amplified(10). In this study the aim was to evaluate the molecular diagnosis of sepsis using PCR amplification of 16 S rRNA in newborns with risk factors for sepsis or those who have clinical evidence of sepsis. We also compared the results of PCR with blood culture and other markers of sepsis screen [total leucocyte count (TLC), absolute neutrophil count (ANC), immature/total neutrophil count ratio (I/T ratio), peripheral blood smear, micro-ESR and C-reactive protein (CRP)].

Subjects and Methods

At level II neonatology unit of a tertiary care referral hospital 100 newborns with 1 major or 2 minor risk factors for sepsis as per ACOG guidelines(11) were included in the study. The major risk factors for inclusion were: (*i*) premature rupture of membrane (PROM)>24 hrs; (*ii*) premature onset of labor <37 week; (*iii*) Chorioamnionitis; (*iv*) Intrapartum maternal fever >38ºC and the minor risk factors for inclusion were: (*i*) PROM >12 hrs, but <24 hrs; (*ii*) prematurity; (*iii*) very low birth weight (<1500 gms); (*iv*) intrapartum maternal fever >37.5ºC but <38ºC; (*v*) multiple gestations; and (*vi*) apgar score at one minute <5 .

Soon after birth, 1 mL of venous blood was drawn for blood culture and PCR each. Also 5 mL of venous blood was collected for TLC, DLC, peripheral blood smear, micro-ESR and CRP. The sepsis screen consisting of TLC $<5 \times 10^9$/L, ANC $<1.5 \times 10^9$/L, I/T ratio >0.2, micro-ESR and CRP was carried out. The sepsis screen was considered positive if I/T ratio >0.2 and CRP was positive(12). The newborns developing symptoms and signs of sepsis later (within 07 days) were also investigated by PCR and blood culture as per the above protocol. The gold standard for diagnosis of sepsis was positive blood culture.

The control group consisted of 30 cases of normal neonates admitted to the postnatal ward who had neither overt evidence of sepsis nor any of the risk factors. They were evaluated for sepsis with sepsis screen and subjected to PCR amplification for 16-S RNA (861 base pair).

Presence of bacterial DNA in blood samples was evaluated by amplifying the DNA region encoding 16 rRNA (861 base pair) using the following primers: F-5AGAGTTTGAT-CCTGGCTCAG-3' (15) and R-5'GGACTACCAGGGTACTT AAT-3'(15). A small volume of the sample (2μ) was mixed in the PCR reaction(12), (briefly, 50 mM KCI, 10 mM Tris-HCL (pH-8), 1.5 mM $MgCl_2$, 200 µL each d NTP, 60 pM each primer and 2.5U Taq polymerase, in a final volume of 100 μL. The PCR was carried out using whole blood (2μL) as the sample for DNA templates(13).

The Taq polymerase was then added and the eppendorf tubes were placed in the Automatic Thermal Cycler. The amplification used an initial modifying denaturation step (03 min at 94ºC and 03 min at 55ºC for 3 times) followed by 30 cycles (1 min at 95ºC, 1 min at 54ºC and 1 min at 72º C). A final extension cycle of 72ºC for 07min was carried out. Each PCR run was carried out including a known positive control; the kit control DNA produced a 600 bp band. The amplified DNA was separated by agarose gel electrophoresis stained with ethidium bromide and visualized under UV trans-illuminator. Analysis was carried out keeping blood culture as the gold standard.

Results

The study population comprised of 100 newborns who fulfilled the ACOG criteria for

probable sepsis from a population of 1923 live births at a large tertiary care hospital who presented consecutively. The prevalence of maternal risk factors was PROM (18%), maternal fever (10%), foul discharge per vaginum (6%) and multiple gestations (4%).

The mean birth weight of the neonates in the study group was 1829 ± 608 grams. The average age of diagnosis of sepsis (based on blood culture results) was 3.6 days and ranged from 2-7 days. In only one neonate intravascular catheter was used and 11 (11%) newborns required some resuscitation. The mean birth weight of the neonates in the control group was 2310 ± 516 grams. The prevalence of neonatal risk factors was prematurity (15%), VLBW (07%) and low APGAR score (11%).

The 06 paramecters of sepsis screen were studied in the study as well as the control group. Of the 100 neonates in the study group I/T ratio of >0.2 and positive CRP was observed in 24 cases (24%), 12 cases (12%) had TLC <5000/cumm, presence of toxic granules in 6% cases and two cases each (4%) fulfilled the criteria for ANC and micro-ESR.

Blood culture yielded positive results in 9 (9%) of the study group and had a sensitivity of 69.2% for diagnosis of neonatal sepsis. Lumbar puncture was performed in all septic neonates with positive blood culture and only 02 (2/9) had abnormal cytology. All CSF cultures were sterile. In comparison with that the sepsis screen had sensitivity of 100%, specificity of 83.5% and negative predictive value of 100% (*Table I*). PCR amplification for 861 bp was positive in 13 cases (13%). This included 04 cases with positive sepsis screen but negative blood culture. PCR was positive in all blood culture positive cases. Thus PCR is proved to have sensitivity of 100%, specificity of 95.6% and negative predictive value of 100%. The lower specificity of PCR is because of all the 04 cases with positive PCR but sterile cultures being true positives and not false positives and can be explained by the lower sensitivity of the gold standard. Of the 15 neonates with positive sepsis screen but negative blood culture, PCR was positive in 4 cases (*Table II*).

In the study group with risk factors for sepsis, 24 cases (24%) required antibiotics for 14-21 days and in remaining 76 cases (76%) antibiotics were stopped based on clinical condition, sterile blood culture and negative PCR for 861 bp bands. All the neonates had a normal outcome.

Discussion

Infections in the neonate are most important cause of mortality and hospitalizations in the neonatal practice. Early recognition of sepsis in neonates is difficult. Early diagnosis and timely treatment of neonatal infections is essential(14). For obvious reasons there is a tendency to over investigate and over treat neonates for suspected sepsis. Sepsis screen and the blood culture have been the diagnos-

TABLE I–*Utility of sepsis screen for diagnosis of Neonatal Sepsis.*

Sepsis screen	Blood culture	
	Positive	Negative
Positive	9	15
Negative	0	76

TABLE II–*Utility of PCR in Diagnosis of Neonatal Sepsis.*

PCR	Blood culture	
	Positive	Negative
Positive	9	04
Negative	0	87

Key Messages

- Sepsis screen is a useful test in clinical side room setting for ruling out neonatal sepsis.
- Blood culture remains the gold standard, but has limitation of delay period of 48-72 hours.
- Polymerase chain reaction with 100% sensitivity and 95.6% specificity, once available in most tertiary centers can help in early and accurate diagnosis.

tic pillars but their sensitivity and specificity is far from 100%(8,10,13).

In this study the incidence of early onset neonatal sepsis is 3.1 per 1000 live births that is low compared to the incidence reported by Mathur et al and Mondal, *et al.*(13-17). Sepsis screen is a useful tool for evaluation of sepsis with the sensitivity of 100%, specificity of 83.5% and negative predictive value of 97.4%. Mathers, *et al.* and Pourcyrous, *et al.* have reported similar results(17,18). With the sensitivity of 69.2% and specificity of 100%; blood culture alone would have missed another 04 (30%) cases. The low sensitivity in this study in comparison to 85% by Visser, *et al.* is likely to be related to the use of antibiotics in large number of mothers (68%) in the antepartum period(19).

PCR with sensitivity of 100% and specificity of 95.6% is a useful tool because of the short time required to reach confirmation. The PCR has shown 100% correlation with microbiological methods in all infected cases and was positive in four additional cases with positive sepsis screen but with sterile blood cultures. In a similar study Laforgia, *et al.* studied 33 newborn infants at risk for early onset neonatal sepsis and found 29 negative blood cultures and 4 positive blood cultures. All cases with positive blood culture yielded 861 base pair bands. Among cases with negative blood culture 2 cases resulted in amplification of 861 base pair bands(13).

Unnecessary treatment with antibiotics could be avoided in 76 cases of newborns with sterile blood culture and negative PCR.

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REFERENCES

1. Placzek MM, WhiteLaw A. Early and late neonatal septicemia. Arch Dis Child 1983; 58: 728-731.
2. Freedman RM. Ingram DL, Gross MB, Ehrenkranz RA. Warshaw JB, Baltimore RS. A half century of neonatal sepsis at Yale. Am J Dis Child 1981; 135: 140-144.
3. Gluck L, Wood H F, Fousek MD. Septicemia of the newborn. Pediatr Clin North Am. 1966; 13: 1131-1147.
4. Jahanke S, Bartirome G, Massles MG. Perinatal white blood cell counts in the diagnosis of neonatal infections. J Perinatol 1985; 5: 50-54.
5. Akenzua GI, Hui YT, Milner R, Zipursky A. Neutrophil and band counts in the diagnosis of neonatal infections. Pediatrics 1974; 54: 38-42.
6. Barner R, Neimeyer CM, JU Funke A, Schwab C, Raw U, *et al*. Plasma levels of G-CSF, IL-l, IL-6, IL-8, TNF alpha and ICAM in early onset neonatal sepsis. Pediatr Res 1998; 44: 469-477.

7. Kuster H, Weisis M, Willeitner AE, Detelefsen S, Jeremias I, Zbojan J, *et al.* IL-1 receptor antagonist and IL-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet 1998; 352: 1271-1277.

8. Stoker NG. The polymerase chain reaction and infectious disease hopes and realities. Trans Roy Soc Trop Med Hyg 1990; 84: 755-758

9. Angert ER, Clements KD, Pace NR. The largest bacterium. Nature 1993; 362: 239.

10. McCabe KM, Khan G, Zhang YR, Mason E O, McCabe ERB. Amplification of bacterial DNA using highly conserved sequences, automated analysis and potential for molecular triage of sepsis. Pediatrics 1995; 95: 165-169.

11. Guerina NG. Bacterial and fungal infections. In: Manual of Neonatal Care. Eds Cloherty JP, Stark AR. 4th Ed. Philadelphia; Lippincott Raven; 1997. p. 273-300.

12. Philip AGS. The changing face of neonatal infection: experience at a regional medical center. Pediatr Inf Dis J 1994; 13: 1098-1102.

13. Laforgia N, Coppola B, Carbone R, Grassi A, Mautone A, lolascon A. Rapid detection of neonatal sepsis using polymerase chain reaction. Acta Ped 1997 86: 1097-1099.

14. Mathur NB. Neonatal Sepsis. Indian Pediatr 1996; 33: 663-674.

15. Chugh K, Aggarwal BB, Kaul VK, Arya SC. Bacteriological profile of neonatal septicemia. Indian J Pediatr 1998; 55: 961-965.

16. Mondal GP, Raghavan M, Bhat BV, Srinivas S. Neonatal septicemia among inborn and out born babies in a referral hospital. Indian J Pediatr 1991; 58: 529-533.

17. Mathers NJ, Pohlandt F. Diagnostic audit of C-reactive protein in neonatal infection. Eur J Pediatr 1987; 146: 147-151.

18. Pourcyrous M, Bada HS, Korones B, Barrett F F, Jennings W, Lockey T. Acute phase reactants in neonatal bacterial infections. J Perinatol 1991; 11: 319-325.

19. Visser VE, Hall RT. Lumbar puncture in the evaluation of neonatal sepsis. J Pediatrics 1980; 96: 1063-1067.