INCIDENCE OF NEONATAL SEPSIS IN A SAMPLE OF IRAQI NEWBORNS

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ABSTRACT

Neonatal sepsis is a common cause of morbidity and mortality among newborns in developing countries. This study aimed to investigate the causative pathogens other than viruses and the predisposing factors for neonatal sepsis among Iraqi newborns. A total of 150 suspected neonates from three Hospitals in Baghdad/Iraq were enrolled in this study. According to time onset, sepsis was divided into early onset sepsis (EOS) and late onset sepsis (LOS). Blood samples were collected from each neonate, then bacterial and fungal detection were achieved through routine culturing, biochemical tests and API system. Anti-toxoplasma IgM and IgG antibodies were investigated by enzyme linked immune-sorbent assay (ELISA). The overall incidence of neonatal sepsis among suspected neonates was 54.67%. Preterm infants, low body weight (LBW), urinary tract infection of the mother and cesarean section delivery seemed to predispose to neonatal sepsis. Staphylococci were predominant in both EOS and LOS sepsis; however, large percentage of these bacteria were coagulase negative in LOS. Overall, gram positive bacteria were more frequent than gram positive bacteria. Fungi and *Toxoplasma* appeared to have less or no significant role in neonatal sepsis among Iraqi newborns. These data indicate the diversity of pathogenic bacteria associated with neonatal sepsis. Every effort should be considered for early detection of the pathogens to avoid the mortality.

KEYWORDS: neonatal sepsis, bacteria, predisposing factors, *Toxoplasma*

INTRODUCTION:

Neonatal sepsis is the presence of pathogenic microorganism in the bloodstream, cerebrospinal fluid (CSF), urine or any other sterile tissue. Bacterial and viral infections are the most common causes of neonatal sepsis; however, protozoa and fungi may play a major role in this regard (Satar *et al.*, 2012). In most cases, the neonates acquire the infectious microorganisms directly from mother's blood, skin or vaginal secretions prior or intrapartum. In these instances, the so called early onset infection or early onset sepsis (EOS) is applied. This usually occurs during the first 7 days of life, or, in case of very low birth weight (VLWB) infants, during the first 3 days of life (Mukhopadhyay *et al.*, 2012). On the other hand, the neonate may acquire the infection from the surrounding environment (nosocomial or community sources) intra- or postpartum. In such cases, the late onset sepsis (LOS) may develop in which the infection is demonstrated after 7 days of life (Camacho-Gonzalez *et al.*, 2013). In all cases, the random using of antibiotic exacerbated the emerge of multidrug resistance bacteria (Hassan, 2015; Hassan *et al.*, 2016) which significantly increased the incidence of neonatal sepsis.

Several factors (whether related to the mother or to the baby itself) can predispose for neonatal infections. In a case-control, Chinese study involved 147 newborns and 588 controls Jiang and Ye

(2013) demonstrated that cesarean section (C/S) delivery, premature rupture of fetal membrane, young mother, urban residence, parity, placental abnormalities and abnormal fetal position are the major predisposing factors for neonatal infection.

Clinically, it is difficult to identify the neonatal infection, because the clinical signs could be identical to those of other very dangerous pediatric diseases such as necrotizing enterocolitis, perinatal asphyxia, and hyaline membrane disease (English *et al.*, 2004). These signs usually manifested by apnea, tachycardia (or bradycardia), jaundice, cyanosis fever (or hypothermia), vomiting and diarrhea (Shah and Padbury, 2014).

This study aimed to evaluate the major causative pathogens and predisposing factors for neonatal infection among Iraqi newborns.

MATERIALS AND METHODS

The Study Cases: This case-control study has enrolled a total of 150 neonates who were admitted to Baghdad City Hospital, Al-Imamain Al-Kadhumain Teaching Hospital and Pediatric Teaching Hospital during the period from January to March 2017. Inclusion criteria were age between 1 to 28 days, and having evidences suggestive for sepsis which are the presence of risk factor (such as preterm), and the presence of clinical manifestations of sepsis (like fever, poor reflex and apnea). The study

was approved by constitutional reviewing council / College of Medicine/Al-Nahrain University. A consent letter was obtained from the neonate's mother explaining the details of the study. Demographic and clinical data were either extracted from the hospital record, or obtained directly from the neonate's mother.

Blood Sample Collection: Depending on the neonate's age, about 1.5 to 3 ml of venous blood were collected and divided into two parts: the first one (whole blood with EDTA) was used for blood culture, while the second part (in plain tube) was used for serological tests.

Bacteria Isolation and Identification: Whole blood samples were directly inoculated into brain-heart infusion broth tubes that were incubated an aerobically for 5 days at 37ºC with continuous agitation. An aliquot was then taken by sterile syringe for gram staining. A subculture on blood agar, mac Conkey agar (incubated aerobically for 24hrs at 37ºC) and /or chocolate agar (incubated at 5% CO_2 conditions), were conducted

The cultured bacteria were identified firstly depending on the morphological characteristics of the colony: size, shape, color, odor and pigment production. The identification of Enterobacteriaceae family was performed according to the biochemical tests indicated in the scheme of Farmer *et al*. (1986). Catalase and coagulase tests were used for the differentiation between staphylococci and enterococci, and between pathogenic and non-pathogenic staphylococci respectively. The final identification of the bacterial isolates was conducted using API20E system for Enterobacteriaceae, API Staph System for identifying 23 species of staphylococci, API 20 Strep System and API Candida according to the procedure suggested by the manufacturing company (Bio-Merieux/France).

Detection of Anti-toxoplasma IgG and IgM Antibodies: Serum levels of anti-toxoplasma IgG and IgM antibodies were detected by enzyme linked immune-sorbent assay (ELISA) with two ready kits for IgG and IgM (BioCheck Inc./ USA) according to the manufacturer's instructions. Briefly, 100µl of diluted sera, calibrator, and controls were dispensed into the appropriate wells of the microplate which was incubated for 30 min at 37ºC. After complete washing, 100µl of enzyme conjugate were dispensed into each well, and incubated as in the previous step. After washing, 100µl of TMB reagent were added to each well, and the plate was incubated at 37ºC for 15 min. Stop solution (100µl) was added and left for 30sec., after which the optical density was read at 450nm within 15 min using a microtiter plate reader (Titertek Multiskan/ Filand).

RESULTS

Sepsis was confirmed in 82 neonates (54.67%) by positive culture of blood samples. The baseline characteristics of the affected neonates and their mothers are shown in table 1. It is obvious that factors of preterm, LBW, urinary tract infection of the mother, C/S delivery and leaking liquor could be considered predisposing factors for neonatal sepsis. None of the mother gave positive result for anti-toxoplasma IgM antibodies, while 10 women (12.2 %) were positive for anti-toxoplasma IgG antibodies.

Table 1: Maternal and Neonates data

Average age in days (range)	9.86(1-28)
Gender	
Male	44(53.66%)
Female	38(46.34%)
Gestational age	
Full term	29(35.37%)
Preterm	53(64.63%)
Body weight at birth	
$\geq$ 2500grams	34(41.64%)
< 2500 grams	48(58.54%)
Previous abortion of the mother	
Yes	33(40.24%)
No	49(59.76%)
Urinary tract infection of the mother	53(64.54%)
Yes	29(35.37%)
No	
Mode of delivery	
Normal vaginal delivery	34(41.64%)
Cesarean section	48(58.54%)
Mother status for anti-toxoplasma IgG Antibodies	
Positive	10(12.2%)
Negative	72(87.8%)
Leaking liquor	
Yes	56(68.29%)
No	26(31.71%)
Dwelling	
Urban	59(71.95%)
Rural	23(28.05%)

The most obvious clinical manifestations of affected neonates are presented in table 2. About two-thirds of the patients had fever, and more than

half of them had developed jaundice, while other symptoms were recorded only in few neonates.

Table 2: Clinical manifestation of affected neonates

Manifestation	Positive, No. (%)	Negative, No. (%)
Fever	64(78.05)	18(21.95)
Eye discharge	2(2.44)	80(97.56)
Meningitis	2(2.44)	80(97.56)
Jaundice	45(54.88)	37(45.12)
Apnea	6(7.32)	76(92.68)
Pneumonia	9(10.98)	73(89.02)
Diarrhea	5(6.1)	77(93.9)
Hydrocephaly	6(7.32)	76(92.68)
Skin rash	3(3.66)	79(96.34)

Based on the age of neonate at which infection occurred, sepsis was recognized as early onset sepsis (EOS) in 44 cases (53.66%) and late onset sepsis (LOS) in 38 cases (46.34%).

Table 3 shows the types and frequencies of different bacteria isolated from blood samples of the affected neonates with EOS. Overall, gram negative bacteria were slightly more prevalent than gram positive bacteria. Staphylococci were the most prevalent bacteria with 17 cases, 8 of them were coagulase-negative staphylococci, followed by *Acinetobacter spp.* and *Pseudomonas spp.* Species like *Klebsiella* and *Aeromonas* were not frequent.

Table 3: Bacterial infection in neonates with early onset sepsis (total number 44 cases)

Bacteria	No of cases (%)
Staphylococcus spp.	17(38.64)
S. aureus	9(20.45)
S. lentus	3(6.82)
S. hemolytica	1(2.27)
S. auricularis	1(2.27)
S. cohnii	1(2.27)
S. xylosus	2(4.55)
Acinetobacter bumannii	6(13.64)
Pseudomonas spp.	6(13.64)
P. aeruginosa	5(11.36)
P. capacia	1(2.27)
Escherichia coli	4(9.09)
Serretia ficaria	3(6.82)
Streptococcus spp.	2(4.55)
S. pyogenes	1(2.27)
S. pneumoniae	1(2.27)
Citrobacter freundii	2(4.55)
Pantoea spp.	2(4.55)
Klebsiella pneumoniae	1(2.27)
Aeromonas hydrophila	1(2.27)

Although staphylococci remain the most prevalent bacteria isolated from neonates with LOS (Table 4) with 13 cases, most isolates (10) were coagulase positive staphylococci represented by *S. aureus.* The second most prevalent bacteria were streptococci with 6 cases, most of which were *S. pyogenes*. Again gram negative bacteria were more prevalent with *Pseudomonas spp.*, *Citrobacter spp.*, and *Serretia spp.* were the most abundant. The fungus *Candida albicans* reported only in one case within this group.

Table 4: Microbial infections in neonates with late onset sepsis (total number 38 cases)

Bacteria/Fungi	No of cases (%)
Staphylococcus spp.	13(34.21)
S. aureus	10(35.71)
S. lentus	1(2.63)
S. hemolytica	1(2.63)
S. xylosus	1(2.63)
Acinetobacter bumannii	1(2.63)
Pseudomonas spp.	5(13.16)
P. aeruginosa	4(10.53)
P. oryzihabitans	1(2.63)
Serretia spp.	4(10.53)
S. ficaria	2(5.26)
S. plymuthica	2(5.26)
Streptococcus spp.	6(15.79)
S. pyogenes	5(13.16)
Veridans streptococci	1(2.63)
Citrobacter freundii	4(10.53)
Klebsiella pneumoniae	3(7.89)
Enterobacter	1(2.63)
Candida albicans	1(2.63)

ELISA results in neonates were identical to their mothers in that no neonates were positive for anti-toxoplasma IgM antibodies, while 10 neonates were positive for antitoxolasma IgG antibodies among whom 6 with EOS and 4 with LOS.

DISCUSSION

This study revealed a relatively high incidence of neonatal sepsis among the candidate neonates. Much lower incidence rates were recorded in many other developing countries like Egypt (Shehab Eldin *et al.*, 2015), Cameroon (Chiabi *et al.,* 2011) and Tanzania (Kayange *et al.*, 2010). In a retrospective study in China reported overall incidence of neonatal sepsis of 2.27%. These variations in the incidence are referred to many factors, the most important of which are the standard measures of health care and the criteria for selection of suspec-

ted neonates to be investigated for culture (Li *et al.*, 2013).

In this series, several factors were confirmed to predispose to neonatal sepsis although there was no control group. These factors, mostly, either have a detrimental effect on the immune response of the newborn (such as reduced gestational age and low body weight), or they increase the exposure of the newborn to the pathogenic microorganisms (like UTI of the mother and leaking of the liquor and C/S) (Mercer *et al.,* 1997). For example, Utomo *et al.* (2010) found that newborns delivered by C/S have 1.89-fold higher risk to develop sepsis than those delivered through normal vaginal delivery.

The clinical manifestations of the affected neonates vary considerably according to the involved organs, the pathogen, gestational age, age of the neonate and several other conditions. Fever and jaundice are the most frequent clinical signs reported in this study. Hefer *et al.* reported that full term newborns are more likely to have fever when they encounter bacterial infection. Preterm newborns, on the other hand, are more likely to have hypothermia for such infection, because they have a temporary difficulty with temperature control especially in the first 2 days (Hofer N, Müller, 2012). In an Iranian study, Maamouri *et al.* (2013) found that bacterial infection is a significant cause of hyperbilirubinemia and jaundice. The mechanism of jaundice in neonatal sepsis is not fully understood, but it may be attributed to liver involvement by infection or blood hemolysis.

Early onset sepsis was found to be more frequent than LOS in the current study. This is in agreement with many previous studies (Afsharpaiman *et al.*, 2012; Cecelia *et al.*, 2011; Shah *et al.*, 2012). However, several other studies reported opposite results (Shaw *et al.*, 2007, Kayange *et al.*, 2010, Shehab El-din *et al.*, 2015). As there is general agreement that EOS is resulted from vertical transmission of bacteria, while LOS is attributed to the horizontal transmission (Bizzarro *et al.*, 2005), it can be deduced that maternal source of infection exceeded that from nosocomial or other sources of horizontal infection in this study. In this regard, LBW preterm infants, which represent more than half of the cases, are at particular risk for LOS because of the immaturity of immune system, use of indwelling catheter, prolonged mechanical ventilation and prolonged hospitalization (Stoll *et al.*, 2002). Thus, when we consider the full-term newborns, most infections are vertically transmitted.

Sepsis caused by gram-negative bacteria was more frequent than that caused by gram-positive bacteria in both EOS and LOS. These results disagree with many previous studies (Shokry *et al.*, 2007; Kholi-Kochhar *et al.*, 2011; Leal *et al.*, 2012; Li *et al.*, 2013; Shehab El-din *et al.*, 2015) which reported the predominance of gram-positive cocci, especially coagulase-negative staphylococci (CoNS). In fact, the diversity of microorganisms involving in neonatal sepsis varies from one geographical region to another. Furthermore, it changes over time even in the same region (Shrestha *et al.*, 2010), and it is not uncommon to find a predominance of different bacterial species in different eras. This implies the need for continuous investigations for a wise use of antibiotics.

There is a great similarity in bacterial profile between EOS and LOS in this study. For example, *Staphylococcus* spp. followed by *Pseudomonas* spp. were the most frequent bacteria in both EOS and LOS. However, most isolates of staphylococci in LOS were CoNS which indicates a nosocomial infection by these bacteria. Some bacterial species were recorded in EOS but not in LOS such as *Escherichia coli* and *Pantoea* spp. and vice versa such as *Veridans streptococci and Enterobacter.* However, these bacteria had little importance.

Only one case in LOS was found to be infected with *Candida albicans* which implies a minor significance of fungi in neonatal sepsis. In this regard, Benjamin *et al.* demonstrated that LBW, male gender, formulas feeding, and antibiotic treatment are the main risks for neonatal infection with this fungus (Benjamin *et al.*, 2006). On the other hand, none of the affected neonates gave a positive result for anti-toxoplasma IgM antibodies which means that there was no active infection with this parasite. The positive results for 10 neonates to anti-toxoplasma IgG antibodies could be explained by the transplancental transmission of these antibodies from the mothers to their infants during pregnancy. The exact number of mother who bear these antibodies support this explanation.

Conclusion: These data indicate the diversity of bacterial causes of neonatal sepsis among Iraqi newborns. A proper and early detection of the causative pathogen could prevent detrimental effect and reduce the mortality rate. Further studies inclu-

ding viral infection causes, and larger sample size are required.

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