Study of Hematological Parameters in Neonatal Septicemia

A thesis project submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Submission Date: JULY, 2010

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This is to certify that, the thesis "Study of Hematological Parameters in Neonatal Septicemia" submitted to the Department of pharmacy, East West University, Mohakhali, Dhaka for the partial fulfill of the requirements for the degree of Bachelor of pharmacy (B.Pharm) was carried out by Shahnaz Parvin (ID: 2006-2-70-051) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and other facilities available of this connection are duly acknowledged.

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ACKNOWLEDGEMENTS

First and foremost, all extol and commendation should go to almighty "ALLAH" the most merciful who enabled me in completing this work soundly and orderly.

I would like to express thanks to my supervisor Mrs. Nishat Nasrin, Lecturer, East West University. She has helped me a lot by giving me her precious time and directed me properly through a guideline to clear my confusion about my study.

Besides, I am grateful to co- supervisor Dr Forhad Monjur Assistant professor of ICH (Institute of child Health) and SSFH (Shishu Sasthya Foundation Hospital), Mirpur Dhaka for his guidance, criticism and encouragement during the preparation of this study. Mrs Farhana Rizwan, Senior lecturer, EWU and Mr. Assaduzzaman, Senior lecturer, EWU, also helped me a lot through their suggestion. I gratefully acknowledge to Pro-vice chancellor Professor Muniruddin Ahmed and Chairperson Professor Dr. Chowdhury Faiz Hossain for their inspiration in my study.

I express my sincere gratitude to my caring parents for guiding me all through my life, including that for my research project. I am very grateful to my brother, sisters and friends, who encouraged me enormously. Finally I am thankful to all of my friends who were involved in this research enthusiastically.

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List of abbreviation

CSF	Cerebro Spinal Fluid
CONS	Coagulase Negative Saphylococcus
CRP	C-Reactive Protein
CRP #1	C-Reactive Protein first measurement
CRP #2	C-Reactive Protein second measurement
CRP #3	C-Reactive Protein third measurement
CD-116	Cluster Differentiation-116
EOS	Early Onset Sepsis
ELISA	Enzyme Linked Immuno Sorbent Assay
ESR	Erythrocyte Sedimentation Rate
FC	Fragment Crystallizable
GCSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte-Monocyte Colony Stimulating Factor
GBS	Group B Streptococci
HSV	Herpes Simplex Virus
I:T ratio	Immature : Total ratio
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMR	Infant Mortality Rate
IFN	Interferon
IL	Interleukin
KCH	Kanti Children Hospital

LOS	Late Onset Sepsis
LPS	Lipopolysaccharide
NK	Natural Killer cell
PC	Phospho Choline
PAF	Platelet Activating Factor
PMN	Polymorphonuclear cell
PROM	Premature Rupture Of Membrane
PCT	Procalcitonin
SAP	Serum Amyloid Component
SCANU	Special care Neonatal Unit
	Systemic Inflammatory Response
SIRS	Syndrome
SPSS	Statistical Package for Social Science
TLR	Toller Like Receptor
	Tribhuvan University Teaching
TUTH	Hospital
TNF	Tumor Necrosis Factor
US	United States
WBC	White Blood Cell

Abstract

Neonatal septicemia is a significant cause of morbidity and mortality in neonates mostly in developing countries. Neonatal sepsis requires rapid and accurate diagnosis as well as treatment for the improved outcome.. So an effective, simple clinical prediction tool is needed for the treatment decision. Our study was accomplished to analyze various hematological parameters of sepsis and in combination to formulate a guideline for the diagnosis of neonatal sepsis. The study was done in the special care neonatal unit (SCANU) of the ICH (Institute of child health) and SSF (Shishu sasthya foundation) hospital, Mirpur over a period of one year commencing from July 2009 to May 2010. A total of 43 neonates were taken who were suspected of neonatal septicemia. Blood samples for culture were taken aseptically before starting antibiotic therapy. Data on demographic characteristics of mothers, children and laboratory tests, results of blood cultures were collected. The results of the study showed that 53% patients were male and 43% patients were female. The ratio of male to female was 1.15: 1. Out of 32 neonates 3 (9 %) had positive blood culture for *Pseudomonas* species and *Streptococcus* species. Thus there were only 3 cases of proven sepsis. Out of 43 patients, Leucopenia (26 %), neutropenia (12%) neutrophilia (14%), leukocytosis (2%), and thrombocytopenia (12%) were observed in neonates with septicemia. Among 19 patients, CRP test was positive in 7 (37%) cases. The result of the study showed that CRP test is the most significant parameters for diagnosis of neonatal septicemia. So CRP test should be used for the diagnostic evaluation of the neonates with suspected sepsis as it is a very good screening test for the early detection of septicemia.

CHAPTER 1

Introduction

1.1. Overview:

Sepsis is one of the most common causes of neonatal mortality. It is also one of the major causes of neonatal deaths in developing countries. Sepsis related mortality is largely preventable with rational antimicrobial therapy and aggressive supportive care.

The infectious agents associated with neonatal sepsis have changed over the past 50 years. *Staphylococcus aureus* and *Escherichia* coli *(E. coli)* were the most common bacterial infectious hazards for neonates during the 1950s in the United States. Over the ensuing decades, Group B *Streptococcus* (GBS) replaced *S aureus* as the most common gram-positive organism that caused early-onset sepsis. During the 1990s, GBS and *E coli* continued to be associated with neonatal infection; however, coagulase-negative *Staphylococcus epidermidis* is now more frequently observed. Additional organisms, such as, *Listeria monocytogenes. Chlamydia pneumoniae, H. influenzae, Enterobacter aerogenes*, and species of *Bacteroides* and *Clostridium* have also been identified in neonatal sepsis.

Neonatal sepsis may be classified according to the time of onset of the disease: early onset septicemia (EOS) and late onset septicemia (LOS). GBS and gram-negative enteric organisms (predominantly *Escherichia coli*) account for most cases of early-on *Staphylococci* account for 30 to 60% of late-onset cases and are most frequently due to intravascular devices (particularly umbilical artery or vein catheters). *E. coli* is also becoming increasingly recognized as a significant cause of late-onset sepsis, especially in very LBW infants.

The most common risk factors associated with early onset neonatal sepsis include maternal group B *Streptococcus* (GBS) colonization (especially if untreated during labor), premature rupture of membranes (PROM), preterm rupture of membranes, prolonged rupture of membranes, prematurity, maternal urinary tract infection, and chorioamnionitis.

Other factors associated with or predisposing to early onset neonatal sepsis include maternal fever greater than 38°C, maternal urinary tract infection, poor prenatal care, poor maternal nutrition, low socioeconomic status, recurrent abortion, maternal

substance abuse, low birth weight, difficult delivery, birth asphyxia, meconium staining, and congenital anomalies. Risk factors implicated in neonatal sepsis reflect the stress and illness of the fetus at delivery, as well as the hazardous uterine environment surrounding the fetus before delivery.

Late onset sepsis is associated with risk factors like prematurity, central venous catheterization (duration of >10 d), nasal cannula or continuous positive airway pressure (CPAP) use, H_2 blocker/proton pump inhibitor use and gastrointestinal tract pathology.

Physical examination of the baby and the laboratory tests help to diagnose neonatal sepsis. The physical examination includes assessing the body temperature, heart rate, breathing, etc. The laboratory tests aim at finding out the bacteria/virus that has caused infection. Blood tests that are performed on the infant, consist of WBC count, platelet count, blood culture, etc. Chest X-rays and urine tests are performed, when infection due to bacteria is suspected.

Neonatal sepsis should be treated at the earliest possible time because the immune system of an infant is not completely developed and the infection may be fatal. Antibiotics are recommended when diagnosis of neonatal sepsis is not yet confirmed. This can help prevent further complications. Further treatment depends on the result of the laboratory tests. In case of infections caused due to GBS and *E. coli* medications like ampicillin and gentamicin are given. These medications help in curing early-onset neonatal sepsis. Intravenous immune globulin replacement, granulocyte transfusion is the treatments given, if the infection is too severe. If the baby is given the proper treatment, it can recover soon from the infection.

This study was accomplished on neonatal septicemia patients to evaluate the hematological parameters. The result of this study is consistent with the other studies regarding neonatal septicemia. This study will help to determine the most significant parameters in diagnosis of septicemia.

1.2. Introduction

Of the estimated 130 million infants born each year worldwide, 14 million die in the first 28 days of life. Three-quarters of neonatal deaths occur in the first week, and more than one-quarter occur in the first 24 hours **(World health report 2005)**. Neonatal deaths account for 40% of deaths under the age of 5 years worldwide. Therefore, efforts to achieve the UN Millennium Development Goal 4 of reducing childhood mortality by two-thirds by 2015 are focused on reducing neonatal deaths in high-mortality countries **(Loo S, 2002)**.

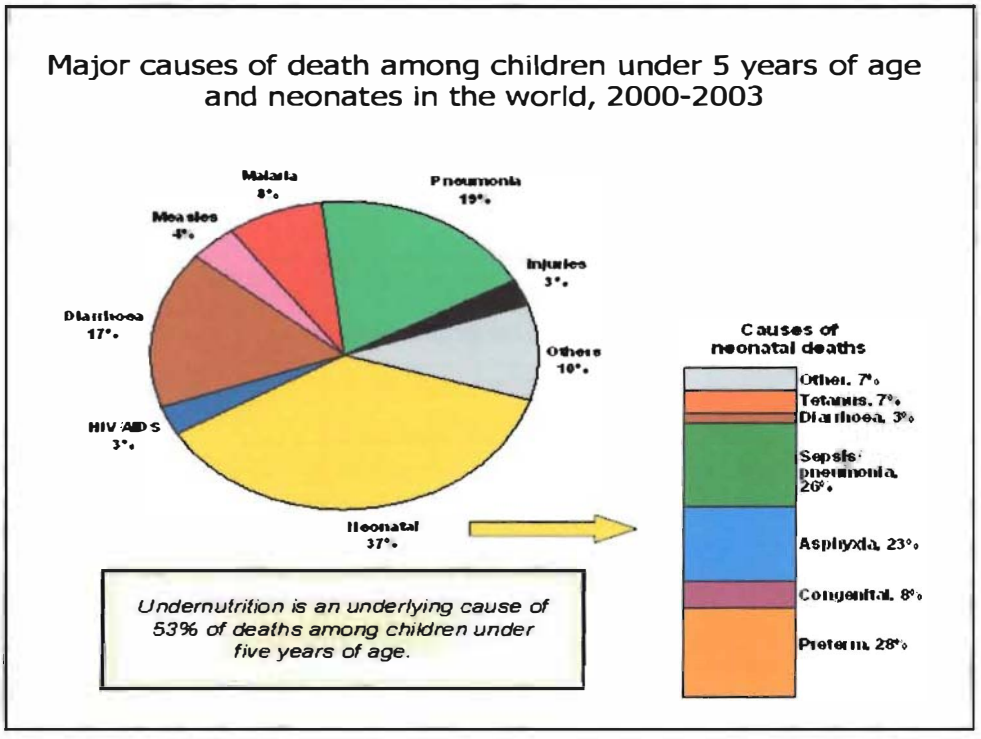

Fig. 1.1: Major causes of death among children and neonates

Major causes of neonatal mortality are diseases associated with preterm birth and low birth weight (LBW) and lethal congenital anomalies. Neonatal mortality is highest during the first 24 hr of life and accounts for 65% of all infant deaths (deaths before 1 year of age).

Perinatal mortality is influenced by prenatal, maternal, and fetal conditions and by circumstances surrounding delivery. Perinatal deaths are associated with intrauterine growth restriction (IUGR); conditions that predispose the fetus to asphyxia, such as placental insufficiency; severe congenital malformations; and overwhelming early-

onset neonatal infections. Postneonatal mortality refers to deaths between 28 days and 1 yr of life. Historically, these infant deaths were due to causes outside the neonatal period, such as SIDS, infections (respiratory, enteric), and trauma. **(Gotoff SP, 2002)**.

Table 1.1. Major Causes of Perinatal and Neonatal Mortality **(Gotoff SP, 2002)**.

FETAL	PRETERM	FULL TERM
Placental insufficiency	Severe immaturity	Congenital anomalies
Intrauterine infection	Respiratory distress syndrome	Birth asphyxia, trauma
Severe congenital malformations (anomalies)	Intraventricular hemorrhage	Infection
	Congenital anomalies	Meconium aspiration pneumonia
Umbilical cord accident	Infection	Persistent pulmonary hypertension (PPHN)
Abruptio placentae	Necrotizing enterocolitis	
Hydrops fetalis	Bronchopulmonary dysplasia (BPD)	

Bangladesh has a neonatal death rate that is substantially high. The neonatal mortality rate was 53.5 per 1,000 live births. The originating causes of death were pre-maturity/low birth-weight (30%), difficult labour (16%), unhygienic birth practices (16%), others (4%), and unknown (34%). The direct causes were sepsis (32%), asphyxia (26%), tetanus (15%), respiratory distress (6%), others (6%), and unknown (14%). According to the prevailing causes of neonatal deaths, implementation of intervention programmes, often in the community, that do not depend on highly-technical training or sophisticated equipment should be implemented **(Chowdhury M, 2005)**.

Infections are the single largest cause of neonatal deaths globally. Neonatal sepsis is a bacterial infection is considered to be an important cause of neonatal mortality. Bacterial organisms causing neonatal sepsis may differ among countries. However, in

most developing countries, Gram-negative bacteria remain the major source of infection **(Dawodu et al., 2002)**. In addition, bacteria 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 **(Motara, et *al*, 2005)**.

1.3. Neonatal sepsis

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms that have been used to describe the systemic response to infection in newborn infants. There is little agreement on the proper use of the terms that is whether it should be restricted to bacterial infection, positive culture or severity of illness **(Gotoff SP, 2002)**.

The application of the terminology to septic newborns needs careful assessment (i.e., age related reference values for blood pressure, heart rate, respiratory rate and leukocyte count). Furthermore, the application of a staging system (including sepsis, severe sepsis, septic shock and multiple organ dysfunction syndromes) may not be best approach to disease or risk stratification in the newborn **(Chiesa C, 2004)**.

Currently, criteria for neonatal sepsis include documentation in a newborn infant with a serious systemic illness in which non-infectious explanation for the abnormal pathophysiological states is excluded or unlikely **(Gotoff SP, 2002)**.

1.4. Definition of Neonatal sepsis

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms that have been used to describe the systemic response to infection in newborn infants. There is little agreement on the proper use of the terms that is whether it should be restricted to bacterial infection, positive culture or severity of illness **(Gotoff SP, 2002)**.

1.5. Classification of neonatal sepsis

Neonatal sepsis can be divided into two main classes depending on the onset of symptoms related to sepsis.

1.5.1. Early onset sepsis

Early onset sepsis usually presents within the first 72 hours of life. In severe cases, the neonate may be symptomatic in utero (fetal tachycardia, poor beat to beat variability) or within a few hours after birth. The source of infection is generally the maternal genital tract. Clinically, neonates usually present with respiratory distress and pneumonia. Presence of some perinatal risk factors has been associated with an increased risk of early onset sepsis. Recommendations from developed countries suggest that presence of two risk factors should be considered an indication for starting antibiotics **(Jeeva M, 2008).**

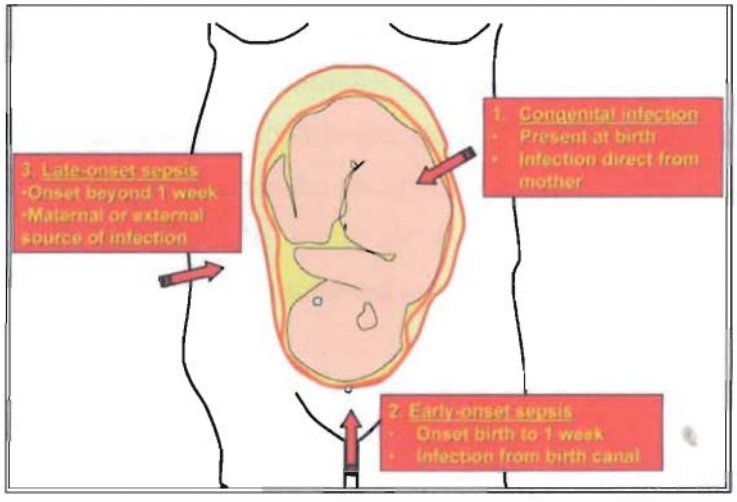

Fig. 1.2. Causes of neonatal septicemia **(Joshi S, 2005).**

1.5.2. Late onset sepsis

Late onset sepsis usually presents after 72 hours of age. The source of infection is either nosocomial or community-acquired and neonates usually present with septicemia, pneumonia or meningitis. Various factors that predispose to an increased risk of nosocomial sepsis include NICU admissions, low birth weight, prematurity, invasive procedures, parenteral fluid therapy, ventilation and use of stock solutions. Factors that may increase risk of community-acquired late onset sepsis include poor hygiene, poor cord care, bottle-feeding and prelacteal feeds. Breast-feeding, on the other hand, prevents infection in neonates **(Jeeva M, 2008).**

1.6. Etiology

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram negative organisms are more common and are mainly represented by *Klebsiella, Escherichia coli, Pseudomonas*, and Salmonella. Of the Gram positive organisms, *Staphylococcus aureus, coagulase negative staphylococci* (CONS), *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are most commonly isolated. Group B streptococcus (GBS) is generally rare in developed countries. In most of the African studies, the incidence is low, with the exception of South Africa. In Asia GBS is also reported to be extremely rare. In South America GBS incidence is comparable to the West. It is not known whether these variations reflect true differences in pathogens across the world, reflecting an epidemiological transition in some countries, or whether it reflects an epidemiological artefact linked to the fact that most EOS babies die at home before reaching the health facilities and they do not appear in the statistics. Neonatal surveillance in developed countries generally identifies GBS and *E coli* as the dominant EOS pathogens and CONS as the dominant LOS pathogen followed by GBS and *Staphylococcus aureus.*

In developed countries, EOS disease is often more severe and case fatality rate is greater than it is for LOS disease. As the latter is usually caused by CONS, the associated morbidity and mortality are low. In developing countries, this may not be the case: in some series, LOS disease has a neonatal sepsis, and methicillin resistant organisms (methicillin resistant Staph aureus (MRSA) are widespread Vancomycin is often not affordable. The experience in the western world suggests that this may change in the future **(Vergnano S, *et al,* 2004)**.

In Nepal neonatal sepsis is likely to be the result of infection by gram positive bacteria such as *Staphylococcus, Streptococcus* and gram negative organisms such as *Klebsiella, Enterobacter* and *Salmonella* **(Manandhar DS, 2003)**. A study done by Shrestha B. M. at Kanti Children Hospital, found the predominance of gram negative organisms in 60.5% cases with *E. coli* being the commonest isolate **(Shrestha BM, 2000)**.

In the study at a tertiary-level pediatric hospital in Dhaka, Bangladesh, late-onset neonatal septicemia was found to be more common than early-onset disease, in contrast to other reports in which early-on et septicemia generally has been more common. *E.coli* and *Klebsiella* was the most common organisms responsible for neonatal septicemia in a tertiary care center in Bangladesh. Most of the gram negative isolates were sensitive to gentamicin and third generation cephalosporins **(Chowdhury A, 2002).**

1.7. Risk factors for early Onset Neonatal Sepsis

Perinatally acquired neonatal bacterial infection usually manifests within the first three days of life and is also termed as early onset sepsis.. A good history especially maternal history and elicitation of risk factors are of great importance in the assessment of individual baby and the need for empirical antibiotic therapy. Risk factors are additive and presence of more than two risk factors increases the risk of sepsis manifold. Most of the risk factors defined are from the west where group B streptococcus is the etiological agent for early onset sepsis **(Dutta A, 2007).** Some of the risk factors are discussed a follows.

1.7.1. Prolonged rupture of membranes:

When membranes have ruptured prematurely before 37 weeks' gestation, a longer latent period precedes vaginal delivery, increasing the likelihood that the infant will be infected. The relationship between duration of membrane rupture and neonatal infection is inversely related to gestational age. Therefore, the more premature an infant, the longer the delay between rupture of membranes and delivery, and the higher the likelihood of neonatal sepsis **(Ann L, 2010).**

The risk of sepsis in newborns born to mothers with rupture of membranes for more than 24 hours has been reported to be 1 percent compared to a baseline incidence of 0.1 to 0.5 percent **(Dutta A, 2007).**

A study by Seaward et al found that more than 6 vaginal digital examinations, which may occur as part of the evaluation for PROM, were associated with neonatal infection even when considered separately from the presence of

chorioamnionitis.There was a three-fold increase in the incidence of sepsis when membranes ruptured for 24 hours prior to delivery **(Basavaraj M , 2002)**.

1.7.2. Chorioamnionitis

It clinically manifests with maternal fever, abdominal tenderness, increased WBC count and erythrocyte sedimentation rate , and premature rupture of the membranes >48 hours before delivery,foul smelling or purulent amniotic fluid and fetal tachycardia or silent where in there is histologic evidence of inflammation, but no symptoms or signs. Chorioamnionitis increases the risk of sepsis by 2 to 3 times. If PROM is associated with chorioamnionitis, the risk of sepsis increases by four-fold **(Dutta A, 2007)**.

1.7.3 Maternal GBS status

- The most common etiology of neonatal bacterial sepsis is GBS. Nine serotypes exist, and each is related to the polysaccharide capsule of the organism. Types I, II, and III are commonly associated with neonatal GBS infection. The type III strain has been shown to be most highly associated with CNS involvement in early-onset infection.
- The GBS organism colonizes the maternal GI tract and birth canal. Approximately 30% of women have asymptomatic GBS colonization during pregnancy. GBS is responsible for approximately 50,000 maternal infections per year in women, but only 2 neonates per 1000 live births are infected. Women with heavy GBS colonization and culture results that are chronically positive for GBS have the highest risk of perinatal transmission. Also, heavy colonization at 23-26 weeks of gestation is associated with prematurity and low birth weight. Colonization at delivery is associated with neonatal infection. Intrapartal chemoprophylaxis of women with positive culture results for GBS has been shown to decrease the transmission of the organism to the neonate during delivery **(Ann L, *et al*, 2010)**.

1.7.4. Prematurity and low birth weight

Preterm babies are deficient in immunoglobulin concentration, complement function and phagocytic activity. They have 2 to 10 times higher risk of developing sepsis than

term infants. Chorioamnionitis may coexist and may trigger for preterm labor. Association of chorioamnionitis and low birth-weight increases the risk of sepsis to 16% compared to association with normal weight babies **(Dutta A, 2007)**.

Prematurity is associated with infection from cytomegalovirus (CMV), herpes simplex virus (HSV), hepatitis B, toxoplasmosis, *Mycobacterium tuberculosis*, *Campylobacter fetus*, and *Listeria* species. Preterm infants are more likely to require invasive procedures, such as umbilical catheterization and intubation **(Ann L, *et al*, 2010)**.

1.7.5. Perinatal asphyxia

Asphyxia is associated with depressed immune function. In addition; several interventional procedures increase the risk of infection. Presence of low Apgar score along with prolonged rupture of membranes has shown to increase the risk of infection by 4 percent and 27 percents as studied by Geme and Knudsenetal respectively **(Dutta A, 2007)**.

1.7.6. Male gender

Boys have 2 to 6 time higher risk of development of neonatal sepsis than girls **(Dutta A, 2007)**.

1.7.7. Other factors

Maternal fever, genitourinary tract nfection, poor socioeconomic condition and feeding artificial milk are other risk factors attributed for sepsis **(Dutta A, 2007)**.

1.8. Pathogenesis

Whether the infants who are exposed to potentially pathogenic organism will develop sepsis or ot is determined by maternal, environmental and host factors. Exposure to the micro-organism may occur in the following ways **(Dear P, 1999)**.

1.8.1. Transplacental

Certain infective agents have an inherent ability to penetrate the barrier, often damaging the placenta **(Dear P, 1999)**.

1.8.2. Ascending infection

Ascent of vaginal organisms into the uterine cavity occurs from the vagina and cervix through microscopic leak in the amniotic membrane or through frankly ruptured membrane **(Dear P, 1999)**. The risk of perinatal sepsis according to Trucker, is about 1-2% after the term PROM. Rupture of membrane without complications for more than 24 hours prior to delivery is associated with 1% increase in the incidence of neonatal sepsis; however when the chorioamnionitis accompanies, the incidence of neonatal infection rises by four times. 26IIn a report presented in the Perinatal Symposium, California, the frequency of sepsis associated with PROM and culture positive GBS showed to be 33 to 50% **(Permanente K, 2003)**.

1.8.3. Intrapartum

Vaginal delivery inevitably results in surface contamination during the passage through the birth canal and causes the colonization of skin and gut. The risk of transmission increases when the density and number of sites of maternal colonization increases **(Dear P, 1999)**.

1.8.4. Postnatal

Spread of infection from the postnatal environment is very common. People are the main source of such contamination **(Dear P, 1999)**.

Many prepartum and intrapartum obstetric complications have been associated with increased risk of infection in the newborn, the most significant of which are premature onset of labour, PROM, chorioamnionitis and maternal fever **(Freij BJ, 1994)**.

In one of the study of 963 pregnancies complicated by PROM, the incidence of clinical sepsis increased from 2% among infants born within 23 hrs of membrane rupture to 7% and 11% among those delivered 24 to 47 hrs and 48 to 71 hrs after the membrane rupture respectively. The incidence of infection has been estimated to be 8.7% for infants born to mother with PROM (>24 hrs) and clinical chorioamnionitis (**Freij BJ, 1994**).

The barrier to infection is provided by the integrity of placenta and membranes, the low pathogenicity of most colonizing organisms and the relative competence of

baby's defence mechanism. Neonatal infection occurs when one or other of these factors are altered **(Dear P, 1999)**.

Colonization of the upper respiratory tract occurs rapidly and 90% of infants have positive pharyngeal culture by third day, with CONS being the commonest organism. Skin colonization is very rapid with the number of bacteria increasing 100 fold during the first week. The umbilicus, perineum and axilla are most heavily colonized. Most infants become colonized without becoming infected but various host factors or the pathogenicity of the organisms and its load are important in causation of sepsis in newborn **(Dear P, 1999)**. The systemic inflammatory cascade is initiated by various bacterial products. These bacterial products gram negative bacteria – endotoxin, exotoxins, proteases, formyl peptides; gram positive bacteria- endotoxin, super antigens (toxic shock syndrome toxin, streptococcal pyrogenic exotoxin A), enterotoxin, hemolysins, peptidoglycans and lipotechoic acid] binds to cell receptor on the hosts macrophages and activates regulatory proteins. Endotoxin activates the regulatory proteins by interacting with several receptors. The CD receptors pool in the LPS binding protein complex on the surface of the cell and then the TLR receptors translate the signal into the cells **(Chamberlain N R, 2000)**.

The proinflammatory cytokines produced are Tumor Necrosis Factor (TNF), Interleukin 1, 6, 12 and Interferon Gamma (IFN-γ). These cytokines can act directly to affect organ function or they may act indirectly through secondary mediators. The secondary mediators include nitric oxide, thromboxane, leukotriens, and platelet activating factor, prostaglandins and complement. These primary and secondary mediators cause the activation of the coagulation cascade, the complement cascade and production of prostaglandins and leukotriens. These products lower the perfusion of organs and can lead to multiple organ system failure **(Chamberlain N R, 2000)**.

1.9. Pathology

Bacterial fragments and endotoxin or exotoxins stimulate monocytes and neutrophils to produce variety of inflammatory mediators. The simultaneous activation of complement, coagulation and fibrinolytic cascades leads to the formation of vasoactive and proinflammatory mediators such as prostaglandin E2, free radicals,

oxide and PAF (platelet activating factor). These mediators either singly or sequentially lead to adhesion and diapedesis of polymorphonuclear cells into the tissue giving rise to clinical features seen in sepsis syndrome and septic shock **(Haque KH, 1998)**.

Sepsis may indicate an immune system that is severly compromised and unable to eradicate pathogens. Cells of the innate immune system recognize messengers through pattern recognition receptors called Toller like Receptors (TLRs), which are resistant to endotoxin because of mutation in the toll like receptor 4 gene (TLR4). These TLR4 mutations have been identified in human and may make person more susceptible to infections. Again, polymorphism in TNF receptor, interleukin-1 receptor, FCγ receptors, TLRs and cytokine genes may determine the concentrations of inflammatory and anti-inflammatory cytokines production. The risk of death during the sepsis has been linked to genetic polymorphism for TNF-α and TNF-β as well. Such polymorphism may be used to identify patients at high risk of development of sepsis and organ dysfunction during infection **(Hotchkiss RS, 2003)**.

Clinical presentation and assessment of the neonate

Early recognition of serious infection in the neonate is essential because of extreme urgency with which the risk of permanent morbidity and mortality can develop **(Dear PR, 1991)**.

Physical findings

These non-specific clinical signs of early sepsis are also associated with other neonatal diseases, such as respiratory distress syndrome, metabolic disorders, intracranial hemorrhage and traumatic delivery. Therefore neonatal sepsis should be diagnosed by excluding other disease process **(Bellig LL, 2004)**. The important symptoms and signs of neonatal sepsis are:

Refusal to feed

Refusal to feed occurs in most of the infants. In a study done by Jaiswal et al, refusal to feed was most prominent (66%) in infants with neonatal sepsis **(Jaswal R S, 2003)**.

Gastrointestinal symptoms

Vomiting, diarrhoea and abdominal distension are important symptoms of neonatal sepsis. **(Jaswal R S, 2003).**

Lethargy

Jaiswal et al reported lethargy as a feature of septicemia in 42% of infants **((Jaswal R S, 2003)**

Temperature instability

A temperature below 36oC or above 37.8oC sustained for more than one hour must be regarded as possible infection. About 50% of infected newborns have temperature more than 37.8oC (axillary) **(Gotoff SP, 2002).**Temperature instability is observed with neonatal sepsis and meningitis either in response to pyrogens secreted by the bacterial organisms or from sympathetic nervous system instability **(Bellig LL, 2004).**

Ictrerus

Icterus is one of the important clinical signs of sepsis in newborns **(Gotoff SP, 2002)** Basu K. et al reported that septicaemia caused jaundice in 4.75% of cases **(Basu K, 2002).**

Respiratory distress

Respiratory distress is the most common symptom occurring in 90% of patients with sepsis **(Guerina NG, 1998).** Cyanosis, apnoea and dyspnoea are the classic signs of neonatal lung disease **(Greenough A, 1999).**

Seizure

Seizures are late features of neonatal meningitis **(Dear P, 1999).**

Cardiovascular dysfunction – Tachycardia of > 160 beats/min usually suggests early sepsis. Other signs are mottling and poor capillary refill time **(Gotoff SP, 2002).**

Umbilical discharge

Omphalitis is a unique neonatal infection resulting from inadequate care of the umbilical cord. The umbilical stump is colonized by bacteria from the maternal

genital tract and the environment. Omphalitis may spread to the umbilical or portal vessels, the liver and peritoneum, often resulting in sepsis **(Gotoff SP, 2002)**.

Cutaneous manifestations:

Petechiae and pustules may have infectious cause. DIC with petechiae and bleeding from puncture site is late sign of sepsis **(Gotoff, SP 2002)**.

1.11. Diagnostic investigations of neonatal sepsis

The diagnosis of neonatal sepsis is difficult to make solely on historical and clinical ground. Laboratory evaluation assists in the diagnosis and confirmation of neonatal sepsis. No single laboratory test has been found to have acceptable sensitivity, specificity for predicting or excluding infection **(Guerina NG, 1998)**. So far there is no rapid and reliable test for confirmation of diagnosis of neonatal sepsis. The treatment for sepsis is generally started when clinical findings are supported by indirect early markers of neonatal infection **(Singh M, 1991)**. The laboratory parameters evaluated as the indicators of infections are followings:

1.11.1 Haematological tests

Total leukocyte count has low predictive value for the diagnosis of neonatal sepsis because of wide range of normal count. Leukopenia (<5,000/cmm) or leukocytosis (>20,000/cmm) is usually associated with neonatal sepsis **(Singh M, 1997)**. During the first three days of life, leukopenia and neutropenia have good sensitivity and specificity of 67%, 90% and 78%, 80% respectively **(Berger C, 1994)**. Beyond 3 days of age, leucocytosis and neutrophilia have sensitivity and specificity of 74%, 56% and 67%, 65% respectively. The ratio of immature to mature neutrophill (I: T ratio) of more than 0.2 is relatively sensitive indicator of sepsis. I: T ratio has sensitivity and specificity of 78% and 73% respectively **(Ang AT, 1994)**.

When the I: T ratio is greater than 0.2, this indicates that there is a "left shift." This left shift means that there are more immature neutrophils than mature neutrophils circulating around in the bloodstream.

A neutrophil is a type of white blood cell that defends the body against organisms that cause infection. When infection is present the neutrophils migrate out of the capillaries and into the infected site, where they ingest and destroy the pathogens causing the infection. When the demand for the neutrophils exceeds the supply in circulation, immature neutrophils are released into the blood to help fight off the infection. This is labeled a "left shift" and indicates that an infection may be present. As the infection diminishes and neutrophils are replenished, a "shift to the right" occurs, indicating that everything is back to normal **(Singh M, 1997)**.

Platelet count

Platelet count falls below 100X109/L in obviously septic infants. The sensitivity and specificity of thrombocytopenia for the diagnosis of septicemia was reported as 65% and 77% respectively. In a study obtaining haematolotical score by a complete blood count, differential leucocyte count, total leucocyte count, band form count and platelet count the hematological score of >3 has the sensitivity of 86% and negative predictive value of 96% **(Manucha V, 2002)**.

ESR measurement

ESR is a sensitive but non-specific indicator of infection **(Haque KH, 1998)**. The ESR is a crude quantitative measurement of fibrinogen concentration in plasma. When fibrinogen level increases as in response to an acute inflammatory stimulus; there is increased adhesion of erythrocytes leading to agglutination, rouleaux formation and increased rate of sedimentation. The micro ESR value of >15mm/hr is suggestive of infection. ESR cannot reliably distinguish the microbial etiology of acute inflammatory process and may take even longer time to rise **(Hilliard NJ, 2002)**.

Blood culture

Identification of the causative organism may be made by blood culture. Blood for culture should be obtained from a peripheral vein with aseptic precautions in an amount of 1-2ml. The sensitivity of single blood culture in identifying septicemia is 73% **(Freij BJ, 1994)**. Two blood cultures from different sites increases the yield and decreasing false positive result **(Gotoff SP, 2002)**.

In a study of 254 clinically suspected cases of neonatal bacteremia Parikh and Singh; 1995, found that blood culture was positive in 119 (47%). In another study done by Pouncyrous et al; found 27% of blood culture was positive with 69% of gram positive and 29% of gram negative organisms. 48 In similar study by Kaiser et al; on evaluation of blood culture results from late onset sepsis found that 10.2% cases were positive for bacterial isolation **(Kaiser JR, 2002)**.

1.11.5. Chest X ray:

The chest x ray was performed after the clinical evaluation in suspected sepsis. The radiographic findings consistent with sepsis includes pulmonary infiltration, pleural effusion, pneumatosis intestinalis or intraperitoneal free air. **(Benitiz W E, 1998)**.

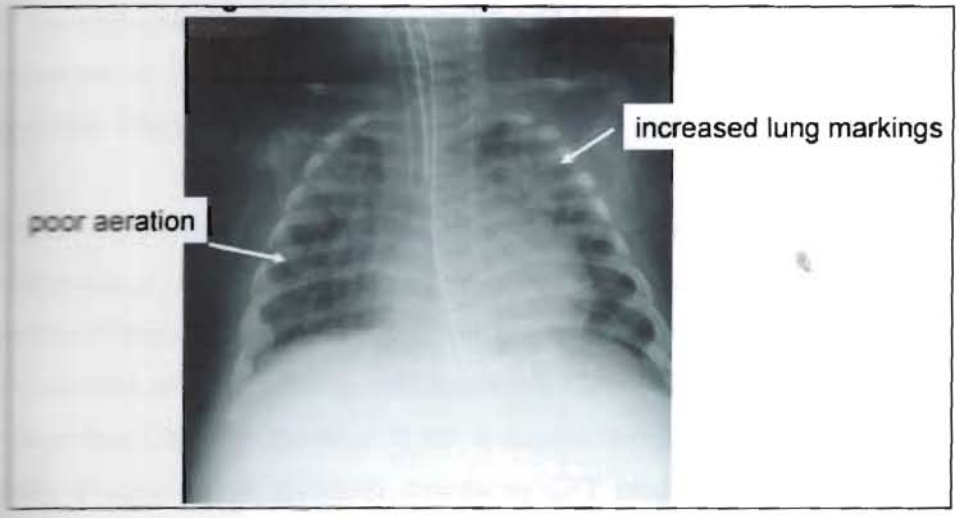

Fig 1.3. CXR of a septic neonate **(Merck M, 2000).**

1.11.6. CSF analysis

CSF examination is indicated in all septic neonates with history of seizure, as meningitis occurs in one third of neonatal sepsis cases **(Hague KH, 1998)**. In case of neonatal meningitis, the ratio of CSF glucose to blood glucose is less than 50%. A positive culture of pathogenic bacteria in the CSF remains the gold standard for diagnosis of bacterial meningitis, CSF examination is positive in most of the cases with late onset sepsis **(Dear P 1999)** Whereas Kaiser et al on evaluation of CSF

culture, found only 5.4% to be positive of bacterial isolation in late onset sepsis. **(Kaiser JR, 2002).**

1.11.7. Urine culture

Urine culture should be done in cases of late onset sepsis. Urinary tract infection is confirmed when there is more than 105 colony forming units/ml of freshly collected urine **(Haque KH, 1998).**

1.11.8. C-reactive protein (CRP)

C-reactive protein was first described by Tillet and Francis in 1930 at Rockefeller University. **(Hengst JM, 2003)** .C-reactive protein is a serum glycoprotein produced by the liver exclusively during acute inflammation.Interleukin-1, interleukin-6 and TNF are mediators for the synthesis of CRP by hepatocytes. It causes rapid increase in concentration of up to 1000 fold in response to tissue damage and inflammation **(Pepys MB, 2003).**

In healthy adult volunteer blood donor, the median concentration of CRP is 0.8 mg/l, but following an acute phase stimulus, values may increase from less than 50μg/l to more than 500mg/l, that is 10,000 fold. The plasma half life of CRP is about 19 hours and is constant under all conditions of health and disease. So that the sole determinant of circulating CRP concentration is the synthesis rate, which directly reflects the intensity of pathological processes stimulating CRP production. When the stimulus for increased production completely ceases, the circulating CRP concentration falls rapidly, at almost the rate of plasma CRP clearance. An acute phase CRP value shows diurnal variation and are unaffected by eating. Liver failure impairs CRP production, but no other intercurrent pathologies and very few drugs reduce CRP values unless they also affect the underlying pathology providing the acute phase stimuli **(Manandhar DS, 2000).**

1.11.8.1. CRP in Neonatal sepsis

Da silva et al, while reviewing the use of CRP as a tool for diagnosis of neonatal sepsis concluded that CRP is probably the best available diagnostic test **(Da Silva O, 1995).** Since the protein is produced by the fetus and the neonate and does not pass the placental barrier, it can be used for the early detection of neonatal sepsis. As

biological half life of CRP is only 24 hours, CRP accurately parallels the activity of the inflammatory process and its concentration decreases much faster than ESR or any acute phase parameter which is useful in providing appropriate treatment.

A level of 10mg/L has consistently been shown to be the most reliable cut off value to indicate sepsis. (Groves A, 2003) CRP remains normal up to 24-48 hours after the onset of signs of infection and is useful in excluding the diagnosis of sepsis in neonates receiving the antibiotic therapy. A very high CRP (>100mg/L) is more likely to occur in bacterial than viral infection, and normal CRP level is unlikely in the presence of bacterial infection. However intermediate CRP levels (10-50mg/L) may be seen in both bacterial and viral conditions **(Reeves G, 2001)**.

Table 1.2. Components of the hematological test and the abnormal values **(Joshi S, 2005)**.

Components	Abnormal value
Total leukocytes count	<5000/mm
I/T ratio	>0.2
Micro-ESR	>15 mm in 1st hour
C-reactive protein(CRP)	>1mg/dl

1.12. Management

1.12. 1. Supportive care

The purpose of supportive care is to normalize the temperature, stabilize the cardiopulmonary status, correct hypoglycemia and prevent bleeding tendency .The septic neonate should be nursed in a thermo neutral environment. If hypothermic, the temperature should be raised using a heat source. An intravenous line should be established. A dextrose bolus will help correct hypoglycemia which is often present in septic infants. Vitamin K should be given to prevent bleeding. Oxygen should be provided if the infant is having retractions, grunt or cyanosis. Apneic neonates should be given physical stimulation and bag-mask ventilation, if required. Enteral feeds are avoided if infant is very sick or has abdominal distension. Appropriate maintenance intravenous fluids are administered. In neonates with sclerema, exchange transfusion

with fresh whole blood may be contemplated. There is no role of intravenous immunoglobulin therapy in neonatal sepsis. **(Dutta A, 2007)**. Following supportive care are taken-

1. Provide warmth, ensure consistently normal temperature
2. Start intravenous line.
3. Infuse normal saline 10 ml/kg over 5-10 minutes, if perfusion is poor as evidenced by capillary refill time (CRT) of more than 3 seconds. Repeat the same dose 1-2 times over the next 30-45 minutes, if perfusion continues to be poor.
4. Infuse glucose (10 percent) 2 ml/kg stat.
5. Inject Vitamin K 1 mg intramuscularly.
6. Start oxygen by hood or mask, if cyanosed or grunting.
7. Provide gentle physical stimulation, if apneic.
8. Provide bag and mask ventilation with oxygen if breathing is inadequate.
9. Avoid enteral feed if very sick, give maintenance fluids intravenously
10. Consider use of dopamine if perfusion is persistently poor.
11. Consider exchange transfusion if there is sclerema.

Antimicrobial therapy

There cannot be a single recommendation for the antibiotic regimen of neonatal sepsis for all settings. The choice of antibiotics depends on the prevailing flora in the given unit and their antimicrobial sensitivity. This protocol does not aim to provide a universal recommendation for all settings but lays down broad guidelines for the providers to make a rational choice of antibiotic combination. Decision to start antibiotics is based upon clinical features and/ or a positive septic screen. However duration of antibiotic therapy is dependent upon the presence of a positive blood culture and meningitis **(Dutta A, 2007)**.

Choice of antibiotics

Empirical antibiotic therapy should be unit-specific and determined by the prevalent spectrum of etiological agents and their antibiotic sensitivity pattern. Antibiotics once started should be modified according to the sensitivity reports. The empirical choice of antibiotics is dependent upon the probable source of infection. For infections that

are likely to be community-acquired where resistant strains are unlikely, a combination of ampicillin or penicillin with gentamicin may be a good choice as a first line therapy. For infections that are acquired during hospital stay, resist t pathogens are likely and a combination of ampicillin or cloxacillin with gentamicin or amikacin may be instituted. In nurseries where this combination is ineffective due to the presence of multiple resistant strains of klebsiella and other gram-negative bacilli, a combination of a third generation cephalosporin (cefotaxime or ceftazidime) with amikacin may be appropriate. 3rd generation cephalosporins have very good CSF penetration and are traditionally thought to have excellent antimicrobial activity against gram negative organisms. Hence they were considered to be a good choice for the treatment of nosocomial infections and meningitis. However, recent reports suggest that at lea t 60-70% of the gram-ve organisms are resistant to them **(Zaidi AK, 2005)**. Moreover, routine use of these antibiotics might increase the risk of infections with ESBL (extended spectrum beta-lactamase) positive organisms. Therefore it is preferable to use antibiotics such as piperacillin-tazobactam or methicillin/vancomycin in units with high incidence of resistant strains. A combination of piperacillin-tazobactam with amikacin should be considered if pseudomonas sepsis is suspected. Penicillin resistant staphylococcus aureus should be treated with cloxacillin, nafcillin or methicillin. Addition of an aminoglycoside is useful in therapy against staphylococcus. Methicillin resistant staphylococcus aureus (MRSA) should be treated with a combination of ciprofloxacin or vancomycin with amikacin. Ciprofloxacin has excellent activity against gram-negative organisms also; however, it does not have good CSF penetration. It may be used for the treatment of resistant gram-negative bacteremia after excluding meningitis. For sepsis due to enterococcus, a combination of ampicillin and gentamicin is a good choice for initial therapy. Vancomycin should be used for the treatment of enterococcus resistant to the first line of therapy **(Dutta A, 2007)**.

Reserve antibiotics

Newer antibiotics like aztreonam, meropenem and imipenem are also nowavailable in the market. Aztreonam has excellent activity against gram-negative organisms while meropenem is effective against most bacterial pathogens except methicillin resistant staphylococcusaureus (MRSA) and enterococcus. Imipenem is generally avoided in neonates because of the reportedincrease in the incidence of seizures

following its use. Empirical use of these antibiotics should beavoided; they should be reserved for situations where sensitivity of the isolated organism warrants its use. **(Dutta A, 2007).**

1.12.3. Adjunctive therapy

Exchange transfusion (ET): Sadana *et al* **(Sadana S, *et al*, 1997)** have evaluated the role of double volume exchange transfusion in septic neonates with sclerema and demonstrated a 50% reduction in sepsis related mortality in the treated group

Intravenous Immunoglobulin (IVIG): Non-specific pooled IVIG has not been found to be useful **(Jenson HB, 1998).**

Granulocyte-Macrophage colony stimulating factor (GM-CSF): This mode of treatment is still experimental **(Goldman S, 1998).**

1.13. Global Epidemiology

In developing countries, neonatal mortality (deaths in the first 28 days of life per 1000 live births) rate is high. Most of these deaths occur in the first week of life, most on the first day **(WHO, 2001)**. In contrast, neonatal mortality for developed countries is in the region of five.

A number of other factors in addition to geographic region influence the rate of neonatal infection. Socioeconomic status, maternal age and sex influence the prevalence of maternal infection.There may be hospital to hospital variability even in the same geographical area and this may be related to environmental condition of neonatal unit, perinatal care, conduction of labour and prematurity.The gastrointestinal tract is the major site of asymptomatic colonization with both group B Streptococcus and E. coli for mother and infant, other being genitourinary tract. Between 40 to 70% of infants where mothers are colonized at delivery becomes colonized with GBS **(Vergnano S, *et al*, 2004)**. The incidence of neonatal sepsis varies according to geographic regions and from one hospital to another and the community in the same geographic area. The incidence varies from 1-4 cases per 1000 live births in developed countries with considerable fluctuation over time and

with geographical location **(Gotoff SP, 2002)**. The incidence in developing countries is as high as 10-15 per 1000 live births **(Haque KH, 1998)**. In the US, the incidence of culture proven sepsis is approximately 2 per 1000 live births **(Bellig LL, 2004)**. The incidence of neonatal sepsis is 9.8 per 1000 live births in South India. **(Kuruvilla KA, 1998)**.

1.14. Age and sex incidence

Early onset neonatal sepsis is clinically apparent within 6 hours of birth in >50% of neonates, the great majority presents within the first 72 hours of life. Males have approximately two fold higher incidence of sepsis than females, suggesting the possibility of a sex linked factor in host susceptibility **(Gotoff SP, 2002)**. In a study conducted in Kanti Children Hospital, showed a preponderance of male septicaemic neonates with the male: female ratio being 1.8:1 **(Shrestha BM, 2000)**.

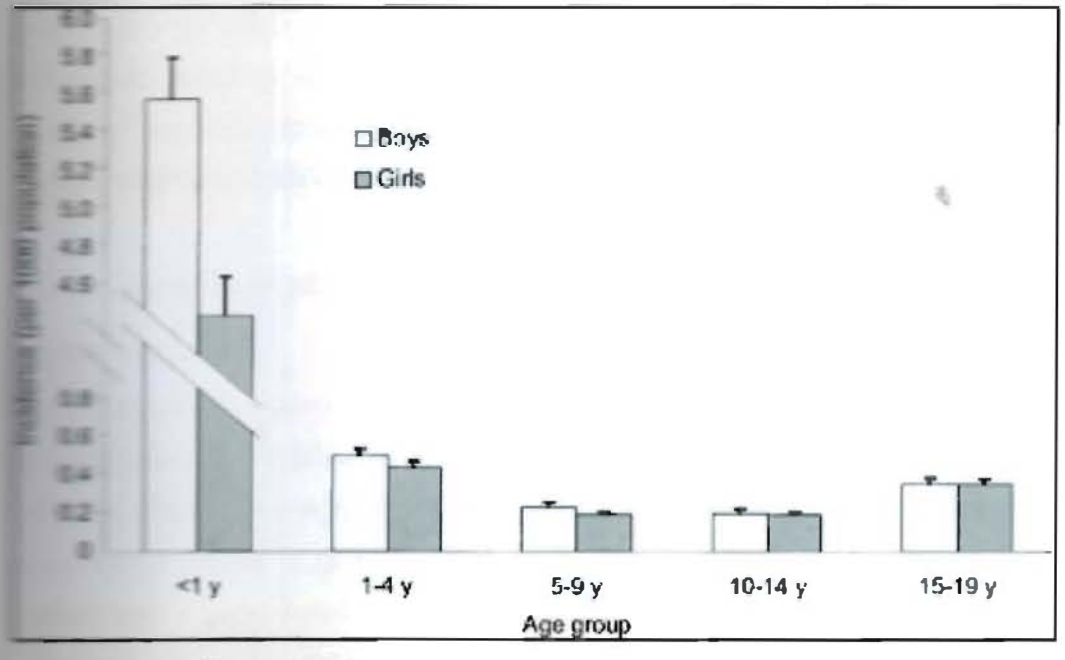

Figure 1.4. Incidence of severe sepsis by age and sex.

The incidence was highest in the youngest patients and decreased until late adolescence (15–19 years old). A total of 48% of all patients were less than 1 year old, and 27% were admitted at birth. The incidence was significantly higher in boys than girls among infants and children aged 1–4 and 5–9 years; 95% confidence intervals are shown by error bars **(Scott R, *et al*, 2002)**.

1.15. Mortality and morbidity

The mortality rate due to neonatal sepsis may be as high as 50% for the infants who are not treated. Infection is a major cause of mortality during the first month of life, contributing to 13-15% of all the neonatal death. Neonatal meningitis is responsible for 4% of neonatal death **(Bellig LL, 2004)**. The infant mortality rate has declined in Nepal in the last few years (1987-2001).The National figure of IMR in the year 1988-1989 was 107 per 1000 **(Janssens R, 1992)**. According to Demographic Health Survey, the neonatal mortality of 49.5 per 1000 live births constitutes 63% of infant mortality rate in Nepal **(Manandhar DS, 2000)**.

1.16. Mortality rate of sepsis in Bangladesh

In Bangladesh, neonatal mortality rate is 42/1000 live births and accounts for two-thirds of the infant mortality. **(UNICEF, 2005)**. Although a 40% reduction of neonatal mortality was achieved over the past two decades, it still remains high compared to the developed countries **(Lawn JE, *et al*, 2005)** It is estimated that 30% of neonatal deaths is contributed by prematurity/ LBW in Bangladesh, the direct causes of mortality being sepsis (32%), asphyxia (26%), tetanus (15%), respiratory distress (6%) while 14% remains unknown. **(Chowdhury EM, 2005)**.

1.17. Significance of hematological tests for confirmation of neonatal septicemia:

The diagnosis of neonatal sepsis is difficult to make solely on historical or clinical ground. Laboratory evaluation is essential in the diagnosis and confirmation of infection. There is no rapid and reliable test for confirmation of diagnosis yet. The treatment for sepsis is generally started when clinical findings are supported by indirect early markers of infection **(Singh M, 1991)**. Positive culture of blood, CSF or urine are the gold standard for confirming sepsis, however in considerable proportion of neonates at risk of infection, culture result may be influenced by previous antibiotic exposure **(Rodrigo I, 2002)**.

The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby. There is an urgent need to know whether the baby has sepsis to institute treatment as early as possible **(Chiesa C, *et al*, 2004)**. No

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The diagnosis of neonatal sepsis is difficult to make solely on historical or clinical grounds. Laboratory evaluation is essential in the diagnosis and confirmation of infection. There is no rapid and reliable test for confirmation of diagnosis yet. The treatment of sepsis is generally started when clinical findings are supported by laboratory markers of infection **(Singh M, 1991)**. Positive culture of blood, CSF or urine are the gold standard for confirming sepsis, however in considerable proportion of neonates at risk of infection, culture result may be influenced by previous antibiotic therapy **(Rodrigo I, 2002)**.

The evaluation of tests for neonatal sepsis is important because the infection may present a very serious threat to the baby. There is an urgent need to know whether the baby has sepsis to institute treatment as early as possible **(Chiesa C, *et al*, 2004)**. No

single laboratory test has been found to have enough specificity and sensitivity and therefore laboratory confirmation must be used in conjunction with risk factors and clinical signs. These tests include culture of blood, urine and cerebrospinal fluid, leukocyte profile, platelet count, acute phase reactants (ESR, C reactive protein), latex agglutination tests, or counter immune electrophoreses, and Polymerase Chain Reaction (PCR) **(Loo S, 2002)** C reactive protein (CRP) synthesis increases within (4-6) hrs, doubling every 8 hrs thereafter, and peaking at 36-50 hrs after the onset of inflammation. CRP level remains elevated with ongoing inflammation and tissue destruction, but with resolution they decline rapidly because of short half life (4 to 7 hrs), so it parallels the degree of injury and repair, thereby supporting its value as an acute measure of disease activity.CRP is found in serum of normal healthy person in very low concentration < 0.02 mg/dl, in most cases not exceeding 6 mg/dl) **(Isaac man D, 2002)**.

There is a wide range of reported sensitivities and specificities for CRP in the detection of neonatal sepsis. In this study CRP was positive in 78% of culture positive cases with sensitivity 78.1%, specificity 68.7%.The study concluded that a positive CRP provide highest sensitivity and a combination of thrombocytopenia with positive CRP provide highest specificity in the diagnosis of neonatal sepsis **(Mahfuza S, *et al*, 2006)**. Thrombocytopenia was seen frequently in sepsis. This could result from increased platelet destruction, sequestration secondary to infections, failure in platelet production due to decreased number of megarkaryocytes or damaging effects of endotoxin on the platelets.

Multiple studies have examined total leucocyte count, immature to total leucocyte ratio, platelet count, and CRP and shown that, these routine investigations either have low sensitivity and specificity or varying delayed responses early in the course of infection. Leukocyte indices and CRP are considered to be late markers of neonatal sepsis. Isolation of bacteria from the central body fluid is the standard and most specific method used to diagnose neonatal sepsis **(Ng PC, *et al*, 1997)**.

The sole use of culture to diagnose neonatal infection has limitations as it may take 24 to 72 hrs to obtain culture reports. In a Study conducted by Kenneth C ***et al***, Twenty-two percent were positive for bacterial growth, yielding gram-negative bacilli (GNB)

and gram-positive cocci (GPC) in almost equal proportion of 25 % of blood culture **(Kenneth C, 2006).**

In the developed world *Coagulase Negative Staphylococcus* CONS is the leading cause of late onset sepsis, while in the developing world, gram negative organisms still predominate.(**Rodrigo I , 2002**). In a etiological study of neonatal sepsis carried out by Anwar et al in Pakistan, in early onset sepsis, gram positive and gram negative organisms were almost equal; while majority of infections were due to gram negative organisms in late onset sepsis (**Anwer S,*et al*, 2000).**

In a study carried out by Kuschel C, common organism causing neonatal sepsis are CONS (28%), *Staphylococcus* (19%), *Streptococcus agalactae* (10%) and *E. coli* (5%) along with others – *Streptococcus pneumoniae, Haemophilus influenzae* and gram negative organisms (20%) **(Kuschel C, 2003).**

Recently, serum procalcitonin (PCT) has been reported as a measurable laboratory marker in inflammatory response to infection in some studies. This factor is the preformed of calcitonin that although is not measurable in serum of normal individuals, but its titer increases during microbial, fungal, and parasitic infections **(Reinhart K, 2000).**

Aims and Objectives

1.18.1. General objective

This study was designed to evaluate the neonatal and maternal clinical manifestations and their hematological parameters, which can be used to formulate a scoring system in predicting the probability of neonatal sepsis.

1.18.2. Specific objective

1. To evaluate neonatal septicemia by performing the blood culture.

2. To determine which of the hematological parameters, namely: the white blood count (WBC) or total leukocyte count (TLC), total neutrophil count (TNC), lymphocytes, immature cells, immature to total neutrophil cells (I/T) ratio, immature to mature cells (I/T) ratio, absolute neutrophil count, nucleated red blood cells (NRBC) and platelet count, are significant in predicting sepsis.

3. To evaluate C reactive protein (CRP) levels for the diagnosis of neonatal infection.

Significance of the study

Infection is one of the major problems in neonates. Neonatal septicemia constitutes an important cause of morbidity and mortality amongst neonates in developing country. The use of safe and effective antimicrobial drugs has significantly contributed to the decrease in neonatal mortality. So rapid identification of the disease is plays a very important role.

Neonatal sepsis, especially in its early stages, may be difficult to diagnose because of its nonspecific clinical symptoms. Although blood culture is the gold standard for the diagnosis of sepsis, culture reports would be available only after 48-72 hours. As the prognosis for sepsis largely depends on early identification and treatment, these neonates are subjected to extensive diagnostic evaluation and empiric treatment. The usefulness of a scoring system based on the clinical manifestations of the neonate and mother supported by their hematological parameters can provide information in determining the probability of sepsis in neonates.

Rapid identification of neonatal sepsis could serve as the basis for a more rational approach to antibiotic use. A significant decrease in the use of antibiotics may prevent the emergence of resistant organisms, decrease the chance of side effects and minimize cost. This study will help to evaluate significant hematological parameters and combine those parameters with clinical manifestations. Thus, the result of the study is expected to improve treatment of neonatal septicemia which ultimately will help to improve the disease management process.

CHAPTER 2

Materials and Methods

2.1 Type of study

It was a prospective type study.

2.2 Place of study

The study was done in the Special care neonatal unit (SCANU) of the ICH and SSF hospital, Mirpur. The SCANU has 25 beds and equipped with incubators, phototherapy units, pulse oximeter, room heater, sucker machines, glucometer, pediatric laryngoscope, oxygen supply system and other common neonatal treatment facilities. But the unit is lacking essential equipments like mechanical ventilator and blood-gas analyzer.

2.3 Study population

A total of 43 cases were taken of the neonates who were suspected of neonatal sepsis.

2.3.1 Inclusion Criteria

i. Neonates admitted in the special care neonatal unit (SCANU)
ii. Neonates treated for suspected sepsis.

2.3.2 Exclusion Criteria

Neonates with extreme prematurity, Respiratory distress syndrome and gross congenital anomalies were excluded.

2.4. Research Approach

After getting the approval of the research proposal from the honorable faculty members, formal permission was obtained from the competent authorities of "Shishu sasthya foundation" (SSF).

2.5 Sampling Technique: In this study, purposive sampling technique was followed

2.6. Study period

Study period was one year commencing from July 2009 to May 2010. To complete the study in time a work schedule was prepared depending on different task of the study. The four months were spent on board meeting for literature review, selection of topic, development of the protocol. Subsequent months spent on official

correspondence, data collection, data analysis, report writing and submission of report.

2.7. Data analysis

All the data were checked after collection. Then data were entered into excel sheet. The results were shown in bar, pie chart and calculated the percentage of the parameters of neonatal sepsis patient

CHAPTER 3

Results

3.1. Percentage of male and female neonates with septicemia

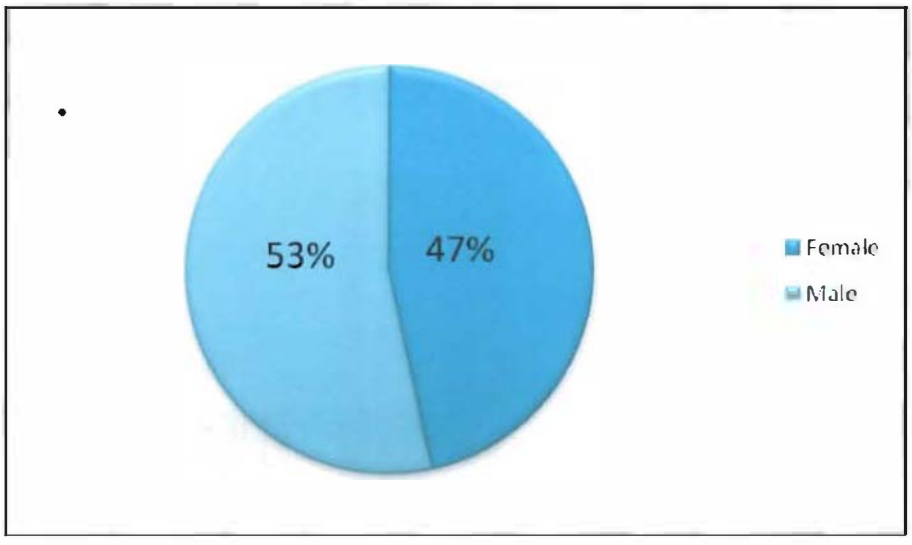

Fig 3.1: Percentage of male and female neonates with septicemia.

The figure shows that among 43 neonates 53% patients are female and 47% patients are male. The ratio of male and female is 1.15: 1.

3.2. Distribution of Age range of mothers having neonate with Septicemia

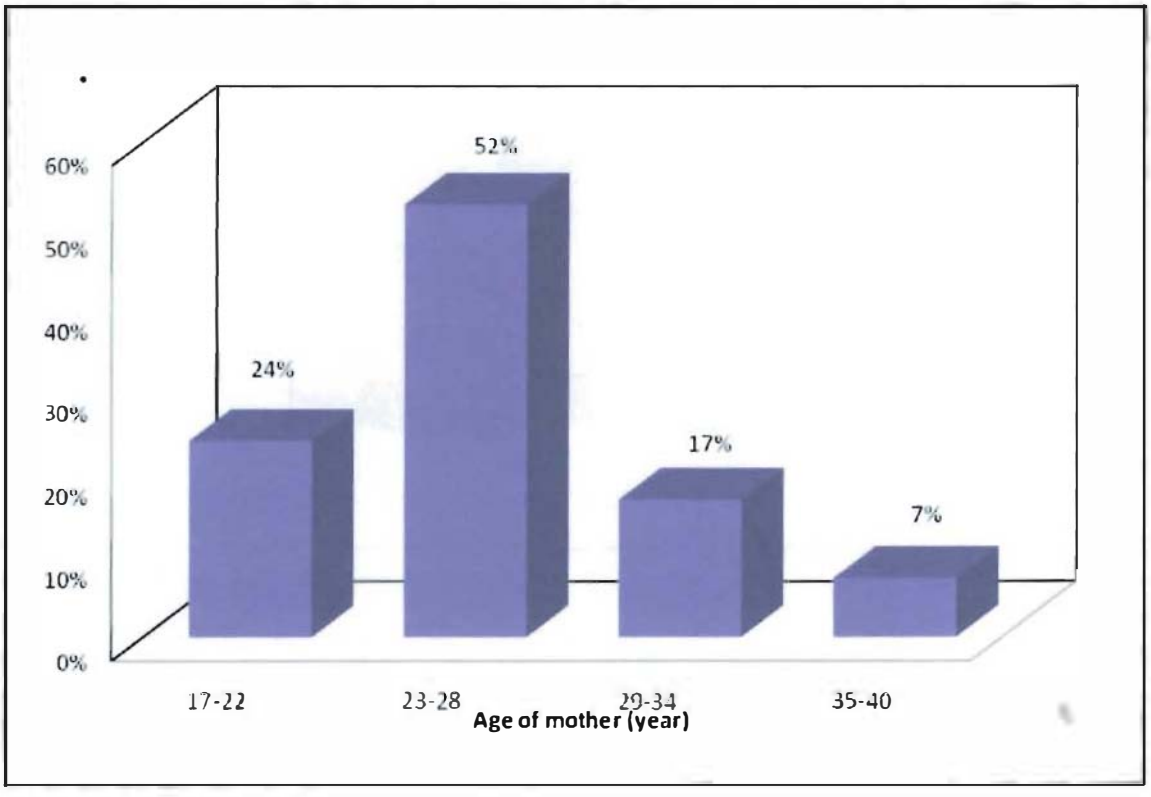

Fig 3.2: Percentage of Age range of mothers having neonate with Septicemia

This figure shows that among 43 mothers 52% have neonates with septicemia and age range of those mothers is 23-28 years.

3.3. Percentage of Blood culture test in septicemia patients

ıia patients.

Fig 3.3: Perc

This figure shows tha ıeucopenia and 74% have no
negative results in blc
were not performed.

3.4. Percentage of Leucopenia in septicemia patients

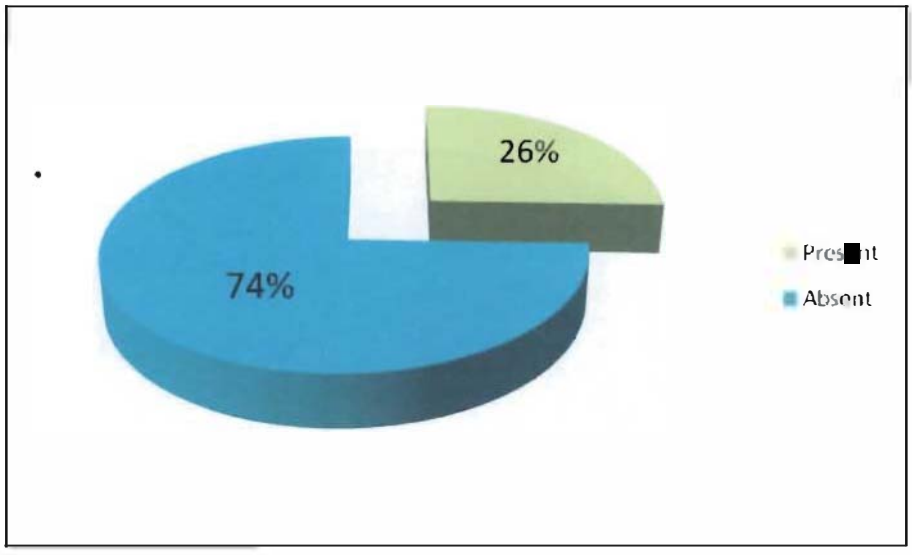

Fig 3.4: Percentage of Leucopenia among septicemia patients.

This figure shows that among 43 patients 26% have leucopenia and 74% have no leucopenia.

3.5. Percentage of Leucocytosis in septicemia patients

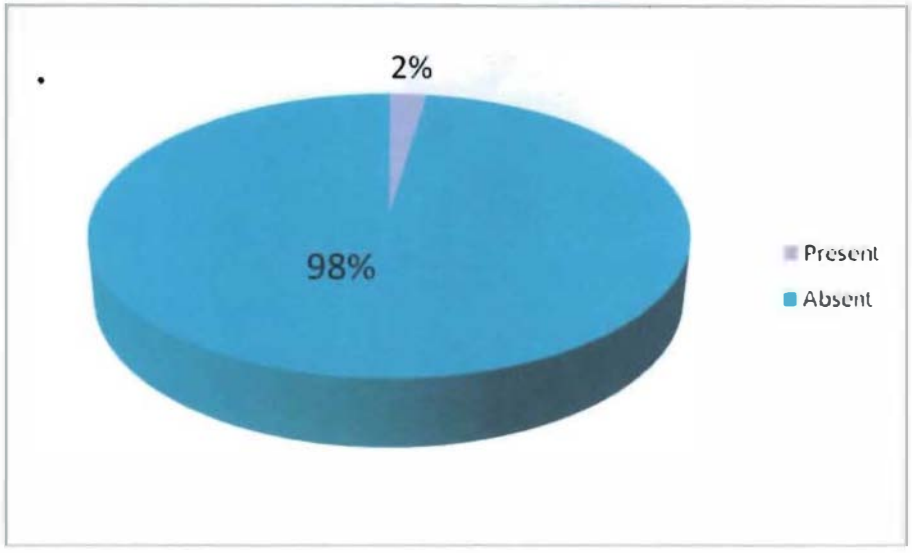

Fig 3.5. Percentage of Leukocytosis in patients with septicemia.

Th figure shows that among 43 patients 2% patients have leukocytosis and 98% have no leukocytosis.

.6. Percentage of Neutropenia in septicemia patients.

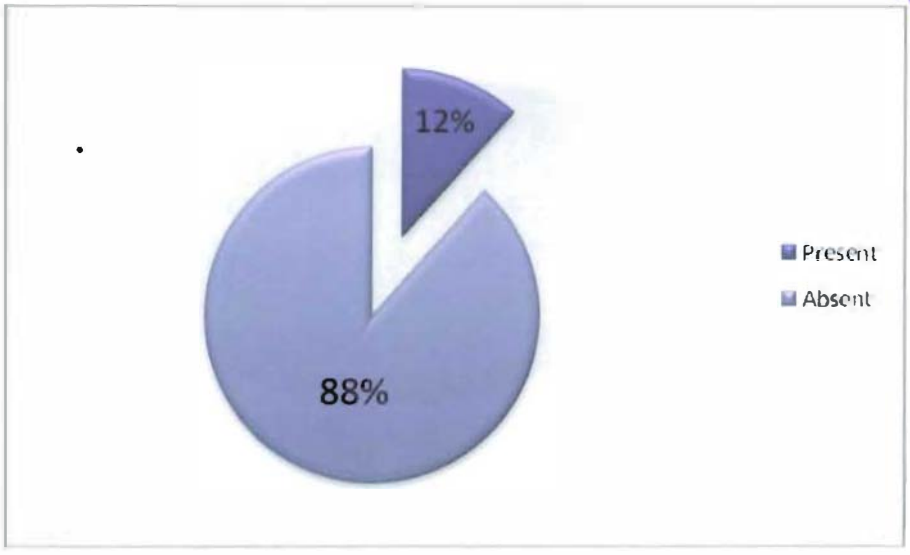

Fig 3.6: Percentage of neutropenic patients with septicemia.

The figure shows that among 43 patients 12% patients are neutropenic.

3.7. Neutrophilia in septicemia patients

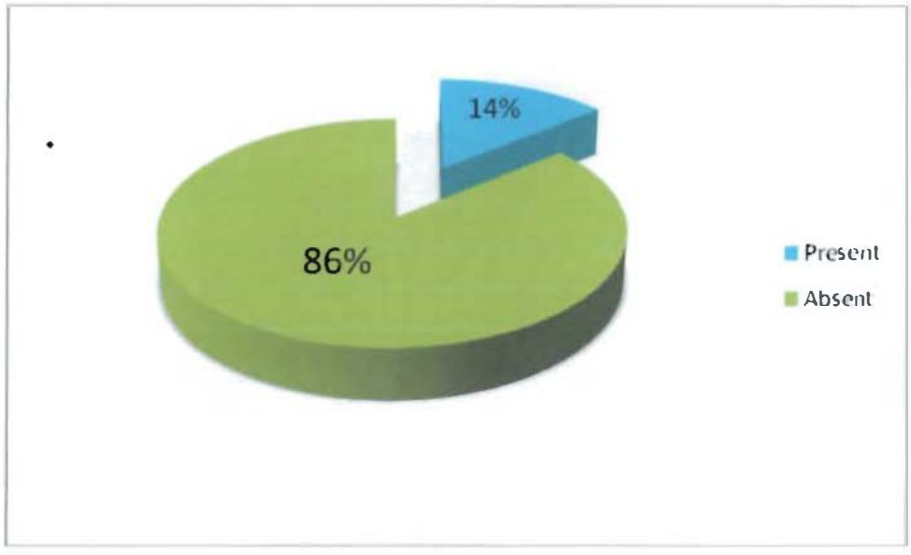

Fig 3.7: Percentage of septicemia patients having neutrophilia

The figure shows that 14% patients have neutrophilia and 86% have no neutrophilia.

. Percentage of Thrombocytopenia in septicemia patients.

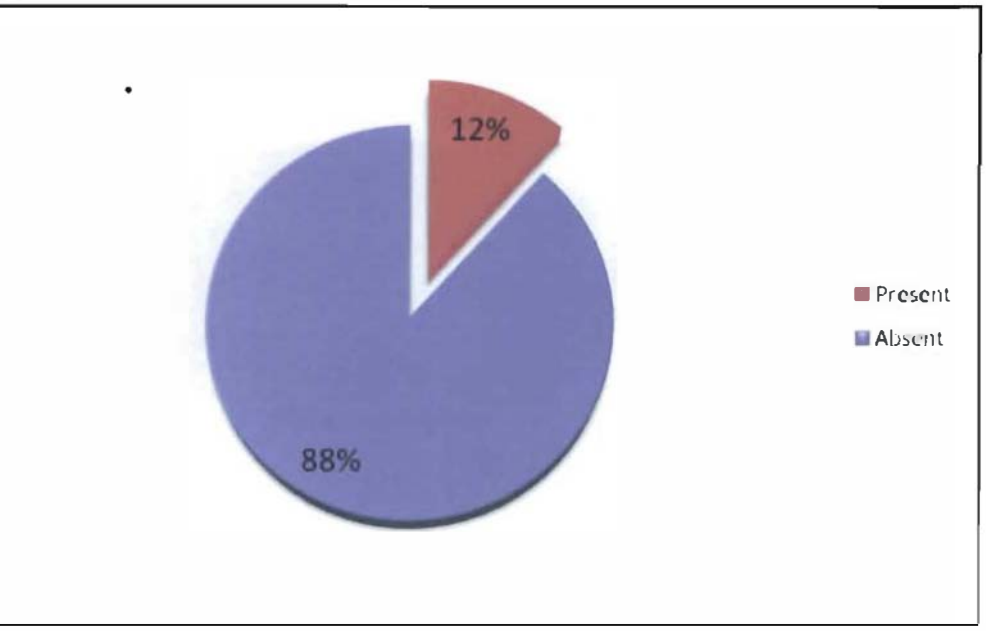

Fig 3.8: Percentage of septicemia patients having thrombocytopenia

figure shows that among 43 patients 12% have thrombocytopenia and 88% have thrombocytopenia.

3.9. Percentage of R.B.C count in septicemia patients

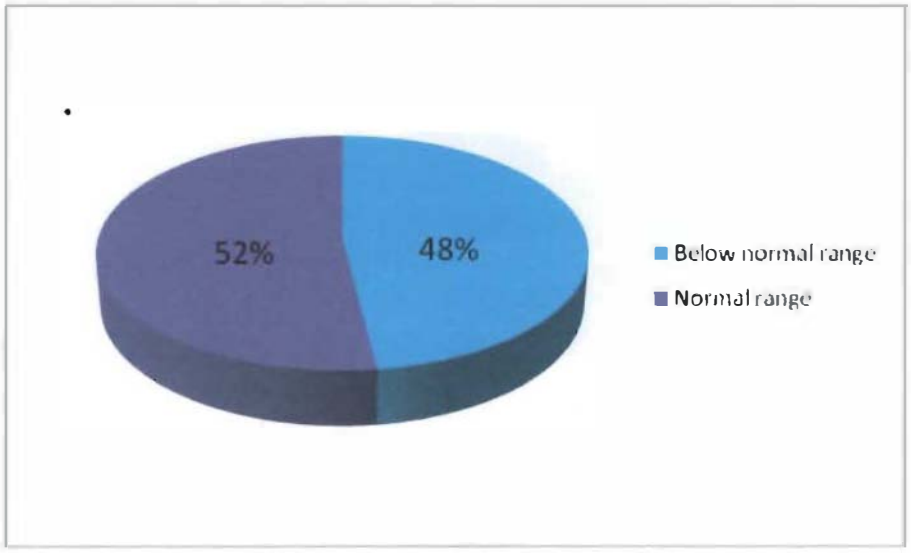

Fig 3.9: Percentage of R.B.C count in septicemia patients

The figure shows that among 43 patients 48% have R.B.C below the standard range.

3.10. Percentage of septicemia patients giving CRP test.

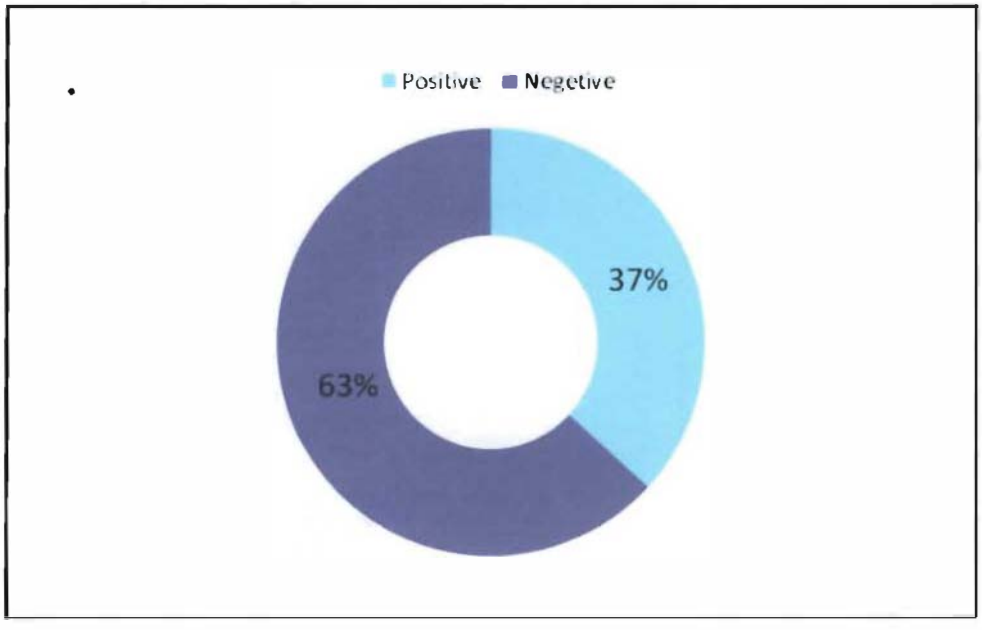

Fig 3.10: Percentage of septicemia patients giving CRP test.

The figure shows that among 19 patients 37 % have positive and 63% patients have negative result in CRP test. In case of the rest 24 patients, CRP test was not performed.

CHAPTER 4

Discussion and Conclusion

This was a prospective study on neonatal septicemia where data were collected over a period of one year. Using data from this study we demonstrated various hematological parameters and blood culture to find out the significant parameter which can be useful for predicting the probability of neonatal septicemia.

Of the 43 neonates included in the present study, 23 (53%) cases were male and 20 (43%) were female. The ratio of male: female was 1.15: 1 which is lower than the study conducted in TUTH with male: female ratio being 1.8:1 **(Shrestha BM, 2000).** Sholl B J and Kliegman R.M have reported approximately two fold higher incidence of sepsis in male than female suggesting the possibility of a sex linked factor in host susceptibility **(Gotoff SP, 2002).** The almost equal male: female ratio in our study may be because of less number of cases.

In the study about 9% cases with neonatal septicemia had positive blood culture. A review showed that, positive culture ranged from 8% to 73% in the diagnosis of potential sepsis **(Chiesa C, *et al*, 2004).** Our low blood culture positivity rate may be due to administration of antibiotic to the mother immediately before birth of the baby. Moreover negative blood cultures do not always exclude sepsis. Cases with negative blood culture have been reported with fatal illness and postmortem evidence of infection. A high rate (56%) of positive blood culture was reported by Sharma et al, **(Sharma PP, 1987)** which is much higher than our study.

When bacteria are isolated from blood culture then it is categorized as proven sepsis and probable sepsis is identified by radiological and laboratory tests and at least three clinical features. In our study there were only 3 cases of proven sepsis and rest of the cases were probable sepsis which is very lower than other experiments.

The normal range of total leukocute leukocyte count for neonate are 10000-26000 /cumm(at birth),7000-23000/cumm (Day 3), 5000-19000/cumm (1 month). Normal leukocyte range for calculating neutropenia or neutrophilia are 4000-14000 /cumm (at birth), 3000-5000/cumm (Day 3), 3000-9000/cumm (1 month). We used the guideline of (ICH) Institute of Child Health for calculating all parameters.

Total leucocyte count was abnormal in 28% cases. Leucocytosis was seen in 2 % of cases while leucopenia was seen in 26% cases. Squire et al also observed that 5% cases had leucocytosis and 27% had leucopenia **(Emerson WA, 1970)**. This confirms the view that total leucocyte count by itself has little diagnostic utility. The diagnostic and prognostic values of leucopenia when present however cannot be under-estimated. Philip et al found it specific indicator of sepsis with a specificity of 94% **(Christeinsen RD, 1981)**.

Neutropenia appears to be highly specific indicator of sepsis **(Carl GE, *et al*, 1984)**. The diagnostic value and significance of neutropenia has been attested to by several workers. But in our study neutropenia was present in 12% of population and neutrophilia was observed in 14% of cases. It has been observed that neutrophilia does occur in bacterial infection in newborn but it usually occurs later in the course of illnss and hence is not a reliable early indicator of sepsis **(Arthem G, et al 1985)**.

RBC count was observed and found that 48% patients have R.B.C count below the standard range. Thrombocytopenia was present only in 12% of cases which showed less significance in predicting the disease.

In our study CRP test was positive in 37% cases. Benitz et al showed strong correlation of positive CRP measurement with both early and late onset sepsis. Our result is lower than the other studies **(Benitz et al 1998)**. It could be due to less number of cases (12) in our study compared to large study population. This error may be corrected by increasing the number of cases. Stephan S et al indicated that serial CRP levels are a useful marker for guiding duration of antibiotic therapy in suspected neonatal infection **(Stephan S, *et al*, 1997)**.

As the study had to be completed in a limited time, the sample size was small. The results of some parameters were lower than those obtained from other experiments. By increasing the numbers of cases this error may be minimized.CRP and blood culture were not measured in all the cases of the study. These were the limitations of the study.

Conclusion

Neonatal sepsis, especially in its early stages, may be difficult to diagnose because of its nonspecific clinical symptoms. Because the prognosis for sepsis largely depends on early identification and treatment, these neonates are subjected to extensive diagnostic evaluation and empiric treatment. The investigations based on the clinical manifestations of the neonate and mother supported by their hematological parameters can provide information in determining the probability of sepsis in neonates.

In this study, significant hematological parameters of neonatal septicemia were studied and compared with other investigations. The outcome of this study may provide important information for future in depth study as well as may help to combine different hematological parameters in predicting the probability of neonatal sepsis by clinical tests. It is recommended that more subjects will be included in future studies wherein there will be a control group composed of healthy, asymptomatic neonates and a test group composed of neonates with probable sepsis or proven sepsis.

CHAPTER 5

References

Ang AT, Honk A, Chia SE. (1990). *The usefulness of CRP and I/T ratio in early diagnosis of Infections in Asian newborns.* In: Journal of.Singapore Paediatric Society , 32(3-4): 159-63

Ann L, Anderson B.(2010). *Neonatal sepsis.* Retrieved from the web on 2nd April. http://emedicine.medscape.com/article/978352-overview

Anwer S, Mustafa S, Pariyani S, Ashraf S, Taufiq K .(2000) . *Neonatal sepsis: an etiological study*, Journal of Pakistan Medical Association , 50 (3): 91-4

Arthem G, Charles (1985). *Neonatal blood cell count in health and disease* , 106: 462.

Basavaraj M, Kerur B, Vishnu B, Harish S, Habeebullahand C, Kumar U.(2006). Indian Journal of Pediatrics, 73

Basu K, Das PK, Bhattacharya R, Bhowmik PK. (2002). *A new look on neonatal jaundice*. JIMA, 100 (8)

Bellig LL, 2004. *Neonatal sepsis.* Retrieved on 2nd April from- http://www.emedicine.com, Inc.

Benitiz WE, Han M Y, Madan A, Ramachandra P (1998). *Serial Serum C-reactive protein levels in the diagnosis of neonatal infection*,102 (4)

Berger C, Uehlinger J, Jhelfi D, Blau N, Fanconi S. (1995). *Comparison of C-reactive protein and white blood cell count with differential in neonates at risk for septicaemia.* Europian Journal of Pediatrics , 154 (2):138-144.

Carl GE, Athen JW, Wintrobe MM. (1984). *The reverse granulopoiesis in normal man* , 24: 780

Chiesa C, Panero A, Osborn JF, Simonetti AF, and Pacifico L. (2004) *Diagnosis of neonatal sepsis: A clinical and laboratory challenge.* Clinical chemistry, 50: 279-287

Christeinsen RD, Bradey PP, Rothenstein G.(1981). The leucocyte left shift in clinical and experimental neonatal sepsis. J Pediatr , 98: 101.

Costello A, Francis V, Byrne A, 2001.*The state of the world's newborns.* Acta Paediatr, 82:687–9.

Chowdhury EM, Akter HH, Chongsuvivatwong V and Geater FA .(2005) . *Neonatal mortality in rural Bangladesh: An exploratory study.* Journal of Health Population; 23(1): 16-24.

Chowdhury M. (2005). *Neonatal mortality in Bangladesh* . Retrieved from the web on

2nd April.
http://centre.icddrb.org/pub/publication.jsp?classificationID=30&pubID=5986

Da SO, Oblsson A, Kenyon C. (1995) *Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review.* Pediatr Infect Dis J. 14(5): 362-6

Dawodu A, Al Umran K, Danso K (2002). *A case study of neonatal sepsis in very low birth weight infants.* N Engl. J. Med. 347: 240-247

Dear P .(1999). *Infection in the newborn.* In: Rennie JM, Roberton NRC (eds) *Textbook of Neonatology.* Churchill livingstone, (3) 1109-1144

Dutta A K. (2007). *Diagnosis and management of Neonatal sepsis.* Advanced in Pediatric.Jaypee Brothers Medical publishers Delhhi ,76-77

Emerson WA, Zieve PD & Krevan JR. (1970) Hematological changes in identifying in septicemia. John Hosp Med J 1970, 126: 69.

Freij BJ, Mc Cracken GH. (1994). *Acute infections.* Avery GB, Fletcher MA, MacDonald MG (eds). *Neonatology: pathophysiology and management of the newborn,* 4th edition. Philadelphia : J. B Lippincott company:1082-1108

Goldman S, Ellis R, Dhar V, Cairo MS. (1998). *Rationale and potential use of cytokines in the prevention and treatment of neonatal sepsis.* Clin Perinatol 25:699-710

Gotoff SP. (2002).Infections *of the neonatalinfant. In*: Behram RE, Kliegman, Nelson Textbook of pediatrics 16th ed. Philladelphia: WB Saunders company ; 538-52

Guerina NG. (1998). *Bacterial and fungal infections.*In: Cloherty JP and Stark AR. (eds).Manual of neonatal care.4th edition; Philadelphia : Lippincott Williams and Wilkins ; 271-276.

Greenough A, Roberton NRC,(1998). Acute respiratory disease in the Newborn. In: Rennie JM, Roberton NRC (eds). Textbook of Neonatalogy. 3rd e. Churchill Livingstone ; 481-495.

Groves A. (2003) *C - reactive protein in neonatal sepsis.* Newborn Services Clinical guidelines Roberton NRC (eds). *Textbook of Neonatalogy.* 3rd e. Churchill Livingstone ; 481-495

Haque KH . (1998). *Infection and immunity in the Newborn.* In: Campbell AG and McIntosh N (eds). Forar and Arneils's Text Book of Paediatrics. Fifth edition; Churchill Livingstone ; 273-289

Hengst JM. (2003). C-*reactive proteins in infants with suspected sepsis.* In: Advances

in Neonatal Care.: W. B. Saunders. 3 (1): 3-13.

Hilliard NJ, Waites KB. (2002) . *C-reactive protein and ESR: What can one test tell you that the other can't?* (CRP and ESR) Contemporary pediatrics.

Hotchkiss RS, Karl IE 2003. Medical progress: *The pathophysiology and treatment of sepsis*. NEJM ; 348(2):137-150.

Isaac man D, 2002. Burke B. *Utility of serum C-reactive protein for detection of occult bacterial infection in children.* Archives of Pediatrics and Adolescent Medicine , 156: 903-909.

Janssens R. (1992). *Infant Mortality.* In: Presernc C (ed).Children and women of Nepal, A situation analysis .National planning commission, HMG, UNICEF M Nepal; 6-62

Jaswal R S, Kaushal R K, Goel A, Pathania K. 2003. *Role of C-reactive protein in deciding duration of antibiotic therapy in neonatal septicemia.* Indian Pediatrics, 40: 800-883.

Jenson HB, Pollock HB. (1998*). The role of intravenous immunoglobulin for the prevention and treatment of neonatal sepsis*. Semin Perinatol, 1998; 22:50-63

Jiang JH, Chiu NC, Huang FY, Kao HA, Hsu CH, Hung HY, Chang JH, Peng CC.(2004). Department *of Pediatrics*, Mackay Memorial Hospital, Taipei, Taiwan, ROC.

Joshi S .(2005). *Serial Serum C-Reactive Protein levels in the Diagnosis of Neonatal infection.* Retrived from the web www.healthnet.org.np/gsdl/collect/thesis/index/assoc/...dir/doc.pdf

Loo S. (2002). *Neonatal Sepsis*. Hawaii Journal of Pediatrics; 10 (3): 49-55.

Kaiser JR, Cassat JE, Lewno MJ. *Sensitivity of 48 hour cultures*. In: Kairalla AB (eds): *Neonotes Journal Club*. Journal of perinatology; 22(6): 445-447

Karthikeyan G, Premkumar K, (2001). *Neonatal sepsis: Staphylococcus aureus as the predominant pathogen.* Indian journal of pediatrics, 68(8):715-7

Kenneth C Iregbu, Olufumilayo Y Elegba, and Iretiola B Babaniyi, (2006). *Bacteriological profile of neonatal septicaemia in a tertiary hospital in Nigeria.* Retrieved on 2nd April from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1831882/

Kuruvilla KA, Pillai S, Jesudason M, Jana AK. (1998). *Bacterial profile of sepsis in a neonatal unit in South India*. In: Indian Pediatrics; 35: 851-858.

Kuschel C. (2003).*Antibiotics for neonatal sepsis*. In: Newborn services Clinical guidelines.

Lawn JE, Cousens SN, Wilczynska K, 2005. *Estimating the causes of four million neonatal deaths in the year : statistical annex*. In: The world health report. Geneva: WHO; 2005.

Mahfuza S, Chowdhury A. (2006). *Sensitivity and Specificity of C-Reactive Protein (CRP) and Thrombocytopenia in the Diagnosis of Neonatal Sepsis.* In. Bangladesh J child health , 29 (2): 41-45.

Manandhar DS,(2000). *Practical strategies for improving perinatal health in Nepal.* In: Preceding of the 20th All Nepal Medical Conference.

Manroe BL, Wienberg AG, Rosenfield CR. (2002). *Differential leucocyte count in the assessment and outcome of early onset of neonatal B streptococcal disease.* Journal of Pediatrics , 91: 632.

Manucha V, Rusia U, Sikka M, Faridi MMA, Madan N. (2002). *Utility of haematological parameters and c-reactive protein in the detection of neonatal of sepsis* In: Journal of paediatrics and child health ; 38(5): 459-64

Marik PE, (2002). *Definition of sepsis: not quite time to dump SIRS?* Crit care med, 30 (3): 706-708.

Motara F, Ballot DE, Perovic O. (2005). *Epidemiology of neonatal sepsis*. Southern Afr. J. Epidemiol. Infect. 20: 90-93.

Merck M. (2000). *Neonatal Sepsis: Infections in Neonates.* Retrieved from the web: http://ajrccm.atsjournals.org/cgi/content/full/167/5/695

Jeeva M ,Agarwal R , Ashok K,Paul R. (2008). *sepsis in new born.* Retrieved from the Web: http://www.newbornwhocc.org/pdf/sepsis_innewborn.pdf

Ng PC, Cheng S.(1997). *Diagnosis of late onset neonatal sepsis with cytokine adhesion molecule*; 77:221-27.

Permanente K, 2003.*Sepsis in Newborn Infants*. In: Proceedings of the 19th Annual *Neonatal – Perinatal Symposium*, June 19, Anaheim, California. Pediatrics, 49(22)

Pepys MB, Hirschfield GM. (2003) . *C- reactive protein: a critical update*. J.clin. Invest. 111(12): 1805-1812

Reinhart K, Karzai W, Meisner M , 2000. *Procalcitonin as a marker of the systemic inflammatory response to infection.* Intensive Care Med ; 26(9):1193-200.

Riedemann N.C, GUORF, Ward PA.2003. The enigma of sepsis. J. Clin. Invest;

2003; 112 (4): 460-467.

Rodrigo I. (2002) .*Changing patterns of neonatal sepsis.* SriLanka journal of child Healths 2002: 3:3-8

Rubin LG, Sanchez PJ, Siegel J, Levine G, saiman L, Jarvis WR,2002.*Evaluation and treatment of neonates with suspected late onset sepsis:* A survey of neonatologists practices. Pediatric 2002; 110(4): 42

Sadana S, Mathur NB, Thakur A.(1997). *Exchange transfusion in septic neonates with sclerema*:. Indian Pediatr, 34:20-5

R. Scott Watson, Joseph A. Carcillo, Walter T. Linde-Zwirble, Gilles Clermont, Jeffrey Lidicker and Derek C. Angus. (2002).*The Epidemiology of Severe Sepsis in Children in the United States.* Retrieved from the web. http://ajrccm.atsjournals.org/cgi/content/full/167/5/695

Sharma PP, Halder D, Dutt AK. (1987). *Bacteriological profile of neonatal septicemia.*Indian Pediatric; 24:1011-17

Shrestha BM. (2000). *Prevalence of meningitis among neonates with septicaemia.* Thesis submitted for Doctor of Medicine, Pediatrics, TUTH :65

Siegel JD. (1994). *Bacterial and viral infections of the newborn.* In: Oski FA DeAngelis CD, Feigin RD, McMillan JA. (2001) Warshaw JB(eds). *Principles and practice of pediatrics*, 2nd ed; JB Lipppincott company. : 516-525

Singh M. (1991). *Perinatal Infections* In: Care of the Newborn. 4th ed; New Delhi: Sagar publication ; 168-171

Stephan E, Gering B, Bartmann P, Hogel J, Pohlandt F.(1997) . *C-reactive protein is a useful marker for guiding duration of antibiotic therapy in suspected neonatal infection.* Pediatrics, 99(2): 216-221

Tankhiwale S S, Jagtap PM, Khadse RK, Jalgaonkar SV. (2001).*Bacteriological study of pyogenic meningitis with special reference to C-reactive protein.* Indian Journal of Medical Microbiology, 19(3): 159-160

UNICEF. *The State of the World's Children. (2005).* New York: UNICEF 2005.

Vergnano S, Sharland M, Kazembe P, Mwansambo C. (2004). *Neonatal sepsis: an international perspective.* Retrived from the web on May 12. http://fn.bmj.com/content/90/3/F220.full

World health report (2005): *Make every mother and child count* .Geneva: WHO; 2005

aidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA.(2005). Hospital-acquired.neonatal infections in developing countries. Lancet ;365:1175-88.

Zeeshan A; Ghafoor T; Waqar T; Ali Salman; Aziz S; Mahmud .(2005). *Diagnostic value of C- reactive protein and haematological parameters in neonatal sepsis.*Retrived frpm the web on 7 the May. http://www.researchprofiles.collexis.com/jhu/pubDetail.asp?t=pm&id=15808093&n=AZIZ%2C+SHAHID&u_id=3587

Zipursky A, Jabar H, (1978). *Hematology of bacterial infection in newborn infants.* Clin Hematol, 7: 175.

ANNEXURE

Hematological Parameters in Neonatal sepsis

ıpital ID:……Patient ID:……..Date:……Admission e:……

ne of the ient:……..Age:……Sex:……M/F

her's name:……..Mother's name: ……Age of mother:…..Parity:…

ıress: Mobile number:

ıenatal problems: Fever/Rash/Drug reaction/Others(specify)……

de of delivery: Normal/LUCS **Place of delivery**: Home/Hospital

th weight:…….. kg **Rupture of membrane**:……hours before birth

io economic status:…… Maturity: < 37 week / 37 week / > 37 week

Premature(days)/ Mature /post mature(days)

lostrums feeding: Y/N **Ex. Breast feeding**: Y/N **EDT**:…….. ..**DOB**:……..

ıgnosis of sepsis:

-Fever:……Y/N

-Reluctant to feed:…… Y/N

-Lethargy:…….. Y/N

-Abd. Distension:……...Y/N

-Vomiting:……...Y/N

-Others:

ceipt No:…… **Ref No**:……

ematological Parameters:

- **Blood culture**: +ve (Organism:……..) /-ve **Blood group**:…….
- **Blood picture**: a. Hb%…… b. CRP…… ESR/MicroESR……
- **Total count of RBC** :……..
- **Total count of WBC**:……..
- **Dif. Count of WBC**: P…… S…… L……..M……E……B…….
- **Platelet count**:……..
- **I/T ratio**:……..
- **Antibiotic**: Y/N
- **Outcome**: Improved/ Referred / Death