ROLE OF SERIAL C-REACTIVE PROTEIN (CRP) IN RELATION TO TOTAL LEUCOCYTE COUNT, PLATELET COUNT & BLOOD CULTURE FOR EARLY DIAGNOSIS OF NEONATAL SEPTICEMIA IN DEVELOPING COUNTRIES

Chiranjib Barua[1] Md Nurul Anwar[2] Md Shahidullah[3] Sharmila Barua[4] Nobel Chowdhury[5]

Summary

Neonatal septicemia is a clinical syndrome of systemic illness accompanied by bacteremia occuring in the first 28 days of life. Neonatal septicemia is one of the major causes of neonatal death in developing countries. Early diagnosis and treatment can prevent neonatal mortality and morbidity. The present study includes: 1) usefulness of CRP (C-reactive protein), Total Leucocyte Count, Platelet Count and Blood Culture in early diagnosis of Neonatal Sepsis, 2) significance of serial CRP in diagnosis of neonatal sepsis. 3) the prognostic value of CRP in neonatal sepsis. This is a prospective study done in neonatal ward, Chittagong Medical College Hospital and carried out from January 2008 to January 2011. Sample size was 300. One hundred fifty neonates with suspected sepsis as cases and 150 healthy babies as control were enrolled in this study. Seventy two percent of cases neonates were preterm and low birth weight. Common risk factors for neonatal septicemia which were identified in this study; preterm (72%), low birth weight (72%), premature rupture membrane (60%), chorioamnionitis (26%) and maternal urinary tract infection (16%). Out of 150 cases of suspected neonatal sepsis total 80.7%% had raised CRP, in initial sample 70.39% were CRP positive and in 2nd sample additional 9.31% case were CRP positive . In control group 91% were CRP negative. CRP was positive in 100% of culture proven sepsis. Sensitivity of CRP was 80.67% and specificity of CRP was 76.44%. Leucocytosis was observed in 7% of cases and leucopenia was found in 11% of cases. In 82 % cases leucocyte count was found normal. In control group, 95% had normal leucocyte count and 5% had leucocytosis but no leucopenia. Sensitivity of leucocyte count was 18% and specificity was 20.68%. Thrombocytopenia was found in 28% of case group. Out of 150 cases only 15.33% yielded growth of organisms in blood culture. Klebsiella was the most common pathogen isolated which was followed by E.coli and Strph. aureus. Sensitivity of blood culture was 15.33% and specificity was 100% Therefore serial CRP can be taken as alternative method for diagnosis of neonatal sepsis specially in developing countries where blood culture is not readily available.

Key word

CRP; neonatal sepsis; premature rupture membrane; chorioamnionitis.

1. Associate Professor of Neonatalogy
Chittagong Medical College, Chittagong
2. Professor & Dean
Faculty of Biological Sciences
Chittagong University, Chittagong
3. Chairman of Neonatalogy
Bangabandhu Skeikh Mujib Medical University, Dhaka
4. Associate Professor of Obstetrics & Gynaecology
Chittagong Medical College, Chittagong
5. Medical Officer of Neonatalogy
Chittagong Medical College & Hospital, Chittagong

Correspondence : Dr Chiranjib Barua
email : baruachiranjib@yahoo.com

Introduction

Neonatal septicemia is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first 28 days of life [1]. Neonatal sepsis may be categorized as early onset, late onset and late onset sepsis [2]. Early onset sepsis occurs after birth to 7 days of life and is acquisition of microorganisms from the mother. It may occur through transplacental infection or an ascending infection from the mother's genitourinary tract. The infant may acquire the microbe by passage through a microbes colonized at birth canal during delivery. In global perspective the microorganisms most commonly associated with early onset of infection include group B Streptococci, Escherichia coli, Heomophilus influenzae, Listeria monocytogenes [3]. Late onset sepsis occurs at 7-28 days of life and is acquired from the care giving environment. Organisms that have been implicated in causing late onset sepsis include coagulase-negative Staphylococci, Staphylococci aureas, E.coli, Klebsiella, Pseudomonas, Enterobacter, Candida, Streptococcus and anaerobes [4]. Non typeable H. influnzae sepsis has been identified in neonates, especially premature neonates. Candida are increasing important cause of late onset neonatal sepsis [2].

Early onset sepsis occurs after birth to 7 days of life and is acquisition of microorganisms from the mother [1,2]. Late onset sepsis occurs at 7-28 days of life and is acquired from the care giving environment. The incidence of neonatal sepsis is 5 to 8 per 1000 birth, the highest rates occur in LBW, perinatal asphyxia, maternal infection and congenital abnormality babies [2]. Culture positive neonatal sepsis in the USA is 0.7% but in very low birth weight infants under prolonged intensive care the rate of culture proven sepsis may be high as 30% [5]. In the developing world neonatal sepsis is one of the commonest cause of perinatal mortality in the developing world [6]. Mortality rate of early onset neonatal sepsis is 2 to 40% and late onset neonatal sepsis is 2 to 20% [2]. Definitive diagnosis of neonatal sepsis is based on positive blood or cerebrospinal fluid(CSF) culture, which both take at least 24 to 48 hours and are often falsely negative. Determination of C-reactive protein (CRP), Total Leucocyte Count and Platelet Count have been used to establish or rule out neonatal infection and to aid the decision of to terminate unwanted antibiotic therapy [7,8,9,10]. CRP produced by the liver under the influence of IL1 when inflammation is present. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production.

Aims and objectives

General objective: Reduction of neonatal morbidity and mortality by early diagnosis of Neonatal sepsis.

Specific objectives:

1) To find out the role of CRP, Total Leucocyte Count, Platelet Count and blood culture in early diagnosis of neonatal sepsis.

2) To detect the significance of serial CRP in diagnosis of neonatal sepsis.

3) To find out the prognostic value of CRP in neonatal sepsis.

Inclusion criteria:1) Preterm & Term neonates.2) Normal birth weight, low birth weight & very low birth weight neonates.3) Clinical features of sepsis.

Criteria of control group: Term/Pre-term, normal birth weight/low birth weight, without any evidence of sepsis, asphyxia or history of maternal infection.

Exclusion criteria: 1) Very sick neonates 2) Congenital abnormality 3) Perinatal asphyxia 4) Birth trauma.

Procedure: First blood sample (3 ml) collected at the day of admission (excluding 1st 24 hours of life) and another sample (1.5 ml) of blood drawn at 5th of day of admission. Proper hand washing was done with soap and water and subsequently hands were dried by using sterile (autoclaved) small towel. 3ml of blood from each patient were collected from single venipuncture. One ml of blood was taken into a clean and dry test tube for estimation of C-reactive protein. Another 1ml of blood was taken in a dry clean small vial containing anticoagulant for estimation of leucocyte and platelet count and rest 1ml of blood was introduced at bed side into blood culture bottle. Second sample of blood sent for CRP estimation only. CRP value 6 mg/L or more was taken as CRP positive.

Ethical aspects: Permission from parents were taken before enrolling each baby in this study and discussed clearly.

Statistical Analysis: Data are presented as the percentage of total number of observations. SPSS for windows (Vs.11) was used for the analysis of data t-test and x2 –test were used where appropriate .P<0.05 was used as the minimum level of significance.

Results

Table I: Distribution of weight in study groups and comparison between normal birth wt. & low birth wt. babies.

Birth weight	Case (n=150)	Control(n=150)	P value
1000- <1500 gm	48 (32%)	9 (6%)	
1500- <2500 gm	60 (40%)	48 (32%)	< 0.001
≥ 2500 gm	42 (28%)	93 (62%)	
<2500 vs ≥ 2500 gm	5.93/p<0.001		

Table I, it was seen that infections occurred more frequently in <2500gm group Chi-Square test value is 5.93, P value < 0.001 which is statistically significant.

Table II: Platelet count in case and control group.

Platelets count	Case (n=150)	Control (n=150)
Normal (150000-300000/mm^3)	108(72%)	150(100%)
Thrombocytopenia (< 150000/ mm^3)	42(28%)	0

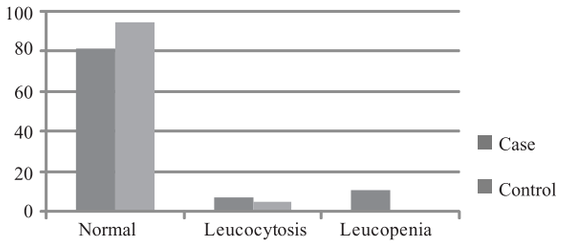

Fig 1 : Leucocyte status in case and control groups.

Fig 1 shows leucocytosis, leucopenia and normal leucocyte counts. Seven percent leucocytosis and 11% leucopenia were recorded in the present study but 82% cases showed normal leucocyte count.

Table III : Blood culture in case and control groups.

Culture in Study Groups			Study Groups case	control	Total	x^2 test	P value
Culture	Positive	Count	23	0	23		
		% within Study Groups	15.3%	0.0%	7.7%	24.91	<0.001
	Negative	Count	127	150	277		
		% within Study Groups	84.7%	100.0%	92.3%		
Total		Count	150	150	300		
		% within Study Groups	100.0%	100.0%	100.0%		

Table III shows out of 300 enrolled neonates 23 (15.3%) cases were culture positive. P value is<.001 which is statistically highly significant.

Table IV: Organisms isolated from Blood Culture in case group(n=23).

Organism isolated	Number	Percentage
Klebsiella	13	56.52
E. coli	7	30.43
Staphy. Aureus	3	13.05

Table-IV shows that Gram Negative Klebsiella was the commonest organism isolated in blood culture. E. coli and Staphylococcus aureus were also common organisms.

Table V : C-reactive protein (CRP) in study groups.

CRP	Study Groups		Study Groups case	control	Total	x^2 test	P value
	CRP	Positive Count	121	25	146		
		% within Study Groups	80.67%	16.7%	48.7%		
		Negative Count	29	125	154	122.96	< 0.001
		% within Study Groups	19.33%	83.3%	51.3%		
Total	Count	150	150	300			
	% within Study Groups		100.0%	100.0%	100.0%		

Table-V shows that majority of cases (80.67%) were CRP positive . Only 19.33% cases were CRP negative.

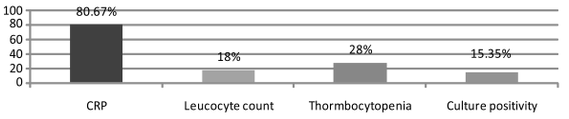

Fig 2 : Comparative scenario among CRP, leucocyte count, thrombocytopenia and blood culture.

Fig 2 shows among three lab. investigations CRP positivity was high (80.67%) thrombocytopenia (28%), leucocytosis and leucopenia (18%). Only 15.35% cases were culture positive.

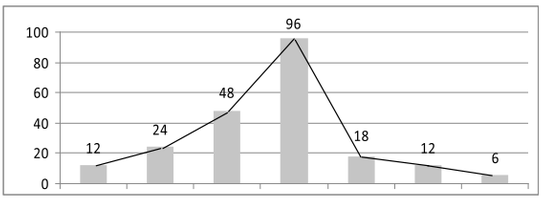

Fig 3 : CRP in day 1 and day 5(mg/dl).

Fig 3 shows high CRP values gradually decline to normal in case group in day 1 and day 5. It indicates prognostic significance of CRP.

Discussion

Septicemia was found more common in male (55%) than female. male-female ratio was 1.2:1[12]. Neonatal sepsis observed more in low and very low birth weight babies [1,12]. Out of 150 cases of suspected neonatal sepsis 80.67% had raised CRP. In culture proven sepsis 100% cases had raised CRP [13,11]. In this study, at initial blood sample CRP positive was 70.39% and in second sample 9.31% were CRP positive but in control group only 16.7% had raised CRP. Forty three per cent of initial sample CRP values were 18mg/dl or more and decline to 12mg/dl or to 6mg/dl in second sample who were under antibiotic treatment. Such finding indicates prognostic significance of CRP [15]. Leucocytosis was observed in 7% of cases and leucopenia was found in 11% of cases. Though in 82% patients leucocyte count was within the normal limit. Significance of leucocyte count in the diagnosis of sepsis is low. In control group, 95% had normal leucocyte count and only 5% had leucocytosis but no leucopenia [14]. Thrombocytopenia was observed in 28% cases. Thrombocytopenia was not found in control group [2,17]. Sensitivity of CRP was 80.67% and specificity was 78.44%, sensitivity of blood culture was recorded 15.33% though specificity was high 100%. Sensitivity of leucocyte count (leucocytosis and leucopenia) was18% but specificity also low (20.68%). So, CRP was more

sensitive investigation tool than other laboratory tests. A negative CRP is important than a positive CRP value in that it excludes infection with a high certainty. Out of 150 neonates with suspected septicemia, the present study showed only 15.33% had growth in blood culture. In control group blood culture yielded no growth [14]. Among the isolated organisms, 87% were gram-negative and rest13% were gram positive. The predominance of gram negative organisms in neonatal septicemia is similar to the observation of other investigations from Bangladesh, India, Pakistan and Nepal [15,16]. Klebsiella was the commonest pathogen isolated (56.52%) followed by E. coli [6,15,13].

Conclusion

In neonatal sepsis, there is no significant change of leucocyte count but thrombocytopenia is not uncommon. CRP is easier to measure, cheaper and the result is available earlier. Serial CRP is one of the most sensitive tests for early diagnosis of neonatal sepsis especially in developing countries.

Disclosure

All the authors declared no competing interest.

References

1. Gotoff SP. Infection of the neonatal infant. In: Behrman RE, Kliegman RM, Jenson HB. Nelson textbook of pediatrics. Philadelphia: WB Saunders Company. 2000;16;538-549.

2. Mery TC, Oct.2009. Neonatal sepsis.National Institute of Health. Online Medical Library.

3. Placzek MM and Whitelow A; Early and late neonatal septicemia. Arch Dis Child 1983; 58: 728-731.

4. Stoll BJ, Gordon T and Korones SB. Early onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. Journal of Pediatric 1996; 129:72-80.

5. Peter D; Infection in the newborn. In: Rennie Jm, Roberton NRC, eds. Textbook of Neonatology. London: Churchill Livingstone :1999;3:1109.

6. Gupta P, Murali MV, Faridi MMA, Kaul PB, Ramchandran VG and Talwar V; Clinical profile of Klebsiella septicemia in neonates. Indian J Pediatr; 1993; 60: 565-572.

7. Tessin I, Trollfors B and Thringer K. Incidence and aetiology of neonatal septicemia and meningits in Western Sweden. Acta Paediatr Scand 1975; 79: 1023-1030.

8. Boo NY and Chor CY. Six-year trend of neonatal septicemia in a large Malaysian maternity hospital. J Pediatr Child Healt, 1994;30:23-27.

9. Guerina NG; Bacterial and fungal infection. In: Cloherty JP and Stark AR, eds. Manual of neonatal care. Philadelphia: Lippincott-Raven Publishers, 1998;4:271-299.

10. Polin RA and Geme JWS. Neonatal sepsis. Adv Paediatr Infect Dis 1992;7: 25-60.

11. Magudumana MD, Ballot DE, Copper PA, Cory BJ, Viljoen E and Carter AC; Serial IL-6 measurements in the early diagnosis of neonatal sepsis. J Trop Pediatr ; 2000; 46:267-277.

12. Reghavan M, Mondal GP, Bhat BV and Srinivasan S. Perinatal risk factors in neonatal infections. Indian J Pediatr ; 1992; 59: 335-340.

13. Chiesa C, Panero A, Osban JF, Simonetti AF. Diagnosis of Neonatal Sepsis.Clinical Chemistry 2004;50: 279-287.

14. Chaturvedi P, Agrawal M and Narang P; Analysis of blood culture isolates from neontaes of a rural hospital. Indian Pediatr 1989; 26: 460-465.

15. Bhutta ZA, Naqbi SH, Muzaffar T and Farroqui BJ; 1991. Neonatal sepsis in Pakistan. Acta Paediatr Scand ; 1991;80:596-601.

16. Karki BM and Parija SC. Analysis of blood culture isolates from hospitalized neonates of Nepal. SE Asia J Trop Med Pub Health . 1999;30: 546-548.

17. Ayenger V, Madhulika and Vani SN. Neonatal sepsis due to vertical transmission from maternal genital tract. Indian J Pediatr 1991, 58: 661- 664.