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Clinical assessment of association of C-reactive protein and blood culture in neonatal sepsis cases

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Abstract

Blood culture remains the gold standard and goes hand in hand with the clinical symptoms in arriving at the diagnosis. But it is time consuming, requires well equipped laboratory and trained personnel. And not all the cases of neonatal sepsis show culture positivity. In case of neonatal sepsis the culture positivity is only moderate. So development of novel early diagnostic markers of sepsis like pro-calcitonin, interleukin-, C-reactive protein etc., help in early assessment of infection, reducing the mortality and sequelae. They are now used in conjunction with blood culture in diagnosing sepsis in neonates. All these variables help in very early diagnosis and in prompt institution of anti-biotic therapy only when indicated thus avoiding their indiscriminate usage. This helps the neonate to bypass the unnecessary adverse effects of anti-biotic administration, makes the treatment cost effective and prevents the emergence of multi- drug resistant strains. Hence based on above findings the present study was planned for Clinical Assessment of Association of C-Reactive Protein and Blood Culture in Neonatal Sepsis Cases.

The present study was planned in Department of Pediatrics, Nalanda Medical College, Patna, Bihar, India. The present study was conducted from March 2015 to Oct 2015. In the present study 50 cases of the suspected neonatal septicemia were enrolled and evaluated with different parameters.

The data generated from the present study concluded that biomarkers like CRP may serve as an important tool in reducing the mortality and morbidity among neonates diagnosed from sepsis. Also biomarkers like CRP may serve as an important tool in reducing the mortality and morbidity among neonates.

Keywords: c-reactive protein, blood culture, biomarkers, neonates, septicaemia, etc

Introduction

Neonatal sepsis is a clinical syndrome of bacteremia characterized by systemic signs and symptoms of infection in the first month of life. Neonatal sepsis encompasses systemic infections of the newborn including septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infection of the newborn.

Neonatal sepsis may be categorized as early onset (day of life 0-3) or late onset (day of life 4 or later). Of newborns with early-onset sepsis, 85% present within 24 hours (median age of onset 6 hours), 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature neonates.

Early-onset sepsis is associated with acquisition of microorganisms from the mother. Infection can occur via hematogenous, transplacental spread from an infected mother or, more commonly, via ascending infection from the cervix. Organisms that colonize the mother's genitourinary (GU) tract may be acquired by the neonate as it passes through the colonized birth canal at delivery. The microorganisms most commonly associated with early-onset infection include the following [1]: Group B Streptococcus (GBS), Escherichia coli, Coagulase-negative Staphylococcus, Haemophilus influenza, Listeria monocytogenes.

Trends in the epidemiology of early-onset sepsis show a decreasing incidence of GBS disease following the widespread adoption of prenatal screening and treatment protocols [2, 3, 4].

In a study involving 4696 women, prenatal cultures showed a GBS colonization rate of 24.5%, with a positive culture rate of 18.8% at the time of labor [5]. As many as 10% of prenatally culture-negative women were found to have positive cultures at the time of labor. In the study, intrapartum antibiotic prophylaxis occurred appropriately in 93.3% of cases, with 0.36 of 1000 infants developing early-onset GBS disease [5].

Trends in late-onset sepsis show an increase in coagulase-negative Staphylococcal sepsis, with most isolates showing susceptibility to first-generation cephalosporins [2]. The infant's skin, respiratory tract, conjunctivae, gastrointestinal tract, and umbilicus may become colonized via contact with the environment or caregivers.

Pneumonia is more common in early-onset sepsis, whereas meningitis and bacteremia are more common in late-onset sepsis. Early-onset sepsis is 10 to 20 times more likely to occur in premature, very low birthweight infants [6]. Premature infants often have nonspecific, subtle symptoms; considerable vigilance is therefore required in these patients so that sepsis can be identified and treated in a timely manner.

The infectious agents associated with neonatal sepsis have changed since the mid-20th century. During the 1950s, S aureus and E coli were the most common bacterial pathogens among neonates in the United States. Over the ensuing decades, Group B Streptococcus (GBS) replaced S aureus as the most common gram-positive organism causing early-onset sepsis.

Currently, GBS and E coli continue to be the most commonly identified microorganisms associated with neonatal infection. Additional organisms, such as coagulase-negative Staphylococcus epidermidis, L monocytogenes, Chlamydia pneumoniae, H influenzae, Enterobacter aerogenes, and species of Bacteroides and Clostridium have also been identified in neonatal sepsis.

Meningoencephalitis and neonatal sepsis can also be caused by infection with adenovirus, enterovirus, or coxsackievirus. Additionally, sexually transmitted diseases (eg, gonorrhea, syphilis, herpes simplex virus [HSV] infection, cytomegalovirus [CMV] infection, hepatitis, human immunodeficiency virus [HIV] infection, rubella, toxoplasmosis, trichomoniasis, and candidiasis) have all been implicated in neonatal infection.

Bacterial organisms with increased antibiotic resistance have emerged and have further complicated the management of neonatal sepsis [7]. The colonization patterns in nurseries and personnel are reflected in the organisms currently associated with nosocomial infection. In neonatal intensive care units (NICUs), infants with lower birth weight and younger gestational ages have an increased susceptibility to these organisms.

S epidermidis, a coagulase-negative Staphylococcus, is increasingly seen as a cause of nosocomial or late-onset sepsis, especially in the premature infant, in whom it is considered the leading cause of late-onset infections. Its prevalence is likely related to several intrinsic properties of the organism that allow it to readily adhere to the plastic mediums found in intravascular catheters commonly required for the care of these infants.

The bacterial capsule polysaccharide adheres well to the plastic polymers of the catheters. Also, proteins found in the organism (AtlE and SSP-1) enhance attachment to the surface of the catheter. The adherence creates a capsule between microbe and catheter, preventing C3 deposition and phagocytosis [8, 9].

Biofilms are formed on indwelling catheters by the aggregation of organisms that have multiplied under the protection provided by the adherence to the catheter. Slimes are produced at the site from the extracellular material formed by the organism, which provides a barrier to host defense as well as to antibiotic action, making coagulase-negative staphylococcal bloodstream infection (BSI) more difficult to treat. The toxins formed by S epidermidis have also been associated with necrotizing enterocolitis.

In addition to being a cause of neonatal sepsis, coagulase-negative Staphylococcus is ubiquitous as part of the normal skin flora. Consequently, it is a frequent contaminant of blood and cerebrospinal fluid (CSF) cultures. When a culture grows this organism, the clinical presentation, colony counts, and the presence of polymorphonuclear neutrophils (PMNs) on Gram staining of the submitted specimen often help differentiate true infection from contaminated culture specimens.

In addition to the specific microbial factors mentioned above, numerous host factors predispose the newborn to sepsis [10]. These factors are especially prominent in the premature infant and involve all levels of host defense, including cellular immunity, humoral immunity, and barrier function. Immature immune defenses and environmental and maternal factors contribute to the risk for neonatal sepsis, morbidity, and mortality, particularly in preterm and/or very low birthweight (VLBW) infants [10, 11]. There may also be a genetic association [10].

PMNs are vital for effective killing of bacteria. However, neonatal PMNs are deficient in chemotaxis and killing capacity. Decreased adherence to the endothelial lining of blood vessels reduces their ability to marginate and leave the intravascular space to migrate into the tissues. Once in the tissues, they may fail to degranulate in response to chemotactic factors.

Furthermore, neonatal PMNs are less deformable and thus are less able to move through the extracellular matrix of tissues to reach the site of inflammation and infection. The limited capacity of neonatal PMNs for phagocytosis and killing of bacteria is further impaired when the infant is clinically ill. Finally, neutrophil reserves are easily depleted because of the diminished response of the bone marrow, especially in the premature infant [12].

Neonatal monocyte concentrations are at adult levels; however, macrophage chemotaxis is impaired and continues to exhibit decreased function into early childhood. The absolute numbers of macrophages are decreased in the lungs and are likely decreased in the liver and spleen as well. The chemotactic and bactericidal activity and the antigen presentation by these cells are also not fully competent at birth. Cytokine production by macrophages is decreased, which may be associated with a corresponding decrease in T-cell production [13].

Although T cells are found in early gestation in fetal circulation and increase in number from birth to about age 6 months, these cells represent an immature population. These naive cells do not proliferate as readily as adult T cells do when activated, and they do not effectively produce the cytokines that assist with B-cell stimulation and differentiation and granulocyte/monocyte proliferation.

Formation of antigen-specific memory function after primary infection is delayed, and the cytotoxic function of neonatal T cells is 50%-100% as effective as that of adult T cells. At birth, neonates are deficient in memory T cells. As the neonate is exposed to antigenic stimuli, the number of these memory T cells increases.

Natural killer (NK) cells are found in small numbers in the peripheral blood of neonates. These cells are also functionally immature in that they produce far lower levels of interferon gamma (IFN-γ) upon primary stimulation than adult NK cells do. This combination of findings may contribute to the severity of HSV infections in the neonatal period.

The incidence of culture-proven early-onset sepsis in the United States is approximately 0.3-2 per 1000 live births. Of the 7%-13% of neonates who are evaluated for neonatal sepsis, only 3%-8% of those screened will have culture-proven sepsis. This disparity arises from the cautious approach to management of neonatal sepsis [11].

Because early signs of sepsis in the newborn are nonspecific, diagnostic studies are often ordered and treatment initiated in neonates before the presence of sepsis has been proven. Moreover, because the American Academy of Pediatrics (AAP) [12, 13], the American College of Obstetricians and Gynecologists (ACOG) [14], and the Centers for Disease Control and Prevention (CDC) [15] all have recommended sepsis screening or treatment for various risk factors related to Group B Streptococcus (GBS) infections, many asymptomatic neonates now undergo evaluation and are exposed to antibiotics.

Mortality from untreated sepsis can be as high as 50%,

leading many clinicians to err on the side of treating asymptomatic infants based on historical and maternal risk factors alone. This approach has been questioned in the past several years as more evidence emerges on the deleterious impact of unnecessary antibiotic exposure, including interference with the establishment of breast feeding, alternations in gut microbiome, increases in the incidence of childhood obesity, and development of antimicrobial resistance, amongst others [16].

Blood culture remains the gold standard and goes hand in hand with the clinical symptoms in arriving at the diagnosis. But it is time consuming, require well equipped laboratory and trained personnel. And not all the cases of neonatal sepsis show culture positivity. In case of neonatal sepsis the culture positivity is only moderate. So development of novel early diagnostic markers of sepsis like pro-calcitonin, interleukin-, C-reactive protein etc., help in early assessment of infection, reducing the mortality and sequelae. They are now used in conjunction with blood culture in diagnosing sepsis in neonates. All these variables help in very early diagnosis and in prompt institution of anti-biotic therapy only when indicated thus avoiding their indiscriminate usage. This helps the neonate to bypass the unnecessary adverse effects of anti-biotic administration, makes the treatment cost effective and prevents the emergence of multi- drug resistant strains. Hence based on above findings the present study was planned for Clinical Assessment of Association of C-Reactive Protein and Blood Culture in Neonatal Sepsis Cases.

Methodology

The present study was planned in Department of Pediatrics, Nalanda Medical College, Patna, Bihar, India. The present study was conducted from March2015 to Oct 2015 In the present study 50 cases of the suspected neonatal septicemia were enrolled and evaluated with different parameters.

1-2 mL of blood collected aseptically was inoculated into blood culture bottle containing 5 mL of Brain Heart Infusion Broth. Blood culture bottles were incubated at 37°C aerobically. After overnight incubation blood culture bottles were examined for indicators of growth like turbidity, gas production, haemolysis or discrete colonies on the surface of sedimented red cells. If any of these were present subculture was done on to blood agar and MacConkey agar. If indicators of growth were not present primary subculture was done after 48 hours of incubation on blood agar and MacConkey agar. If no growth occurred on plates after overnight incubation, bottles were incubated further and observed daily for indicators of growth till 7 days. A final subculture was done at the end of day 7 or at appearance of indicators of growth which ever was earlier. The colonies grown on blood agar and MacConkey agar were identified by conventional methods according to the standard laboratory protocol, including colony morphology, Gram staining and biochemical reactions [17]. CRP estimation was done by Latex Agglutination Card test. CRP was reported as positive if agglutination particles were detected and negative if no particles were seen. Samples positive for CRP were further subjected to CRP estimation using Automated Clinical Chemistry Analyser (ERBA Diagnostics Mannheim GmbH- Germany) [18].

All the patients were informed consents. The aim and the objective of the present study were conveyed to them. Approval of the institutional ethical committee was taken prior to conduct of this study.

Following was the inclusion and exclusion criteria for the present study.

Inclusion Criteria: Cases of the suspected neonatal septicemia.

Exclusion Criteria: Babies who had suffered from birth asphyxia, birth weight less than 1500 grams, extremely premature (less than 32 weeks of gestation) and neonates who were already given antibiotics were excluded from the study.

Results & Discussion

Neonatal sepsis is a serious condition. Prompt treatment is required to reduce mortality and morbidity. Clinical presentation is nonspecific hindering early diagnosis. High index of suspicion is needed for early diagnosis. C-reactive protein was first described in 1930 by Tillet and Francis at Rockefeller University. C-reactive protein is an acute phase and an inflammatory marker that is synthesized in the liver in response to inflammatory cytokines and plays a major role in innate immunity. The level of C-reactive protein rises rapidly with a peak level in 6 hours, even up to thousands folds during an acute response. It has a short half-life of 19 hours, so the level falls rapidly once the source is removed [19]. Thus CRP level is also a useful marker in determining the duration of antibiotic therapy. These features distinguish CRP to other acute phase proteins and with availability of rapid assay method, it has a potential importance in neonatal sepsis. Unlike blood culture, CRP level is not affected by prior antibiotic therapy [20], so may be particularly useful in developing countries like India, where a significant number of neonates may have been given antibiotics by local unqualified doctors before presentation at the hospital.

CRP passes the placenta only in very low quantities; therefore, any elevation in the neonate always represents endogenous synthesis [21]. De novo hepatic synthesis starts very rapidly after a single stimulus with serum concentrations peaking around 48 hours [22]. CRP level can be assayed by both quantitatively and qualitatively. Quantitative method provides rapid, highly sensitive and specific result but more expensive and required more technical expertise, so it is mostly used in developed countries and well-equipped modern hospitals [23]. The qualitative method provides very rapid but less specific result, it has the advantage of being simple and easier to perform and interpret and as such can be performed at the patients bed side or side laboratory [24]. It is also less expensive and requires less skill. The qualitative method may, therefore, be more feasible in resource poor countries and where there may be no laboratory services or trained manpower. In neonates, there are many non-infectious causes where CRP level is elevated, eg- maternal and perinatal distress, maternal fever during labour, stressful delivery, prolonged rupture of membranes, prolonged labor, meconium aspiration syndrome, neonatal hypoxia, intraventricular hemorrhage, pneumothorax, surfactant application and tissue injury.

Table 1: Demographic Details

Parameters	No. of Cases
Age	
Less than 1 day	2
1 – 3 days	5
4 – 7 days	20
8 – 28 days	23
Birth Weight	
Less than 1 kg	1
1.0 – 1.5 kg	10
1.5 – 2.5 kg	35
More than 2.5 kg	4
Gestational Age	
Pre-term	32
Term	17
Post Term	1
Total	50

Table 2: Signs & Symptoms

Symptoms	No. of Cases
Refusal for feed	32
Lethargy	20
Poor cry	10
Diarrhea	4
Vomitting	4
Fever	4
Excessive crying	2
Signs	
Jaundice	14
Pyoderma	8
Hypothermia	6
Cyanosis	6
Abdominal distention	4
Seizures	4
Conjunctivitis	4
Vomiting	4
Fever	4
Apnea	2
Tachypnea	2
Excessive crying	1
Poor capillary refill	1

Table 3: Comparison of blood culture and CRP

Parameters	Blood Culture Positive	Blood Culture Negative
CRP Positive	35	10
CRP Negative	3	2
Total	38	12

Table 4: CRP in relation to organisms isolated

Parameters	Blood Culture Positive	Blood Culture Negative
Gram Negative Bacteria	23	5
Gram Positive Bacteria	15	7
Total	38	12

The bacteriological spectrum causing neonatal sepsis shows marked geographic variation. They may vary at different times in the same place. Any micro-organism that colonise the maternal genito-urinary tract or gastrointestinal tract may result in intra-partum as well as post-partum infection.

Shaw CK et al. from Nepal in the year 2007 reported that Staphylococcus aureus was the most frequent organism ensued by Klebsiella and E. coli in causing sepsis in neonates [25]. Ziba mosaiebi et al. (2003) from Iran reported that the most common organism isolated from neonatal sepsis was Klebsiella followed by Staphylococcus aureus, E. coli, Pseudomonas, Acinetobacter [26].

Klebsiella was the commonest organism causing EOS while Staphylococcus aureus was the commonest isolate in LOS. Rahman et al. from Pakistan (2002) reported that the most common organism isolated from sepsis in neonates was Escherichia coli followed by Staphylococcus, Pseudomonas and Klebsiella [27].

Gram negative organisms are more frequently responsible for sepsis in neonates (65-85%) when compared to the Gram-positive organisms. Commonly found isolates are Klebsiella, E. coli, Pseudomonas, Staphylococcus aureus, Enterobacter, Citrobacter, Proteus and Group B Streptococcus.

Mechanisms for elimination of the organisms are activated when bacteria gain access into the blood stream of the neonate. Normally the bacteria are efficiently killed by the monocyte-macrophage system. However, sometimes it may develop systemic inflammatory response syndrome and progress into sepsis. The earlier belief that the bacteria and their components like endotoxins (lipo-polysaccharides) of Gram-negative bacteria and lipo-teichoic acid of Gram-positive bacteria were responsible for the direct toxic effects is now being replaced. Recent studies show that the pathological effects of sepsis generated by the bacterial infection are mainly mediated by the interaction of the inflammatory cytokines activated in response to these microbial components in the vascular compartment.

Most forms of tissue injury trigger the cytokine mediated response resulting in dramatic elevation of the circulating levels of CRP, a normal plasma protein. So it is considered a prototype acute phase reactant playing an important role in innate immunity. This plasma protein is synthesized in the hepatocytes during infection and inflammation. Darren Thompson et al. (1999) reported that the cation (Ca2+) mediated specific binding of the phosphocholine ligand in addition to failure to detect any polymorphism due to its conserved structure depicts essential role of CRP in diagnosing sepsis [28].

It belongs to the pentraxin family, which is a small, well conserved and evolutionarily ancient group. It has 224 amino acids with a molecular weight of 25 kDa and has annular pentameric disc shape. It was discovered in 1930 by Tillet and Francis from the serum of the patients with acute inflammation. It was named so due to its reaction with the 'C'- polysaccharide of Streptococcus pneumoniae. It has been frequently used in investigating and monitoring neonatal sepsis [29].

Marnell et al. (2005) reported that C-reactive protein is an indicator for acute inflammation and infection [30]. According to Sierra et al. (2004) CRP raises up to thousand folds in case of systemic infections caused by all bacteria [31]. Chauhan Setal B et al. (2012) reported that CRP shows a rapid rise within 12 to 24 hours of sepsis and have further incremental increase thereafter [32]. According to Rodwell RL et al. (1993) C-reactive protein has practical added advantage over the other biological markers of sepsis as serum levels of CRP is not influenced by prior antibiotic administration [33].

The newborn infant is susceptible to infection due to immaturity of both natural and acquired immune systems. Compared to developed nations, the incidence of neonatal sepsis in India is quite high accounting for 40% of all neonatal deaths. The presentation of sepsis is usually by a

variety of subtle nonspecific clinical signs and symptoms which may at times evade even the most skilled Paediatrician. The newborn responds to the stress of sepsis in a stereotyped manner. So Sepsis must be considered as a strong possibility for any clinical deterioration in a neonate unless the event is explained by other causes. Neonatal septicemia causes considerable morbidity and mortality. So diagnosis of the infection gains paramount importance as earlier diagnosis reduces morbidity. Blood culture remains the gold standard in not only identifying the infecting organism but also provides vital information regarding the antibiotic sensitivity pattern so that proper usage of drugs can be made.

Conclusion

The data generated from the present study concluded that biomarkers like CRP may serve as an important tool in reducing the mortality and morbidity among neonates diagnosed from sepsis. Also biomarkers like CRP may serve as an important tool in reducing the mortality and morbidity among neonates.

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