

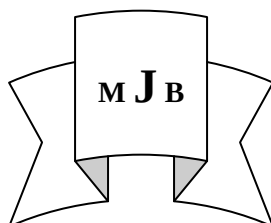
Bacteriological and Hematological Study on Neonatal Sepsis

Khamael Adnan Baqir

Habeeb S. Naher*

Hilla Teaching Hospital, Hilla, Iraq.

*College of Medicine, University of Babylon, Hilla, Iraq.



Abstract

This study aimed to detect the bacterial types which cause neonatal sepsis in addition to identifying the risk factors that result in the infection of this disease. The subjects of the study comprised (50) neonates ranging in age between one to ninety days from both sexes who admitted to the Preterm Unit and Intensive Care Unit (ICU) in the Babylon Hospital for Pediatric and Gynecology, from October 2011 to March 2012. The blood samples were examined for the WBC count and the C-reactive protein as signs for the infection. Then, a blood culture was made on blood agar, MacConkey agar, and nutrient agar media to identify the bacterial types causing the infection. The results indicated that Early Onset Sepsis is more common than Late Onset Sepsis and Gram negative bacteria are the main cause for sepsis. Abnormal WBC count and positive CRP are strongly associated with blood culture proven septicemia.

الخلاصة

أستهدفت هذه الدراسة الكشف عن الأنواع البكتيرية المسببة لتعفن الدم في الأطفال حديثي الولادة و تحديد عوامل الخطورة الموهبة للأصابة. شمل البحث دراسة ٥٠ طفل من كلا الجنسين حديثي الولادة الراقدين في وحدة الخدج و وحدة الأنعاش في مستشفى بابل للولادة و الاطفال خلال مدة ستة اشهر (من تشرين الأول ٢٠١١ الى اذار ٢٠١٢) تراوحت أعمار الأطفال ما بين (١-٩٠ يوما) أجري على عينة الدم حساب عدد كريات الدم البيض و تحديد البروتين النشط كدلائل على وجود اصابة ثم تمت زراعته على الاوساط (أكار الدم ،ماككونكي اكاروالاكار

المغذي) للكشف عن الانواع البكتيرية المسببة للاصابة. اظهرت النتائج ان تعفن الدم المبكر أكثر شيوعا من تعفن الدم المتأخر وان البكتيريا الموجبة لصبغة غرام هي السبب الرئيسي لتعفن الدم. وان معظم الاطفال أظهروا نتيجة موجبة للبروتين النشط وتغيرا في حساب عدد كريات الدم البيض.

Introduction

Sepsis neonatorum is a term used to describe any systemic bacterial infection documented by a positive blood culture in the first month of life. Neonatal septicemia remains one of the main causes of mortality and morbidity despite the considerable progress in hygiene, introduction of new and potent antimicrobial agents and advanced measures for diagnosis and treatment. Up to 10%, infants have infections in the first month of life, the

matter which results in 30-50% of total neonatal deaths in developing countries [1]. These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of premature birth and low birth weight. It is known that risk factors related to neonatal bacterial sepsis are complex; They include interaction of maternal-fetal colonization, transplacental immunity and physical and cellular defence mechanisms of the neonate [4]. The incidence of neonatal bacterial sepsis

depends on geographic area and may vary from country to country as well as within the same country. In the developing countries, neonatal mortality, resulting from all causes of neonatal sepsis, is about 34 per 1000 live births, occurring mainly in the first week of life, whilst it is only 5 per 1000 live births in developed countries [5]. Neonatal mortality is about 34 per 1000 live births in Asia, 42 per 1000 live births in Africa and 17 per 1000 live births in Latin America[6] versus relatively low rates being reported in the United States and Australasia which is about 6–9 per 1000 live birth and in Europe 0.3-3 per 1000 live births [7]. Bacterial organisms have developed increased drug resistance over the last three decades and management of neonates with sepsis has become a major problem [8]. On the other hand Group B *Streptococcus* (GBS) has been the most frequent ethological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality rates. In addition, bacterial organisms causing neonatal sepsis have developed increased drug resistance to commonly used antibiotics, making its management a challenge for both the public and private health sectors [9]. It is agreed that neonatal sepsis is classified as either early onset sepsis (EOS) (0-7 day of age) or late onset sepsis (LOS) (7-28 days of age) [6]. A few papers distinguish between very early onset (within 24 hours), EOS (24 hours to six days), and LOS (more than six days to 30days)[10]. Neonatal sepsis can be defined both clinically and/or microbiologically, by positive blood and/or cerebrospinal fluid/urine cultures. According to[6], clinical criteria for diagnosis of neonatal sepsis are any of the signs listed below imply high suspicion of severe bacterial infection.

- Respiratory rate > 60 breaths/min (tachypnea).
- Grunting .
- Temperature >37.7oC or <35.5oC (hypothermia).
- Lethargic or unconscious
- Not able to sustain sucking.
- Tachycardia.
- Convulsion.

World Health Organization (WHO) estimates that globally there are about five million neonatal deaths per year, ninety-eight percent of them occur in developing countries in the first week of life [3]. In developing countries, neonatal mortality (deaths in the first 28 days of life per 1000 live births) from all causes is about thirty-four ; most of these deaths occur in the first week of life, mostly on the first day [5] (according WHO 2001 Estimates). Neonatal mortality in Asia is about 34, in Africa about 42, and in Latin America and the Caribbean about 17 per 1000 live birth, although there are wide variations between different countries in these regions as well as within the countries themselves. The most common causes of death in the neonatal period are neonatal infections (32%) and injuries (29%), and prematurity (24%) [5].

Maternal, infant and environmental factors all contribute to the pathogenesis of NNS, at a time when bacteria may invade the newborn via a number of routes. These routes are the following:

1. Intrauterine Infection

The intrauterine infection occurs due to apparent or inapparent maternal bacteremia with transplacental transmission to the fetus. Although bacterial transplacental infections are rare, a number of organisms have been associated with this mode of infection. *Listeria monocytogenes* septicaemia is an example of such infections [14]. In

addition to transplacental transmission, a fetus may be infected by organisms from vagina invading the amniotic fluid through the cervix with or without intact membrane [15]. The most common organisms found in the amniotic fluid and vagina are *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and Group -B- beta haemolytic *Streptococcus* is also occasionally present in the vaginal flora.

2. Intra Partum Infection (Ascending Infection)

These infections are acquired just before or during delivery with vertical transmission of the microorganisms from mother to newborn infant. Amniotic fluid infection may be asymptomatic or may produce maternal fever, with or without local or systemic signs of chorioamnionitis [14]. The fetus may swallow bacteria present in the fluid or may aspirate them. Furthermore, the organisms may gain access to the blood through the skin, mucous membranes of the eyes, ears or intestinal tract [14] & [16].

3. Postpartum Infection (Nosocomial or Community)

Following birth, bacterial organisms may be acquired from the delivery room or in the newborn nursery via the main pathways, namely the respiratory and gastrointestinal tracts. After birth, the skin and umbilical cord become an important alternative route for the entrance of bacteria into the systematic circulation. The umbilical stump is a frequent site for cutaneous infection leading to septicaemia[22].

Materials and Methods

Patients: During the period of October 2011 and March 2012 a total of 50 neonates who admitted to the preterm unite and intensive care unit (ICU) in

Babylon Hospital for children and Maternity were investigated for early onset sepsis (0-7 days of age)and late onset sepsis (>7-90 days of age) all were investigated. Written informed consent was obtained from their parents/guardians. Following detailed clinical examination, neonates with suspected sepsis having any one of the clinical symptoms and signs as outlined above were considered.

Blood Samples:

Using aseptic technique by applying Povidone iodine and 70% alcohol at the site of vein puncture, 5 ml venous blood was drawn from the antecubital or femoral vein by the attending nurse. 2.5 ml of blood was inoculated directly onto brain heart broth and incubated for 24 hr for culture and the remaining 2.5ml blood was used for white blood cell count and C-reactive protein. The specimens were transported within one hour to the Core Laboratory.

Culture and Identification:

All blood cultures were incubated in brain heart infusion broth at 37°C and inspected daily for 3 days for presence of visible microbial growth by observing any of one of the following: turbidity, haemolysis, gas production and coagulation of broth. Subculturing were made during 3 successive days into neutrient agar, blood agar, and macConkey agar. The neutrient agar, blood agar, and macConkey agar plates were incubated under aerobic conditions for 24 hr. All positive blood cultures were identified by their characteristic appearance on their respective media, Gram staining reaction and confirmed by the pattern of biochemical reactions using the standard methods[17]. Members of the family enterobacteriaceae were identified by indole production, H₂S production,

citrate utilization, motility test, urease test, oxidase, carbohydrate utilization tests and other tests using API 20E identification kits. For gram-positive bacteria; coagulase, catalase, bacitracin and optochin susceptibility tests, and other diagnostic tests were used. Blood culture which showed no microbial growth within 3 days was reported as negative culture, while any growth during routine subculturing on nutrient, blood, and MacConkey agar considered to be positive culture.[17]

Qualitative determination of C-reactive protein:

The CRP-latex is a slide agglutination test for qualitative detection of CRP in human serum. Latex particles coated with goat IgG are agglutinated when mixed with samples containing CRP. [23]

White Blood Cell Count:

Counting of WBC in peripheral blood sample was done by auto-analyzer. After sampling of blood into EDTA tube approximately 50 μ of blood drawn by auto-analyzer to give the result on the screen within few minutes.

Results and Discussion

The results of blood culture may take about 4 days, necessitating initial empirical treatment of suspected septicemia. Among 50 samples of blood obtained from neonates of different ages, only 10 samples revealed positive culture. *E.coli* accounted for 30% while *S.aureus*, *Pseudomonas* species and *Enterobacter* accounted 20% for each. *Salmonella* was the least in 10% only. Figure (1).

Age and Sex in Septicemia:

Distributions of investigated neonates for bacterial sepsis according to the age, sex and type of septicemia are shown in. Of the 50 patients, 32 (64 %) were males

and 18 (36%) were females resulting in an overall female to male ratio of 1: 1.7. A total of 29 (58%) neonates presented with EOS and 21 (42 %) neonates presented with LOS Table (1). The results indicated that early onset sepsis (EOS) was more common than late onset sepsis (LOS), which is in agreement with the reports from other developing countries e.g. in Iran (77.5% vs.22.5%) [2] and Bangladesh (70.7 vs. 29.3%) [18], but in contrast with reports from Saudi Arabia (39% vs. 61%) [19], Pakistan (42% vs.58%) [20] and Libya (31 vs.69%) [21] where late onset sepsis is more common. The possible explanation for low frequency of LOS in this study might be the early discharge policy in the hospital since the newly delivered is mother discharged from hospital within 3 hr for normal vaginal delivery and after 24hr for cesarean section. Of the 50 neonates, 31 (62%) were preterm (gestational age less than 37 weeks), 17 (34%) were born in the midwife, 34 (68%) were delivered by normal vaginal delivery. Approximately, 33 (66%) neonates with sepsis had low birth weight (<2500 g) and 7 (14%) neonates had very low birth weight (< 1500 g).

C - reactive protein (CRP):

Estimation of C-reactive protein in 50 neonates admitted with clinical findings was positive in 33 (66%) and the remaining 17(34%) were negative as indicated in figure(2). Those neonates with positive CRP reveal positive bacterial culture in 10 cases. This mean that (30.30%) of positive CRP had positive bacterial culture. Measurement of the acute phase response is a helpful indicator of the presence and extent of inflammation or tissue damage and response to treatment. Franz *et al.*2001 suggested that the combination of IL-8

and CRP appeared to provide a more reliable method in the early diagnosis of neonatal sepsis. CRP assay is sensitive and fairly precise small rises in serum level and can often be detected before any clinical feature become apparent. Whilst as a tissue damaging process resolves the serum level rapidly decreases toward the normal rang. The outcome of neonates with infections is strongly related to their appropriate diagnosis and management. Diagnosing of neonatal infection, however, is a challenge, since clinical signs and symptoms are often nonspecific for a particular infection. As a consequence, deciding whether to treat or not, balancing optimal patients care with aspects such as possible adverse events or antibiotic resistance, may be difficult. In line with this idea, the recognition of the risk factors for neonatal infections is extremely relevant in the clinical setting,

since it contributes to the diagnostic reasoning and supports clinical decisions.

White Blood Cell Count (WBC count):

Among the 50 neonates admitted with suspected cases of sepsis, 28(56%) had normal white blood cell count (5000 20,000/mm³), 8(16%) high WBC count (>20,000/mm³) and 14(28%) had low WBC count (<5000/mm³) Table (2). The white blood cell count (WBC) and differential count are useful for assessing a neonate who may have sepsis and for evaluating a neonate being treated for proven sepsis. The bone marrow reserves of leukocytes in a newborn are relatively small compared to those of older children and adults and leukopenia (low WBC count) occurs more frequently as sign of overwhelming infection.

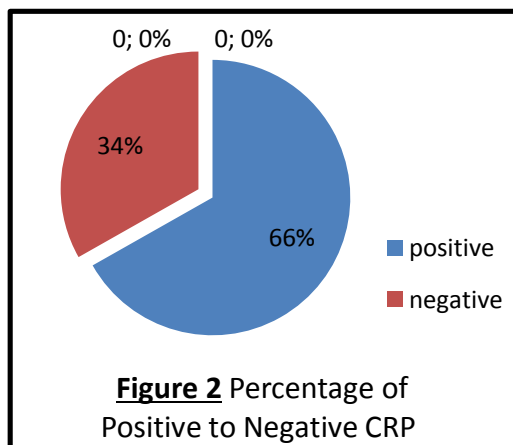
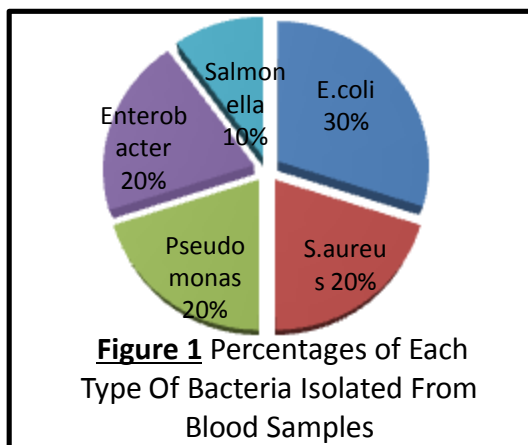


Table 1 Distribution of 50 neonates with suspected sepsis according to the sex, age and type of sepsis.

Category			
	Neonates with EOS (0-7 days of age) No. (%)	Neonates with LOS sepsis (>7-90 days of age) No (%)	Total (0-90 days of age) No (%)
Male	21	11	32(64%)
Female	8	10	18(36%)
Total	29(58%)	21(42%)	250

Table 2 White blood cell counts in neonates admitted with suspected cases of sepsis

WBC Count/mm3	No. (%)
5000-20,000 (Normal)	28 (56%)
>20, 000 (High)	8 (16%)
< 5000 (Low)	14(28%)
Total	50

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