Risk Factors Assessment and Clinical Profile of Neonatal Sepsis in Blood Culture Proven Neonates.

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ABSTRACT

Neonatal sepsis refers to infection occurring within the neonatal period i.e. the first 28 days of life for a term baby, and up to 4 weeks beyond the expected date of delivery in a preterm baby. Neonatal sepsis is still a major cause of morbidity and mortality during the first year of life. Assessment of risk factors and clinical profile of neonatal sepsis was done on 42 cases with blood culture proved neonatal sepsis of total 102 cases with clinically suspected neonatal sepis admitted to the Neonatal Intensive Care Units (NICUs) in El Menoufia and El Kalyoubia governorates hospitals versus 30 age and sex matched healthy controls. Complication during labour, Birth weight and Gestation age were the common risk factors were associated with the development of neonatal sepsis where Respiratory distress, Lethargy, Poor feeding, abdominal distension, Apnea and Cyanosis were the most common clinical signs represent clinical profile of neonatal sepsis.

Keywords: Neonatal sepsis; Risk factors; Clinical profil; BACT/ALERT automated blood culture ystem.

INTRODUCTION

Sepsis has been called the hidden public health disaster **(Angus, 2010).** The international consensus definition has recently defined sepsis as" life threatening organ dysfunction caused by a dysregulated host response to infection" and septic shock as "a sub set of sepsis in which particularly profound circulatory, cellular and metabolic abnormalities are associated with a great risk of mortality than with sepsis alone" **(Singer et al, 2016).**

In Europe, every year, 157 000 people die for this systemic multi-organs failure as a consequence of bacterial or fungal infection **(Goto & Al-Hasan; 2013).** Every year 2.6 million neonates die; three fourths of deaths occur in the first week of life, and almost all (99%) in low- and middle-income countries **(Wang et al, 2014).** In fact 75% of mortality occurs in developing countries is due to under-recognition of illness **(Obiero et al, 2015).** There are many risk factors for development of neonatal sepsis including unsafe place of delivery or unclean delivery, prolonged rupture of membranes more than 24 hours, maternal pyrexia, chorio-amnionitis (foul smelling liquor), prolonged labor and perinatal asphyxia **(Khinchi et al, 2010).** Maternal fever within 2weeks prior to delivery, meconium stained amniotic fluid (MSAF) and instrumental delivery **(Shah et al, 2006).**

The fetal risk factors are low birth weight, gestation and Apgar score immature immune system, decreased phagocyte activity of white cells,

decreased production of cytokines and weak humoral immunity. The natural skin barrier is thin and weak **(Shah et al, 2006).**

Neonatal sepsis is divided into early-onset sepsis (if symptoms start before 72 h of life) and late-onset sepsis (if symptoms start afterward). Various cutoff points have been used to defined sepsis onset type, it ranges from 48 h to 7 days, but most epidemiological studies use 72 h **(Shane& Stoll; 2014).** Early-onset sepsis (EOS) is generally caused due to the pathogens acquired from the mother or during birth and also the vaginal tract **(Kumar & Baht, 2015).**

The common risk factors for EOS include prematurity, maternal Group B *Streptococcus* (GBS) infection, chorioamnionitis, immunological immaturity, premature rupture of membranes, prolonged rupture of membranes (more than 18 h), maternal urinary tract infections (UTI), intra-amniotic infections, and multiple pregnancies **(Camacho-Gonzalez et al, 2013).** Although there are a number of microorganisms causing these early-onset infections, GBS infection and *Escherichia coli* (E. coli) constitute about 90% of them **(Fleming et al, 2012).**

Late-onset sepsis (LOS) is generally after 72 h of birth and is mainly caused by pathogens from environment, which invades through the skin and nasal channel of the newborn **(Kumar & Baht, 2015).** Frequent pathogens associated with LOS are *Staphylococcus aureus*, Coagulase negative *Staphylococci* (CoNS), *Haemophilus influenzae*, *E. coli*, *Klebsiella*, *Pseudomonas*, and *Candida* spp **(Fleming et al, 2012).** In general, the most prevalent gram-positive organisms causing neonatal sepsis include *Staphylococcus aureus*, CoNS, *Enterococcus*, and *Listeria monocytogenes*. Other than E coli, the most common gram-negative organisms are *Klebsiella*, *Enterobacter*, Citrobacter, and Pseudomonas **(Weston et al 2011).**

MATERIAL AND METHODS

This study was conducted on 102 neonates suspected to have clinically diagnosed neonatal sepsis admitted to Neonatal Intensive Care Units (NICUs) in 4 distinct hospitals from Menoufia and El Kalyoubia governorates were enrolled. These NICUs and corresponding patients' numbers were:-

1- Neonatal intensive care unit (NICU), National Liver Institute hospital-Menoufia University, from which 24 cases were included.

2- Neonatal intensive care unit (NICU), Menoufia University hospital, from which 41 cases were included.

3- Neonatal intensive care unit (NICU), Benha University hospital, from which 30 cases were included

4- Neonatal intensive care unit (NICU), Berket El Saba central hospital – Ministry of Health and Population, from which 7 cases were included.

These patients were evaluated for the following risk factors:-

1- Maternal risk factors: Age of the mother, education level, socioeconomic status, Parity, mode of delivery (cesarean, vaginal, instrumental), premature rupture of the membranes (PROM), meconium stained amniotic fluid (MSAF), maternal fever in the third trimester two weeks before delivery, pregnancy induced hypertension (PIH), Pre eclamptic toxemia (PET)/ Eclampsia, foul smelling amniotic fluid, antepartum hemorrhage and maternal chronic diseases and medications

2- Fetal risk factors: Gestational age ,sex of the baby, birth weight, Apgar score at one minute, birth asphyxia and complicated labor (birth trauma, laryngeal intubation, invasive procedures) and prolonged labor.

The neonates were evaluated for the following clinical manifestations from which Clinical profile of neonatal sepsis will be concluded:-

Respiratory distress, lethargy, fever, poor feeding, abdominal distention, apnea, vomiting, hypothermia, cyanosis, irritability and convulsions.

The sepsis work up were done and dependent on positivity of at least two of the following laboratory screening tests to identified the patients to had clinical neonatal septicemia.

The investigations are:-

i. Total leukocyte count < 5000/mm3 or ≥ 25,000 at birth or ≥ 30,000—12–24 h or ≥ 21,000—Day 2 onwards.

ii. Band cell count ≥ 20%

iii. Micro ESR ≥ 15 mm/ 1st hour

iv. C-reactive protein > 6 mg/dL

v. Absolute neutrophil count <1500/mm^3

The clinically diagnosed cases with neonatal sepsis will be undergone convential blood culture test with automated BACT/ALERT® 3D 60 instrument (BioMérieux, INC. USA) bloodculture system to confirm the diagnosis of neonatal sepsis.

Results

In this study, from 102 clinically diagnosed neonatal sepsis, 42 cases were blood culture proven bacterial neonatal sepsis confirmed with the detection of bacterial growth with BACT/ALERT automated blood culture system. **Table (1)** represent bacteria detected with conventional blood culture in 42 neonates out of 102 clinically diagnosed neonatal sepsis patients.

Table (1): Types of bacteria isolated with BACT/ ALERT automated blood culture system.

Organisms	No	percent
1- Bacterial		
Klebsiella	20	47.6%
Staphylococcus aureus	5	11.9%
Coagulase negative staphylococcus **(CoNs)**		
- *Staphylococcus epidermedis*	3	7.1%
- CoNS	2	4.8%
- *Staphylococcus lugdunensis*	1	2.4%
Streptococcus	1	2.4%
E coli	2	4.8%
Enterococci	3	7.1%
Enterobacter	4	9.5%
Acinatobacter	1	2.4%
Total	42	100%

Table (2): Various maternal and neonatal risk factors in blood culture proven sepsis subgroup.

Parameter	Variables	Cases (n=42)	Control (n=30)	Statistical significance
Maternal age	<20 ≥ 20	5 37	1 29	**P = 0.194**
Educational status	Illiterate Literate or more	2 40	3 27	**P = 0.388**
Socioeconomic status	Poor Moderate or more	0 42	1 29	**P= 0.233**
Parity	Primi Multi	9 33	6 24	**P= 0.883**
Mode of delivery	Vaginal Instrumental/LCS	11 31	4 26	**P= 0.185**
PROM	Yes No	5 37	1 29	**P= 0.194**
MSAF	Yes No	1 41	1 29	**P = 0.808**
Maternal fever	Yes No	0 42	0 30	**P = 0.00**
PIH	Yes No	2 40	2 28	**P = 0.727**
PET/Eclampsia	Yes No	4 38	0 30	**P = 0.08**
FSAF	Yes No	0 42	0 30	**P =0.00**
APH	Yes No	5 37	0 30	**P = 0.05**
Maternal chr.dis.& medications	Yes No	5 37	1 29	**P = 0.194**
Birth weight **Mean± SD (Grams)**	< 2.5 ≥ 2.5	21 21 2214.5 ± 818.3 (n=42)	7 23 2784.3 ± 509.3 (n=30)	**P = 0.02** **P = 0.118**
Apgar score (1 minute)	< 7 ≥ 7	25 17	7 23	**P = 0.002**
Gestation age **Mean± SD (Weeks)**	Preterm Full term	20 22 35.69 ± 2.7	7 23 37 ± 1.66	**P = 0.03** **P = 0.02**
Sex	Male Female	18 24	19 11	**P = 0.08**
Birth asphyxia	Yes No	5 37	0 30	**P= 0.05**
Complications during labour	Yes No	9 33	0 30	**P= 0.006**

Table (3): Clinical profile of blood culture proven sepsis subgroup.

Parameter	Variable	Cases (n=42)	Control (n=30)	Statistical significance
Respiratory distress	Yes No	31 11	8 22	**P < 0.0001**
Lethargy	Yes No	35 7	9 21	**P < 0.0001**
jaundice	Yes No	35 7	29 1	**P= 0.07**
Fever	Yes No	6 36	2 28	**P= 0.31**
Poor feeding	Yes No	39 3	8 22	**P < 0.0001**
Abdominal distension	Yes No	23 19	3 27	**P < 0.0001**
Apnea	Yes No	18 24	1 29	**P = 0.0002**
Vomiting	Yes No	4 38	1 29	**P= 0.308**
Hypothermia	Yes No	1 41	0 30	**P= 0.394**
Cyanosis	Yes No	20 22	1 29	**P < 0.0001**
Irritability	Yes No	2 40	0 30	**P= 0.225**
Convulsions	Yes No	0 42	0 30	**P= 0.00**

Table 2 represents statistically significant maternal and fetal risk factors associated with the development of neonatal sepsis. The significant risk factors were birth weight, gestational age including its mean ± SD, Apgar score at 1 minute after birth and complication during labour.

Table 3 represents statistically significant clinical signs and symptoms associated with neonatal sepsis. These significant signs and symptoms were respiratory distress, lethargy, poor feeding, abdominal distension, apnea and cyanosis.

Discussion

Our study was designed to detect maternal and fetal risk factors associated with the development of neonatal sepsis and its clinical profile in blood culture proven neonatal sepsis patients.

From 102 clinically diagnosed neonatal sepsis patients, blood culture proven neonatal sepsis were detected in 42 cases. The bacteria which were isolated

and its corresponding cases numbers were shown in **Table (1)**.

Klebsiella microorganism were the most frequent bacteria isolated from clinically diagnosed neonatal sepsis patients; in 20 cases represent 47.6% of 42 blood culture proven sepsis patients. In study by **Kayange et al**, it was reported that *Klebsiella pneumoniae* was the commonest isolate recovered from infected neonates **(Kayange et al, 2010).** Also, in tertiary care institutions in India, *Klebsiella pneumoniae* is the most frequent bacterial isolate, followed by Staphylococcus aureus **(Tripathi & Malik, 2010).** CoNS were isolated from 6 cases represent 14.3% of 42 blood culture proven sepsis patients **Table (1).** The CoNS isolates were 6 cases; 3 cases for *Staphylococcus epidermedis*, one case for *Staphylococcus lugdunensis* and 2 cases were ill defined CoNS bacteria. CoNS infections in neonatal sepsis are usually secondary to *Staphylococcus epidermidis*, other strains such as *Staphylococcus capitis*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis* have also been reported **(Camacho-Gonzalez et al, 2013).** There was an important role of CoNS as pathogens in neonatal infections has been recognized **(Piette & Verschraegen, 2009).** *Staphylococcus aureus* was isolated from 5 cases represents 11.9% of 42 blood culture proven sepsis patients **Table (1).** *S aureus* causes blood stream infections and sepsis, skin and/or soft-tissue/wound infections, osteoarthritis (which can be multifocal), and less commonly, central nervous system infections such as meningitis and ventriculitis **(Polin & Saiman, 2003).** *Enterobacter* were isolated from 4 cases which represent 9.5% of 42 blood culture proven sepsis patients **Table (1).** *Enterobacter* spp, such as *E cloacae*, *E agglomerans*, and *E sakazakii*, are less common causes of hospital-acquired infections in NICUs. *Enterobacter* spp are multi-drug resistant pathogens and can cause sepsis and meningitis in preterm infants. Infections with these pathogens are associated with high mortality rates **(Polin and Saiman, 2003).** *Enterococci* was isolated from 3 cases which represents 7.1% of 42 blood culture proven sepsis patients **Table (1).** *Enterococcal* EOS is usually mild compared with LOS and is characterized by either a mild respiratory illness or diarrhea without a focal infection. *Enterococcus faecalis* is more frequently isolated than *Enterococcus faecium* **(Camacho-Gonzalez et al, 2013)**.

Escherichia coli was isolated from 2 cases which represent 4.8% of 42 blood culture proven sepsis patients **Table (1).** Epidemiologic surveillance has noted the

emergence of *E. coli* as an important pathogen associated with EOS, especially among VLBW infants **(Shane & Stoll, 2014).** *Streptococcus and Acintobacter*; was isolated in one case which represent 2.4% of 42 blood culture proven sepsis patients for each bacteria **Table (1).** GBS remains the most frequent pathogen of early-onset infection, there has been a shift from GBS to *E. coli* as the most important pathogen associated with early-onset infection in preterm and very low birthweight infants. Maternal GBS bacteriuria, indicative of a heavy burden of GBS colonisation, represents a notable risk for acquisition of neonatal GBS infection **(Shane et al, 2017).**

In this study, low birth weight and gestational age were significant risk factors for the development of neonatal sepsis. The incidence rate of neonatal sepsis is 3- to10-fold higher in preterm infants than in full term normal birth weight infants **(Stoll, 2007).** The incidence rate of nosocomial infections is more much higher among preterm infants <1500 g, admitted at Neonatal Intensive Care Units (NICUs) (20-25%) and increases with decreasing gestational age and birth weight and has been reported to be 43% for infants of 401-750 g, 28% for infants 751-1000 g, 15% for infants 1001-1250 g and 7% for infants 1251-1500 grams **(Stoll, 2007).** In addition to, low birth weight (LBW, <2500gms), has been associated with an increased risk of EOS **(Tripathi& Malik, 2010)**. Agar score at one minute after birth was a significant risk factor for the development of neonatal sepsis. this was in agree with, **Palazzi et al,** who stated that, Fetal distress may be one of the earliest signs of sepsis even before delivery in an infected fetus. Fetal tachycardia in the second stage of labour and a low Apgar score at birth have also been associated with peripartum infections **(Palazzi et al, 2006).** A low Apgar score, has been correlated with sepsis and associated adverse outcomes in the neonatal period **(Roper et al, 2007).** Complications during labour were a significant risk factor for the development of neonatal sepsis. These complications may include, preterm labor, premature rupture of the membranes at any time during gestation, prolonged rupture of membranes, chorioamnionitis, prolonged labor, intrauterine scalp electrodes, and traumatic delivery are factors were associated with neonatal sepsis **(Nizet & Klein, 2010).** Procedures during pregnancy, such as cervical cerclage and amniocentesis, which disrupt the integrity of amniotic cavity, may also increase the rates of intra-amniotic

infection and subsequent neonatal sepsis **(Simonsen et al, 2014).**

The significant signs and symptoms of neonatal sepsis were respiratory distress, lethargy, poor feeding, abdominal distension, apnea and cyanosis **Table (2).**

Respiratory distress and apnea were a significant clinical signs associated with blood culture proven neonatal sepsis. Initial symptoms of neonatal sepsis might be few and could include apnoea alone or tachypnoea with retractions, nasal flaring, grunting, or tachycardia **(Shane et al, 2017).** Respiratory distress include tachypnea, grunting, nasal flaring, and retraction of respiratory muscles can be the sole manifestation of neonatal sepsis with or without pneumonia and can be confused with transient tachypnea (TTN) of newborn initially **(Martin et al, 2011).** Also, lethargy was a significant clinical sign associated with blood culture proven neonatal sepsis. Lim et al. reported a high incidence of "poor activity," presumably lethargy and increased respiratory effort in the presence of respiratory distress **(Lim et al, 2012).** Poor feeding was a significant clinical symptom associated with neonatal sepsis in Infant with neonatal sepsis may have poor feeding, temperature instability, irritability, lethargy, respiratory distress, apnea, abdominal distension, jaundice, and/or tachycardia **(Stoll et al, 2011).** Abdominal distension was a significant clinical sign associated with blood culture proven neonatal sepsis. Abdominal distension is a common feature of generalized sepsis and is usually secondary to functional ileus, though must be distinguished from necrotizing enterocolitis (NEC). Abdominal palpation may be uncomfortable for the baby, especially if the abdomen is tense. Bowel sounds may be relatively silent in both NEC and functional ileus. An abdominal X rays radiograph (AXR) may be helpful in the differentiation between septic ileus and NEC **(Bedford Russell, 2015).** Cyanosis was a significant clinical sign associated with blood culture proven neonatal sepsis. Cardiac symptoms of neonatal sepsis may include cyanosis, oxygen desaturation, bradycardia, poor perfusion, reduced capillary refill, and hypotension. It is important to realize that subtle changes in respiratory status of newborns, temperature instability, or feeding problems can be the first signs of a life-threatening infection (**Simonsen et al, 2014).** Preterm infants will often have apnea, bradycardia, and cyanosis as the first sign of infection **(Lim et al, 2012).**

Conclusions

the most frequent bacteria isolated with conventional blood culture was *Klebsiella* which represent 47.6% of bacteria detected in blood culture proven neonatal sepsis patients. Followed by CONS, *S. aureus*, *Enterobacter*, *Enterococci*, *E coli*, *Streptococci* and *Acinatobacter* which represent 14.3%, 11.9%, 9.5% ,7.1%, 4.8%, 2.4% and 2.4% respectively. The common risk factors associated with the development of neonatal sepsis in blood culture proven sepsis patients were gestational age, low birth weight, Agar score at one minute and complications during labour while respiratory ditress, apnea, letharg, poor feeding, abdominal distension and cyanosis were the significant clinical sign and symptoms.

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