B.L.D.E (DEEMED TO BE UNIVERSITY)

SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA, KARNATAKA

TO STUDY THE SIGNIFICANCE OF miRNA 23 b AS A MARKER FOR NEONATAL SEPSIS.

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LIST OF ABBREVIATIONS USED

EOS- Early Onset Sepsis

LOS- Late Onset Sepsis

IL- Interleukins

TLR- Toll Like Receptors

SNP- Single Nucleotide Polymorphism

IAP- Intrapartum Antibiotic Prophylaxis

DIC- Disseminated Intravascular Coagulation

SIRS- Systemic Inflammatory Response Syndrome

PROM- Premature Rupture of Membranes

AMR- Antimicrobial Resistance

GBS- Group B Streptococci

ELBW- Extremely low birth weight

VLBW- Very low birth weight

NICU- Neonatal Intensive Care Unit

E-coli- Escherichia coli

CONS- Coagulase Negative Staphylococci

CMV- Cytomegalovirus

HSV- Herpes Simplex Virus

MDR- Multi Drug Resistant

NEC- Necrotizing Enterocolitis

DIC- Disseminated Intravascular Coagulation

WBC- White Blood Cells

I/T- Immature/ Total Neutrophil ratio

CRP- C Reactive Protein

PCT- Procalcitonin

LP- Lumbar Puncture

CSF- Cerebro Spinal Fluid

IVH- Intraventricular Hemorrhage

PVL- Periventricular Leukomalacia

RDS- Respiratory Distress Syndrome

HIE- Hypoxic Ischaemic Encephalopathy

CPAP- Continuous Positive Airway Pressure

HFOV- High Frequency Oscillatory Ventilation

HFNC- High Flow Nasal Cannulation

IEM- Inborn Errors of Metabolism

PN- Parenteral Nutrition

VRE- Vancomycin resistant Enterococci

MRSA- Methicillin resistant Staphylococcus aureus

TNF- Tumor Necrosis Factor

NF- kB- Nuclear Factor kappa B

rt-PCR - reverse transcriptase Polymerase Chain Reaction

RNA- Ribonucleic acid

DNA- Deoxyribonucleic acid

ddCt- delta delta Ct method

NICHD- National Institute of Child Health and Human Development

CRT- Capillary Refill Time

AF- Anterior Fontanelle

ESBL- Extended Spectrum beta-Lactamase

DAMP- Damage associated molecular pattern

PAMP- Pathogen associated molecular pattern

INTRODUCTION

A systemic inflammatory response to infection in newborns, especially those born within the first 28 days of life, is known as neonatal sepsis, a potentially fatal clinical disease. It is a major cause of morbidity and mortality worldwide, particularly in areas with little resources, where healthcare systems struggle to detect it early and treat patients effectively[1]. Neonatal sepsis is divided into two categories: early onset sepsis (EOS) and late onset sepsis (LOS).

Due to their young immune systems' limited capacity to fight infections, newborns are especially vulnerable. Prematurity, low birth weight, prolonged membrane rupture, maternal illnesses, and invasive treatments in the neonatal intensive care unit (NICU) are risk factors for neonatal sepsis.

Neonatal sepsis prevalence varies greatly throughout the world, with lower and middle-income nations reporting greater rates because of inadequate prenatal care and restricted access to medical interventions[2].

Sepsis is still a difficult problem despite improvements in neonatal care because of delayed diagnosis, antibiotic resistance, and the high prevalence of sequelae and long-term impairment such as neurodevelopment delays[3]. With an emphasis on its epidemiology, classification, pathophysiology, clinical presentation, diagnostic techniques, therapy, and preventative strategies, this paper attempts to offer a thorough overview on neonatal sepsis and effective management and prompt treatment.

NEED FOR THE STUDY

Neonatal sepsis refers to a serious condition that occurs in the first month of a baby's life. It is a major cause of morbidity and mortality globally, particularly in developing countries. Despite advancements in medical technology and treatments, sepsis remains a significant health concern for newborns.

The condition can be challenging to diagnose as the symptoms are often non-specific and can be mistaken for other conditions. Early recognition and prompt treatment are crucial to improve the chances of survival for infants with sepsis.

It affects the quality of life of the neonatal period. It is divided into two types according to onset time: early-onset sepsis (EOS), which is the first 72 hours of life, then late onset sepsis, it develops after 72 hours. Infant and late fetal deaths are key factors when assessing a country's level of social protection. The treatment of neonatal sepsis is mostly empirical antibiotics, it usually targets mostly the infecting organisms and not the cytokines or the inflammatory markers. Any ideal approach should be antimicrobial and anti-inflammatory. Therefore, identifying the inflammatory cascade and the resulting "cytokine storm" in neonatal sepsis is crucial.

Most common organisms in neonatal sepsis are bacterias, fungal or viruses. Diagnostic tool for sepsis purely relies on clinical workup, which has its own limitations where its purely observational and prone to bias. Blood cultures are the "gold standard" to identify bacterial infections in the bloodstream. However, it is a cumbersome process which is prone to multiple errors as they are limited to large sample collection, contamination and false negative values. An empirical antibiotic usage is the most common approach in the neonatal sepsis especially in our clinical practice. In suspected bacterial infection, a concoction of multiple antibiotics is often used which is absolutely unnecessary and prolongs the treatment in newborns. Increasing the emergence of multi resistant strains, but delaying and stopping

antibiotic use in these septicaemia newborns will also be catastrophic as it would lead to disease progression. In addition, caring for newborns in specific hospital departments takes a toll on human and financial resources.

MicroRNAs (miRNAs), class of small single-stranded non-coding regulatory RNAs of about 19 to 22 nucleotides, are involved in a wide range of biological processes which have opened a whole new window of hope to the diagnosis, and even treat, innumerable diseases. miRNA binds to specific mRNA molecules to inhibit the expression of target genes or to degrade mRNA, which then contributes to cell proliferation, differentiation, development, metabolism, apoptosis and other physiological activities. The major aim of this topic is to minimise the door to diagnosis time of sepsis and early detection and accurate treatment of neonates.

AIMS AND OBJECTIVES OF THE STUDY

- To determine the efficacy of using haemoculture broth to evaluate microRNA.
- To study the significance of miRNA 23 b as a marker for neonatal sepsis.

REVIEW OF LITERATURE

Sepsis still remains one of the prevalent acute bacterial infections among infants and neonates. There is a high cumulative incidence of sepsis, which can often be the first sign of severe morbidity, during the childhood years especially among neonates. It has been observed that sepsis is incidentally diagnosed in 25- 40% of blood culture positivity.

Early detection of sepsis by clinical parameters, culture or microscopic processes is critical because failure to diagnose sepsis especially in young populations can have serious and rapid complications. Rapid diagnostic tests therefore are imperative which are inexpensive, rapid and non-culture based approaches, can be a sensitive method for quick and accurate diagnosis.

The IL10 (rs1800896) G allele is linked to higher IL-10 release, which may be a risk factor for septic shock in pneumococcal infection, according to a study on septic shock by Schaaf et al., whereas the IL10 (rs1800872) A allele is linked to lower stimulated IL-10 release and higher mortality. Gram-positive bacteria were responsible for most late-onset infections, whereas Gram-negative bacteria remained the most common pathogens linked to early-onset sepsis, according to the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network. They discovered that TLR2 rs3804099 was linked to Gram-positive infections, while one IL10 polymorphism (rs1800896) was linked to Gram-negative infections [4].

Another TLR2 SNP, TLR2 T-16933A (rs4696480), which is located at position 5 of the TLR2 gene, was investigated in a cohort of sepsis patients in a related study by Sutherland et al. They discovered a correlation between the A allele with the emergence of sepsis and Gram-positive cultures. Research on the TLR2 Arg753Gln amino acid variation (rs5743708) indicates that it may make people more susceptible to specific Gram-positive infections. There is no information on the functional significance of TLR2 (rs3804099). Overall, this suggests that genetic variation in

TLR2 may affect sepsis risks; however, further research is required to elucidate this possible relationship in order to ascertain whether rs3804099 or another SNP associated with it is causal.[5].

In one study, four potential miRNAs miR-146a, miR-223, miR-155, and miR-132 were examined for expression levels in one study. Because miRNAs originating from cells similar to monocytes and endothelial cells exhibited variable expression during microbial infections, the circulating miRNAs were chosen. Ceppi M et.al(2009)[6].

According to numerous studies, MiR-223 is a blood cell-specific miRNA that plays important roles in granulocyte differentiation, myeloid lineage development, and the inhibition of red blood cell differentiation. Additionally, it was shown that MiR-223 is essential for controlling the pattern of macrophage polarization, which shields mice from diseases including diet-induced inflammation and insulin resistance.[7-10].

Through the activation of NF-kB and the production of pro-inflammatory cytokines, TLR5, which recognizes bacterial flagellin in both Gram-positive and Gram-negative bacteria, plays a crucial role in modulating responses to this bacterial antigen. Research indicates that TLR5 is involved in pulmonary epithelial responses and may make people more vulnerable to pneumonia caused by flagellated pathogens. Both the sepsis and control groups showed significant TLR5 (rs5744105) analysis. Hawn et al. showed that TLR5 is activated by flagellated bacteria but not by non-flagellated bacteria, suggesting that flagellin is a particular ligand for TLR5.[11].

DEFINITION AND EPIDEMIOLOGY

Definition

Neonatal sepsis refers to a systemic inflammatory response syndrome (SIRS) caused by bacterial, viral or fungal pathogens in neonates, particularly during the first 28 days of life. It is broadly categorised into early-onset sepsis (EOS), which occurs within the first 72 hours after birth and late-onset sepsis (LOS), which occurs after 72 hours[12]. The presence of sepsis related clinical symptoms accompanied by conclusive tests and proof of infection is also included in the criteria. While LOS is frequently linked to postnatal environmental exposure, especially in neonatal intensive care units (NICUs), EOS is generally induced by vertical transmission from the mother during labor or delivery[13].

Epidemiology

The United Nations has suggested that worldwide, the greatest contributors to the death of newborns are infections as well as sepsis. In the review conducted by the Global Burden of Disease Study, it was reported that sepsis along with infections account for approximately 15% of the deaths after childbirth on a global level with lower and middle income countries having a higher ratio. While the Postnatal period is arguably the most critical period for mothers and baby's health due to the high cost of living, painful hospital stay, complicated diseases as well as the already existing unnatural traditions, low income regions suffer greatly from lack of health services and highly rated maternal's ill-health such as chorioamnionitis and prolonged rupture of membrane rupture, hence explaining their higher rates than low income countries. The estimated incidence of EOS in high income countries is 0.77–1.3 per 1,000 live births, while LOS incidence is higher due to extended hospital stays and invasive procedures in NICUs[2].

Regional and Socioeconomic Disparities

In low-resource settings, neonatal sepsis often goes under diagnosed due to limited diagnostic capability and management is heavily reliant on empirical antibiotic treatments. In these regions, sub-Saharan Africa and south Asia are the most affected, with neonatal sepsis contributing to about 20-30% of neonatal death[14]. There are significant disparities due to socio economic factors such as maternal malnutrition, inadequate antenatal visits and deliveries conducted at home.

Emerging Trends

In high income countries, the incidence of neonatal sepsis is comparatively lower because of improved maternal care and infection control, but novel challenges such as antimicrobial resistance (AMR) and an increase in multi drug resistant organisms pose a problem. NICUs have been facing an increasing treatment dilemma with the predominant pathogens of *Klebsiella pneumoniae*, *Escherichia coli*, and *coagulase negative staphylococci*[15].

To improve the neonatal survival rate, these gaps need to be addressed through better maternal care, provision of widely accessible preventive measures, and advancement in rapid diagnostic technology to enable proper management of neonatal sepsis.

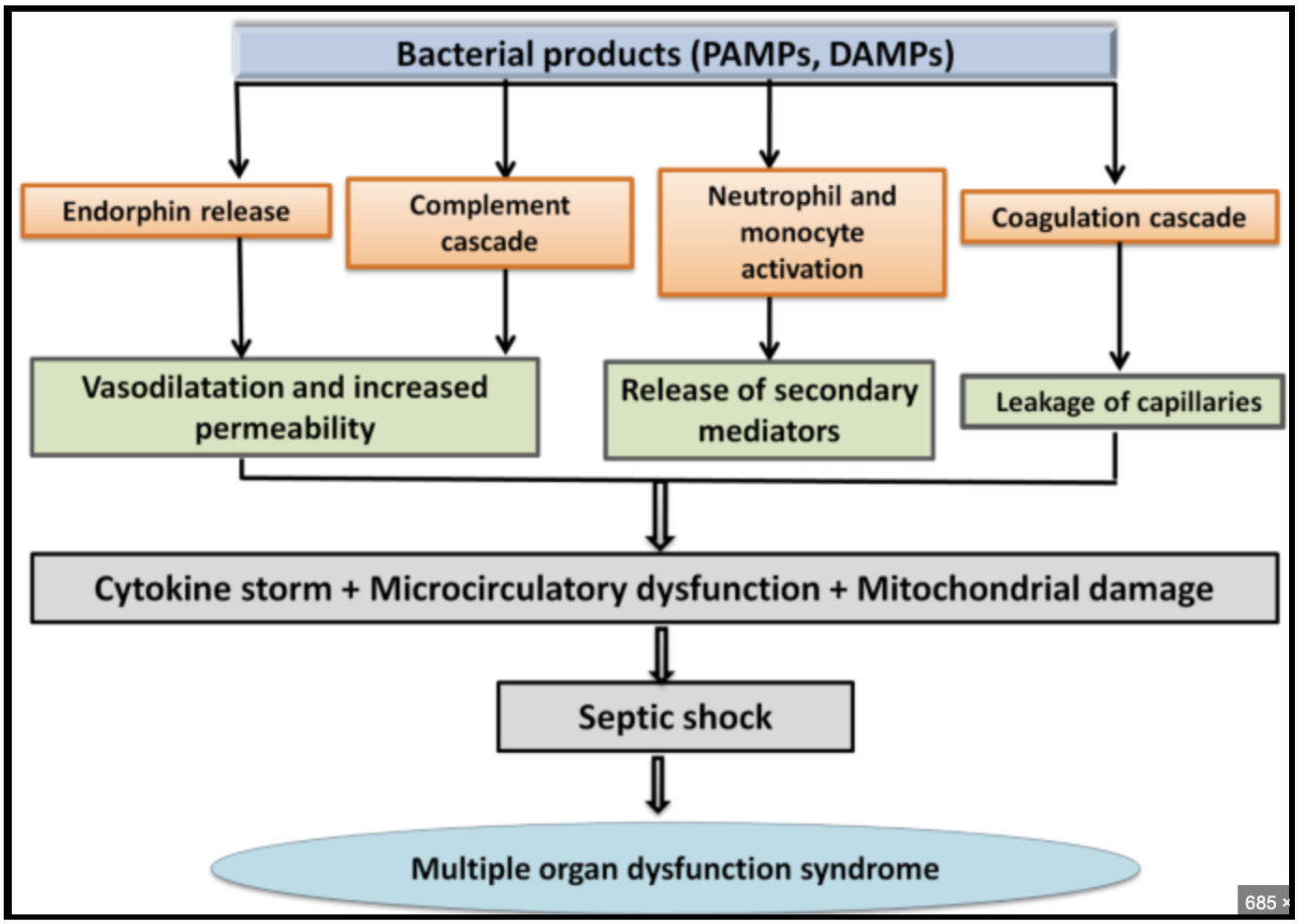


Figure 1: Pathophysiology of neonatal sepsis[16]

PATHOPYSIOLOGY

Neonatal sepsis is a life-threatening condition where the pathogens enter the neonate through either vertical transmission or horizontal transmission and creates an immune response in the body by activation of the innate immunity through neutrophils, macrophages and complement pathway. As the neonates are currently adapting to the external environment and their defence mechanisms are still immature, there is a cascade of events through inflammatory cytokines, leading to widespread inflammation. There is increased vascular permeability which results in leakage of fluids contributing to hypotension and shock. Later widespread and uncontrolled reactions could affect various organ systems of the neonate and lead to Disseminated Intravascular Coagulation (DIC) and death.

CLASSIFICATION

Neonatal sepsis is classified into two primary categories: early-onset sepsis (EOS) and late-onset sepsis (LOS). These categories are defined based on the onset of symptoms, the mode of transmission and the most common causative organisms. In classifying sepsis, diagnostic and therapeutic approach would be easier.

1. Early Onset Sepsis (EOS)

EOS occurs within the first 72 hours of life, although some studies extend the window to the first 7 days[13]. It is primarily associated with vertical transmission of pathogens from the mother to the neonate during labor or delivery.

Mode of Transmission:

- Ascending infections from the maternal genital tract.
- Transplacental infections during pregnancy.
- Pathogens acquired during passage through the birth canal.

Common Pathogens:

- *Acenetobacter bauminii,*
- *Escherichia coli,*
- *Klebsiella pneumoniae,*
- *Listeria monocytogenes,*
- Group B Streptococcus (GBS).

2. Late Onset Sepsis (LOS)

LOS typically presents after 72 hours of life and is more commonly observed in neonates who require prolonged hospital stays, particularly in neonatal intensive care units (NICUs). LOS is often caused by nosocomial or community acquired infections[17].

Mode of Transmission:

- Horizontal transmission from the hospital environment, healthcare workers or caregivers.
- Contaminated medical devices, such as intravenous catheters and ventilators.

Common Pathogens:

- *Coagulase-negative staphylococci (CONS),*
- *Klebsiella species,*
- *Pseudomonas aeruginosa*,
- *Candida.*

3. Other Subcategories of Sepsis

Host characteristics and type of infections are some of the other parameters looked at, other than the EOS and LOS. It would be ideal to include these specific characteristics.

- Sepsis in Preterm vs. Term Neonates

Preterm neonates have are prone to severe sepsis as they have weak immune response and undergo invasive procedures and increased NICU stay. The full term

neonates however have better outcomes due to mature immune responses and fewer comorbidities.

- Fungal vs. Bacterial Sepsis

While preterm neonates who undergo invasive procedures are more to fungal sepsis due to biofilm formation, the most common organism being candida species. The majority of sepsis is attributed to bacterial sepsis with variation in the organism causing it for early and late onset sepsis.

Significance of Classification

The classification of neonatal sepsis gives us an overall insight to type of organism, the disease severity and the essential treatment required. Antimicrobial resistance poses a growing threat in the management of neonatal sepsis. Multi drug resistant (MDR) organisms such as Klebsiella pneumoniae and Acinetobacter species are increasingly reported, particularly in low resource settings. These pathogens limit the efficacy of first line antibiotics, necessitating the use of broad-spectrum or last resort agents, which may further exacerbate resistance[15]

ETIOLOGY

Neonatal sepsis is caused by wide range of microbes such as bacteria, fungal and viral. These vary on the environmental factors, geographical location and onset of sepsis.

a. Early-Onset Sepsis (EOS)

EOS is most commonly caused by bacteria acquired during delivery or from the maternal genital tract. The leading pathogens are Group B Streptococcus (GBS), *Streptococcus agalactiae* which remains the most common cause of EOS in many parts of the world, particularly in regions with limited access to maternal screening and prophylaxis programs. *Escherichia coli* (E. coli) is responsible for EOS in preterm neonates and is often associated with high mortality[18]. Lastly *Listeria monocytogenes*, though rare, this pathogen is associated with transplacental infection and severe complications such as meningitis.

Term and late preterm neonates (more than 34 weeks period of gestation)	**Preterm neonates (less than 34 weeks period of gestation)**
GBS *Escherichia coli*	*Escherichia coli* GBS
Listeria monocytogens *Staphylococcus aureus* *Coagulase-negative staphylococci* Enterococcus sp. Gram negative bacteria(including Klebsiella, Enterobacter)	*Listeria monocytogens* *Staphylococcus aureus* Coagulase-negative staphylococci Enterococcus sp. Gram negative bacteria(including Klebsiella, Enterobacter)

Table 1. Etiology of early-onset neonatal sepsis.

b. Late-Onset Sepsis (LOS)

1. Bacterial Pathogens

LOS is typically caused by hospital acquired pathogens that colonise or invade neonates during prolonged NICU stays. Common organisms include are Coagulase-negative Staphylococci (CoNS) like *Staphylococcus epidermidis* is the most frequent cause of LOS, particularly in preterm infants with central venous catheters[17]. Gram negative Pathogens like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and Acinetobacter species are significant contributors to LOS, often in the setting of antimicrobial resistance. Enterobacter species are also associated with NICU outbreaks.

2. Fungal Pathogens

The most common cause of fungal sepsis is Candida species, particularly *Candida albicans* and *Candida parapsilosis*. It is more common in extremely low birth weight (ELBW) infants who have prolonged exposure to antibiotics or invasive devices. Fungal infections are associated with higher mortality and long-term neurodevelopmental impairments[19].

3. Viral Pathogens

Neonatal sepsis can also result from viral infections, albeit this is less common, seen especially in premature or immunocompromised newborns. The Cytomegalovirus (CMV), which is frequently contracted by breast milk or transplacentally, is a common viral disease. Perinatal or postnatal transmission of the Herpes Simplex Virus (HSV) is linked to widespread illness with a high fatality rate if treatment is not received. Enteroviruses cause widespread systemic illnesses through horizontal transmission.

Bacteria	**Fungi**	**Viruses**
Gram-positive bacteria: - *Coagulase-negative staphylococci* - *Staphylococcus aureus* - GBS - Enterococcus sp. **Gram-negative bacteria:** - *Escherichia coli* - Klebsiella - Enterobacter - Citrobacter - Pseudomonas - Serratia - Acinetobacter	*Candida albicans* *Candida parapsilosis*	*Cytomegalovirus* *Herpes Simplex Virus* Enterovirus

Table 2. Etiology of late-onset neonatal sepsis.

RISK FACTORS

A number of environmental, maternal, and neonatal variables influence the acquisition of pathogens:

Maternal Factors:

- GBS colonisation in the vaginal tract is a maternal factor.
- Pregnancy related illnesses, such as urinary tract infections (UTIs).
- Prolonged rupture of membranes and chorioamnionitis.

Neonatal Factors:

- Extremely low birth weight and premature birth.
- Use of intrusive tools like central venous catheters and endotracheal tubes.

Environmental Factors:

- Poor hand hygiene among caregivers.
- Contaminated equipment and NICU surfaces.

CLINICAL PRESENTATION

Neonatal sepsis is often subtle and non-specific, making early recognition and diagnosis challenging. The symptoms can vary based on the timing of onset, the causative organism and the overall health status of the neonate[1].

Neonates with sepsis often exhibit non specific signs that overlap with other neonatal conditions. Key features include:

- Temperature instability: Hypothermia or hyperthermia may occur, with hypothermia being more common in preterm neonates.
- Feeding intolerance: Refusal to feed, poor sucking or vomiting.
- Lethargy or irritability: Neonates may be difficult to arouse or excessively fussy.
- Respiratory distress: Grunting, nasal flaring, tachypnea, apnea or cyanosis.

EOS typically presents within the first 72 hours of life and is associated with vertical transmission of pathogens. The clinical features often reflect systemic involvement, including:

- Respiratory Symptoms: Respiratory distress syndrome (RDS) due to pneumonia or sepsis associated lung injury.
- Cardiovascular Instability: Hypotension, pallor, delayed capillary refill and shock.
- Neurological Signs: Seizures, bulging fontanelle or altered tone suggestive of meningitis.
- Septicaemia

LOS presents after 72 hours of life and is associated with hospital or community acquired infections. The features often include localised and systemic signs, such as:

- Fever or Hypothermia: More prominent in LOS compared to EOS.
- Localised Infections: Signs of skin or soft tissue infections, such as cellulitis or abscesses.
- Signs of meningitis, such as irritability, seizures or a bulging fontanelle.
- Abdominal Symptoms: Feeding intolerance, abdominal distension or necrotizing enterocolitis (NEC).
- Device Associated Infections: LOS is commonly linked to the use of invasive devices (e.g., central lines, ventilators), with clinical signs including catheter site infections or endotracheal tube colonisation[17].

Clinical Features by System Involvement:

a. Cardiovascular System

- Tachycardia or bradycardia.
- Poor perfusion, weak pulses or mottling of the skin.
- Cardiogenic shock in severe cases.

b. Respiratory System

- Grunting, retractions and nasal flaring.
- Episodes of apnea, particularly in preterm neonates.

c. Neurological System

- Lethargy, irritability, seizures or altered consciousness.
- Hypertonia or hypotonia.

d. Gastrointestinal System

- Vomiting, diarrhoea or feeding intolerance.
- Necrotizing enterocolitis in severe LOS cases.

e. Haematological System

- Pallor or jaundice.
- Petechiae, purpura or signs of disseminated intravascular coagulation (DIC).

Red Flag Symptoms:

- Persistent hypotension unresponsive to fluid resuscitation.
- Prolonged capillary refill time (>3 seconds).
- Oliguria or anuria.
- Severe metabolic acidosis.

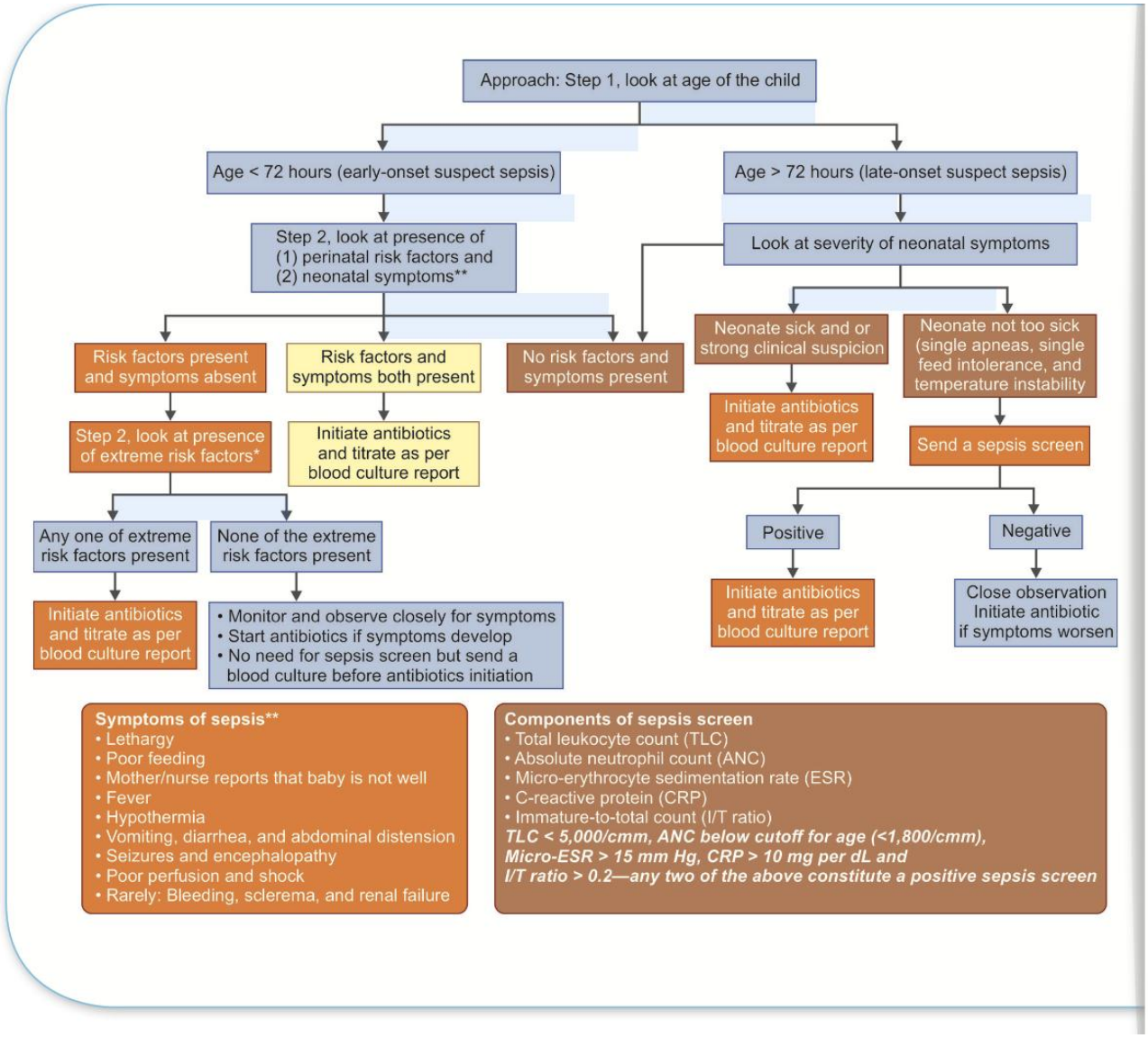


Figure 2: Approach to a neonate with sepsis[20]

DIAGNOSIS

The diagnosis of neonatal sepsis relies on a combination of clinical evaluation, laboratory testing, microbiological cultures, and imaging studies. As clinical signs are often nonspecific, an early and accurate diagnosis is crucial to initiating treatment and reducing mortality[21].

1. Clinical Evaluation

A detailed history and physical examination play a key role in the early detection of neonatal sepsis.

- History: Maternal factors such as prolonged rupture of membranes (PROM), maternal fever, chorioamnionitis, and prenatal infections increase the risk of neonatal sepsis[22]. Additionally, neonates with low birth weight or prematurity have a higher susceptibility to infection.

- Physical Examination: Key systemic signs of neonatal sepsis include temperature instability, lethargy, poor feeding, respiratory distress, tachycardia and hypotension[23]. Localised signs such as umbilical erythema, skin pustules or signs of meningitis(e.g., bulging fontanelle, irritability) should raise concern for infection.

2. Laboratory Testing

Laboratory tests aid in diagnosing neonatal sepsis, identifying causative pathogens and monitoring disease progression[24].

a. Blood Cultures

Blood cultures remain the gold standard for diagnosing neonatal sepsis. A positive culture confirms infection and allows for targeted antibiotic therapy[25].

- Timing & Technique: Blood should be drawn before starting antibiotics to improve detection rates. At least 1 mL of blood per sample is recommended for accurate results[26].

- Interpreting Results: A positive blood culture from two separate sites strongly suggests sepsis. However, false-negative results can occur due to low bacterial counts or prior antibiotic exposure[27].

b. Complete Blood Count (CBC) & Inflammatory Markers

- White Blood Cell(WBC) Count: Leukocytosis(WBC > 30,000/mm³) or leukopenia(WBC < 5,000/mm³) suggests sepsis but is not definitive[23].

- Neutropenia & Immature to Total(I/T) Neutrophil Ratio: An I/T ratio > 0.2 is highly suggestive of infection[24].

- C-Reactive Protein(CRP) & Procalcitonin: CRP: Rises within 6-12 hours of infection and is useful for monitoring response to treatment. However, it has low specificity[25] and Procalcitonin (PCT) is more specific to bacterial infections.

c. Lumbar Puncture (LP) for Meningitis

Indications: LP is performed when meningitis is suspected, especially in late-onset sepsis(LOS)[28].

CSF Findings: Elevated WBC count(>30 cells/mm³ in term neonates, >20 cells/mm³ in preterm neonates), low glucose(<40 mg/dL) and high protein(>150 mg/dL) suggest bacterial meningitis[26].

3. Microbiological Testing

Microbiological tests help identify the source of infection and guide treatment.

- Urine Cultures.

- Tracheal & Nasopharyngeal Cultures.

- Umbilical & Skin Swabs.

4. Radiologic Imaging

Imaging studies assist in diagnosing pneumonia, necrotizing enterocolitis (NEC), and intracranial complications.

• Chest X-ray: Used to assess for pneumonia or respiratory distress syndrome (RDS)[28].

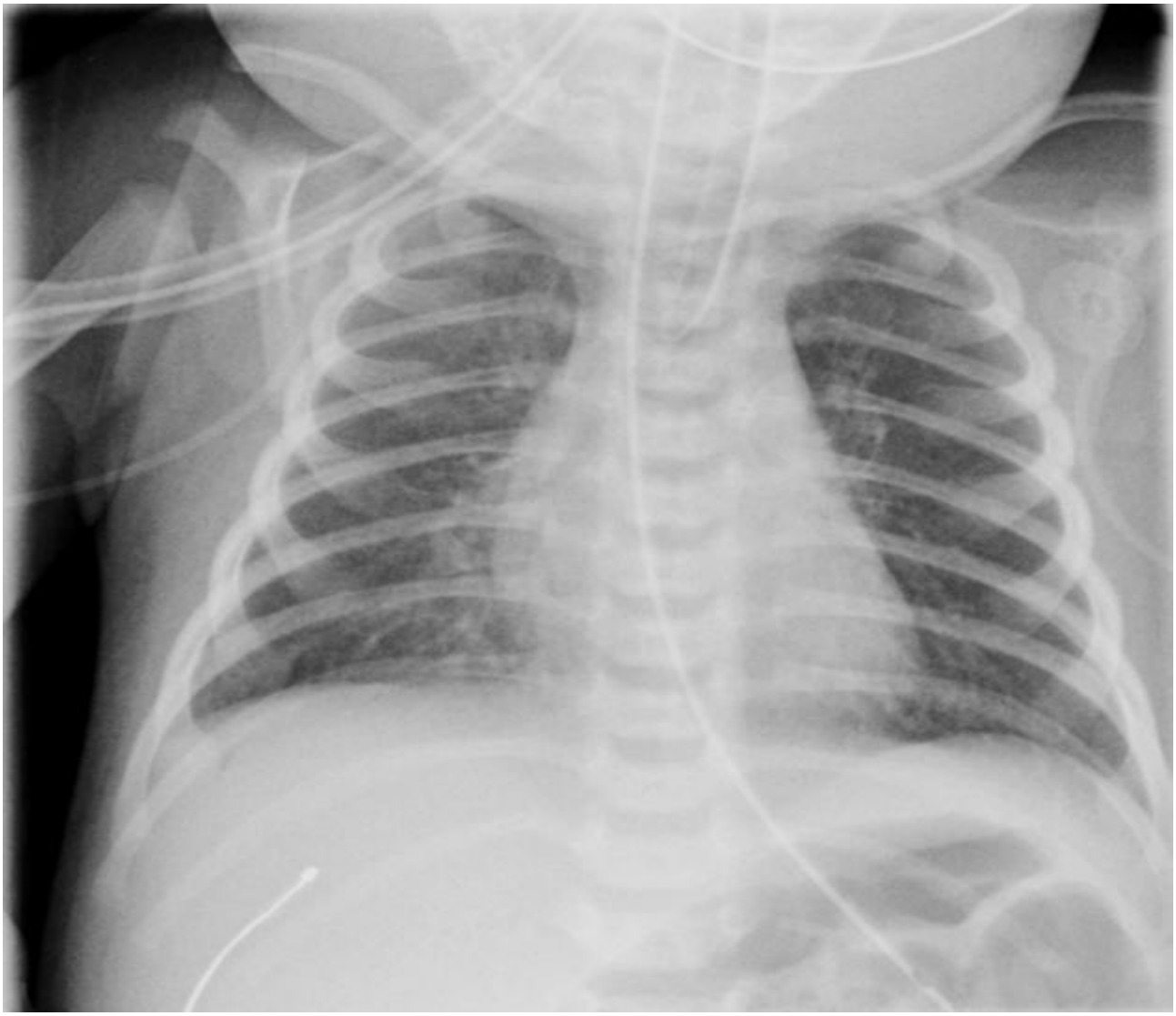
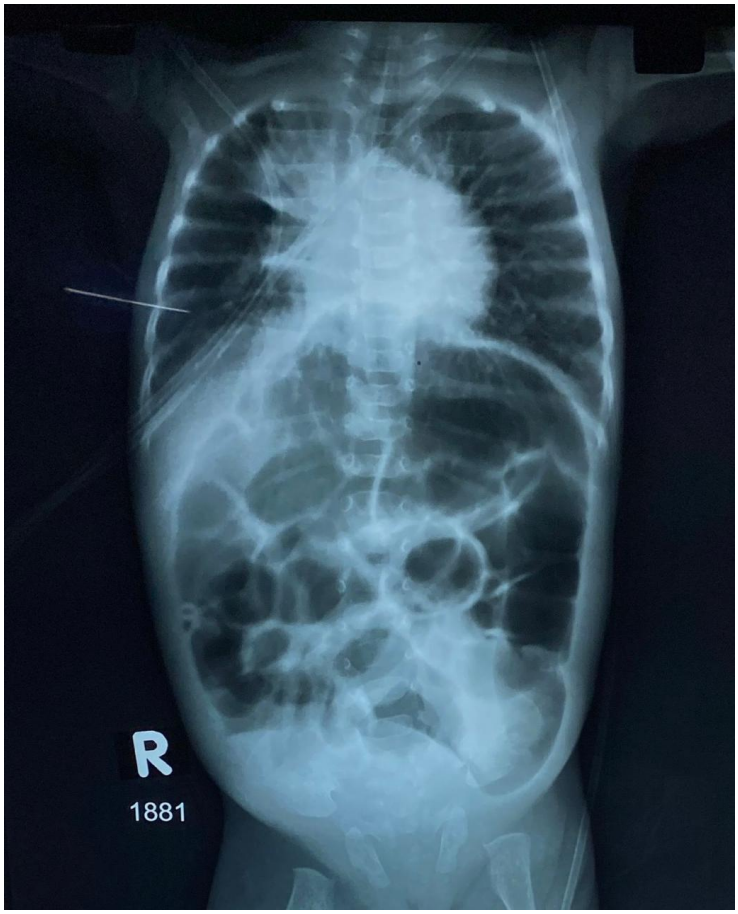


Figure 3: Chest X-ray of neonatal pneumonia[29]

• Abdominal X-ray & Ultrasound: Indicated in suspected NEC, showing signs like



pneumo-bilia, air in intestines, intramural necrosis.

Figure 4: Abdominal X-Ray of Necrotising Enterocolitis

• Neurosonogram: Used to rule out intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL) in preterm.

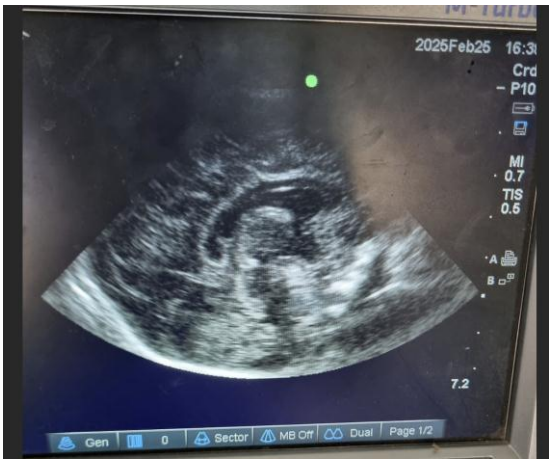


Figure 5: Neurosonogram showing Intraventricular hemorrhage in a neonate

DIFFERENTIAL DIAGNOSIS

- Hypoxic Ischemic Encephalopathy (HIE): Presents with lethargy, poor tone and seizures but lacks infectious markers.

- Inborn Errors of Metabolism (IEM): Features vomiting, metabolic acidosis and hypoglycaemia but does not respond to antibiotics.

- Respiratory Distress Syndrome (RDS): Can mimic pneumonia, but RDS occurs in preterm neonates and improves with surfactant therapy.

- Necrotizing Enterocolitis (NEC): Presents with feeding intolerance, bloody stools and abdominal distension, requiring imaging confirmation.

MANAGEMENT

The management of neonatal sepsis requires a comprehensive approach that includes early identification, immediate empirical antibiotic therapy, supportive care, and targeted treatment based on microbiological results. Early intervention is essential to prevent serious complications, such as septic shock, organ dysfunction, and death[30].

1. Initial Assessment and Stabilisation

Neonates suspected of having sepsis require immediate stabilisation to prevent rapid deterioration. Initial management includes:

- Vital Signs Monitoring.
- Fluid Resuscitation.
- Oxygen Therapy.
- Inotropic Support.

2. Empirical Antibiotic Therapy

Those neonates, have been suspected to have sepsis must receive empirical antibiotic therapy as soon as possible, even before culture results are available. The antibiotic selected depends on the pathogen suspected and the gestational age of the newborn. The suspected organism for Early Onset Sepsis(EOS) is commonly Group B Streptococcus(GBS) in the first world countries and Escherichia coli in resource limited setting, first line antibiotics mostly used are ampicillin and cefotaxime or gentamicin. And for the Late Onset Sepsis(LOS) usually the common pathogens include coagulase-negative staphylococci(CoNS) and Staphylococcus aureus, requiring coverage with ampicillin and cefotaxime. Whereas for fungal sepsis, if we are suspecting Candida species, anti fungal treatment with amphotericin B or fluconazole is recommended. It is crucial to start empirical antibiotic therapy ideally one hour within sepsis suspicion.

3. Supportive Care

In addition to antibiotics, neonates with sepsis require multi system supportive care to prevent complications[31]. This includes:

Respiratory Support– Infants with respiratory distress may require oxygen therapy, heat humidified high flow nasal cannula(HFNC), continuous positive airway pressure (CPAP), mechanical ventilation or high frequency oscillatory ventilation (HFOV).

Nutritional Support– Due to feeding intolerance, some neonates require parenteral nutrition (PN) until they can tolerate breast milk or formula[32].

Laboratory Monitoring– Continuous monitoring of the blood parameters would prove useful.

4. Targeted Therapy Based on Microbiological Results

Antibiotic stewardship is required for antibiotics escalation or de escalation. Broad spectrum antibiotics must be tapered once culture and sensitivity results are available to reduce antibiotic toxicity and resistance. The duration of therapy will vary based on the infection severity. It is usually 7–10 days for EOS and 10–14 days for LOS. In complex cases(e.g., multi-drug-resistant organisms), consultation with an infectious disease specialist is recommended[33].

5. Surgical Intervention

Surgical intervention is only indicated in select few cases where the infection is usually contained and easily resectable. For example necrotizing enterocolitis (NEC) where severe cases would require surgical resection of bowel tissue that is necrotic. Abscess drainage– This is a collection of localised pus in the neonate and would require incision and drainage. Sometimes central venous catheters are prone for

fungal sepsis as they form a biofilm layer when in situ for longer duration, hence removal and reinsertion of the new catheters is a must in timely manner.

6. Monitoring and Follow-Up Care

Close monitoring and follow-up would be absolutely essential to prevent long-term complications, hence in Neonatal Intensive Care Unit(NICU) monitoring the vital signs continuously is a must, laboratory evaluation and clinical assessment guide management[34]. Long term follow up of the survivors of neonatal sepsis, especially preterm infants, require neurodevelopment assessment to detect potential delays in neurological growth and development. Long term neurological effects, hearing loss and growth failure require ongoing multidisciplinary care.

7. Prevention of Neonatal Sepsis

Preventative strategies are a must in neonatal sepsis as it is extremely dangerous life threatening condition and requires Intrapartum Antibiotic Prophylaxis(IAP) where the pregnant women colonised with GBS must receive IV penicillin at labor onset to prevent infecting the neonate. Strict infection control measures such as hand hygiene, sterile techniques and isolation protocols reduce the risk of hospital acquired infections[35]. Breastfeeding promotion will provides immunity against neonatal infections.

PROGNOSIS

The prediction of a case of neonatal sepsis still relies on the infant's age and birth weight, diagnosis, the onset of treatment, the causative pathogen and their associated complications. However, there's no doubt that early intervention significantly factors into survival rates. Unfortunately, the long term outlook for infants, particularly those born prematurely, is still fraught with danger. Sepsis continues to be one of the highest contributors to cases of infant mortality across the globe, regardless of advances made in health care[32].

Prognosis Factors at a Glance Gestational Age and Birth Weight: When factoring in age and weight, children born prematurely, along with those with low birth weight, have an underdeveloped immune system which makes them more susceptible to extreme infections. It is a well known phenomenon that infants with extremely low birth weight (ELBW) – i.e. those weighing 1000 grams or less – die from sepsis at a higher proportion than their fully term counterparts[35]. Time to Diagnosis and Treatment: Surviving through a critical health condition is only possible when firstly, appropriate antibiotics are given, and secondly, they are given at the right time. An increase in the time taken to diagnose a condition makes a patient susceptible to more complications like multi-organ failure along with increased mortality rates36. Causative Organism: The infective organism is one of the major determinants of the illness and or the outcome of the disease. Escherichia coli and Klebsiella pneumoniae, being gram-negative bacteria, are linked with more severe infections than the Group B streptococcus organism which is gram-positive.[37].

Complications: Sepsis-related complications such as meningitis, necrotizing enterocolitis (NEC) and intraventricular hemorrhage increase morbidity and long term neurodevelopment impairments[32].

2. Death Rates

Infectious diseases are still an important reason for child mortality considering the ranges of deaths are different depending on the available resources for obstetric care, kind of illness and etc.

Early Onset Sepsis (EOS): For EOS the mortality rates are estimated to be 5-25%, with the highest percentages being among preterm and ELBW infants[36].

Late Onset Sepsis (LOS): The mortality rates for LOS are reported to be around 10%, with some experts estimating rates slightly above 20%. Multi drug resistant fungal and bacterial infections are exceptionally dangerous[37].

3. Long-term Consequences and Neurodevelopment Outcomes

Each of the children surviving neonatal sepsis face the following long enduring problems and difficulties:

Neurodevelopment Impairment: The presence of sepsis is known to be highly associated with cognitive/motor delays and significantly higher among neonates who develop meningitis or suffer from hypoxic ischemic injury[37].

Cerebral Palsy: There is a correlation between severe neonatal infections and the incidence of cerebral palsy, thought to stem from inflammatory insult to the developing brain[37].

Growth and Nutritional Problems: In post septic patients, particularly in post NEC patients, there is a notable incidence of poor feeding and prolonged dependence on nutritional supplements[35].

4. Antibiotic Resistance's Effect

The management of sepsis is made more difficult by the advent of multi drug resistant pathogens. Longer hospital admissions and increased death rates are associated with neonates infected with methicillin resistant Staphylococcus aureus

(MRSA), vancomycin resistant enterococci (VRE), or bacteria that produce extended spectrum beta lactamases (ESBLs)[37].

5. Group B Streptococcus (GBS) Prognosis by Pathogen Type: Although delays raise the risk of neurological sequelae, prompt treatment of GBS sepsis typically produces positive results[35]. Gram-Negative Bacteria: Higher morbidity and death are associated with E. Coli and Klebsiella species infections, especially in preterm neonates[36]. Fungal Sepsis: Candida infections, especially in ELBW newborns, have a mortality rate of over 30% even with anti fungal therapy[37].

Sepsis is still a serious concern in neonatal care, especially for preterm and low birth weight newborns, despite tremendous progress in this area. In order to improve prognosis and lower long-term consequences, early diagnosis, suitable antibiotic therapy, and prophylactic actions are crucial. To significantly improve neonatal sepsis outcomes, more research and international initiatives to fight antibiotic resistance are essential.

PREVENTION

Preventing neonatal sepsis is crucial for reducing infant morbidity and mortality worldwide. Effective prevention strategies encompass maternal health optimisation, stringent infection control practices, early identification of at-risk neonates, appropriate vaccination, antimicrobial stewardship, promotion of breastfeeding, diligent postnatal care, and robust public health measures.

Preventing newborn sepsis requires ensuring good health of the mother and her vaginal flora. Most important methods to prevent is by routinely screening for GBS colonisation between 35 and 37 weeks of pregnancy, colonised mothers can get intrapartum antibiotic prophylaxis, which dramatically lowers the risk of early onset GBS illness in newborns. Using aseptic techniques during invasive procedures, such as catheter insertions, minimises the risk of introducing pathogens[38]. Regular cleaning and disinfection of the NICU environment help eliminate potential reservoirs of infection[39]. Preterm infants are at higher risk for sepsis. Preventative measures such as treating maternal infections and administering antenatal corticosteroids to enhance fetal lung maturity can decrease this risk[40].

Implementing rigorous infection control measures in NICUs is essential to prevent hospital-acquired infections by strict adherence to hand hygiene protocols among healthcare workers is paramount in reducing infection transmission[41].

Proactive measures to identify and manage at-risk infants include: Evaluating maternal and neonatal risk factors, such as chorioamnionitis or low birth weight, facilitates early detection and intervention and administering antibiotics to neonates with identified risk factors can prevent the progression of sepsis[41]

Vaccinations play a pivotal role in preventing infections that could lead to neonatal sepsis: Immunising pregnant women against influenza and pertussis provides passive immunity to the neonate, reducing infection risk[42]. Hepatitis B vaccination at birth stops the infection from spreading vertically[43]. The fight against antimicrobial resistance requires the prudent use of antibiotics. Antibodies and bioactive

substances found in breast milk can prevent infections and lower the risk of sepsis[44]. Prompt management[45] is ensured by early diagnosis of any problems through planned follow-ups.

ROLE OF miRNA-23b IN NEONATAL SEPSIS

MicroRNA-23b (miRNA-23b) is considered as a potential surface marker for neonatal sepsis, low levels are associated with the evolution of sepsis in newborns, it is suggested that it is a key factor in early detection and prognostication of the disease. A decrease in miRNA-23b levels might indicate an greater level of risk in neonates with sepsis.

Anti-inflammatory Effects:

miRNA-23b has been shown to negatively regulate inflammatory responses by targeting key genes in inflammatory pathways, NF-κB signalling pathway (which drives pro-inflammatory cytokine production). It may suppress expression of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β.

Immune Modulation:

Advanced Sepsis Inflammation Results MiR-23b is believed to be able to modulate immune cell activity, especially in monocytes and macrophages, which are already overly stimulated and busy restructuring during sepsis. In preventing subsequent arms of the cytokine storm that worsen sepsis outcomes, miRNA-23b would be beneficial by controlling cell activation.

Potential Biomarker:

Some researchers have pointed out a lesser expression of miRNA-23b in neonates with sepsis as it relates to greater negative clinical outcomes through increased inflammation. This suggests that it may be possible to use miRNA-23b as an indicator for reliable diagnosis or prognosis of neonatal sepsis.

Therapeutic Potential:

Modulators or mimics to restore levels of miRNA-23b would be expected to reduce inflammatory response, protect systemic tissues from injury, and improve chances of survival among children suffering from neonatal sepsis, although, this thinking is quite speculative.

In cases of neonatal sepsis, children who have lower levels of miRNA-23b may suffer from over expression of inflammation with inadequate regulation of immune response. While restoring expression of miRNA-23b could help such children, the precise impact and therapeutic potential can only be ascertained through further clinical studies.

MATERIALS AND METHODS

SOURCE OF DATA

Place of study: NICU Of Shri B M Patil Medical College Hospital and Research Centre, BLDE (Deemed University), Vijayapura, Karnataka.

Duration of study: May 2023 to December 2024.

Type of study: Observational Case Control Study.

INCLUSION CRITERIA:

Neonates less than 72 hours of life with suspected early onset sepsis

EXCLUSION CRITERIA:

Lethal Congenital Anomalous neonates

Not willing to participate in the study

METHOD OF COLLECTION OF DATA

Twenty three newborns admitted in NICU for other reasons and twenty three newborns admitted in NICU for suspected sepsis at BLDE University was considered. From each newborn, a minimum of 3 ml of blood was drawn by standard sterile procedures. The miRNA-23b level in haemoculture was evaluated by RT-qPCR.

Sample size (n):

A Proportion of term neonates, Early onset of sepsis, and Glycemic index, the study required a sample size of minimum 46 patients with 95% level of confidence and 10% absolute precision,

Formula used :

$$n = \frac{z^2\, p*q}{d^2}$$

Where z= z statistic at α level of significance

d^2= Absolute error

P= Proportion rate

q= 100-p

Sample Collection and Methodology

The ethical clearance certificate was obtained from our Institution Ethical Committee once our project was selected, Information was collected from each patient through a pro forma, meeting the objectives of the study.

The purpose of the study was explained to patient attenders in detail, and written consent was taken. Patients suspected with sepsis identified. 3ml Blood samples was collected on admission to NICU; serum was separated and collected in aliquots.

The serum aliquots was frozen at −80°C. Serum isolation was done by centrifugation at 1600 rpm for 15min at room temperature. The supernatant was transferred to Eppendorf tubes. The samples was recentrifuged at 14,000 rpm for 10 min to precipitate cell debris and the supernatants was stored at − 80 °C until the extraction of ribonucleic acid (RNA), the same was repeated for the controls.

RNA Extraction

Total no of samples: 23 sepsis and 23 controls

Total RNA was extracted from 200µl serum samples by using Nucleospin Plasma isolation Kit, these extracted samples were quantified by teckon make multimode plate reader at 260/280 OD for RNA samples purity to get 0.25–8 µg.

Sl.No. Case samples	OD at 260/280	Concentration in ηg/µl
1	1,96	54
2	1,89	65
3	1,92	44
4	1,90	70
5	1,91	136
6	1,98	64
7	1,93	82
8	1,92	73
9	2,01	68
10	1,92	111
11	1,97	64
12	1,87	66
13	2,04	53
14	2,02	65
15	2,15	82
16	1,94	94
17	1,88	49
18	2,09	39
19	1,93	46
20	2,04	100
21	2,6	51,5
22	2,35	85,5
23	1,97	73,9

Table 3: Quantification of RNA by multimode Reader for cases sample

Sl.No. Contol Samples	OD at 260/280	Concentration in ηg/µl
1	2,06	57
2	2,01	81
3	2,24	125
4	2,09	137
5	1,85	104
6	1,96	92
7	1,94	93
8	2,06	84,01
9	2,21	75,02
10	2,01	45,01
10	2,01	45,01
11	2,03	63,02
12	1,96	64,1
13	1,95	75,3
14	1,94	68,01
15	1,97	45,01
16	2,03	85,02
17	1,96	56,01
18	1,98	45,03
19	12,05	95,3
20	2,04	74,1
21	2,01	65,02
22	2,09	74,01
23	2,04	74,05

Table 4: Quantification of RNA by multimode Reader for controls sample

Polyadenylation and Reverse Transcription

For every RNA sample, a cDNA synthesis reaction was prepared for qPCR analysis. To use a standard curve to calculate the absolute level of miRNA levels.

We added the following ingredients in a 0.2 ml tube that is free of RNase:

Poly(A)/cDNA Synthesis Reagent

Reagent	Volume(µl)
mRQ Buffer (2x)	5
RNA sample (0.25–8 µg)	3.75
mRQ Enzyme	1.25
Total Volume	10

In order to inactivate the enzymes, samples were incubated for one hour at 37°C and then stopped for five minutes at 85°C using a thermal cycler.

To get 100 µl, 90 µl of ddH_2O was added to each tube.

The cDNA was prepared for the measurement of miRNA.

Primer designing for miRNAs

The widely used web-based, open-source program PRIMER 3 is used to generate PCR primers. Primers 3 is a bioinformatics tool that assists in creating primers for the target region in the specified nucleotide sequence based on the needs of the user or applications.

MiR-23b - (forward): 5'-TCTCCCTGGCGTCCTCCCTTCG- 3'

(reverse);- 5'-CCTTATCAAGAACACCAACCAGT-3 '

U6: F:5-AAGATCATTGCTCCTCCTGAGC-3 ,

R: 5 -TCCTGCTTGCTGATCCACATC-3

U6 as a reference and control gene.

Quantification of miRNA by qPCR

This was performed by using standard curve method. Additional qPCR amplifications was done(U6 snRNA controls for the ddCt method or cDNA prepared from synthetic miRNA for the standard curve method).

Sample qPCR Reaction

Reagent	**Volume(µl)**
ddH2O	9
TB Green Advantage Premix (2X)	12.5
ROX Dye (50X)	0.5
miRNA-specific primer (10 µM)	0.5
mRQ 3’ Primer (10 µM)	0.5
cDNA	2.0
Total volume	25

Table 5. Sample qPCR Reaction

U6 qPCR Reaction

Reagent	**Volume(µl)**
ddH2O	9
TB Green Advantage Premix (2X)	12.5
ROX Dye (50X)	0.5
U6 Forward primer (10 µM)	0.5
U6 Reerse Primer (10 µM)	0.5
cDNA	2.0
Total volume	25

Table 6. Sample U6 qPCR Reaction

Cycle reactions were established in accordance with the TAKARA's specifications. We created a one-sep QRTPCR kit for the immediate use. We utilized the ABI QUANT 5 Studio instrument for this.

- Denaturation 95°C 10 sec
- qPCR x 40 Cycles
  95°C 5 sec
  60°C 20 sec
- Dissociation Curve
  95°C 60 sec
  55°C 30 sec
  95°C 30 sec

Delta-Delta Ct Method

By comparing two samples to a second RNA that acts as a normalisation standard (such as U6), the delta-delta Ct method (ddCt) approximates the relative levels of miRNA between the two samples. In short, each sample's Ct is determined by amplifying the U6 RNA and the unknown miRNA. This makes it possible to use the ddCt computation to calculate relative levels.

The Standard Curve Method of Absolute Quantification

This technique creates the standard curve by serially diluting a calibrated synthetic miRNA preparation. The plot is then used to calculate the miRNA copy number based on the experiment samples' Ct values.

1. Plotted the average Ct values against the input miRNA copy number on a log scale for the duplicate qPCR reactions of the cDNA samples made from the diluted synthetic miRNA samples.

2. Using the standard curve created in Step 1, extrapolate the matching RNA copy number from the average Ct values for each duplicate experiment sample or sample dilution.

The Nano Quant (infiniteM200) Spectrophotometer was used to measure the absorbance at 260 and 280 nm in order to ascertain the concentration and purity of RNA.

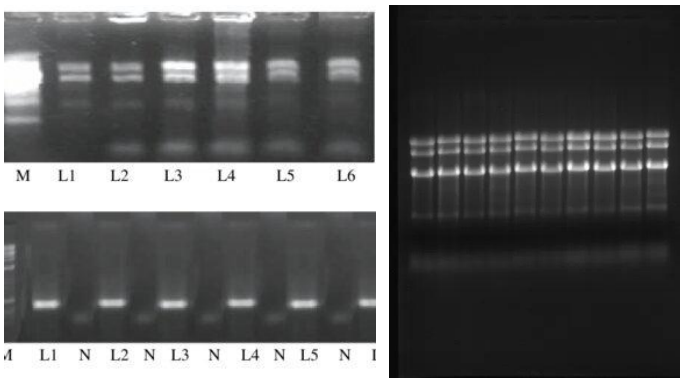


Figure 6: Total RNA from serum sample

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS version 20 statistical software (SPSS, Inc., Chicago, IL) and a P-value of <0.05 is considered statistically significant. All the results are defined as mean ± standard deviation. The independent sample-test was applied to compare the MicroRNAs expression levels, and the other measured parameters between PE group and control group. One-way ANOVA test was applied for comparison between diseased and control groups.

OBSERVATIONS AND RESULTS

Table 7: Distribution of Parity

Group	Parity		Total (%)
	Primi	Multi	
	Number (%)		
Cases	12(52.2)	11(47.8)	23(50)
Controls	12(52.2)	11(47.8)	23(50)
Total	**24(47.8)**	**22(52.2)**	**46(100)**

Out of the total 46 mothers, 24(47.8%) were primigravida group and 22(52.2%) were multigravida group. Among the cases and controls 12(52.2%) were primigravida and 11(47.8%) were multigravida mothers.

Figure 7: Distribution of Parity

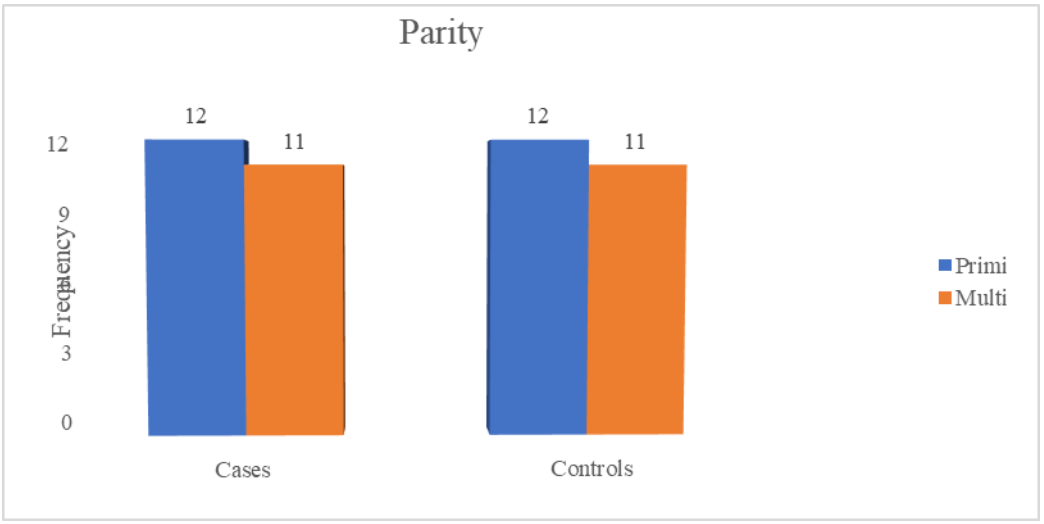


Table 8: Distribution of History of PROM

Group	History of PROM		Total (%)
	Yes	No	
	Number (%)		
Cases	11(47.8)	12(47.8)	23(50)
Controls	3(13)	20(87)	23(50)
Total	**14(30.4)**	**32(69.6)**	**46(100)**

Among the total 46 mothers, 14(30.4%) had a history of PROM and 32(69.2%) did not have premature rupture of membranes(PROM). In the cases group 11(47.8%) had history of PROM and in the controls group 2(13%) had a history of PROM.

Figure 8: History of PROM

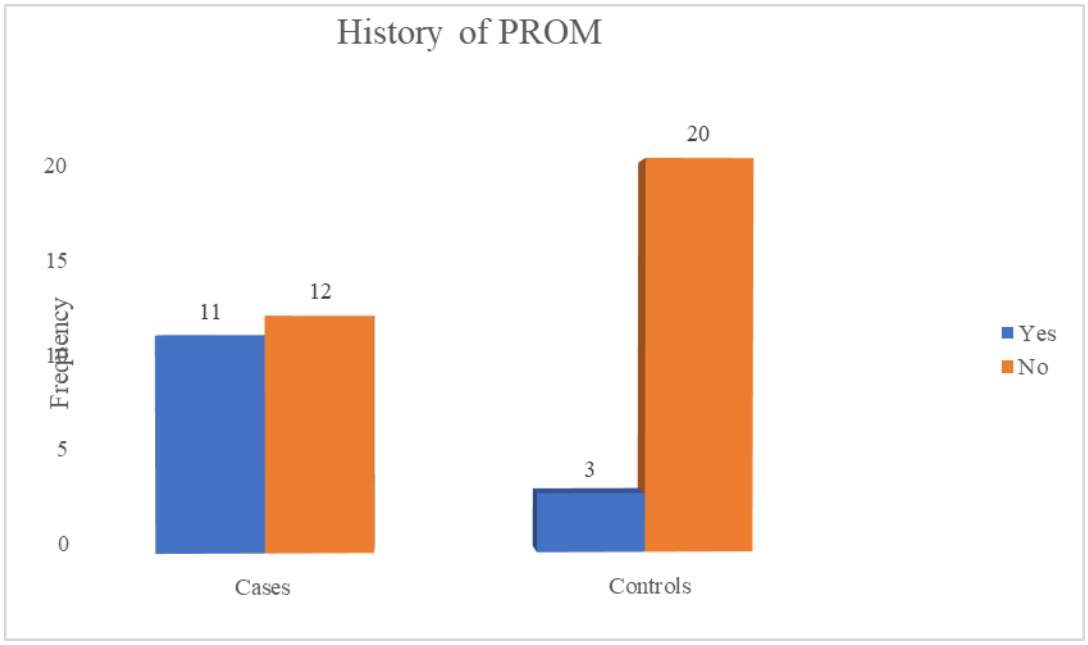


Table 9: Distribution of Mode of delivery

Group	Mode of delivery		Total (%)
	Normal	LSCS	
	Number (%)		
Cases	11(47.8)	12(52.2)	23(50)
Controls	11(47.8)	12(52.2)	23(50)
Total	**22(47.8)**	**24(52.2)**	**46(100)**

Out of the total 46 neonates, 22 babies(47.8%) were delivered via normal vaginal delivery, and 24(52.2%) were delivered through Lower Segment Cesarean Section(LSCS). In both cases and control 11(47.8%) were normal delivery, while 12 (52.2%) were delivered via LSCS.

Figure 9:Distribution of Mode of delivery

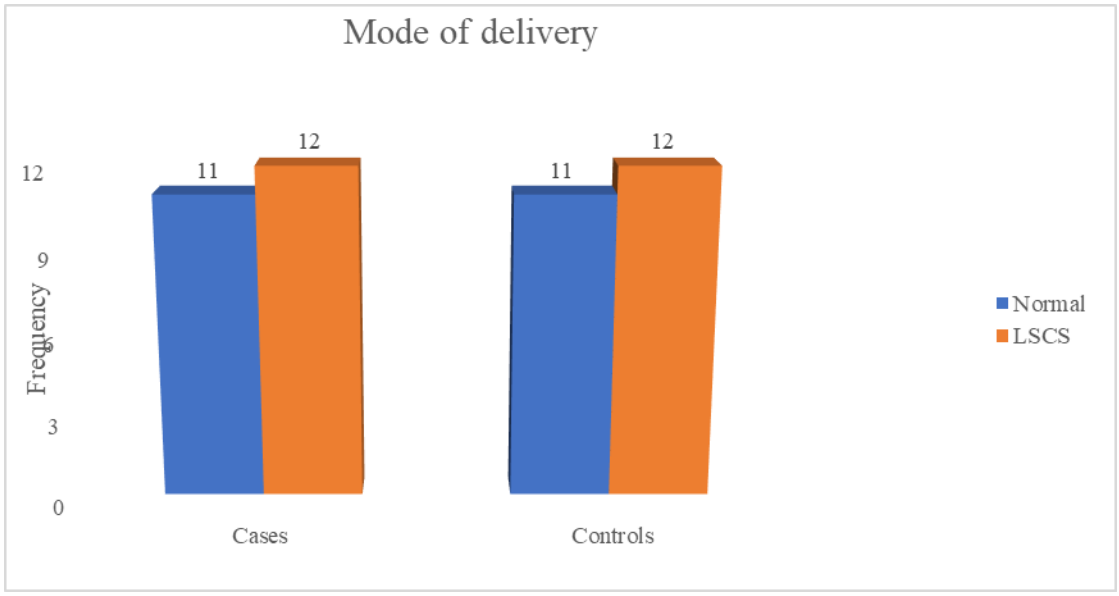


Table 10:Distribution of Blood group of the mothers

Blood group(mothers)	Group		Total
	Cases	Controls	
	Number (%)		
A+	7	2	**9**
AB-	2	2	**14**
AB+	0	1	**1**
B+	8	7	**15**
O+	6	11	**17**
Total	**23**	**23**	**46**

A total of 46 enrolled mothers, majority had B positive blood group(8) in the cases group, while majority of the control group mothers had O positive blood group(11). While in both case and control groups least common blood group was AB positive(0).

Figure 10: Distribution of Blood group of the mothers

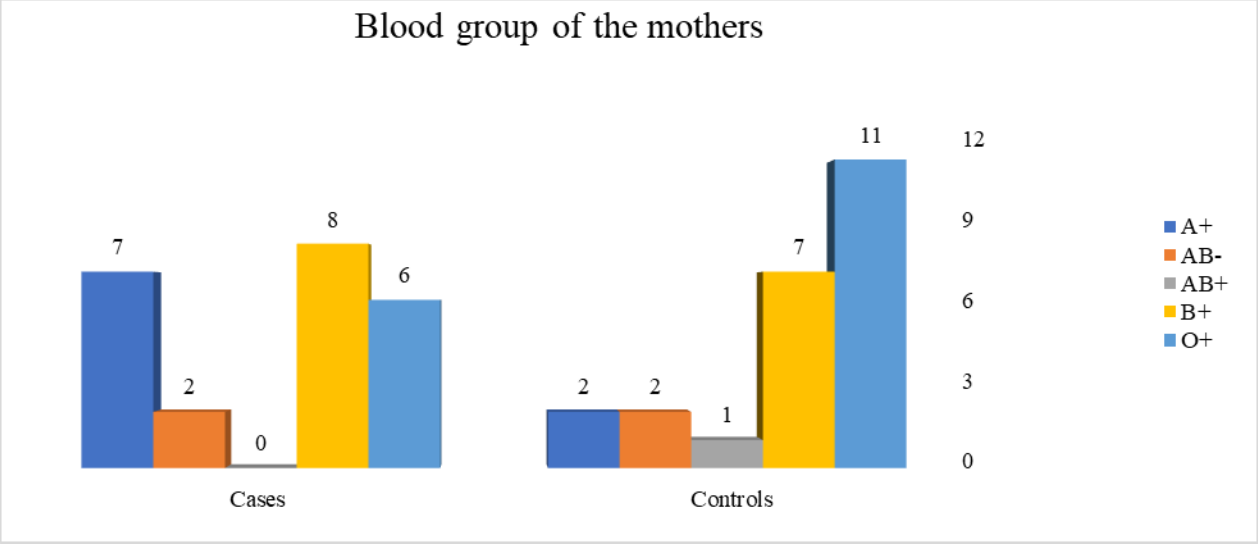


Table 11: Distribution of Place of delivery

Group	Place of delivery		Total (%)
	In born	Out born	
Cases	9(31.9)	14(60.9)	23(50)
Controls	15(65.2)	8(34.8)	23(50)
Total	**24(52.2)**	**22(47.8)**	**46(100)**

Out of the total 46 neonates, 24(52.2%) were Inborn, and 22(47.8%) were Outborn neonates. Amongst the cases, 9 neonates were Inborn and 14 were Outborn. In control group ,15 were Inborn, and 8 were Outborn neonates.

Figure 11: Distribution of Place of delivery

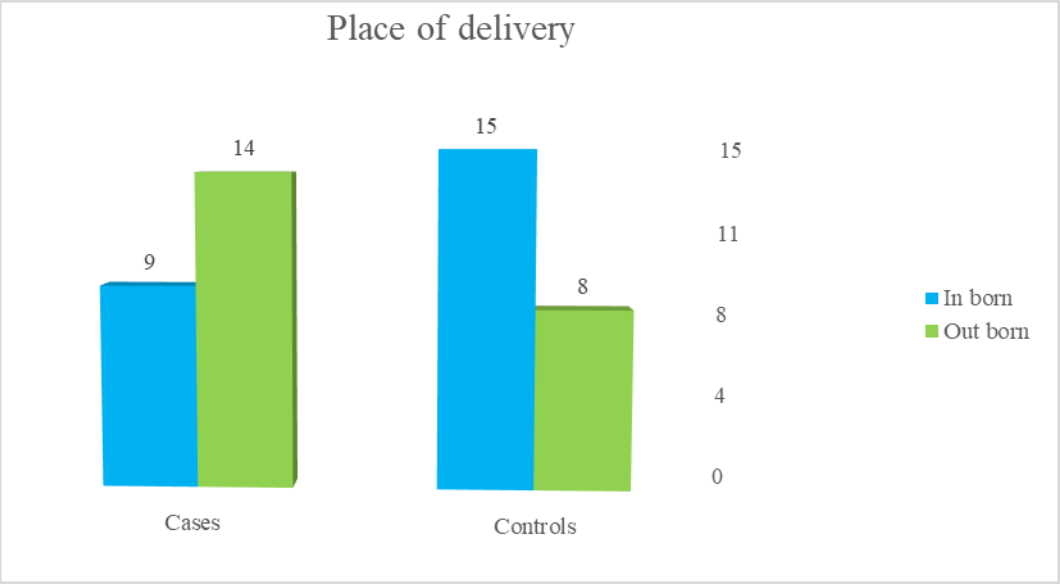


Table 12: Distribution of Birth details of the neonates

Variables	Group	
	Cases	Controls
	Mean±SD	
Birth weight (in kg)	2.48±0.568	2.61±0.596
Head circumference	30.91±1.70	31.98±1.59
Length	51.30±2.22	51.65±1.69

The average birth weight of the neonates in the cases were 2.48±0.56kg and in the controls were 2.61±0.59kg

The average head circumference of the neonates was 30.91±1.70cm in cases group and 31.98±1.59cm in the controls group.

The average length of the neonates was 51.30±2.22cm in the cases group and 51.65±1.69cm in the controls group.

Table 13: Distribution of gender

Group	Gender		Total (%)
	Female	Male	
	Number (%)		
Cases	8(34.8)	15(65.2)	23(50)
Controls	9(39.1)	14(60.9)	23(50)
Total	**17(37.0)**	**29(63.0)**	**46(100)**

A total of 46 neonates were enrolled for the case control study of which 17(37%) were females and 29(63%) were males, mainly constituted the study population. In cases, there were 15(65.2%) males and 8(34.8%) females, and in controls, there were 14(60.9%) males and 9(39.1%) females.

Figure 12: Distribution of gender

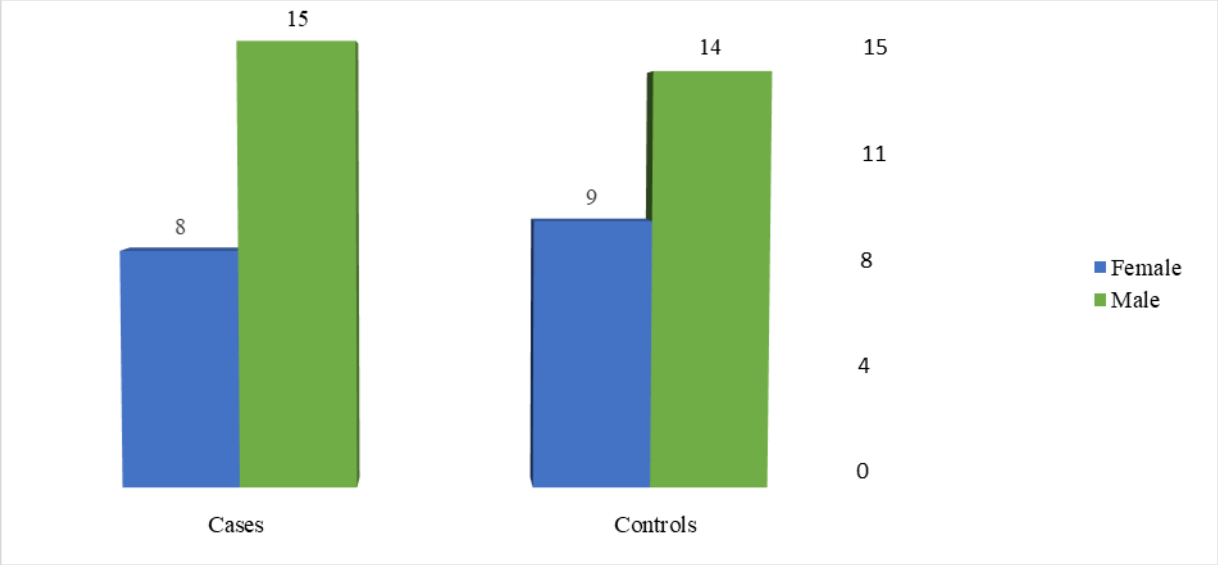


Table 14: Distribution of Neonates that were Febrile

Group	Febrile		Total (%)
	Yes	No	
	Number (%)		
Cases	1(4.3)	22(95.7)	23(50)
Controls	0(0)	23(100)	23(50)
Total	**1(2.2)**	**45(97.8)**	**46(100)**

Only one neonate was febrile at the time of admission while the rest 22 neonates were afebrile in the cases group. All the neonates(23) in the controls group were afebrile at the time of admission.

Figure 13: Distribution of Neonates that were Febrile

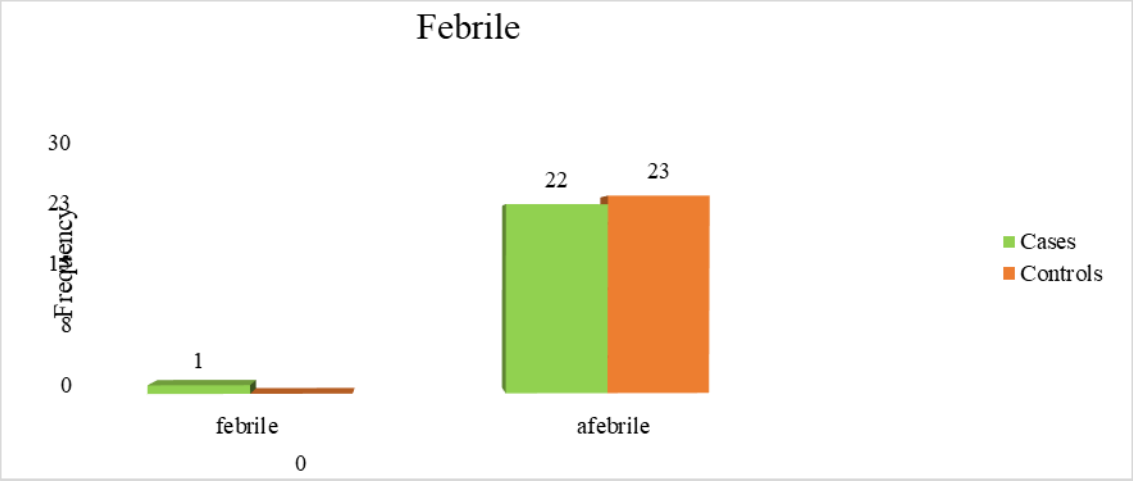


Table 15: Distribution of Indication of admission

Indication of admission	Group		Total
	Cases	Controls	
	Number		
TTNB	2	11	**13**
RDS	4	10	**14**
Birth Asphyxia	10	2	**12**
Birth Asphyxia, Sepsis	1	0	**1**
Sepsis	5	0	**5**
Sepsis, RDS	1	0	**1**
Total	**23**	**23**	**46**

Of the 46 enrolled neonates in this case control study, birth asphyxia is a main component for admission in the cases group, while TTNB and RDS were the main component for admission in the controls group. Also least common cause for admission to NICU is sepsis in both cases and controls.

Table 16: Comparison of vitals between the study group

Variables	Group		p-value
	Cases	Controls	
	Mean±SD		
Heart Rate	150.70±12.56	149.96±12.17	0,840
Respiratory rate	53.00±9.08	48.04±2.90	0,017
SPO2	97.08±3.71	97.69±1.69	0,478

The average heart rate of the neonates in the cases were 150.70±12.56/min and in the controls were 149.96±12.17/min

The average respiratory rate of the neonates were 53.00±9.08/min in cases group and 48.04±2.90/min in the controls group.

The average SPO2 of the neonates was 97.08±3.71% in the cases group and 97.69±1.69% in the controls group.

Table 17: Distribution of Capillary refill time(CRT)

Group	CRT		Total (%)
	<3 Sec	>3sec	
	Number (%)		
Cases	20(87.0)	3(13.0)	23(50)
Controls	23(100)	0(0)	23(50)
Total	**43(93.5)**	**3(6.5)**	**46(100)**

Out of 23 neonates in the cases group, 20(87.0%) had Capillary refill time less than 3 seconds while 3 neonates had CRT greater than 3 seconds. None in the control group had CRT greater than 3 seconds.

Figure 14: Distribution of Capillary refill time(CRT)

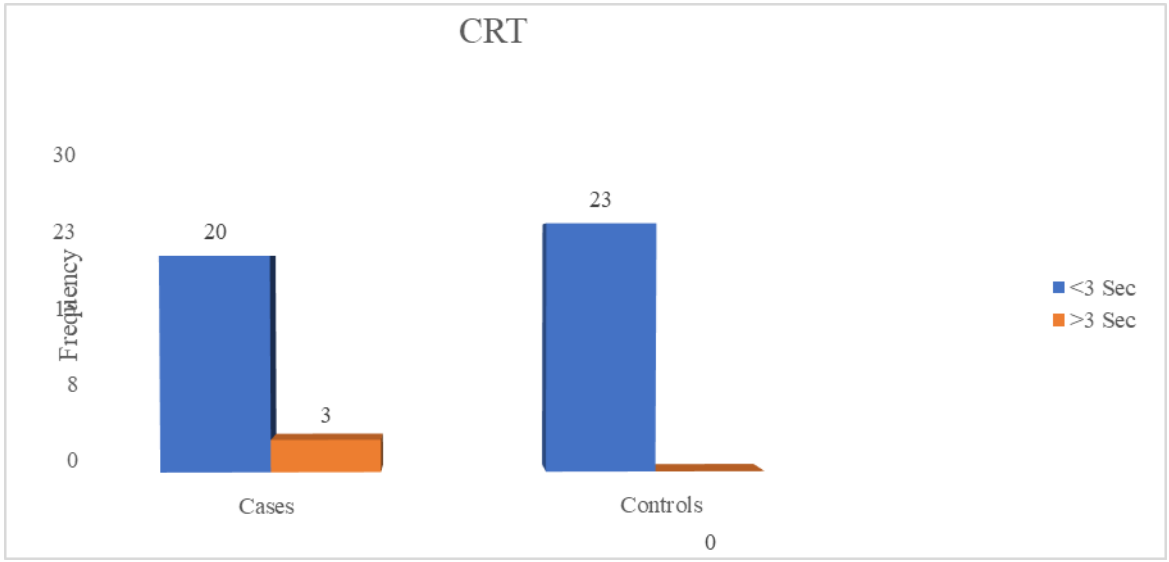


Table 18: Distribution of Anterior Fontanelle(AF)

Group	AF		Total (%)
	At normal level	Bulged	
	Number (%)		
Cases	22(95.7)	1(4.3)	23(50)
Controls	23(100)	0(0)	23(50)
Total	**45(97.8)**	**1(2.2)**	**46(100)**

Only one neonate had bulged anterior fontanelle at the time of admission while the rest 22 neonates had AF at normal level in the cases group. All the neonates(23) in the controls group had normal anterior fontanelle at the time of admission.

Figure 15: Distribution of Anterior Fontanelle(AF)

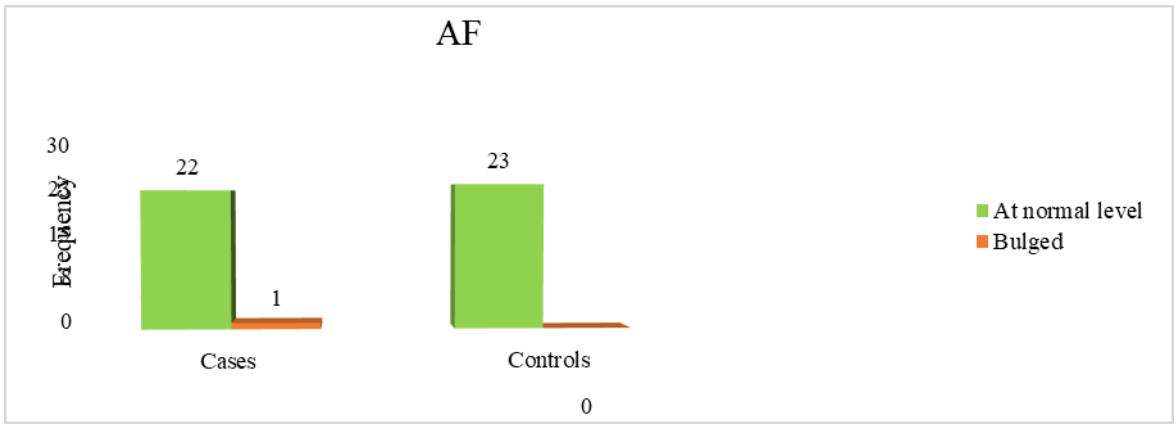


Table 19: Distribution of Blood group of neonates.

Blood group(baby)	Group		Total
	Cases	Controls	
	Number (%)		
A+	8	3	11
AB+	2	3	5
B+	**10**	**8**	**18**
O+	**3**	**9**	**12**
Total	**23**	**23**	**46**

A total of 46 enrolled neonates, majority had B positive blood group(10) in the cases group, while majority of the controls group mothers had O positive blood group(9). While in both case and control groups least common blood group was AB positive(0).

Figure 16: Distribution of Blood group of neonates.

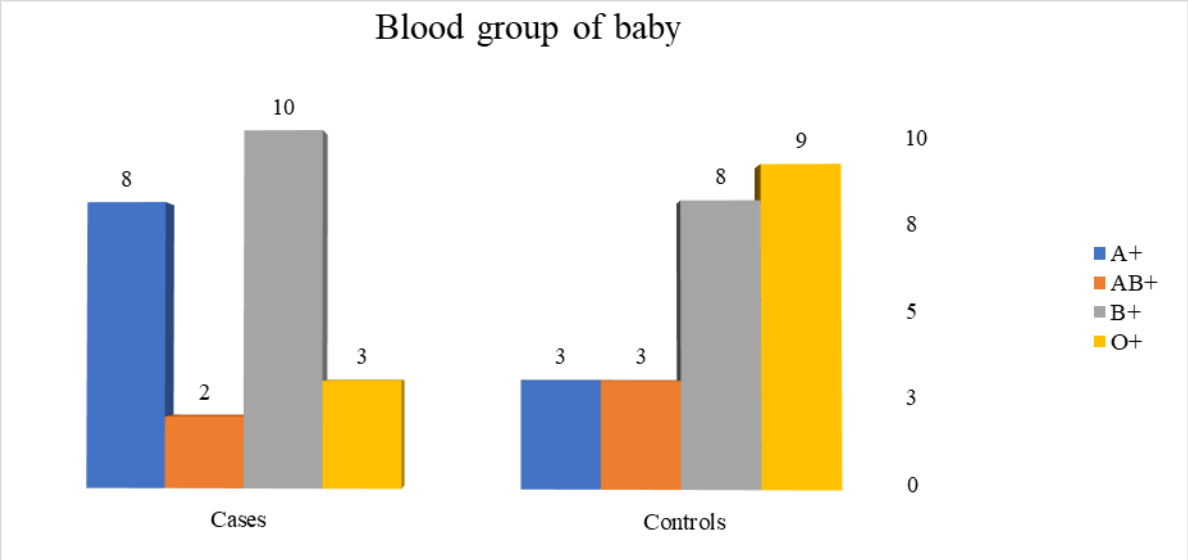


Table 20: Distribution of CRP levels

Group	CRP level(mg/L)		Total (%)
	< 20	>20	
	Number (%)		
Cases	5(21.7)	18(78.3)	23(50)
Controls	23(100)	0(0)	23(50)
Total	**28(60.9)**	**18(39.1)**	**46(100)**

Of the 46 enrolled neonates, all 23 neonates in the controls group had CRP levels below <20 mg/L. Neonates among the cases group 5(21.7%) had CRP level <20mg/L and 18(78.3%) had CRP level >20 mg/L.

Figure 17: Distribution of CRP levels

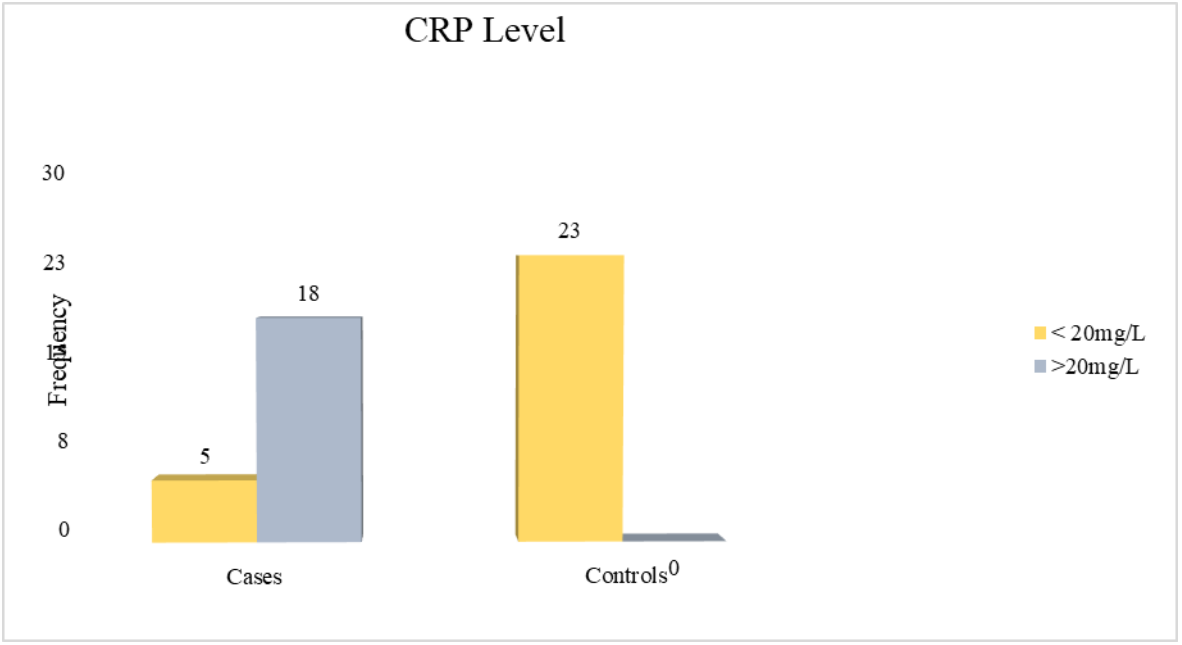


Table 21: Comparison of blood values between the study group

Variables	Group		p-value
	Cases	Controls	
	Mean±SD		
GRBS	106.17±26.46	90.30±14.77	0,016
HB	15.59±3.65	16.27±2.60	0,470
PCV	47.64±11.15	49.47±8.40	0,535
MPV	10.55±0.99	9.53±1.06	0,002
TC	17764.35±9169.79	12499.48±5812.34	0,042
Platelet count	205195.65±126500.51	205195.65±68208.556	0,323

There was a significant difference in random blood sugar levels (GRBS) between the study groups, with neonates in the cases group showing higher GRBS values compared to the controls group(p = 0.016). Additionally, mean platelet volume (p=0.002) and total count (p=0.042) were statistically significant.

Table 22: Mode of respiratory support

Mode of respiratory support at the time of admission	Group		Total
	Cases	Controls	
	Number (%)		
HOOD O_2	4(17.4)	12(52.2)	16(34.8)
HFNC	8(34.8)	7(30.4)	15(32.6)
Conventional ventilator	8(34.8)	2(8.7)	10(21.6)
CPAP	3(13.0)	1(4.3)	4(8.7)
NPO_2	0(0)	1(4.3)	1(2.2)
Total	**23(100)**	**23(100)**	**46(100)**

Among the 23 individuals in the cases group, 4(17.4%) were put on HOOD O_2, 8 (34.8%) were connected to a conventional ventilator, another 8 (34.8%) were put on HFNC and 3 (13.0%) were connected to CPAP. In the controls group, 1 (4.3%) received NP O_2, 12 (52.2%) were put on HOOD O_2, 1 (4.3%) required CPAP, 7 (30.4%) were on HFNC, and 2 (8.7%) were connected to a conventional ventilator. Overall, out of the 46 participants, HOOD O_2 was the most common mode of respiratory support (16 cases), followed by HFNC (15 cases), conventional ventilator (10 cases), CPAP (4 cases), and NP O_2 (1 case).

Table 23: Distribution of Antibiotics used before the blood culture

Antibiotics used before culture	Cases	Controls	Total
Amikacin, Piperacillin and Tazobactum	9	10	19
Cefotaxime, Piperacillin and Tazobactum	1	6	7
Cefotaxime and Amikacin	1	5	6
Meropenem, Vancomycin	5	0	5
Piperacillin and Tazobactum	4	0	4
Amikacin, Piperacillin and Tazobactum, Amphotericin B	1	0	1
Meropenem, Vancomycin, Fluconazole	1	0	1
Tigecycline and linezolid	1	0	1
Total	23	21	44

The major antibiotics used before the blood culture were amikacin, piperacillin, and tazobactam in both the cases and controls group, while the least commonly used antibiotics before blood culture reports were tigecycline and linezolid in both cases and controls group. Antifungals like Amphotericin B and Fluconazole were rarely started before the culture reports.

Table 24: Distribution of Blood culture reports

Blood Culture	Cases	Controls	Total
Acinetobacter baumannii	1	0	1
Coagulase negative staphylococci	3	0	3
E coli	2	0	2
Klebsiella pneumonia	1	0	1
MRSA	5	0	5
Pseudomonas aeruginosa	1	0	1
Serratia marcescens	1	0	1
Staph aureus	1	0	1
Staphylococcus epidermidis	1	0	1
Sterile	7	23	28
Total	**23**	**23**	**46**

Of the 46 enrolled neonates the most commonly isolated organisms from the blood culture report of neonates in the cases group were MRSA(5), coagulase-negative staphylococci(3) and E. coli(2), while 7 neonates in the cases group were sterile. In the controls group, all 23 neonates had sterile blood culture report .

Table 25: Distribution of Antibiotics used after the blood culture

Antibiotics after culture	Cases	Controls	Total
Piperacillin and Tazobactum	4	14	18
Meropenem, Vancomycin	11	1	11
Cefotaxime, Amikacin	1	5	6
Tigecycline, Colistin	5	0	5
Amikacin, Piperacillin and Tazobactum	1	1	2
Colistin, Vancomycin	1	0	1
Total	**23**	**21**	**44**

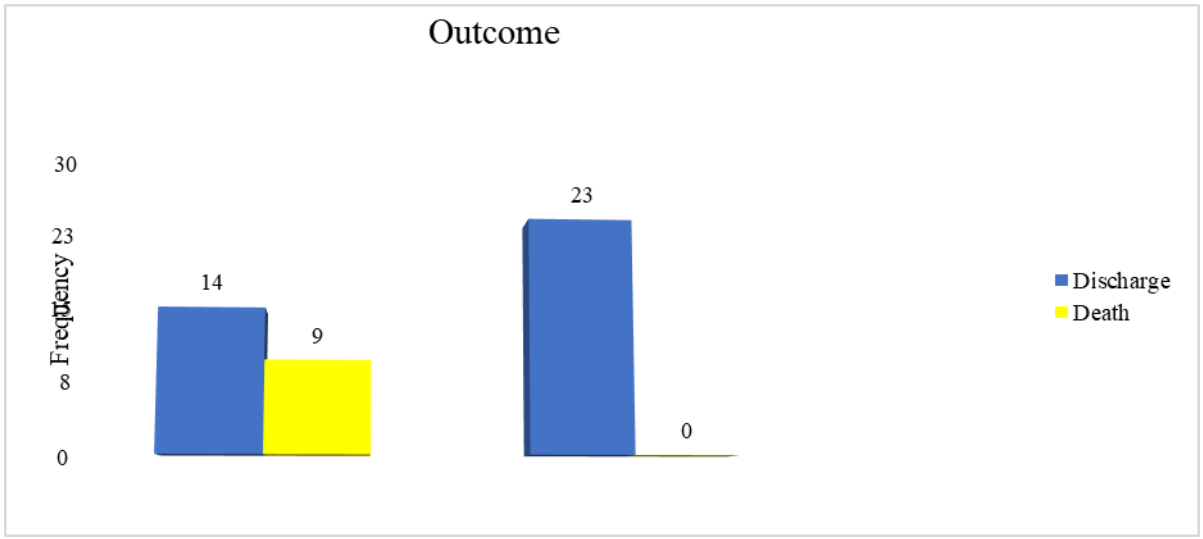
The major antibiotics used after the blood culture were meropenem and vancomycin in the cases while in the controls group piperacillin and tazobactum were commonly used. The least commonly used antibiotics after blood culture reports were tigecycline and colistin in the controls group, while cefotaxime and amikacin were rarely used in the cases group.

Table 26: Distribution of Outcome of the neonates

Group	Out come		Total (%)
	Discharge	Death	
	Number (%)		
Cases	14(60.9)	9(39.1)	23(50)
Controls	23(100)	0(0)	23(50)
Total	**37(80.4)**	**9(19.6)**	**46(100)**

Of the 46 enrolled neonates, 9 neonates in the cases group died, and 14 were discharged. No death occurred in the control group. The average NICU stay of the neonates in the cases group was 9.83±6.59 days and in the controls group neonates 4.91±4.1 days

Figure 18: Distribution of Outcome of the neonates



Observation of the Result:

In this study, a comparison with cases and controls, yielded results as follows: The expression of miRNA23b gene was slightly higher in controls than cases (7.5 fold expression seen in the controls, while only 6.52 fold expression was seen in cases).

SEPSIS							
Sl No	Case No.	Condition	MiRNA 23b	U6			
			Avg. Cq	Avg. Cq	DCq	DDCq	2^ - DDCq
1	1	Case	26.17	16.65	9.52	4.179524	0.055187
2	2	Case	27.87	16.303	11.567	6.226524	0.013355
3	3	Case	27.02	14.02	13	7.659524	0.004946
4	4	Case	29.25	24.48	4.77	-0.57048	1.485014
5	5	Case	28.52	25.02	3.5	-1.84048	3.581282
6	6	Case	30.26	27.02	3.24	-2.10048	4.288509
7	7	Case	31.88	27.35	4.53	-0.81048	1.75379
8	8	Case	29.02	26.03	2.99	-2.35048	5.099926
9	9	Case	28.05	23.02	5.03	-0.31048	1.240117
10	10	Case	24.02	20.06	3.96	-1.38048	2.603543
11	11	Case	28.95	17.81	11.14	-6.01375	64.61289
12	12	Case	25.06	23.02	2.04	-0.98857	1.984217
13	13	Case	24.54	21.52	3.02	-1.38757	2.616376
14	14	Case	28.64	23.22	5.42	-0.95857	1.943385
15	15	Case	29.34	24.34	5	-1.37857	2.600108
16	16	Case	31.36	31.14	0.22	-6.15857	71.43561
17	17	Case	27.04	24.03	1.37	-5.00857	32.19069
18	18	Case	33.68	27.7	5.98	-0.39857	1.318202
19	19	Case	28.43	22.69	5.74	-0.63857	1.556787
20	20	Case	28.97	26.03	2.98	-3.39857	10.54562
21	21	Case	26.63	22.54	4.09	-2.28857	4.885721
22	22	Case	28.36	20.33	8.03	1.651429	0.318325
23	23	Case	27.98	24.23	3.75	0.188571	0.877474
Control							

1	1	Control	33.77	21.59	12.18	6.839524	0.008732
2	2	Control	31.69	27.63	4.06	-1.28048	2.429191
3	3	Control	32.87	24.02	8.85	3.509524	0.087807
4	4	Control	31.19	18.36	12.83	7.489524	0.005565
5	5	Control	29.94	26.72	3.22	-2.12048	4.348374
6	6	Control	29.95	26.13	3.82	-1.52048	2.868857
7	7	Control	27.06	24.33	2.73	-2.61048	6.107052
8	8	Control	36.91	26.97	9.94	4.599524	0.041248
9	9	Control	27.26	24.18	3.08	-2.5475	5.846203
10	10	Control	28.42	24.4	4.02	-1.6075	3.047233
11	11	Control	27.84	17.76	10.08	4.4525	0.045673
12	12	Control	25.35	21.59	3.76	5.801429	0.017931
13	13	Control	29.05	27.63	1.42	-2.31857	4.98838
14	14	Control	32.87	24.02	8.85	2.471429	0.180313
15	15	Control	31.19	18.36	12.83	6.451429	0.011427
16	16	Control	29.94	26.72	3.22	-3.15857	8.929451
17	17	Control	32.31	28.03	4.28	3.561429	0.084704
18	18	Control	33.84	31.33	2.51	-3.86857	14.60683
19	19	Control	32.9	23.08	9.82	3.441429	0.092051
20	20	Control	30.92	24.9	6.02	-0.35857	1.282156
21	21	Control	27.07	23.35	3.72	-2.65857	6.314075
22	22	Control	29.53	25.26	4.27	-2.10857	4.31264
23	23	Control	32.9	23.08	9.82	1.9	0.267943

Table 27. Observation of the results among the cases and controls

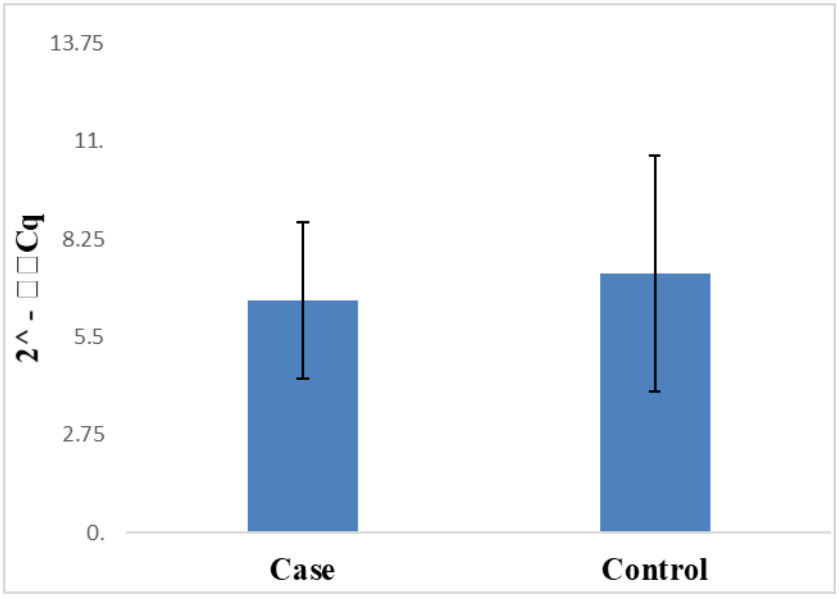


Figure 19: Observation of the results among the case and control

Note:

Samples	Average	SD
Cases	6.520181168	2.2
Control	7.284317	3.3

Table 28: Comparison of miRNA values

Variables	Group				p-value
	Cases		Controls		
	Mean ±SD	Median (Q1, Q3)	Mean± SD	Median (Q1, Q3)	
Avg cq-MiRNA 23b	28.30 ±2.26	28.43(27.02,29.25)	30.64±2.75	30.92(28.42, 32.87)	**0,003**
Avg Cq-U6	22.98 ±4.06	23.22(20.33,26.03)	24.33±3.31	24.33(23.08, 26.72)	**0,226**
ΔCq	5.25± 3.30	4.53(3.02,5.98)	6.31±3.71	4.27(3.22,9.82)	**0,489**
ΔΔCq	-0.78± 3.31	-0.98(-2.28,-0.31)	1.05±3.73	-0.35(-2.31,4.45)	**0,328**
2^(-ΔΔCq)	9.43± 19.65	1.98(1.24,4.88)	2.86±3.71	1.28(0.04,4.98)	**0,328**

The mean and median Avg cq-MiRNA 23b values in the case group were 28.30**±**2.26 and 28.43(27.02,29.25), respectively while in the control group the values were 30.64**±**2.75 and 30.92(28.42,32.87). The results showed a statistically significant difference in the Avg cq-MiR 23b values between the study group(p = 0.003). Control group showed higher values as compared to cases.

The Avg Cq-U6, ΔCq, ΔΔCq, and 2^(-ΔΔCq) values did not show any statistically significant difference between the groups (These are control genes used for comparison).

DISCUSSION

Neonatal sepsis is a serious condition with high morbidity and mortality rate, where door to diagnosis becomes a key factor in the treatment of sepsis. The management of neonatal sepsis should be a wholistic approach, which includes treatment with anti-inflammatory and anti microbial approach. Hence it is of a substantial importance to diagnose the inflammatory cascade and target the particular markers in treating the neonate as a whole. Determining the value of miRNA in the serum becomes a necessary prognosticating and diagnostic marker. In this research miRNA-23b as a biomarker for neonatal sepsis through haemoculture broth was assessed.

Distribution of Parity

Of the 46 enrolled mothers, the Primigravida mothers constituted 52.2% while the Multigravida mothers constituted around 47.8%. The calculated p value is 1.0

A similar study by Katta et al. in 2024 evaluated the sensitivity levels of miRNA-181a, miRNA-23b and miRNA-16 in late-onset neonatal sepsis. The researchers established a diagnostic accuracy rate with miRNA-23b showing clear decreases in sepsis patients achieving an area under the curve of 0.92. The diagnostic potential of miRNA-23b strengthens further because this marker displayed a sensitivity rate of 98% among cases of neonatal sepsis Findings from this study confirmed that sepsis is primarily affecting the term neonates, while it didn't have any significance to the parity of the mother.[46]

Distribution of history of PROM.

Among the total 46 mothers, 14(30.4%) had a history of PROM and 32(69.2%) did not have PROM. In the cases group 11(47.8%) had history of PROM and in the controls group 2(13%) had a history of PROM. The calculated p value is approximately 0.01 which indicates a statistical significance in the mothers who had PROM history, were more prone to sepsis.

Distribution of mode of delivery

In this study out of the total 46 neonates, 22 babies(47.8%) were delivered via normal vaginal delivery, and 24(52.2%) were delivered through Lower Segment Cesarean Section(LSCS). In both cases and control 11(47.8%) were normal delivery, while 12 (52.2%) were delivered via LSCS. Sepsis didn't show much preponderance to the mode of delivery in these enrolled neonates.

Distribution of blood group of mothers and neonates.

A total of 46 enrolled mothers, majority had B positive blood group(8) in the cases group, while majority of the control group mothers had O positive blood group(11). While in both case and control groups least common blood group was AB positive(0).

While that of the neonatal blood group most were B positive and least common blood group was AB positive, which is an incidental finding and is not significant with sepsis.

Distribution of place of delivery.

In this study, a total 46 neonates, 24(52.2%) were Inborn, and 22(47.8%) were Outborn neonates. Amongst the cases, 9 neonates were Inborn and 14 were Outborn. In control group, 15 were Inborn and 8 were Outborn neonates.

The chi square test gives a p value of approximately 0.08. This result is not statistically significant, suggesting that there is a trend in the distribution of inborn versus outborn neonates but the difference is not statistically significant.

Distribution of birth details of the neonates.

The average birth weight of the neonates in the cases were 2.48±0.56kg and in the controls were 2.61±0.59kg, showing not much significance in the weight distribution and predisposition to sepsis.

The average head circumference and length of the neonates remains insignificant for the predisposition to sepsis.

A study by Eva Serna et al found that of 11 late-onset Gram-positive sepsis affected very low birth-weight neonates together with 16 control participants. A total of 217 microRNAs displayed differential expression between the two groups. The researchers conducted a combined analysis which integrated these selected miRNAs with 4297 differentially expressed genes.[47]

A similar study by Fathima et al found that miR-23b expression levels rose significantly in premature and full-term newborns with early onset sepsis ($p < 0.001$ and $p < 0.005$ respectively) while this miRNA showed decreased levels in late onset sepsis for full-term newborns ($p < 0.05$) compared to their respective negative control groups. The research also revealed that newborns suffering from both EOS and LOS presented a strong negative correlation with miRNA 23b and death during EOS based on a correlation coefficient value of -0.96 and p value of 0.0019. The analysis showed that increased miRNA-23b levels had a positive correlation with survival of LOS (correlation coefficient = 0.70, $p = 0.506$).[48]

Distribution of gender

Among the 23 neonates in the cases group, 8 (34.8%) were female, and 15 (65.2%) were male, indicating a higher proportion of males. Among the 23 neonates in the control group, 9 (39.1%) were female, and 14 (60.9%) were male, showing a similar male dominance. Total Distribution: Across both groups, 17 (37.0%) were female, and 29 (63.0%) were male, the p value of approximately 0.76 indicates there is no statistically significant difference in the distribution of gender between the cases and controls.

Distribution of neonates that were febrile.

In this study only 1 neonate was febrile at the time of admission, while the rest 45 neonates were afebrile. Fisher exact test was used to determine the p value which was

1.0 which showed no significance difference in distribution of febrile neonates between the cases and controls.

Distribution of indication of admission.

Birth asphyxia and sepsis together with respiratory distress syndrome (RDS) (4) stood as the primary reasons for admission among the cases while in controls Transient tachypnea of newborns and Respiratory Distress were main reasons for admission.

Birth asphyxia together with sepsis emerged as the predominant factors leading to hospitalisation of the neonates in the intensive care unit according to research by Patel et al. (2019)[49] The findings from Gupta et al. (2021) showed TTNB and RDS appearing often as non-septic neonatal hospitalisation reasons.[50]

Comparison of vitals between the study group.

The respiratory rate is the only variable showing a significant difference between cases and controls, suggesting a possible association with the studied condition. P value of < 0.02, indicating the significance of increased respiratory rate in cases group. Heart rate and SPO_2 levels do not show significant variation, implying no major difference between the groups for these parameters.

Distribution of capillary refill time.

Of the 46 enrolled neonates, only 3 cases had significant increase in capillary refill time. Although there is no statistical significance.

Distribution of Anterior fontanelle.

In this study only 1 neonate had a bulging anterior fontanelle, while the rest 45 neonates had normal anterior fontanelle.

Distribution of CRP levels.

All 23 neonates enrolled for the cases group were CRP positive >20, while the control group were CRP negative <20.

Comparison of blood values between the study group.

BLOOD PARAMETERS	VALUES (CASES)	VALUES (CONTROLS)	P VALUE
GRBS	106.17 ± 26.46	90.30 ± 14.77	0,016
Haemoglobin	15.59 ± 3.65	16.27 ± 2.60	0,470
PCV(packed cell volume)	47.64 ± 11.15	49.47 ± 8.40	0,535
MPV(mean platelet volume)	10.55 ± 0.99	9.53 ± 1.06	0,002
TC(total count)	17764.35 ± 9169.79	12499.48 ± 5812.34	0,042
mean platelet count	205195,65	205195,65	0,323

Table 29: Discussion of blood values between the study group

GRBS, MPV, and TC show significant differences between cases and controls, indicating a possible association with the study condition. HB, PCV, and Platelet Count do not show significant differences, suggesting no major variation between the groups for these parameters.

Distribution of Primary mode of respiratory support.

In our study, the primary mode of respiratory support for the cases were conventional ventilator 8(34.8%) and 8(34.8%) HFNC while the controls group, HOOD O2 12(52.2%) was the most commonly used primary mode of respiratory support. This research signifies that sepsis patients require aggressive invasive respiratory support.

Distribution of Antibiotics used before and after the blood culture.

Most commonly used antibiotics were cefotaxime and amikacin for inborn and outborn neonates were piperacillin-tazobactum and amikacin. Based on the blood culture antibiotics were escalated to meropenem and vancomycin, rarely tigecyclin and colistin. Very rarely antifungals were used in this study.

Distribution of Blood culture reports.

The main pathogens causing sepsis in the neonates included Methicillin-Resistant Staphylococcus Aureus (MRSA) in 5 cases, coagulase-negative staphylococci (CoNS) in 3 cases, with Escherichia coli (E. coli) in 3 cases. 7 out of 23 neonates in the sepsis group yielded sterile blood culture results while all 23 control group neonates obtained sterile results.

The results in the South Indian study by Lakshmi et al done in Tertiary Care Hospital of South India, revealed a different bacterial pattern than this study, here MRSA was the dominant organism.[51] An 8-year retrospective study by Jin Z et al analyzed clinical laboratory features of pathogen distribution in early and late-onset sepsis among newborns. The research identified gram-positive cocci as the most frequent isolated bacterial group because CONS strains dominated at 63.9% of 864 strains tested. Among 19.8% of isolated pathogens Gram-negative bacilli predominated alongside E. coli at 10.8% and followed by Klebsiella pneumoniae at 3.8%. The reported cases of Staphylococcus aureus represented 2.8% while the study neglected to mention the specific prevalence of MRSA. The reported prevalence of MRSA stands as a notable difference between findings in this study. [52]

Distribution of Outcome of the patients.

Of the 46 neonates enrolled in this study, cases group showed significant mortality, 9 neonates succumbed to sepsis and a total of 14 neonates survived in sepsis group and were discharged from the hospital with mild to moderate Neurodevelopmental sequelae. All neonates in the control group survived without experiencing any mortality or morbidity. The NICU treatment period averaged 9.83 ± 6.59 days in the sepsis patients while the control participants stayed an average of 4.91 ± 4.1 days.

A study by Mackay CA, et al[53] which examined neonatal sepsis outcomes in an Australian tertiary NICU observed that late onset sepsis increased both patient mortality rates as well as NICU lengths of stay. The study determined that infants experiencing LOS needed care in the NICU for 101 days for premature birth before 28 weeks gestation and 49 days for 28 to 31 + 6 weeks gestation. The death rate and NICU stay length increased among babies born too early at less than 37 weeks gestational age and those infected with gram-negative bacteria.

Comparison of miRNA values.

In this study, the mean and median Avg cq-MiR 23b values in the case group were 28.30±2.26 and 28.43(27.02,29.25), respectively while in the control group the values were 30.64±2.75 and 30.92(28.42,32.87). The results showed a statistically significant difference in the Avg cq-MiR 23b values between the **study group (p = 0.003)**. Control group showed higher values as compared to cases.

The Avg Cq-U6, ΔCq, ΔΔCq, and 2^(-ΔΔCq) were the control genes used to compare with Avg cq- MiR 23b.

The diagnostic potential of miRNA-23b received investigation through two research studies specifically focused on early-onset sepsis (EOS) and late-onset sepsis (LOS).

The research conducted by Fatmi et al. (2020)[48] showed that the miRNA-23b expression values in sepsis-positive newborns among EOS and LOS subgroups. Author determined the miRNA-23b levels in haemoculture samples by conducting quantitative real-time polymerase chain reaction (qRT-PCR.)[48]

Results suggest that miRNA-23b influences how the immune system reacts early during sepsis based conditions. Studies found decreased miRNA-23b levels during sepsis situations. Such decreased expression levels appear to indicate distinct immunological processes.

Another similar prospective study by Katta et al. (2024)[46] investigated miRNA-23b expression measurements alongside other miRNAs together with their diagnostic ability in identifying LOS. The researcher collected 100 samples from a combined group of 50 patients with uniquely identified LOS and 50 healthy participants. They observed the similar miRNA-23b expression pattern identified by Fatmi et al. in LOS as they documented decreased miRNA-23b levels in sepsis neonates against control neonates. The diagnostic performance of miRNA-23b achieved exceptional results based on the receiver operating characteristic curve analysis which produced an area under the curve value of 0.92. The high sensitivity value of 98% makes miRNA-23b suitable for use as an extremely sensitive biomarker for LOS.[48]

STRENGTHS

1. This paper investigates miRNA-23b as a diagnostic marker for evaluating neonatal sepsis while solidifying the evidence of microRNAs as a significant diagnostic tool.
2. The study incorporates case and control groups to establish a detailed comparison of miRNA-23b expression together with their relationship to neonatal sepsis.
3. The qRT-PCR molecular marker determines miRNA expression accurately which makes the assessment of results more dependable.
4. This research identifies harmful bacterial microbes including MRSA, CoNS and E. coli to determine the common pathogen responsible for neonatal sepsis for the given NICU.
5. The research gap is filled by reporting healthcare outcomes of NICU hospital stays and mortality, which demonstrates the disease severity, connection to miRNA-23b expression levels.
6. The study used solid statistical methods such as ΔCq analysis and p-values which strengthened the validity of the reported results.
7. This research evaluates miRNA-23b as a new diagnostic indicator to enhance available testing methods.
8. The study measures miRNA levels and correlates findings between the results and NICU stay as well as the morbidity and mortality of that individual.
9.There is a chance to diagnose sepsis in individuals even before the onset of symptoms and hence will have an upper hand in diagnosis and appropriate treatment which would markedly reduce the mortality in neonates.
10. The research confirms a possible efficient procedure to evaluate miRNAs by using Haemoculture Broth thus opening new opportunities for laboratory investigations and diagnostic applications.
11. The blood culture of few neonates yielded to be sterile, while other sepsis parameters were positive in the case group, which is definitely misleading and would culminate to under diagnosis of sepsis in neonates.

LIMITATIONS

- Most important limitation of this study was small sample size(n).
- The procedure to obtain and isolate miRNA 23b is cumbersome and slightly expensive.
- The single institution setting where research took place restricts the ability to represent diverse population groups.
- The diagnostic approach would be stronger with a broad miRNA panel than using isolated analysis of miRNA-23b due to restricted examination.

RECOMMENDATIONS

- Future studies are needed with larger sample size, as they will enhance statistical significance while validating research findings.
- The diagnostic accuracy can be enhanced through a combination of miRNA-23b with other miRNAs alongside diagnostic inflammatory markers including CRP and PCT and IL-6.
- Researching the Neurodevelopmental progression of neonates who suffer from sepsis will allow us to understand how miRNA expression influences the neurological outcome of the babies.
- Next-generation sepsis diagnostic methods can become more efficient when miRNA-23b is included as part of standard protocol algorithms.

CONCLUSION

From the present study, it can be inferred that miRNA-23b is a promising diagnostic biomarker for neonatal sepsis. Its significant altered expression in sepsis neonates could facilitate prompt treatment and improved clinical outcome.

However further research with larger cohorts and multi centre collaborations is essential for routine clinical implementations.

SUMMARY

- The purpose of this research was to assess the utility of miRNA-23b as a biomarker that could help diagnose neonatal sepsis at its early stages.
- This research discovered significant differences in miRNA-23b expression patterns between septic newborns and healthy control subjects making it suitable for diagnostic purposes.
- A low expression level of miRNA-23b correlated directly with higher inflammatory activity.
- The most frequently detected microbial pathogens included Methicillin resistant Staphylococcus aureus (MRSA), Coagulase-negative Staphylococci (CoNS) and with Escherichia coli (E. coli).
- Some neonates in the cases had sterile blood culture results, although the neonates showed CRP positivity and clinically sepsis screen was positive.
- Levels of expression of miRNA 23b when reduced is suggestive of bad prognosis and severe inflammatory cascade activation in the neonate that could lead to mortality and morbidity of the neonate.
- The neonates in cases group had an increased mortality rate and on an average spent about 9.83 ±6.59 days in the NICU compared to healthy controls, due to the serious nature of their condition.
- Future clinical practice could use miRNA-23b as an early warning indicator for neonatal sepsis to enable improved treatment interventions. The introduction of this diagnostic and prognostic tool needs additional validation through testing it across multiple research sites before being approved for clinical use.

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ANNEXURE VI

RESEARCH INFORMED CONSENT FORM

BLDE(DEEMED TO BE UNIVERSITY) Shri B.M PATIL Medical

College, Hospital & Research Centre,

Vijayapur-586103.

TITLE OF THE PROJECT: TO STUDY THE SIGNIFICANCE OF MiRNA 23 B AS A MARKER FOR NEONATAL SEPSIS

GUIDE : DR S.S KALYANSHETTAR
PROFESSOR
DEPARTMENT OF PEDIATRICS.

PG STUDENT : DR RUCHI R

RISK AND DISCOMFORTS:

None

BENEFITS:

I understand that my participation in the study will have no direct benefit to me other than the potential benefit of the research and education.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality. Information of sensitive personal nature will not be part of the medical record, but will be stored in the investigations research file.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission. I understand that I may see the photograph before giving the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time; Dr.RUCHI R at the department of Paediatrics is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. S S KALYANSHETTAR may terminate my participation in the study after he has explained the reasons for doing so.

Parents/ Guardians Name:_____

Relationship to the baby: _____

Signature/Thumb impression:_____

Date:_____

Investigator's Name:_____

Signature:_____

Date: _____

INJURY STATEMENT:

I understand that in the unlikely event of injury to child resulting directly from child's participation in this study, if such injury were reported promptly, the appropriate treatment would be available to the child. But no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to _____the purpose of the research,

the procedures required and the possible risks to the best of my ability.

_____ _____

Dr.RUCHI R Date

(Investigator)

PARENTS / GUARDIAN CONSENT STATEMENT:

We confirm that DR. RUCHI R is doing “TO STUDY THE SIGNIFICANCE OF MiRNA 23 B AS A MARKER FOR NEONATAL SEPSIS.” Dr. RUCHI R, has explained to us the purpose of research and the study procedure. We are willing to give as much as information required for the study and consent for investigations and the possible discomforts as well as benefits. We have been explained all the above in detail in our own language and we understand the same. Therefore, we agree to give consent for child’s participate as a subject in this research project.

_____	_____
(Parents / Guardian)	Date
_____	_____
(Witness to signature)	Date

ಪೋಷಕರು / ಗಾರ್ಡಿಯನ್ ಸಮ್ಮತಿ ಹೇಳಿಕೆ:

ಸಂಶೋಧನಾ ಮಾಹಿತಿ ಒಪ್ಪಂದ ಪತ್ರ

ಬಿಎಲ್ ಡಿ ಈ (ಡೀಮ್ ಟು ಬಿ ಯೂನಿವರ್ಸಿಟಿ)

ಶ್ರೀ ಬಿ. ಎಂ. ಪಾಟೀಲ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ವಿಜಯಪುರ – 586103

ಪ್ರಾಜೆಕ್ಟ್ ಶೀರ್ಷಿಕೆ:

“ನಿಯೋನಟಲ್ ಸೆಪ್ಸಿಸ್ ಗುರುತಿಸಲು miRNA-23b ನ ಮಹತ್ವವನ್ನು ಅಧ್ಯಯನ ಮಾಡುವುದು”

ಮಾರ್ಗದರ್ಶಕ:

ಡಾ. ಎಸ್. ಎಸ್. ಕಲ್ಯಾಣಿಶೆಟ್ಟರ್

ಪ್ರೊಫೆಸರ್ ಮಕ್ಕಳ ವಿಭಾಗ

ಪಿಜಿ ವಿದ್ಯಾರ್ಥಿ:

ಡಾ. ರೂಬಿ ಆರ್

ಅಪಾಯ ಮತ್ತು ಅನಾನುಕೂಲಗಳು:

ಪರೀಕ್ಷೆಯ ಸಮಯದಲ್ಲಿ ಅಥವಾ ನನ್ನ ಚಿಕಿತ್ಸೆಯ ಸಮಯದಲ್ಲಿ ನಾನು ಕೆಲವು ನೋವು ಮತ್ತು ಅಸ್ವಸ್ಥತೆಗಳನ್ನು ಅನುಭವಿಸಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಇದು ಮುಖ್ಯವಾಗಿ ನನ್ನ ಸ್ಥಿತಿಯ ಫಲಿತಾಂಶವಾಗಿದೆ ಮತ್ತು ಈ ಅಧ್ಯಯನದ ಕಾರ್ಯವಿಧಾನಗಳು ಚಿಕಿತ್ಸೆಯ ಸಾಮಾನ್ಯ ಕೋರ್ಸ್‌ಗೆ ಸಂಬಂಧಿಸಿದ ಈ ಭಾವನೆಗಳನ್ನು ಉತ್ಪ್ರೇಕ್ಷಿಸುವುದಿಲ್ಲ ಎಂದು ನಿರೀಕ್ಷಿಸಲಾಗಿದೆ.

ಪ್ರಯೋಜನಗಳು:

ಚಿಕಿತ್ಸೆಯ ಸಂಭಾವ್ಯ ಪ್ರಯೋಜನವನ್ನು ಹೊರತುಪಡಿಸಿ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯು ನನಗೆ ಯಾವುದೇ ನೇರ ಪ್ರಯೋಜನವನ್ನು ಹೊಂದಿಲ್ಲ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಗೌಪ್ಯತೆ:

ಈ ಅಧ್ಯಯನದಿಂದ ಉತ್ಪತ್ತಿಯಾಗುವ ವೈದ್ಯಕೀಯ ಮಾಹಿತಿಯು ಆಸ್ಪತ್ರೆಯ ದಾಖಲೆಗಳ ಭಾಗವಾಗುತ್ತದೆ ಮತ್ತು ಗೌಪ್ಯತೆಗೆ ಒಳಪಟ್ಟಿರುತ್ತದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಸೂಕ್ಷ್ಮ ವೈಯಕ್ತಿಕ ಸ್ವಭಾವದ ಮಾಹಿತಿಯು ವೈದ್ಯಕೀಯ ದಾಖಲೆಯ ಭಾಗವಾಗಿರುವುದಿಲ್ಲ ಆದರೆ ತನಿಖೆಯ ಸಂಶೋಧನಾ ಕಡತದಲ್ಲಿ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ. ಡೇಟಾವನ್ನು ವೈದ್ಯಕೀಯ ಸಾಹಿತ್ಯದಲ್ಲಿ ಅಥವಾ ಬೋಧನಾ ಉದ್ದೇಶಕ್ಕಾಗಿ ಪ್ರಕಟಣೆಗಾಗಿ ಬಳಸಿದರೆ, ಯಾವುದೇ ಹೆಸರನ್ನು ಬಳಸಲಾಗುವುದಿಲ್ಲ ಮತ್ತು ಛಾಯಾಚಿತ್ರಗಳಂತಹ ಇತರ ಗುರುತಿಸುವಿಕೆಗಳನ್ನು ವಿಶೇಷ ಲಿಖಿತ ಅನುಮತಿಯೊಂದಿಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ. ಅನುಮತಿ ನೀಡುವ ಮೊದಲು ನಾನು ಫೋಟೋವನ್ನು ನೋಡಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಹೆಚ್ಚಿನ ಮಾಹಿತಿಗಾಗಿ ವಿನಂತಿ:

ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದ ಕುರಿತು ಹೆಚ್ಚಿನ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ; ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ಅಥವಾ ಕಳವಳಗಳಿಗೆ ಉತ್ತರಿಸಲು ಮಕ್ಕಳ ವಿಭಾಗದಲ್ಲಿರುವ ಡಾ. ರೂಚಿ ಆರ್ ಅಧ್ಯಯನದ ಅವಧಿಯಲ್ಲಿ ಪತ್ತೆಯಾದ ಯಾವುದೇ ಮಹತ್ವದ ಹೊಸ ಸಂಶೋಧನೆಗಳ ಕುರಿತು ನನಗೆ ತಿಳಿಸಲಾಗುವುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ, ಅದು ನನ್ನ ಮುಂದುವರಿದ ಭಾಗವಹಿಸುವಿಕೆಯ ಮೇಲೆ ಪ್ರಭಾವ ಬೀರಬಹುದು. ಈ ಸಮ್ಮತಿ ನಮೂನೆಯ ಪ್ರತಿಯನ್ನು ಎಚ್ಚರಿಕೆಯಿಂದ ಓದುವುದಕ್ಕಾಗಿ ಇರಿಸಿಕೊಳ್ಳಲು ನನಗೆ ನೀಡಲಾಗುವುದು.

ಭಾಗವಹಿಸುವಿಕೆ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆಗೆ ನಿರಾಕರಣೆ:

ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ ಮತ್ತು ನಾನು ಭಾಗವಹಿಸಲು ನಿರಾಕರಿಸಬಹುದು ಅಥವಾ ಒಪ್ಪಿಗೆಯನ್ನು ಹಿಂಪಡೆಯಬಹುದು ಮತ್ತು ಪೂರ್ವಾಗ್ರಹವಿಲ್ಲದೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದನ್ನು ನಿಲ್ಲಿಸಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಡಾ. ಡಾ. ಎಸ್. ಎಸ್. ಕಲ್ಯಾಣಶೆಟ್ಟರ್ ಅವರು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಕಾರಣಗಳನ್ನು ವಿವರಿಸಿದ ನಂತರ ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ಕೊನೆಗೊಳಿಸಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಗಾಯದ ಹೇಳಿಕೆ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಮಗುವಿನ ಭಾಗವಹಿಸುವಿಕೆಯಿಂದ ನೇರವಾಗಿ ನನ್ನ ಮಗುವಿಗೆ ಗಾಯವಾಗುವ ಸಾಧ್ಯತೆಯಿಲ್ಲದ ಸಂದರ್ಭದಲ್ಲಿ, ಅಂತಹ ಗಾಯವನ್ನು ತಕ್ಷಣವೇ ವರದಿ ಮಾಡಿದರೆ, ಮಗುವಿಗೆ ಸೂಕ್ತವಾದ ಚಿಕಿತ್ಸೆಯು ಲಭ್ಯವಿರುತ್ತದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಆದರೆ ಆಸ್ಪತ್ರೆಯಿಂದ ಹೆಚ್ಚಿನ ಪರಿಹಾರ ನೀಡುವುದಿಲ್ಲ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನನ್ನ ಒಪ್ಪಂದಗಳ ಮೂಲಕ ಮತ್ತು ನನ್ನ ಯಾವುದೇ ಕಾನೂನು ಹಕ್ಕುಗಳನ್ನು ಬಿಟ್ಟುಕೊಡುವುದಿಲ್ಲ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ನಾನು

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_____ ಸಂಶೋಧನೆಯ

ಉದ್ದೇಶ, ಅಗತ್ಯವಿರುವ ಕಾರ್ಯವಿಧಾನಗಳು ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯಕ್ಕೆ ಸಾಧ್ಯವಾದಷ್ಟು ಅಪಾಯ

Dr. Ruchi R (Investigator) Date

PROFORMA

I. DEMOGRAPHIC INFORMATION

- Name
- Age
- Gender
- IP no
- Contact no

II. PATIENT INFORMATION

- Date and time of delivery
- Admission date
- Obstetric history
- History of PROM
- Mode of delivery
- Delivered at Inborn/ Outborn
- Birth weight
- Mother blood group
- Baby blood group

- Febrile at the time of admission
- Number of days of NICU stay

III. CLINICAL INFORMATION

- Anthropometry
    - Head circumference
    - Length
- On examination
    - Heart Rate
    - Capillary refilling time
    - Anterior fontanelle
    - SpO2
- Systemic examination
    - Cardiovascular system
    - Respiratory system
    - Per abdomen
    - Central nervous system
- Primary mode of respiratory support

- Outcome (Discharge/ Death)

IV. INVESTIGATIONS ON ADMISSION

- GRBS at the time of admission
- Hemoglobin
- Packed cell volume
- Total count
- Platelet count
- Mean platelet volume
- C Reactive Protein
- Blood culture
- miRNA 23b levels

V. TREATMENT INFORMATION

- Antibiotics before culture report
- Antibiotics after culture report

ETHICAL CLEARANCE CERTIFICATE

Azadi Ka Amrit Mahotsav

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 969/2022-23 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "TO STUDY THE SIGNIFICANCE OF miRNA 23 b AS A MARKER FOR NEONATAL SEPSIS."

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.RUCHI R.

NAME OF THE GUIDE: DR. S.S.KALYANSHETTAR, PROFESSOR AND HOD, DEPT. OF PEDIATRICS.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

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SCHOOL OF APPLIED SCIENCES AND TECHNOLOGY,

GENETICS LABORATORY

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INVESTIGATOR BIODATA

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DOB	:	19/12/1992
QUALIFICATION	:	MBBS
KMC REGISTRATION NO	:	115158
PRESENTLY WORKING AS	:	POST GRADUATE STUDENT/JUNIOR RESIDENT

DEPARTMENT OF PEDIATRICS,

SHRI B M PATIL MEDICAL COLLEGE,

HOSPITAL & RESEARCH CENTRE,

VIJAYAPURA – 586103

MASTER CHART

Nam	Mode	Gend	Histor	Obste	Birth v	Da	Date (	Indica	GBS	Blood	CRT	Feb	Hea	Re	SpC	CRP	AF	Antil	No of c	MR	Outc	Infor	Moth	Bab	Place	Hea	Lengt	CVS	RS	PA	CNS	Mod	HB	PC	TC	PLA	MP	Antibiotics used after cu
Bloh	LSC‡	Fema	No	G4P3	3.3kg	#	####	RDS	104	Negati	< 3 se	no	116	48	####	<5.0	At Lev	Amil	14 days		Discharge	B	PO	O	Pl	Outbo	32cı	54cm	s1 s:	BIL	Soft	CITA	g	CDI	15	#	##	##	9.3	Piperacillin and Tazobac
BIO	LSC‡	Male	Yes	G2A1	1.5kg	#	####	TTNB	98	Sterile	< 3 se	no	186	45	####	<5.0	At Lev	Amil	3-4 days		Discharge	O	PC	O	Pl	Inbori	32cı	50cm	s1 s:	BIL	Soft	CITA	g	HFI	16	#	##	##	9.7	Vancomycin, Meropener
BIO	Nvd ‡	Male	No	G3P2	1.5kg	#	####	RDS	106	Sterile	< 3 se	no	148	45	####	5.6	At Lev	Amil	4 days		Discharge	O	PC	O	Pl	Inbori	30cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	20	#	##	##	9.4	Amikacin, Piperacillin ar
BIO	Nvd ‡	Fema	No	Primi	2.2kg	#	####	RDS	106	Sterile	< 3 se	no	170	50	####	7.2	At Lev	Amil	2 days		Discharge	A	PO	O	Pl	Outbo	32cı	56cm	s1 s:	BIL	Soft	CITA	g	HFI	13	#	##	##	10	Piperacillin and Tazobac
BIO	Nvd ‡	Fema	No	Primi	2.5kg	#	####	TTNB	86	Sterile	< 3 se	no	146	55	####	<5	At Lev	Amil	3 days		Discharge	O	PC	O	Pl	Outbo	33.5	52cm	s1 s:	BIL	Soft	CITA	g	HOI	14	#	##	##	9.9	Piperacillin and Tazobac
BIO	LSC‡	Male	No	Primi	3.42kg	#	####	TTNB	112	Sterile	< 3 se	no	142	50	####	<5	At Lev	Cefc	2 days		Discharge	B	PO	B	Pl	Inbori	35cı	52cm	s1 s:	BIL	Soft	CITA	g	NP	16	#	##	##	10	PIPeracillin and Tazobac
Blo	LSC‡	Male	No	G3P2	2.9kg	#	####	RDS	68	Sterile	< 3 se	no	146	50	####	<5	At Lev	Amil	3 days		Discharge	AB	N	A	Pl	Outbo	36cı	48cm	s1 s:	BIL	Soft	CITA	g	HFI	15	#	##	##	10	Piperacillin and Tazobac
BIO	Vagin	Fema	Yes	G3P1	3.7KG	#	####	RDS	94	STERI	< 3 se	no	149	48	####	19.1	At Lev	Amil	11 DAYS		DISCHAR	O	PC	O	Pl	Outbo	32cı	54cm	s1 s:	BIL	Soft	CITA	g	CDI	16	#	##	##	10	Piperacillin and Tazobac
BIO	Vagin	Male	No	G2P1	2.4KG	#	####	Birth /	74	STERI	< 3 se	no	141	48	####	17.4	At Lev	Amil	14 DAYS		DISCHAR	AB	N	B	Pl	Outbo	30cı	52cm	s1 s:	BIL	Soft	CITA	g	CPA	19	#	##	##	10	Piperacillin and Tazobac
BIO	Vagin	Fema	No	PRIM	2.6KG	#	####	Birth /	74	STERI	< 3 se	no	152	52	####	17.8	At Lev	Amil	14 DAYS		DISCHAR	O	PC	B	Pl	Outbo	32cı	52cm	s1 s:	BIL	Soft	CITA	g	HFI	20	#	##	##	9.8	Piperacillin and Tazobac
BIO	LSC‡	Fema	No	G3P,	2.375k	#	####	RDS	98	Sterile	< 3 se	no	158	50	####	<5	At Lev	Cefc	3 days		Discharge	B	PO	AB	Pl	Inbori	32cı	50cm	s1 s:	BIL	Soft	CITA	g	HFI	18	#	##	##	10	Piperacillin and Tazobac
BIO	LSC‡	Male	No	Primi	2.6kg	#	####	RDS	96	Sterile	< 3 se	no	148	50	####	10	At Lev	Cefc	3 days		Discharge	O	PC	A	Pl	Inbori	34cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	17	#	##	##	10	Piperacillin and Tazobac
BIO	LSC‡	Male	No	Primi	2.835k	#	####	TTNB	98	Sterile	< 3 se	no	144	50	####	<5	At Lev	Cefc	4 days		Discharge	O	PC	O	Pl	Inbori	33cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	14	#	##	##	11	Piperacillin and Tazobac
BIO	LSC‡	Fema	No	Primi	2.595k	#	####	RDS	92	Sterile	< 3 se	no	148	45	####	<5	At Lev	Cefc	2 days		Discharge	B	PO	B	Pl	Inbori	32cı	50cm	s1 s:	BIL	Soft	CITA	g	HOI	15	#	##	##	11	Piperacillin and Tazobac
BIO	LSC‡	Male	No	G2p1	2.3kg	#	####	RDS	93	Sterile	< 3 se	no	148	50	####	<5	At Lev	Cefc	3 days		Discharge	A	PO	A	Pl	Outbo	32cı	53cm	s1 s:	BIL	Soft	CITA	g	HFI	10	#	##	##	9.3	Piperacillin and Tazobac
BIO	NVD ‡	Male	No	Primi	2kg	#	####	TTNB	116	Sterile	< 3 se	no	148	50	####	<5	At Lev	Cefc	3 days		Discharge	O	PC	O	Pl	Inbori	30cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	19	#	##	##	9.8	Piperacillin and Tazobac
BIO	Vagin	Fema	No	Primi	3kg	#	####	TTNB	98	Sterile	< 3 se	no	156	45	####	<3	At Lev	Cefc	3 days		Discharge	AB	P	AB	Pl	Inbori	30cı	50cm	s1 s:	BIL	Soft	CITA	g	HOI	15	51	##	##	10	Cefotaxime, Amikacin
BIO	Vagin	Fema	No	PRIM	3.2kg	#	####	TTNB	76	Sterile	< 3 se	no	156	48	####	<3	At Lev	Cefc	2 days		Discharge	B	PO	AB	Pl	Inbori	30cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	20	#	##	##	6.8	Cefotaxime, Amikacin
BIO	LSC‡	Male	No	G2P1	2.8kg	#	####	TTNB	67	Sterile	< 3 se	no	148	46	####	5.6	At Lev	Cefc	2 days		Discharge	B	PO	B	Pl	Inbori	30cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	18	#	##	##	7.8	Cefotaxime, Amikacin
BIO	LSC‡	Male	No	G3P2	2.7kg	#	####	TTNB	67	Sterile	< 3 se	no	148	46	####	5.3	At Lev	Cefc	5days		Discharge	B	PO	B	Pl	Inbori	32cı	52cm	s1 s:	BIL	Soft	CITA	g	HOI	20	#	##	##	7.2	Cefotaxime, Amikacin
BIO	Vagin	Male	No	PRIM	3.2kg	#	####	TTNB	78	Sterile	< 3 se	no	152	46	####	5.4	At Lev	none	1 day		Discharge	O	PC	B	Pl	Inbori	32cı	50cm	s1 s:	BIL	Soft	CITA	g	HOI	15	#	##	##	8.8	none
BIO Ama	Vagi nal	Male	No	PRI MI	3.2K G	10/ 23	10/26/ 24	TTN B	78	Steril e	< 3 sec	No	153	42	####	5.2	At level	Non e	3		Disc har	O POS	B PO	Inbor n	32cı m	51cm	s1 s2	BI L	Sof t	CITA good	HO COI	14	#	##	##	8.9	no ne	
BIO	LSC‡	Male	Yes	G2P1	1.5kg	#	####	RDS	98	Sterile	< 3 se	no	146	46	####	<5.0	At Lev	Amil	3-4 days		Discharge	O	PC	O	Pl	Inbori	32cı	50cm	s1 s:	BIL	Soft	CITA	g	HFI	16	#	##	##	9.7	Cefotaxime, Amikacin

Times	Name	Date	Mode	IP	Gende	Histo	Obste	Birth	Indic	GRBS	B	CRT	Febr	Heal	Resp	SpO2	CRP	AF	Antil	No c	MF	Out	Infor	Mothe	Bab	Pla	Hea	Len	CV	RS	PA	CNS	Mo	HE	PCV	T	PLAT	MPV	Antibiotics used after cultur
####	B/O AI	###	Vagin	#	Male	Yes	PRIMI	1.8 K	Seps	98	C	< 3 se	no	148	42	99%	<5	At Le	Pipe	12	DAYS	DISCHA	A	POS	A	P	Out	30c	52cı	s	1	B/	Soft	C/T	HO	24	61.5	#	####	10.9	Meropenem, Vancomycin
####	B/O SA	###	Vagin	#	Femal	No	PRIMI	3KG	Birth	96	N	< 3 se	no	142	45	98%	42	At Le	Amil	8	DAYS	Discharg	B	POS	B	P	Out	32c	52cı	s	1	B/	Soft	C/T	HO	22	65.1	#	####	11.5	Meropenem, Vancomycin
####	B/O TA	###	LSCS	#	Male	Yes	G2P1I	2.8K	Birth	146	A	> 3 se	no	158	62	92%	157	At Le	Mer	10	days	DEATH	O	POS	B	P	Out	32c	52cı	s	1	B/	Soft	C/T	CO	9	25.6	#	####	11.2	Tigecycline, Colistin
####	B/O VA	###	LSCS	#	Male	No	G2P1I	1.7K	RDS	114	N	< 3 se	no	142	68	98%	10	At Le	Amil	10	DAYS	DISCHA	B	POS	B	P	Inbc	27c	48cı	s	1	B/	Soft	C/T	CP	17	52.2	#	####	10.7	Meropenem, Vancomycin
####	B/O LA	###	LSCS	#	Femal	No	Primi	2.1kg	RDS	114	N	< 3 se	no	145	50	99%	46	At Le	Amil	3	days	Discharg	AB	NEA	A	P	Inbc	32c	52cı	s	1	B/	Soft	C/T	HF	14	42.1	#	####	9.7	Cefotaxime, Amikacin
####	B/O AI	###	LSCS	#	Male	Yes	PRIMI	1.8K	Birth	104	N	< 3 se	no	166	68	98%	56	At Le	Amil	10	DAYS	DEATH	B	POS	AB	Inbc	27c	48cı	s	1	B/	Soft	C/T	CP	15	43.1	#	####	10.9	Meropenem, Vancomycin
####	B/O RI	###	LSCS	#	Male	Yes	PRIMI	2.6K	Birth	140	N	< 3 se	no	142	52	####	17	At Le	Amil	8	DAYS	DISCHA	O	POS	A	P	Out	30c	52cı	s	1	B/	Soft	C/T	HF	13	37.7	#	####	10.8	Vancomycin, Meropenem
####	B/O RI	###	Vagin	#	Male	Yes	G3P2I	2.9K	Birth	98	S	< 3 se	no	148	52	98%	78	At Le	Amil	8	DAYS	DEATH	O	POS	B	P	Out	32c	54cı	s	1	B/	Soft	C/T	HF	13	37.3	#	####	11.9	Tigecycline, Colistin
####	B/O MI	###	Vagin	#	Male	No	PRIMI	3.6K	Birth	94	S	> 3 se	no	142	48	####	>90	At Le	Mer	8	DAYS	DEATH	A	POS	A	P	Out	30c	50cı	s	1	B/	Soft	C/T	CO	16	48.1	#	####	11.3	Colistin, Vancomycin
####	B/O SU	###	Nvd	#	Male	No	Primi	2.1kg	TTNE	86	S	< 3 se	no	156	50	98%	45	At Le	Amil	3	days	Discharg	A	POS	B	P	Out	32c	52cı	s	1	B/	Soft	C/T	HF	16	51.6	#	####	9.8	Piperacillin and Tazobactun
####	B/O LA	###	Vagin	#	Femal	Yes	G3P2I	1.95K	RDS	92	S	< 3 se	no	156	56	####	46	At Le	Cefo	13	days	DISCHA	B	POS	O	P	Inbc	32c	54cı	s	1	B/	Soft	C/T	CO	17	58	#	####	12	Tigecycline, Colistin
####	B/O PA	###	Vagin	#	Femal	Yes	PRIMI	2.4K	RDS	104	S	< 3 se	no	142	57	####	21	At Le	Amil	7	DAYS	DISCHA	O	POS	O	P	Out	32c	50cı	s	1	B/	Soft	C/T	HO	19	62	#	####	10	Piperacillin and Tazobactun
####	B/o sh	###	LSCS	#	Male	Yes	Primi	1.9K	Birth	94	E	< 3 se	yes	142	44	98%	25	At Le	Amil	12	days	Discharg	A	POS	A	P	Inbc	30c	50cı	s	1	B/	Soft	C/T	Cp	16	45.9	#	####	10.5	Meropenem, Vancomycin
####	B/o sur	###	Vagin	#	Male	No	Primi	2.3K	Seps	86	S	< 3 se	no	140	44	96%	41	At Le	Mer	12	days	Death	O	POS	O	P	Out	32c	54cı	s	1	B/	Soft	C/T	CO	10	30.6	#	####	11.5	Tigecycline, Colistin
####	B/o rer	###	LSCS	#	Male	No	G3p2I	3KG	Seps	86	N	< 3 se	no	140	46	99%	>90	At Le	Amil	9	days	Death	B	POS	B	P	Out	30c	50cı	s	1	B/	Soft	C/T	HF	12	36.7	#	####	11.1	Meropenem, Vancomycin
####	B/O AI	###	LSCS	#	Femal	No	G8P2I	2.6K	Birth	108	S	< 3 se	no	156	56	92%	22	At Le	Mer	12	DAYS	DISCHA	A	POS	B	P	Out	32c	50cı	s	1	B/	Soft	C/T	HF	11	34.5	#	####	11.1	Meropenem, Vancomycin
####	B/O SA	###	Vagin	#	Femal	No	G2P1I	3.5K	Birth	104	S	> 3 se	no	176	78	86%	38	Bulge	Mer	2	DAYS	DEATH	A	POS	A	P	Out	32c	56cı	s	1	DE	Soft	C/T	CO	16	43.5	#	####	10.9	Meropenem, Vancomycin
####	B/O BI	###	Vagin	#	Femal	Yes	PRIMI	1.7K	Birth	96	K	< 3 se	no	142	56	####	49	At Le	Tige	30	DAYS	DEATH	B	POS	B	P	Inbc	28c	46cı	s	1	B/	Soft	C/T	CO	14	45.1	#	####	7.6	Tigecycline, Colistin
####	B/O SU	###	LSCS	#	Male	No	G2P1I	2.9K	Seps	86	P	< 3 se	no	139	48	####	<5.0	At Le	Pipe	3	DAYS	DISCHA	O	POS	A	P	Out	32c	52cı	s	1	B/	Soft	C/T	HO	16	54	#	####	9.4	Amikacin, Piperacillin and T
####	B/O de	###	LSCS	#	Femal	No	G3P1I	2 kg	TTNE	84	S	< 3 se	no	182	55	92%	52	At Le	Cefo	2	days	Discharg	AB	NEA	AB	Inbc	33c	50cı	s	1	B/	Soft	C/T	HF	14	43.1	#	####	9.9	Piperacillin and Tazobactun
####	B/O Ar	###	LSCS	#	Male	Yes	G2P1I	3kg	Seps	104	E	< 3 se	no	172	52	98%	58	At Le	Mer	16	days	Death	B	POS	B	P	Inbc	32c	52cı	s	1	B/	Soft	C/T	CO	17	62.4	#	####	10.8	Meropenem, Vancomycin

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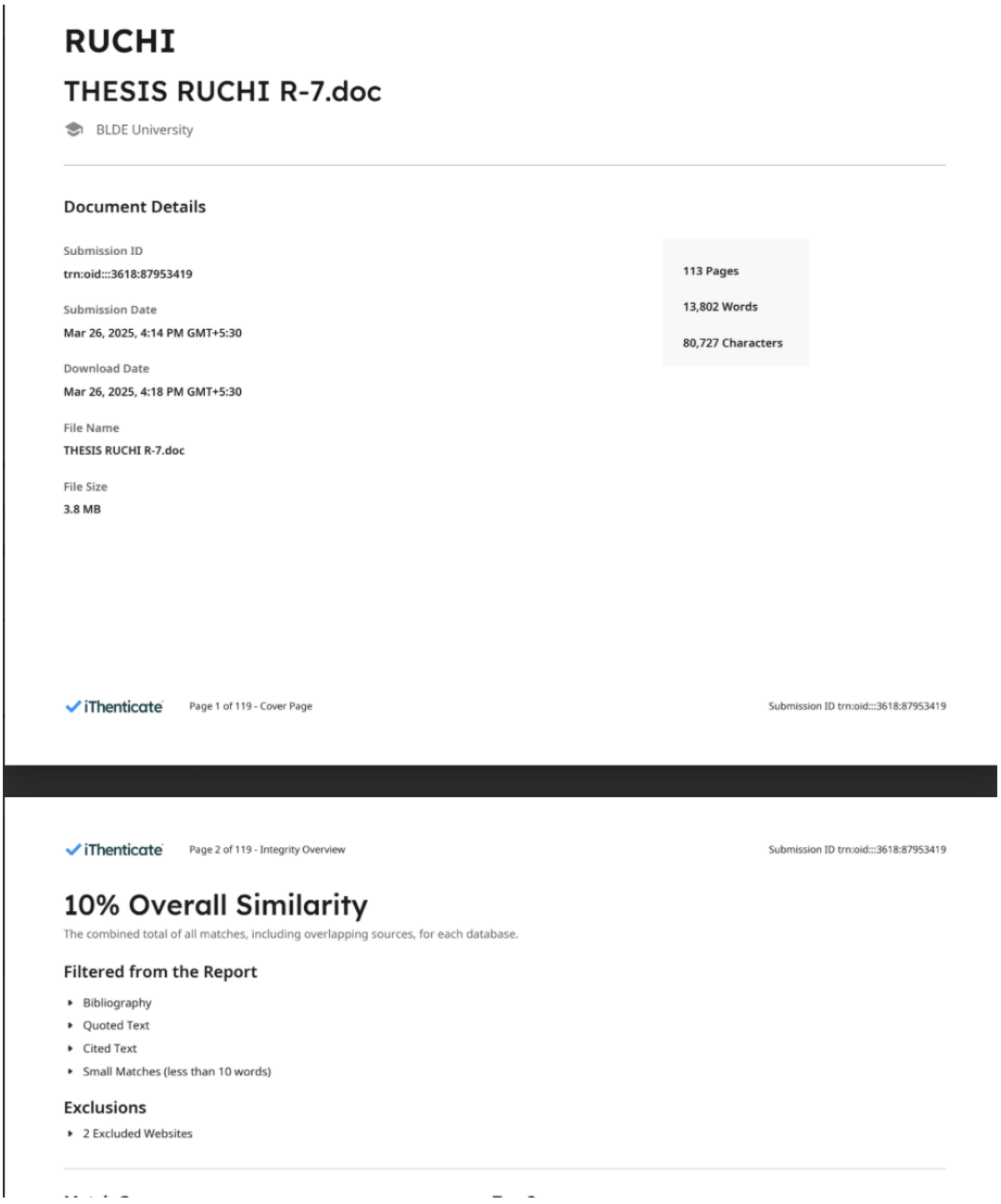

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