"SERIAL SERUM C-REACTIVE PROTEIN LEVELS FOR THE DIAGNOSIS OF NEONATAL INFECTION"

By

Dr. SRINIVAS.H.A

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IN

PAEDIATRICS

Under the guidance of

Dr.PUSHPALATHA.K,M.D.

Professor of Paediatrics

DEPARTMENT OF PAEDIATRICS
SRI DEVARAJ URS MEDICAL COLLEGE
KOLAR-563101

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TAMAKA , KOLAR , KARNATAKA,

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is a bonafide and genuine research work carried out by me

under the guidance of

Dr.PUSHPALATHA.K, M.D.

Professor,

Department Of Paediatrics
Sri Devaraj Urs Medical College, Tamaka, Kolar.

DR.SRINIVAS.H.A

Date:

Place : Kolar .

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH ,

TAMAKA , KOLAR , KARNATAKA

CERTIFICATE BY THE GUIDE

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is a bonafide research work done by

DR.SRINIVAS.H.A

in partial fulfilment of the requirement for the degree of

M.D in **PAEDIATRICS.**

Dr.PUSHPALATHA.K, M.D.

Professor,

Department Of Paediatrics,

Sri Devaraj Urs Medical College,

Tamaka,Kolar.

Date :

Place : Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH ,

TAMAKA , KOLAR , KARNATAKA

CERTIFICATE BY THE CO-GUIDE

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is a bonafide research work done by

DR.SRINIVAS.H.A

in partial fulfilment of the requirement for the degree of

M.D in **PAEDIATRICS.**

Dr.BEENA.P.M, M.D.

Professor & HOD,

Department Of Microbiology,

Sri Devaraj Urs Medical College,

Tamaka,Kolar.

Date :

Place : Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH ,

TAMAKA , KOLAR , KARNATAKA

ENDORSEMENT BY THE HOD & PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled

"SERIAL SERUM C-REACTIVE PROTEIN LEVELS FOR THE DIAGNOSIS OF NEONATAL INFECTION"

is a bonafide research work done by

DR.SRINIVAS.H.A

under the guidance of

Dr.PUSHPALATHA.K, M.D.

Professor of Pediatrics.

Dr.Susheela.C,
Professor and HOD,

Department Of Paediatrics,
Sri Devaraj Urs Medical College,
Tamaka,Kolar.

Date:
Place: Kolar

Dr.M.B.Sanikop,
Principal,

Sri Devaraj Urs Medical College.
Tamaka,Kolar.

Date:
Place: Kolar

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH ,

TAMAKA , KOLAR , KARNATAKA

ETHICAL COMMITTEE CERTIFICATE

This is to certify that the Ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved

DR.SRINIVAS.H.A

Post-Graduate student in the subject of

PAEDIATRICS at

Sri Devaraj Urs Medical College, Kolar

to take up the Dissertation work entitled

"SERIAL SERUM C-REACTIVE PROTEIN LEVELS FOR THE DIAGNOSIS OF NEONATAL INFECTION"

to be submitted to the

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH,

TAMAKA , KOLAR , KARNATAKA

Member Secretary
Sri Devaraj Urs Medical College,
Kolar–563101

Date :

Place : Kolar

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Abbreviations

CSF	Cerebro Spinal Fluid
CONS	Coagulase Negative Saphylococcus
CRP	C-Reactive Protein
CRP 1	C-Reactive Protein first measurement
CRP 2	C-Reactive Protein second measurement
CRP 3	C-Reactive Protein third measurement
CD-116	Cluster Differentiation-116
EOS	Early Onset Sepsis
ELISA	Enzyme Linked Immuno Sorbent Assay
ESR	Erythrocyte Sedimentation Rate
FC	Fragment Crystallizable
GCSF	Granulocyte Colony Stimulating Factor
GM-CSF	Granulocyte-Monocyte Colony Stimulating Factor
GBS	Group B Streptococci
HSV	Herpes Simplex Virus
I:T ratio	Immature : Total ratio
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMR	Infant Mortality Rate
IFN	Interferon
IL	Interleukin
LOS	Late Onset Sepsis
LPS	Lipopolysaccharide
NK	Natural Killer cell
PC	Phospho Choline
PAF	Platelet Activating Factor
PMN	Polymorphonuclear cell
PROM	Premature Rupture Of Membrane
PCT	Procalcitonin
SAP	Serum Amyloid Component
SDUMC	Sri Devaraj Urs Medical College
SIRS	Systemic Inflammatory Response Syndrome
SPSS	Statistical Package for Social Science
TLR	Toller Like Receptor
TUTH	Tribhuvan University Teaching Hospital
TNF	Tumor Necrosis Factor
US	United States
WBC	White Blood Cell

ABSTRACT

BACKGROUND: Neonatal sepsis accounts for the majority of neonatal morbidity and mortality especially in the developing countries including India. Neonatal sepsis requires rapid and accurate diagnosis as well as treatment for the improved outcome. There is an increasing need for careful evaluation of neonatal sepsis in early period.

OBJECTIVE: To evaluate serial serum C reactive protein levels for the diagnosis of neonatal infection.

METHOD: A total of 50 neonates with clinically suspected neonatal infections is evaluated. The neonates are evaluated by thorough history from mother and detailed clinical examinations. The findings are recorded in the Patient record form. Laboratory and radiological evaluation is done for the diagnosis and confirmation of infection. Infants are categorized as having proven sepsis, probable sepsis or no sepsis, without consideration of CRP levels. CRP levels are determined at the initial evaluation and on each of the next two mornings. Sensitivity, specificity and predictive values are calculated for the first and second CRP (1 and 2).

RESULTS: In the present study, 5% case of proven sepsis and 95% cases of probable sepsis is present among CRP1 positive cases with significant correlation ($p<0.05$).While 9.3% cases of proven sepsis and 90.6% cases of probable sepsis is present among CRP1 and 2 positive cases with highly significant correlation ($P<0.001$), indicating that serial CRP monitoring had clinical utility in diagnosis of neonatal sepsis. Further comparative analysis of CRP and culture of the body fluid showed that serial measurement of CRP 1 and 2 showed increase in sensitivity from 25.0% to 100%, NPV from 90% to 100% and PPV was increased from 5.0% to 9.3% but decrease in specificity from 58.69% to 15.21%. The serial CRP1 and 2 measurements showed higher sensitivity and negative predictive value compared to CRP1 alone, in both early as well as late proven and probable sepsis.

CONCLUSION: As the culture positivity rate are low and takes time for growth, serial CRP levels can be used in the diagnostic evaluation of neonates with suspected infection. Further serial CRP levels are more sensitive than single CRP measurement.

KEYWORDS : Neonatal sepsis, C-reactive protein, sensitivity, specificity

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INTRODUCTION

Neonatal sepsis is defined as invasive bacterial infection occurring in first 4 weeks of life.[1]

The incidence of neonatal sepsis varies in different countries. It varies from 2.7/1000 live birth in developed countries to 10-15/1000 live birth in developing countries. Again the incidence in preterm babies is 1/250 live premature births. [2]The incidence of neonatal sepsis varies from 11-24.5/1000 live births in India. [3]The most common cause of neonatal mortality is sepsis, birth asphyxia, prematurity and neonatal tetanus. [4]

The contribution of neonatal sepsis to high morbidity and mortality rate makes it an important subject for research so as to find out the possible solution. [5] Prompt diagnosis of neonatal sepsis is of paramount importance, yet can be difficult because of subtle and nonspecific features. The early signs of sepsis are frequently subtle and nonspecific. [6]

The newborn infant responds to a wide variety of noxious stimuli, (infection, metabolic, respiratory, traumatic) with limited repetition of stereotyped reactions. Many of the manifestations of the sepsis have their counterparts in non-infectious neonatal disorder.[7]Thus the inability to be certain of infection coupled with non-specific signs of life threatening illness in neonates have resulted in widespread use of antibiotics aggravating the problem of antibiotic resistance.[3]

Neonatal sepsis is categorized into early onset sepsis, occurring within 72 hrs of life and late onset sepsis, occurring after 72 hrs of life. [1, 8] The early onset sepsis is commonly caused by microorganisms acquired from the mother before or

during birth (vertically transmitted and perinatally acquired), whereas the late onset sepsis is caused by microorganisms acquired form the environment rather than from the mother (nosocomial and horizontally transmitted). [9]

The predisposing obstetric factors to neonatal sepsis are premature rupture of membrane (PROM >24 hrs) before birth, maternal bleeding, toxemia, maternal infection and presence of chorioamnionitis.[1,7] Prematurity is also another risk factor for infection. [9]

Septicaemia can be suspected in a neonate with sepsis score which includes following signs and symptoms: refusal to feed, abdominal distension, vomiting, fever, lethargy, icterus, poor cry, seizure, diarrhoea, apnoea, poor capillary refill, hypothermia and umbilical discharge. If the neonate has three or more of the above signs or symptoms, septicaemia can be suspected. [3] Respiratory distress is the most common symptom occurring in 90% of neonates with sepsis. [7]

Neonates can also be categorized as having proven sepsis if bacteria are isolated in blood, CSF or urine; probable sepsis if clinical and laboratory findings are consistent with bacterial infection without positive culture; or no sepsis if findings are not consistent with sepsis. [10]

The diagnosis of neonatal sepsis is difficult to make solely on historical or clinical ground. Laboratory evaluation is essential in the diagnosis and confirmation of infection. There is no rapid and reliable test for confirmation of diagnosis yet. The treatment for sepsis is generally started when clinical findings are supported by indirect early markers of infection.[8] Positive culture of blood, CSF or urine are the gold standard for confirming sepsis, however in considerable proportion of neonates at risk of infection, culture result may be influenced by previous antibiotic exposure.[9]

Also, the bacteraemic phase of the illness may be missed by poor timing or blood sample size.[11] The sole use of culture to diagnose neonatal infection has limitations as it may take 24 to 72 hrs to obtain culture reports.[12]

The well-known laboratory parameters indicating infection include WBC count, immature to total WBC ratio (I:T ratio), ESR, CRP and procalcitonin. Similarly in recent years, several new markers of infection have been investigated such as Tumor Necrosis Factor (TNF), Interleukin 6 (IL-6), IL-1 receptor antagonist, Granulocyte Colony Stimulating Factor (GCSF), leukocyte-$\propto_1$ proteinase inhibitor and most recently CD 116 as a cell surface marker. But these markers have not yet made the progress from the laboratory to clinical applications.[13, 14]

C- reactive protein is an acute phase protein belonging to pentraxin family of protein. It is so named as it reacts with the somatic C polysaccharide of streptococcal pneumoniae and was first discovered by Tillet and Frances in 1930.[15] It is exclusively produced in liver and is secreted in increased amounts within 6 hours of an acute inflammatory stimulus. The plasma level can double in every 8 hours, reaching a peak at 24-48 hours of the stimulus.[12, 13] After effective treatment or removal of the inflammatory stimulus, level can fall as rapidly as 5-7 hours.[15]

CRP is present in the serum of normal person at the concentration ranging up to 5mg/l. Since the protein is produced by the fetus and neonate and does not pass the placental barrier, it can be used for the early detection of neonatal sepsis.[16]

The CRP level increases dramatically following the bacterial infection which may be particularly helpful for the diagnosis and monitoring of bacterial septicemia in neonates. The concentration of CRP accurately

parallels the activity of inflammatory process and the concentration decreases much faster than the any other acute phase parameter which is particularly useful in monitoring appropriate treatment of bacterial disease with antibiotics.16

The total WBC count has low predictive value for the diagnosis of neonatal sepsis because of wide range of normal count (5000-20000/cumm). WBC count of <5000/cumm or >20000/cumm is usually associated with neonatal sepsis. [7,17]

The value of CRP is reliable in the 24-48 hrs after onset of infection. In the study carried out by Groves A, a single CRP measurement in 24 hrs of illness has a sensitivity of 93% for the early onset probable sepsis. Other two measurements 24 hrs apart have even greater sensitivity. After obtaining a normal CRP measurement, the probable sepsis is 10 times less likely. Similarly, for two successive normal CRP measurements, the probable sepsis becomes 30 times less likely. [18] In another study, the CRP measurement once has sensitivity of 39.4% and 64.6% respectively for proven and probable sepsis of early onset. Whereas the sensitivity rate for late onset sepsis is 35% and 61.5% for proven and probable sepsis respectively. [10]

Serial serum CRP measurements have the sensitivity of 97.8% and 8.1% for proven and probable sepsis of early onset and 88.9% and 97.5% for proven and probable sepsis of late onset in the diagnosis of neonatal infection. [10] In another study, the serial measurement of CRP drawn every 24 to 48 hrs after the onset of signs of infection have an increased sensitivity between 78.9% to 98%, specificity of 84% to 97% and negative predictive value of 99% in detecting sepsis. [12] As there is no rapid and reliable test yet for the diagnosis of neonatal sepsis, serial CRP measurement can

be used for its early detection as well as exclusion of infection in the neonates.

Rationale of the study

Neonatal sepsis accounts for the majority of neonatal morbidity and mortality especially in the developing countries including India. Neonatal sepsis requires rapid and accurate diagnosis as well as treatment for the improved outcome. There is an increasing need for careful evaluation of neonatal sepsis in early period.

CRP is an acute phase protein produced early in the infection with short half life (only 24 hrs), doesn't pass placental barrier, can be used for early detection of neonatal sepsis. The serial increase in levels following bacterial infections and faster decrease in level following therapy indicating its reactivity to inflammatory process is particularly helpful in the diagnosis and monitoring of bacterial septicemia and appropriate treatment in neonatal sepsis.

The ability of serial CRP levels within the first 48 hrs of suspected sepsis to distinguish neonates from bacterial infection with false negative culture, who may require continuation of antibiotics from those with clinical findings not related to bacterial infection with true negative culture, for which antibiotics can be safely discontinued, have the greatest utility.

So, serial CRP level monitoring will help in early diagnosis and management of neonatal sepsis, initiation and adjustment of antibiotic therapy, reduces length and cost of hospital stay as well as parental anxiety. So far such study has not been carried out in India, thus this study has been proposed.

AIMS AND OBJECTIVES

To evaluate serial serum C reactive protein levels for the diagnosis of neonatal infection.

REVIEW OF LITERATURE

Historical perspective

Sepsis remains a serious cause of morbidity and mortality in the neonates. The definition of sepsis is ever evolving. For nearly a century, sepsis has been defined as the systemic host response to an infection. Even though it has been classified in many ways, there has been little modification on this definition. Originally, sepsis was believed to be associated with the presence of bacteria in the blood (bacteremia) and the terms "sepsis" and "septicaemia" were frequently interchanged in the clinical setting.In1989, Bone et al established a simple definition, which was based on specific clinical symptoms and included a known source of infection [19]. However, the clinical signs presented were frequently not characterized by measurable levels of bacteria in the blood and this discrepancy was first taken into account at a Consensus Conference held by the Society of Critical Care Medicine and American College of Chest Physicians in 1992, when the term "Systemic Inflammatory Response Syndrome" (SIRS) was established for which no definable presence of bacterial infection was required [19, 20, 21].

SIRS is characterized by two or more of the following: 1) fever or hypothermia. 2) tachycardia. 3) tachypnoea 4) abnormal WBC count or increase in immature cells. [19, 20, 21]

Sepsis is defined as SIRS with a documented infection site (documented by positive culture for organisms from that site). Blood culture do not need to be positive. [20, 21, 22]

When sepsis is accompanied by organ dysfunction, hypoperfusion or hypotension; the sepsis is considered as severe.[20, 21] Septic shock is considered

when hypotension develops despite adequate fluid replacement. [20,21] While SIRS, sepsis and septic shock are associated commonly with bacterial infection, bacteremia may not be present. Bacteremia is the presence of viable bacteria within the liquid component of blood. Bacteremia may be transient, as seen commonly after injury to mucosal surface, primary (without an identifiable focus of infection), or more commonly secondary to intravascular or extra vascular focus of infection. [20]

Neonatal Sepsis:

Neonatal sepsis, sepsis neonatorum and neonatal septicemia are terms that have been used to describe the systemic response to infection in newborn infants. There is little agreement on the proper use of the terms that is whether it should be restricted to bacterial infection, positive culture or severity of illness. [11]

The application of the terminology to septic newborns needs careful assessment (i.e., age related reference values for blood pressure, heart rate, respiratory rate and leukocyte count). Furthermore, the application of a staging system (including sepsis, severe sepsis, septic shock and multiple organ dysfunction syndromes) may not be best approach to disease or risk stratification in the newborn. [22]

Currently, criteria for neonatal sepsis include documentation in a newborn infant with a serious systemic illness in which non-infectious explanation for the abnormal pathophysiological states are excluded or unlikely. [11]

Definition:

Neonatal septicemia is defined as a spectrum of disease in infants who are less than one month of age, are clinically ill and have a positive blood culture. [23]

Classification:

The epidemiological studies of neonatal infections usually distinguishes early onset sepsis(EOS) from the late onset sepsis(LOS) with the assumption that early onset sepsis are presumably transmitted perinatally from the mother and late onset sepsis are acquired postnatally from the environmental source. [21, 24] The classification is useful as it facilitates the consideration of common principles of causation, presentation or treatment.[24]

Early onset sepsis occurs within 72 hours of life and is commonly caused by micro-organisms acquired from the mother before or during the birth (vertically transmitted and perinataly acquired). [9, 22] Pre-maturity, chorioamnionitis, maternal infection or colonization, prolonged rupture of membrane are the risk factors for the development of early onset sepsis.[1,7,9,11,24] Early onset sepsis is usually sudden and follows a fulminant course with primary focus of inflammation in the lungs.[24]

Late onset sepsis occurs after 72 hours till 28 days of life. [9, 11, 24] It occurs in approximately 10% of all the neonates admitted to NICU.[25] It is generally caused by micro-organisms acquired from the environment and also called as nosocomial and horizontally transmitted infection. [24]

Incidence:

The incidence of neonatal sepsis varies according to geographic regions

and from one hospital to another and the community in the same geographic area. The incidence varies from 1-4 cases per 1000 live births in developed countries with considerable fluctuation over time and with geographical location. [11] The incidence in developing countries is as high as 10-15 per 1000 live births. [2] In the US, the incidence of culture proven sepsis is approximately 2 per 1000 live births. [26] The incidence of neonatal sepsis is 9.8 per 1000 live births in South India. [27]

Mortality and morbidity:

The mortality rate due to neonatal sepsis may be as high as 50% for the infants who are not treated. Infection is a major cause of mortality during the first month of life, contributing to 13-15% of all the neonatal death. Neonatal meningitis is responsible for 4% of neonatal death. [26, 28]

Age and sex incidence:

Early onset neonatal sepsis is clinically apparent within 6 hours of birth in >50% of neonates, the great majority presents within the first 72 hours of life. [1]

Males have approximately two fold higher incidence of sepsis than females, suggesting the possibility of a sex linked factor in host susceptibility. [11] In a study conducted in Kanti Children Hospital, showed a preponderance of male septicaemic neonates with the male: female ratio being 1.8:1. [31]

Immunity:

The neonate is unable to respond effectively to infectious hazards because of deficits in the physiological response to the infectious agents. Diminished concentrations of the immunologic factors and decreased function are often demonstrated in neonates. [11] The following factors are considered:

a. Immunoglobulins:

Active transport of immunoglobulin G occurs through the placenta with a concentration in a full term infant comparable to that of mother. Fetus can synthesize IgM in response to intrauterine infection. The presence of specific IgG antibody in adequate concentration provides neonates protection against the infection which is antibody mediated. In general infant lacks antibody mediated protection against Escherichia coli and other Enterobacteriaceae. [11] The neonates may receive IgA from breast feeding but does not secrete IgA until 2-5 weeks after birth. [26]

b. Complements:

Fetus begins to synthesize complement compound as early as 1st trimester. Full term newborn have slightly diminished classical pathway of complement activity and moderately diminished alternative pathway activity. Their deficiencies contribute to diminished complement derived chemotactic activity and diminished ability to opsonize certain organisms in the absence of antibody. [11] Usually the killing of organisms, especially the gram negative bacteria is inefficient. [26]

c. Neutrophil:

The neutrophil storage pool in newborn infant is 20-30% of that in adults. [11] The neonatal neutrophil or polymorphonuclear cell which is vital for effective killing of bacteria is defective in chemotaxis and killing capacity. Decreased adherence to the endothelial lining of blood vessels reduces their ability to marginate and leave the intravascular area to migrate into the tissue; they fail to aggregate in response to chemotactic factors. Also neonatal PMNs are less deformable. Therefore they have less ablility to move through the extracellular matrix of tissue to reach the site of inflammation and infection. The limited ability of neonatal PMNs for

phagocytosis and killing of bacteria is impaired when the infant is clinically ill. [26]

d. Monocyte - Macrophage system:

Neonatal monocyte concentration and function are at adult level; however macrophage chemotaxis is impaired and continues to exhibit decreased function into early childhood. Macrophages are decreased in the lungs, liver, and spleen. The chemotactic and bactericidal activity and the antigen presentation by these cells are not fully competent. Cytokine production by macrophage is decreased, which may be associated with a corresponding decrease in T-cell production. [26]

T-cells are found in early gestation in fetal circulation and increase in number from birth to about age of 6 months. Neonates are deficient in T-cells, however the number increases with maturity as the neonate is exposed to antigenic stimuli. These antigenically naive cells do not effectively produce the cytokines that assist with B-cell stimulation, differentiation and with bone marrow stimulation of granulocyte/ monocyte proliferation. [26]

e. Natural Killer (NK) cells :

Natural Killer (NK) cells are found in greater concentrations in the peripheral blood of neonates than in that of adults; however certain antigen expressivity by the cell membrane is diminished, thereby reducing cytotoxic activity. This decreased response has been observed with infection by herpes group of virus in the neonates. [26]

f. Cytokines:

Interferon (IFN) -α and β levels are normal, but IFN - γ synthesis is diminished. IFN - γ level is elevated in infants with neonatal sepsis, but the response may be less consistent than in adults. IL- 6 level is increased in serum of newborns

with neonatal sepsis. [26]

Etiology:

The causative organisms in neonatal infections have varied somewhat in the past 50 years and are related to the introduction of newer antibiotics and development of antibiotic resistance, changes in obstetric management. However there is remarkable similarity at present in predominant organisms causing neonatal sepsis. [17] Group B Streptococcus remained as the most common pathogen in the US from 1970-2000 followed by Coagulase Negative Staphylococcus (CONS), Enterococci, Escherichia coli, Haemophilus influenzae, Streptococcus pneumoniae and Neisseria meningitidis.[32]

Group B Streptococcus and Escherichia coli account for 60-70% of all infection. Several other pathogenes as Staphylococcus aureus, Klebsiella, Enterobacter, Serratia, Salmonella and Pseudomonas species are most frequently isolated from the infants with late onset infections.[29] Stapylococcus species accounts for 30 to 50% of late onset sepsis. [1]

E. coli is the most common gram negative organism causing early onset sepsis.[1] Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae and group A, C and G Streptococcus are the respiratory tract pathogens that occasionally colonize the maternal genital tract and cause early onset neonatal sepsis. [11, 29]

In the developed world CONS is the leading cause of late onset sepsis, while in the developing world, gram negative organisms still predominate. [9] In a etiological study of neonatal sepsis carried out by Anwar et al in Pakistan, in early onset sepsis, gram positive and gram negative organisms were almost equal; while

majority of infections were due to gram negative organisms in late onset sepsis.[33]

In a study carried out by Kuschel C, common organism causing neonatal sepsis are CONS (28%), Staphylococcus (19%), Streptococcus agalactae (10%) and E. coli (5%) along with others - Streptococcus pneumoniae, Haemophilus influenzae and gram negative organisms (20%).[34]

In a study of 96 consecutive inborn neonates with blood culture proven bacterial sepsis in Chennai, Staphylococcus aureus was the predominant pathogen in 61.5%, followed by Klebsiella pneumoniae in 21.9%, E. Coli 13.5% and Streptococci 3.1%. [35]

In a bacteriological study of pyogenic meningitis conducted by Tankhiwale et al, it was found that E. coli, Streptococcus pneumoniae, non fermenters (6.8%) and Staphylococcus aureus (4.25%) were the most common organism with the gram negative bacteria being predominant. [36]

Community based data are limited in Nepal but neonatal sepsis is likely to be the result of infection by gram positive bacteria such as Staphylococcus, Streptococcus and gram negative organisms such as E. coli, Klebsiella, Enterobacter and Salmonella. [37] A study done by Shrestha B. M. at Kanti Children Hospital, found the predominance of gram negative organisms in 60.5% cases with E. coli being the commonest isolate.[31]

Epidemiology

A number of other factors in addition to geographic region influence the rate of neonatal infection. Socioeconomic status, maternal age and sex influence the prevalence of maternal infection. [11] There may be hospital to hospital variability even in the same geographical area and this may be related to environmental condition of neonatal unit, perinatal care, conduction of labour and prematurity. [11]

The gastrointestinal tract is the major site of asymptomatic colonization with both group B Streptococcus and E. coli for mother and infant, other being genitourinary tract. Between 40 to 70% of infants where mothers are colonized at delivery becomes colonized with GBS. [24]

Pathogenesis:

Whether the infants who are exposed to potentially pathogenic organism will develop sepsis or not is determined by maternal, environmental and host factors. Exposure to the micro-organism may occur in the following ways. [24]

a. Transplacental:

Certain infective agents have an inherent ability to penetrate the barrier, often damaging the placenta. [24]

b. Ascending infection:

Ascent of vaginal organisms into the uterine cavity occurs from the vagina and cervix through microscopic leak in the amniotic membrane or through frankly ruptured membrane. [24] The risk of perinatal sepsis according to Trucker, is about 1-2% after the PROM.[38] Rupture of membrane without complications for more than 24 hours prior to delivery is associated with 1% increase in the incidence of neonatal sepsis; however when the chorioamnionitis accompanies, the incidence of neonatal infection rises by four times. [26I]In a report presented in the Perinatal Symposium, California, the frequency of sepsis associated with PROM and culture positive GBS showed to be 33 to 50%. [39]

c. Intrapartum :

Vaginal delivery inevitably results in surface contamination during the passage through the birth canal and causes the colonization of skin and gut.[24] The risk of

transmission increases when the density and number of sites of maternal colonization increases. [24, 32]

d. Postnatal:

Spread of infection from the postnatal environment is very common. People are the main source of such contamination. [24] Many prepartum and intrapartum obstetric complications have been associated with increased risk of infection in the newborn, the most significant of which are premature onset of labour, PROM, chorioamnionitis and maternal fever. [23] In one of the study of 963 pregnancies complicated by PROM, the incidence of clinical sepsis increased from 2% among infants born within 23 hrs of membrane rupture to 7% and 11% among those delivered 24 to 47 hrs and 48 to 71 hrs after the membrane rupture respectively. The incidence of infection has been estimated to be 8.7% for infants born to mother with PROM (>24 hrs) and clinical chorioamnionitis. [23]

The barrier to infection is provided by the integrity of placenta and membranes, the low pathogenicity of most colonizing organisms and the relative competence of baby's defence mechanism. Neonatal infection occurs when one or other of these factors are altered. [24]

Colonization of the upper respiratory tract occurs rapidly and 90% of infants have positive pharyngeal culture by third day, with CONS being the commonest organism. Skin colonization is very rapid with the number of bacteria increasing 100 fold during the first week. The umbilicus, perineum and axilla are most heavily colonized. Most infants become colonized without becoming infected but various host factors or the pathogenicity of the organisms and its load are important in causation of sepsis in newborn. [24]

The systemic inflammatory cascade is initiated by various bacterial

products. These bacterial products [gram negative bacteria - endotoxin, exotoxins, proteases, formyl peptides; gram positive bacteria-endotoxin, super antigens, toxic shock syndrome toxin, streptococcal pyrogenic exotoxin A, enterotoxin, hemolysins, peptidoglycans and lipotechoic acid] binds to cell receptor on the hosts macrophages and activates regulatory proteins. Endotoxin activates the regulatory proteins by interacting with several receptors. The CD receptors pool in the LPS binding protein complex on the surface of the cell and then the TLR receptors translate the signal into the cells. [20]

The proinflammatory cytokines produced are Tumor Necrosis Factor (TNF), Interleukin 1, 6, 12 and Interferon Gamma (IFN-γ). These cytokines can act directly to affect organ function or they may act indirectly through secondary mediators. The secondary mediators include nitric oxide, thromboxane, leukotriens, and platelet activating factor, prostaglandins and complement. These primary and secondary mediators cause the activation of the coagulation cascade, the complement cascade and production of prostaglandins and leukotriens. These products lower the perfusion of organs and can lead to multiple organ system failure. [20]

Pathology

Bacterial fragments and endotoxin or exotoxins stimulate monocytes and neutrophils to produce variety of inflammatory mediators. The simultaneous activation of complement, coagulation and fibrinolytic cascades leads to the formation of vasoactive and proinflammatory mediators such as prostaglandin E_2, free radicals, nitric oxide and PAF (platelet activating factor). These mediators either singly or sequentially lead to adhesion and diapedesis of polymorphonuclear cells into the tissue giving rise to clinical features seen in sepsis syndrome and septic shock. [2]

Sepsis may indicate an immune system that is severly compromised and unable to eradicate pathogens. Cells of the innate immune system recognize messengers through pattern recognition receptors called Toller Like Receptors (TLRs), which are resistant to endotoxin because of mutation in the toll like receptor 4 gene (TLR4). This TLR4 mutation have been identified in human and may make person more susceptible to infections. Again, polymorphism in TNF receptor, interleukin-1 receptor, FCγ receptors, TLRs and cytokine genes may determine the concentrations of inflammatory and anti-inflammatory cytokines production. The risk of death among the sepsis has been linked to genetic polymorphism for TNF-α and TNF-β as well. Such polymorphism may be used to identify patients at high risk of development of sepsis and organ dysfunction during infection. [40]

Clinical presentation and assessment of the neonate

Early recognition of serious infection in the neonate is essential because of extreme rapidity with which the risk of permanent morbidity and mortality can develop. [24]

History:

History about maternal, perinatal and neonatal events that put the infant at increased risk of infection should be considered and asked thoroughly. The risk factors that are associated most highly with neonatal sepsis include premature rupture of membrane (PROM), prolonged rupture of membrane, chorioamnionitis and prematurity. [24, 26]

The predisposing risk factors also associated with neonatal sepsis includes maternal fever greater than 101^{o}F (38.4^{o}C), poor maternal nutrition, meconium stained liquor, low apgar score (< 6 at 1 or 5 minutes), low birth

weight, difficult delivery, birth asphyxia, etc. The predisposing risk factors implicated in neonatal sepsis reflect the stress and illness of the fetus at delivery as well as hazardous uterine environment surrounding the fetus before delivery. [26]

Physical findings:

The early manifestations of neonatal sepsis are usually non-specific and subtle. [1, 6, 11, 24, 26, 28] These non-specific clinical signs of early sepsis are also associated with other neonatal diseases, such as respiratory distress syndrome, metabolic disorders, intracranial hemorrhage and traumatic delivery. Therefore neonatal sepsis should be diagnosed by excluding other disease process. [26]

The important symptoms and signs of neonatal sepsis are:

Refusal to feed - Refusal to feed occurs in most of the infants. In a study done by Jaiswal et al, refusal to feed was most prominent (66%) in infants with neonatal sepsis.[3]

Gastrointestinal symptoms - Vomiting, diarrhoea and abdominal distension are important symptoms of neonatal sepsis. [2, 11, 24]

Lethargy- Jaiswal et al reported lethargy as a feature of septicemia in 42% of infants. [3]

Temperature instability - A temperature below 36^{o}C or above 37.8^{o}C sustained for more than one hour must be regarded as possible infection. [11, 23] About 50% of infected newborns have temperature more than 37.8^{o}C (axillary). [11] Temperature instability is observed with neonatal sepsis and meningitis either in response to pyrogens secreted by the bacterial organisms or from sympathetic nervous system instability. [26]

Ictrerus-Icterus is one of the important clinical signs of sepsis in newborns.[2, 11, 24, 32] Basu K. et al reported that septicaemia caused jaundice in

4.75% of cases. [41]

Respiratory distress - Respiratory distress is the most common symptom occurring in 90% of patients with sepsis.[7]

Cyanosis, apnoea and dyspnoea are also common

Diagnostic investigation of neonatal sepsis:

The diagnosis of neonatal sepsis is difficult to make solely on historical and clinical ground. Laboratory evaluation assists in the diagnosis and confirmation of neonatal sepsis. No single laboratory test has been found to have acceptable sensitivity, specificity for predicting or excluding infection. [7] So far there is no rapid and reliable test for confirmation of diagnosis of neonatal sepsis. The treatment for sepsis is generally started when clinical findings are supported by indirect early markers of neonatal infection. [8] The laboratory parameters evaluated as the indicators of infections are followings:

1. Haematological tests:

Total leukocyte count has low predictive value for the diagnosis of neonatal sepsis because of wide range of normal count. Leukopenia (<5,000/cmm) or leukocytosis (>20,000/cmm) is usually associated with neonatal sepsis. [17] During the first three days of life, leukopenia and neutropenia have good sensitivity and specificity of 67%, 90% and 78%, 80% respectively. [43] Beyond 3 days of age, leucocytosis and neutrophilia have sensitivity and specificity of 74%, 56% and 67%, 65% respectively. The ratio of immature to mature neutrophill (I: T ratio) of more than 0.2 is relatively sensitive indicator of sepsis. [8] I:T ratio has sensitivity and specificity of 78% and 73%. respectively[43, 44]

2. Platelet count:

Platelet count falls below $100x10^9$/L in obviously septic infants. The sensitivity and specificity of thrombocytopenia for the diagnosis of septicemia was reported as 65% and 47% respectively. In a study obtaining haematolotical score by a complete blood count, differential leucocyte count, total leucocyte count, band form count and platelet showed the hematological score of >3 has the sensitivity of 86% and negative predictive value of 96%. [45]

3. ESR measurement:

It is sensitive but non-specific indicator of infection. [2] The ESR is an indirect, quantitative measurement of fibrinogen concentration in plasma. When fibrinogen level increases as in response to an acute inflammatory stimulus; there is greater cohesion of erythrocytes leading to agglutination, rouleaux formation and faster rate of sedimentation. [46] The micro ESR value of >15mm/hr is suggestive of infection. [8, 28] ESR cannot reliably distinguish the microbial etiology of acute inflammatory process and may take even longer time to rise. [46]

4. X ray:

The chest x ray was performed after the clinical evaluation in suspected sepsis. The radiographic findings consistent with sepsis includes pulmonary infiltration, pleural effusion, pneumatosis intestinalis or intraperitoneal free air. [10]

5. Blood culture:

Identification of the causative organism may be made by blood culture. Blood for culture should be obtained from a peripheral vein with aseptic precautions in an amount of 1-2ml. The sensitivity of single blood

culture in identifying septicaemia is 80%. [23] Two blood cultures from different sites increases the yield and in identifying false positive result.[7, 11,23] In a study of 254 clinically suspected cases of neonatal bacteremia Parikh and Singh; 1995, found that blood culture was positive in 119 (47%).[47] In another study done by Pourcyrous et al; found 27% of blood culture were positive with 69% of gram positive and 29% of gram negative organisms. [48] In similar study by Kaiser et al; on evaluation of blood culture results from late onset sepsis found that 10.2% cases were positive for bacterial isolation. [49]

6. CSF analysis:

CSF examination is indicated in all septic neonates, as meningitis occurs in one third of neonatal sepsis cases. [2] In case of neonatal meningitis, the ratio of CSF glucose to blood glucose is less than 50%. A positive culture of pathogenic bacteria in the CSF remains the gold standard for diagnosis of bacterial meningitis, [17] CSF examination is positive in most of the cases with late onset sepsis. [24] Whereas Kaiser et al on evaluation of CSF culture, found only 5.4% to be positive of bacterial isolation in late onset sepsis. [49]

7. Urine culture:

Urine culture should be done in all cases. Urinary tract infection is confirmed when there is more than 10^5 colony forming units/ml of freshly collected urine. [2]

8. C-reactive protein (CRP):

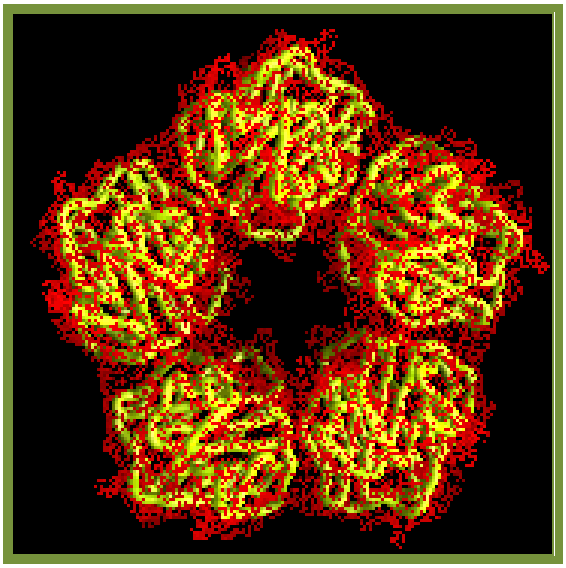
C-reactive protein was first described by Tillet and Francis in 1930 at Rockefeller University.[2,13] C-reactive protein is a serum glycoproteinproduced by the liver exclusively during acute inflammation.[50]Interleukin-1, interleukin-6 and TNF are mediators for the synthesis of CRP by hepatocytes. [12, 13, 16] It causes rapid increase in concentration of up to

1000 fold in response to tissue damage and inflammation. [12, 50, 51]

In healthy adult volunteer blood donor, the median concentration of CRP is 0.8 mg/l, but following an acute phase stimulus, values may increase from less than 50µg/l to more than 500mg/l, that is 10,000 fold. The plasma half life of CRP is about 19 hours and is constant under all conditions of health and disease. So that the sole determinant of circulating CRP concentration is the synthesis rate, which directly reflects the intensity of pathological processes stimulating CRP production. When the stimulus for increased production completely ceases, the circulating CRP concentration falls rapidly, at almost the rate of plasma CRP clearance. Liver failure impairs CRP production, but no other intercurrent pathologies and very few drugs reduce CRP values unless they also affect the underlying pathology providing the acute phase stimulus.[30]

i) Structure of Human C-reactive protein:

CRP belongs to the pentraxin family of calcium dependent ligand binding plasma proteins, the other member of which in human is serum amyloid component (SAP).The human CRP molecule (MW 115, 135) is composed of five identical nonglycosylated polypeptide subunits (MW 23,027), each containing 206 amino acid residues. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry (figure).



Structure of C-reactive protein (Greenhough et al.) [52]

Each protomer has the characteristic "Lectin fold" composed of a two layered B-sheet with flattened jellyroll topology. The ligand- binding site, composed of loops with two calcium ions bound 4 A ^{o} apart by protein side-chains is located on concave face. The other face carries a single helix. The pentraxin family is named for its electron micrographic appearance. [50]

The structure contains a remarkable crystal contact, where the calcium binding loop including Glu-147 from one protomer co-ordinates into the calcium site of a protomer in a symmetry related pentamer, revealing the mode of binding of the principal ligand phosphocholine (PC) and providing information concerning conformational changes associated with calcium binding. A striking structural cleft on the pentameric face opposite to the PC binding site suggests an important functional role, perhaps complement activation. [51]

ii) Functions of CRP:

The function of CRP is related to its role in innate immune system. It recognizes and binds to phosphocholine exposed in damaged cell walls found in

many bacteria, fungi and parasites. [12] It acts like an opsonin for the bacteria, damaged cell wall and nuclear debris for phagocytosis. [12]

It activates complements, binds to Fc receptors and acts as an opsonin for various pathogens. [13] CRP is recognized by C1q and potentially activates the classical complement pathway engaging C3, the main adhesion molecule of the complement system and the terminal membrane attack complex, C5-C9. Bound CRP may also provide secondary binding sites for factor H and there by regulate alternative pathway amplification and C5 convertases. [50] CRP recognizes altered self and foreign molecules based on pattern of recognization. Thus CRP is thought to act as surveillance molecule for altered self pathogens. The recognition provides an early defense and leads to a pro-inflammatory signal and activation of the humoral, adaptive immune system.[15, 50]

iii)Why to measure CRP?

Level of CRP increases very rapidly in response to inflammation, infection and trauma and decreases rapidly with resolution of condition. CRP is present in the serum of normal persons at concentration ranging upto 5mg/L. It is secreted in increased amount within 4-6 hours of an acute inflammatory stimulus. The plasma level can double in every 8 hours reaching a peak at 24-48 hours.[12, 13] After effective treatment or removal of the inflammatory stimulus, level can fall as rapidly as 5-7 hours. [15] Since an elevated level is always associated with pathological changes, determination of CRP is of great value in diagnosis, treatment and monitoring of inflammatory conditions. [13, 16]

iv)CRP in Neonatal sepsis:

Da silva et al, while reviewing the use of CRP as a tool for diagnosis of neonatal sepsis concluded that CRP is probably the best available diagnostic test.

[53] Since the protein is produced by the fetus and the neonate and does not pass the placental barrier, it can be used for the early detection of neonatal sepsis. [16]

As biological half life of CRP is only 24 hours, CRP accurately parallels the activity of the inflammatory process and its concentration decreases much faster than ESR or any acute phase parameter which is useful in providing appropriate treatment. [13, 15, 16]

A level of 10mg/L has consistently been shown to be the most reliable cut off value to indicate sepsis.[18] CRP level is of some use in differentiating between bacterial and viral infection. A very high CRP (>100mg/L) is more likely to occur in bacterial than viral infection, and normal CRP level is unlikely in the presence of bacterial infection. However intermediate CRP levels (10-50mg/L) may be seen in both bacterial and viral conditions. [15]

v)Laboratory methods to measure CRP :

The laboratory methods used to measure serum CRP levels are followings:

A. qualitative

B.semiquantitative

C .quantitative

A. Qualitative testing method:

The qualitative latex agglutination test is the first laboratory method developed to measure CRP. This method measures the presence or absence of agglutination and precipitation indicating whether CRP is present or not in the sample. A positive test result indicates the presence of CRP-ligand complexes formed when CRP binds to cause agglutination and precipitation, where as a negative test result occurs when no agglutination is present. A CRP level more than 6mg/l or more than 10mg/l indicates

a positive result depending on the specific testing kit and reagent being used. [12]

B. Semi-quantitative testing method:

The semi quantitative latex agglutination assay involves the use of serial dilutions of serum and saline. Each is mixed with a latex reagent and observed for the presence of agglutination. The highest dilution in which agglutination is visualized corresponds to an approximate titer or concentration of CRP ligand complexes. The test can be performed in 15-30 minutes and has a reported upper detection level between 6 and 10 mg/dl. Results are reported in ratio or an approximate mg/l concentration of CRP. [12]

C. Quantitative testing method:

Quantitative immunoassay is the most rapid, sophisticated and sensitive method of detecting and measuring CRP. The enzyme linked immuno sorbent assay (ELISA) is based on sandwich principle. The micro liter wells are coated with an antibody, directed towards an epitope of an antigen molecule. [16] The ELISA and immunofluorescent quantitative test uses monoclonal antibodies marked with an enzyme or fluorescent tracer fixed to microwells located within microliter plate or test tube. When diluted human serum is added, CRP binds to the immobilized marked anti CRP antibody forming bound CRP ligand complex. Unbound antibodies are washed from the test tube and fluorescent marked CRP ligand complexes can be visualized and measured under fluroescent microscope. A substrate is added to the enzyme marked CRP ligand complexes, which reacts with the enzyme causing a color reaction. The specimen is read with a spectrophotometer. This method of testing can be performed within 10-15 minutes. [12]

The Quick Read CRP is an immunoturbidimetric test for quantitative determination, based on micro-particles coated with anti-human CRP. CRP present in the sample reacts with the micro particles and the resultant change in the turbidity of the solution is measured by the Quick Read instrument. [53] Whole blood is added to the buffer and the blood cells are haemolyzed. The assay is performed in the same cuvette. The reagents are precalibrated and the calibration provided with each kit. [54]

vi) Sensitivity, specificity and clinical utility of CRP levels :

In early onset sepsis, a single CRP measurement at 24 hours of the illness has 93% of sensitivity for probable sepsis and two measurements 24 hours apart are even better. The likelihood ratio for a single CRP for probable sepsis was 0.10. This means that having obtained a normal CRP one can assume probable sepsis to be 10 times less likely. For the two measures the ratio is 0.03, making probable sepsis 30 times less likely. [18]

In late onset sepsis, the single CRP measurement has a sensitivity of 85% with likelihood ratio of 0.19 (sepsis 5 times less likely) and two separate measures have a likelihood ratio 0.07 (sepsis 14 times less likely).[18]

Berger et al, on prospective comparison of diagnostic value of CRP and WBC count for detection of neonatal septicemia found that during first 3 days of life, CRP has sensitivity and specificity of 75% and 86% respectively in comparison to leucopenia (67%/90%), neutropenia (78%/89%) and IT ratio (78%/73%). But beyond 3 days of age, CRP has sensitivity and specificity of 88% and 87%. [43]

Several studies have revealed increased sensitivity and specificity of serial CRP measurement. Serial measurement of CRP levels done every 24 to 48 hours after the onset of signs of infection have an increased sensitivity

between 78.9% to 98%, specificity of 84% to 97% and negative predictive value of 99% in excluding sepsis. [12] Gonzalez et al, reported serial measurement of CRP have sensitivity of 91% and specificity of 93%.[55] Matesanz et al, showed that sensitivity of CRP is significantly higher than other haematological indices. [56]

In a study carried out by Benitz et al, 1998, on serial measurement of CRP in neonatal infection, CRP #1(initial evaluation) had sensitivity of 39.4% and 64.6% for proven and probable early onset sepsis and 35% and 61.3% for proven and probable late onset sepsis. [10] CRP level at the initial evaluation can be omitted without compromising the diagnostic utility of serial levels obtained during the next 48 hours. Groves A, on a study reported 40% sensitivity of the test at presentation.[18]The CRP levels on the morning after the initial evaluation (CRP #2) has higher sensitivity 92.9% and 85.0% for proven and probable early onset sepsis and 78.9% and 84.4% for proven and probable late onset sepsis respectively. [10] Three serial CRP measurements have even higher sensitivity of 97.8% and 98.1% for proven and probable late onset sepsis. So the CRP level obtained at the time of initial evaluation can be omitted without significant loss of sensitivity. [10]

The predictive value of CRP can be enhanced by serial rather han a single CRP measurement. Serial CRP showed very high predictive value of 91.6% for diagnosis of neonatal sepsis and is better than that of leukocyte indices of CBC. [57]

vii) Limitations in the use of CRP level in neonatal septicaemia:

The timing of CRP measurement is critical to achieve highest sensitivity. A single CRP drawn early in the course of disease has low sensitivity in detecting the presence or absence of infection, because the sampling time may

precede a measurable rise in CRP level; the rise may lag 12 to 24 hours after the onset of symptoms. [12]

CRP can be elevated for reasons other than infectious conditions like meconium aspiration, respiratory distress, fetal hypoxia, intraventricular haemorrhage, immunization, viral illness like invasive HSV can cause false positive result.[12, 18]

CRP is not considered accurate in the pre-term neonates. Level does not always rise above 10mg/l in pre-term neonates or those with overwhelming sepsis, resulting in a false negative test result.[12]The false negative result may occur early in infective episodes, and also in UTI.[18]

The CRP response to CONS is significantly less pronounced than to other community encountered pathogens in neonatal septicaemia. A rise in CRP beyond the third day of empirical treatment should give rise to a suspicion of fungal infection or ineffective antibacterial treatment. [58]

viii)Immunological tests:

The detection of bacterial antigen in blood, CSF or urine confirms the presence of systemic bacterial infection. Counter current immunoelectrophoresis latex particle agglutination and coagulation tests are used for this purpose. [59]In recent years new markers of infections such as Interleukin-6, Interleukin-8, Procalcitonin, Granulocyte colony stimulatory factor, Interleukin-1 receptor antagonist, Leucocyte- proteinase inhibitor and most recently CD-116 as a cell surface marker have been investigated. [13, 14, 24, 59]

In a recent study of some new markers in association with CRP it has been found even more reliable to diagnosis sepsis. In a study of CRP combined with interleukin-6 and procalcitonin in immediate post natal

period it was found that early neonatal infection was associated with significant increases in concentration of all three parameters independent of illness severity. [60]

Another study of comparing the procalcitonin with interleukin-8 and CRP, found that combination of CRP with IL-8 was more reliable than PCT as a test for early diagnosis of bacterial infection in neonates. Measurement of interleukin-8 in combination with CRP reduces the unnecessary antibiotic therapy in neonates. [62]

ix) Radiological studies:

All neonates with suspected sepsis should have a chest X-Ray and abdominal X-Ray is indicated when necrotizing enterocolitis is suspected. [24]

Treatment:

All the neonates with suspected neonatal septicaemia are evaluated using a standard clinical pathway. Neonates are categorized as having proven, probable and no sepsis according to clinical, laboratorial, radiological and culture findings. [10]

Septicaemia is suspected with "sepsis score". If the neonates have three or more than three signs and symptoms, septicaemia can be suspected.[3] Probable sepsis is diagnosed in a neonate with negative culture if it has three or more signs suggestive of sepsis and one or more abnormal laboratory markers. [63] The necessity of early vigorous treatment has resulted in the widespread use of sepsis score to guide treatment and has resulted in improved outcome. [64] Once the diagnosis of sepsis is probable or proven and the appropriate cultures have been taken, antimicrobials should be started.[7,59] The initial choice of antibiotics depends on the knowledge of

prevalent organisms responsible for infection within a geographical area, as well as the pattern of specific antimicrobial susceptibility. The initial therapy for EOS is started with the combination of crystalline penicillin and an aminoglycoside (usually Gentamicin). [7, 9, 59] The choice of antibiotics for use in LOS would depend on the organisms generally responsible for such infections. The combination of an aminoglycoside with cloxacillin (flucloxacillin) has been the standard therapy for suspected LOS. [9] Neonates who are at risk of having nosocomial infection with CONS should be treated with vancomycin and aminoglycoside. [7, 9] Once the culture and sensitivity studies are available, therapy is changed accordingly. [59]

The duration of therapy depends on the clinical signs and symptoms and isolation of organism on blood or body fluid. If the cultures are negative, antibiotics may be discontinued after 48 hours. [2, 9] But if culture is positive or clinical suspicion of sepsis is strong, then even in negative cultures cases antibiotics should be continued.

Measurement of serial CRP concentration in serum is useful in treatment of suspected neonatal sepsis. [10] CRP is found as a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection. If CRP level is less than 10 mg/l, infants are considered unlikely to be infected and treatment is stopped, whereas when CRP level is 10mg/l or greater, infants are considered likely to be infected. CRP is determined daily and treatment discontinued as soon as CRP returns to less than 10mg/l. [65]

Burke, studied serial assessment of CRP to guide antibiotic therapy and reported that CRP estimation correctly identified 99 of 100 infants not requiring further antibiotic therapy, and thus concluded that it is a safe and practical approach to use CRP in suspected neonatal sepsis in a developing

country.[66]

Therapy for most infection should be continued for a total of 7-10 days or at least 5-7 days after a clinical response has occurred. The course of treatment for meningitis caused by GBS is usually 14 days and for a minimum of 14 days after sterilization of the CSF in gram negative meningitis. [9, 11] Adjunctive therapies have been used in the treatment of neonatal sepsis such as granulocyte transfusion, exchange transfusion, intravenous immunoglobulin and administration of haemopoetic growth factors (G-CSF and GM-CSF). [9]

Prevention and screening of neonatal sepsis:

The risk of bacterial infection in asymptomatic newborn is low. Evidence based observation and treatment protocols could be defined based on maternal risk factor, initial neonatal examination and laboratory findings.[67]

Intrapartum antibiotics are most effective when administered at least 4 hours before delivery and when at least two doses have been given. [9] Selective intrapartum administration of antibiotics to women in labour has been shown to be effective in preventing infections. Mothers should receive intrapartum antibiotics if they have any of the following risk factors:

a. chorioamnionitis

b. prematurity

c. antepartum temperature>38 ^{o}C

d. PROM > 18 hours

e. history of group B streptococcus bacteraemia or rectovaginal carrier during pregnancy

Asymptomatic infants with maternal risk factors should be kept in observation for perinatal infection till appropriate duration up to 24 hours, and may require laboratory evaluation.Critical observation period is the first 6 hours after birth. [68]

Symptomatic infants require laboratory evaluations and antibiotic therapy should be initiated. Nosocomial infections account for an increasingly large proportions of neonatal infections. Meticulous attention to hand washing is the most effective measure in reducing hospital infection. Hand washing should not only be done before and between handling the infants but also after handling potentially contaminated sites. Again proper staffing, adequate space in neonatal units, controlling admissions, aseptic care of catheters and appropriate limitation of antibiotic usage are some of the other measures which are helpful in reducing hospital acquired infections. [9]

METHODOLOGY

Study design:

This is a prospective case series study carried out between December 2008 to November 2009 in the Neonatal unit, Department of Pediatrics, R. L. Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Medical College (SDUMC), Kolar.

Sample size: A total of 50 neonates with clinically suspected neonatal infections is evaluated.

INCLUSION CRITERIA:

Neonates up to 4 weeks of age with birth weight more than 1500g and clinically suspected neonatal infections.

EXCLUSION CRITERIA:

a. Neonates ≤ 32 weeks gestational age.

b. Neonates with weight ≤ 1500 g.

c. Neonates with major congenital anomalies.

d. Neonates requiring assisted ventilation and who have undergone surgery.

Method of collection of data:

The neonates are evaluated by thorough history from mother and detailed clinical examinations. The findings are recorded in the Patient record form.

Neonatal sepsis is suspected in the following maternal conditions:

a. Prolonged rupture of membrane (>18 hrs.)

b. Foul smelling liquor.

c. Meconium stained liquor.

d. Intrapartum fever >38 C.

Neonatal sepsis is suspected in the neonates with the sepsis score which includes following signs and symptoms. The neonates with 3 or more of these signs and symptoms are suspected to have neonatal sepsis.

a. Refusal to feed.

b. Tachypnea.

c. Lethargy.

d. Seizures.

e. Fever.

f. Hypothermia.

g. Icterus.

h. Cyanosis.

i. Apnoea.

j. Pallor.

k. Umbilical discharge.

l. Abdominal distension.

m. Others – Vomiting, diarrhoea, skin(pustule, petechiae)

Following laboratory and radiological evaluation are done for the diagnosis and confirmation of infection

a. Serial Measurements of serum C-reactive proteins.

b. Complete blood count with differential count, band forms and I/T Ratio.

c. Blood culture and sensitivity.

d. Urine culture and sensitivity.

e. CSF analysis and culture.

f. Chest X-ray.

g. Others, if required. (E.g. Pus culture)

Infants are categorized as having proven sepsis (bacteria isolated from blood, cerebrospinal fluid, or urine culture), probable sepsis (clinical and laboratory findings consistent with bacterial infection without a positive culture), or no sepsis (clinical and laboratory findings not consistent with sepsis), without consideration of CRP levels. Infants whose blood cultures yielded skin flora but who demonstrated no other signs of bacterial infection are not considered to have sepsis. CRP levels are determined at the initial evaluation and on each of the next two mornings. Sensitivity, specificity and predictive values are calculated for the first and second CRP. When neonatal sepsis is suspected, antibiotics are started empirically considering the obstetric factors, sepsis score, WBC count or any positive radiological findings. The choice of antibiotics is Ampicillin and Gentamicin initially, which is changed later if needed according to sensitivity pattern in culture positive sepsis or if worsening of signs and symptoms. The neonates are followed up daily till discharge.

a) Measurement of CRP

Immunostat CRP(a latex agglutination slide test for detection of CRP in Serum) a product of Diagnova was used.

All reagents and samples are equilibrated to room temperature.

Step1: 50 µL of undiluted serum or control is added to circles on test slide.

Step2: 2 drops of latex reagent dispensed to each circle

Step3: mixing sticks used to mix and spread the reagents over the entire area of circle.

Step4: test slides gently rocked and rotated for 2 min only and examined for agglutination immediately.

Interpretation of results:

A positive result is interpreted by the development of clearly visible agglutination. It indicates CRP content in the sample of 6 mg/L or greater.

It is possible that a very strongly positive sample may show a prozone effect. If this suspected, add a drop of the control serum to the circle containing and the suspected sample/reagent mixture. Mix and rotate for further 2 min. if the result becomes positive, the original sample contained less than 6 mg/L CRP. If the results remain negative the original sample should be diluted 1 in 20 and retested.

SEMI QUANTATIVE TEST

1) Serial doubling dilution of the sample to be tested is prepared with filtered normal saline.
2) Each dilution tested with reagent till the last dilution , where a positive result is obtained.

Titres reported as per the table below

Agglutionation upto the serum dilution	Approx. CRP concentration in mg/l
Undiluted	6
1:2	12
1:4	24
1:8	48
1:16	96

The CRP 1 level measurement is done at the time of admission. CRP 2 is done after 24 hours of admission. Several studies reported, in almost all cases

CRP 3 level were positive, subsequently after positive CRP 2 level measurement. So If CRP 2 is negative, CRP 3 is measured subsequently but if the CRP 2 is positive, CRP 3 is not performed considering it to be positive.

b) Total WBC count:

A total count of <5000/cu.mm or >20000/cu.mm is taken as abnormal and considered suspicious of sepsis.

c) Blood culture and sensitivity:

For the blood culture, 1ml of blood is drawn from a peripheral vein after cleansing the site with iodine and alcohol and letting it dry. Blood is collected in a bottle containing 10ml of brain heart infusion (in the dilution of 1:10). This is then incubated at 37^{o}C under aerobic condition and subculture is made on Mac Conkey agar media after 24 hrs of incubation. If any growth occurred at 48 hrs, it is again subcultured in Mac Conkey agar. Cultures are taken as sterile if no growth occurred at 96 hrs. Anaerobic cultures are not done.

Organisms are identified by standard biochemical and enzymatic reactions in case of any growth. The antimicrobial sensitivity of the isolates towards the various antimicrobial discs is done by modified Kirby-Bauer disc diffusion technique.

d) CSF analysis and culture:

CSF analysis is done in all cases. CSF is obtained by lumbar puncture performed aseptically with a 24 gauze disposable needle. Sample is transported promptly to the laboratory for analysis and kept for culture and sensitivity.

e) X-ray:

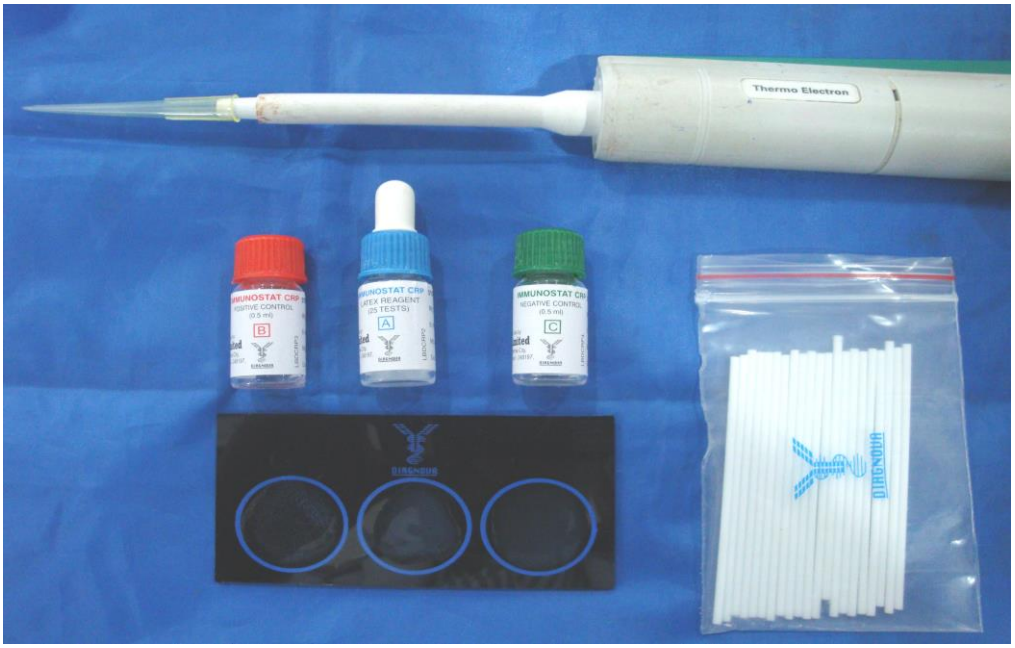
X-ray is done in neonates with clinical findings suggestive of respiratory distress and abdominal distension.

f) Urine culture:

Urine culture is done in all cases by suprapubic puncture method.

Statistical methods:

Data collected on predesigned proforma for each individual case was analyzed using computer based SPSS software. The data were analyzed by Chi - Square test. P value < 0.05 was considered as statistically significant.



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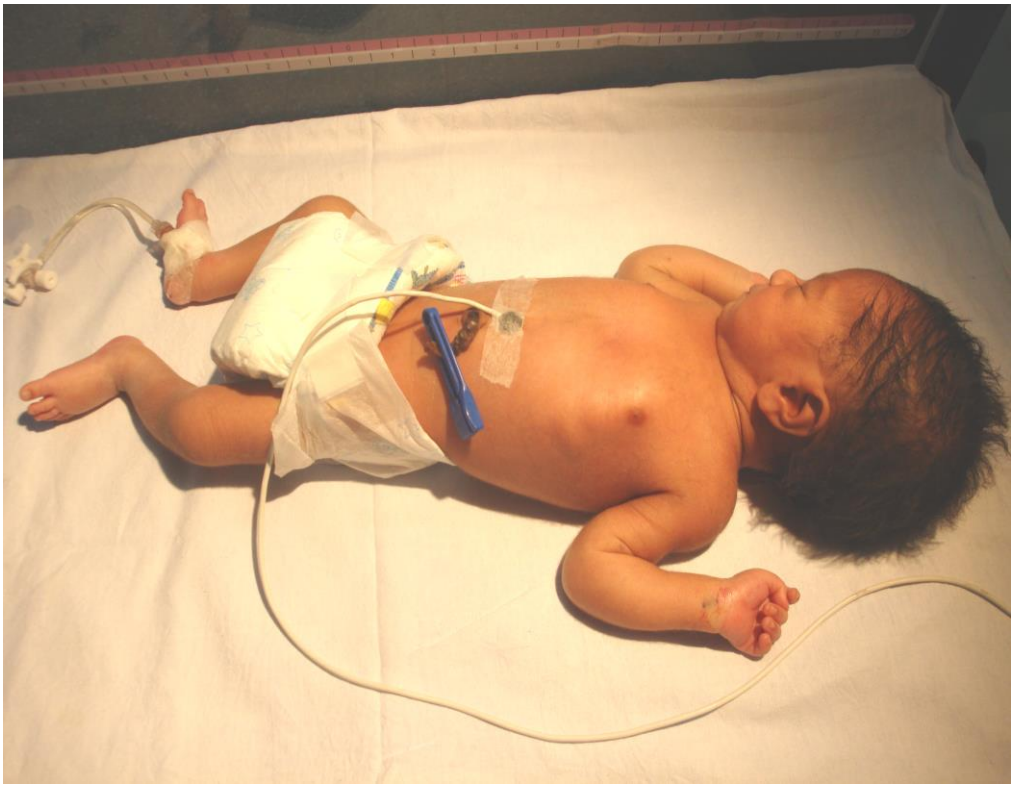

NEONATE WITH SUSPECTED NEONATAL INFECTION

OBSERVATIONS AND RESULTS

In the study conducted between December 2008 to November 2009 in Neonatal unit, Department of Pediatrics, R. L. Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Medical College (SDUMC), Kolar, 50 neonates fulfilling the inclusion criteria were included.

Out of 50 neonates, 31(62%) are male and 19(38%) are female. According to the age of onset of sepsis, neonates are classified to have early onset sepsis (onset at less than 3 days of life) or late onset sepsis (onset after 3 days of life). There are 22 cases of early onset sepsis and 28 cases of late onset sepsis.

Onset of sepsis and Sex Distribution

Table 1: Onset of sepsis and Sex incidence in study population (n=50)

Onset of sepsis	Male		Female	
	No. of Patients	%	No. of Patients	%
Early onset sepsis(<3days)	17	34	5	10
Late onset sepsis (> 3 days)	14	28	14	28
Total	31	62	19	38

Majority (62%) were male neonates. 44% had EOS and 56% had LOS. 77% of EOS occurred in male neonates where as equal incidence of LOS was found in both sexes.

Onset and type of sepsis

Table 2: Onset and type of sepsis in study population (n=50)

Type of Sepsis	EOS		LOS	
	No. of Patients	%	No. of Patients	%
Proven sepsis	2	4	2	4
Probable sepsis	18	36	23	46
No sepsis	2	4	3	6
Total	22	44	28	56

Majority (82%) had probable sepsis, 8% had proven sepsis and 10% had no sepsis.

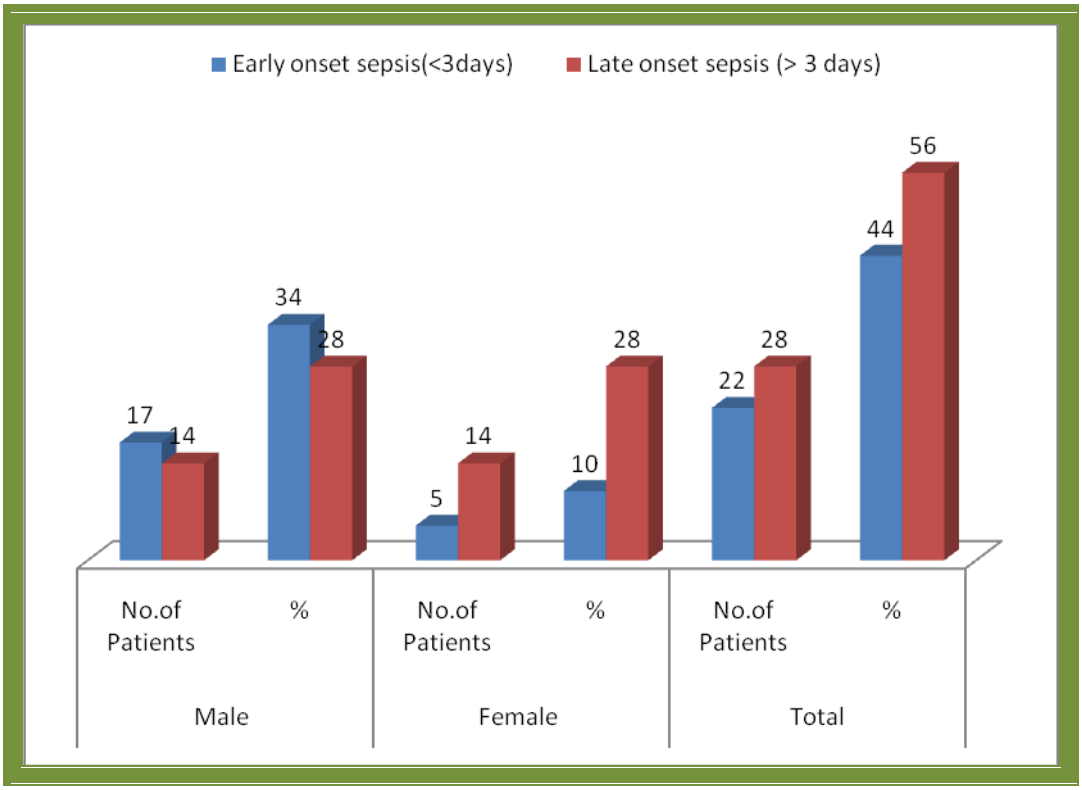

Fig 1. Bar diagram showing Onset of sepsis and sex distribution in study population

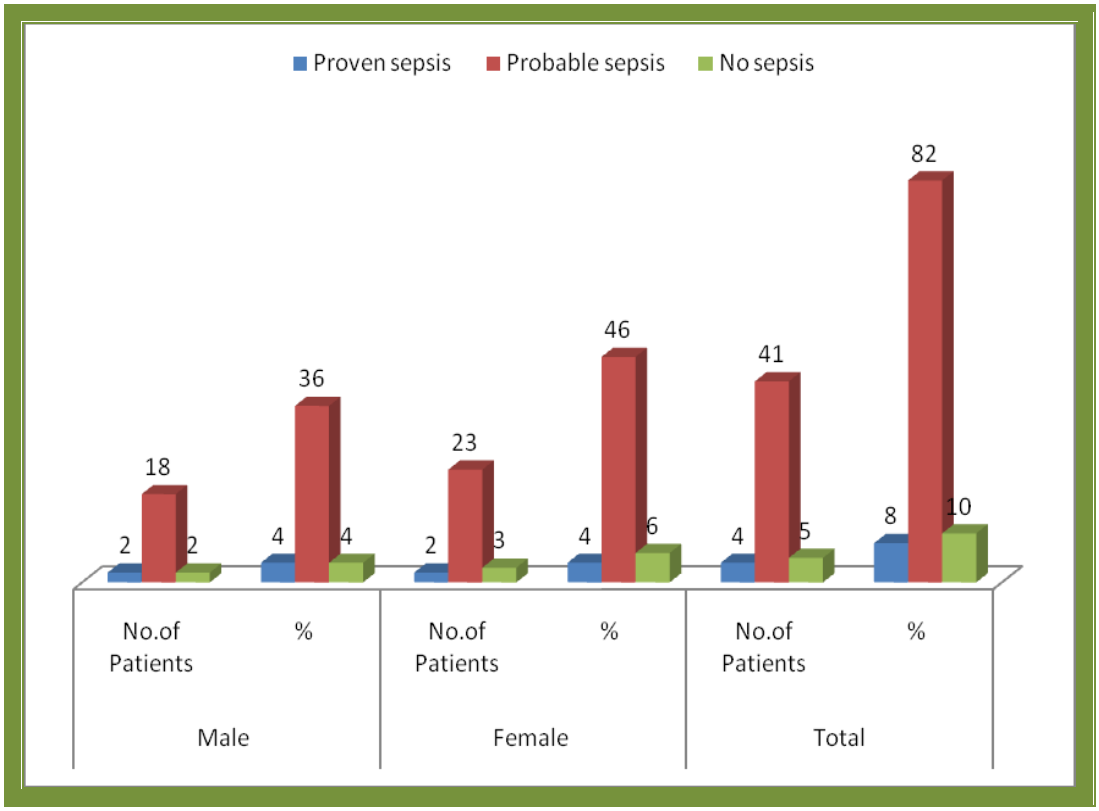

Fig 2. Bar diagram showing onset and type of sepsis in study population.

Table 3: Serial CRP Measurements and onset of sepsis in the study population (n=50)

Type	CRP (1 and or 2)		Total	
	Negative	Positive	No. of Patients	%
Early onset	3	19	22	44
Late onset	4	24	28	56
Total	7	43	50	100

$p > 0.05$ (Pearson chi – square test)

The statistical analysis between positive CRP measurement and age of onset of sepsis shows no significant relationship ($p>0.05$)

Investigations (Culture)

Table 4. Types of organism isolated in the blood, urine and CSF culture of the study population.

Organism isolated	Early onset			Late onset			Total	
	Blood Culture	CSF Culture	Urine	Blood Culture	CSF Culture	Urine	No. of Patients	%
Staphylococcus Aureus (MSSA)	0	0	0	0	0	1	1	2
CONS	1	0	0	0	0	0	1	2
P.Aeruginosa;	0	0	0	0	1	0	1	2
NFGNB	1	0	0	0	0	0	1	2
Total	2	0	0	0	1	1	4	8

There was equal distribution of Gram positive and Gram negative organisms isolated from body fluids.

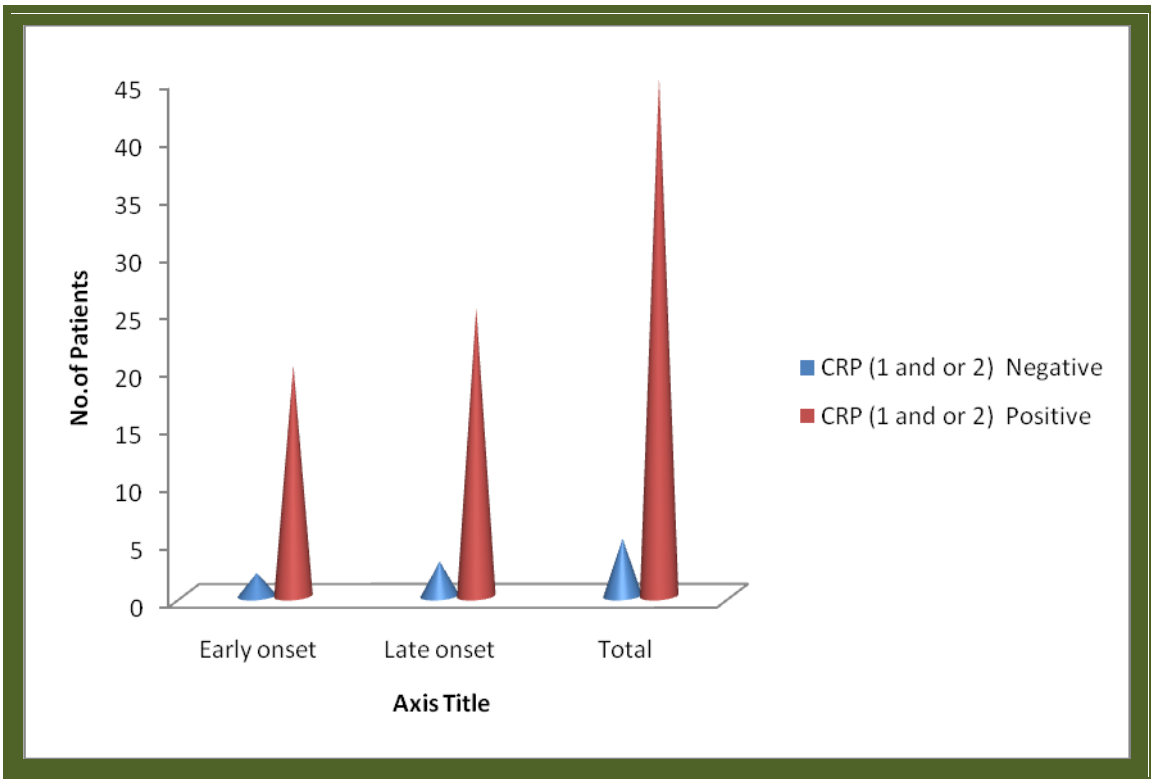

Fig 3. Bar diagram showing Serial CRP Measurements and onset of sepsis

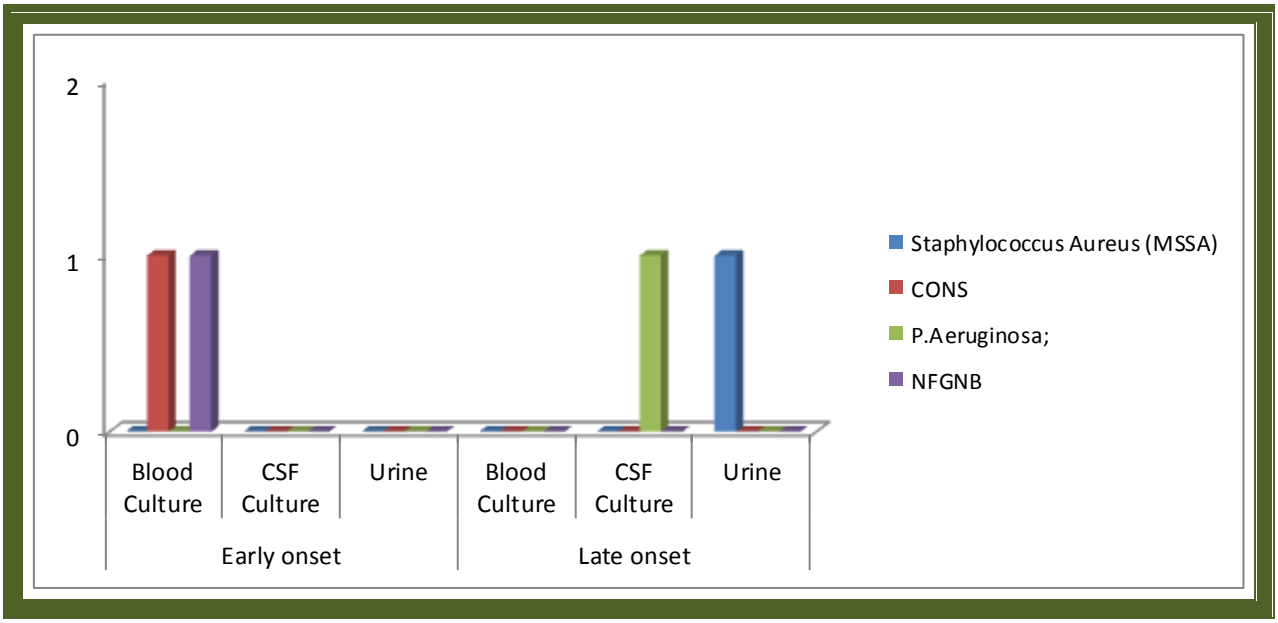

Fig 4. Bar diagram showing type of organism isolated in culture

In 43 cases out of 50, CRP measurement is positive (either CRP1 or 2 >6 mg/l). CRP1 alone is positive in 20 cases but subsequent serial CRP2 measurement is positive in remaining 23 cases. Among the CRP positive cases, 19 are the cases of early onset sepsis and 24 are of late onset sepsis. Similarly 4 CRP positive cases fall in the group of proven sepsis, 39 in probable sepsis and no cases in no sepsis group.

Table 5: Serial CRP Measurements and type of sepsis in the study population (n=50)

	CRP (1 and or 2)		Total	
	Negative	Positive	No.of Patients	%
Proven sepsis	0	4	4	8
Probable sepsis	2	39	41	82
No sepsis	5	0	5	10
Total	7	43	50	100

CRP (1 and or 2) is positive in 100% of culture proven sepsis, 95% of probable sepsis and negative in 100% of cases of no sepsis.

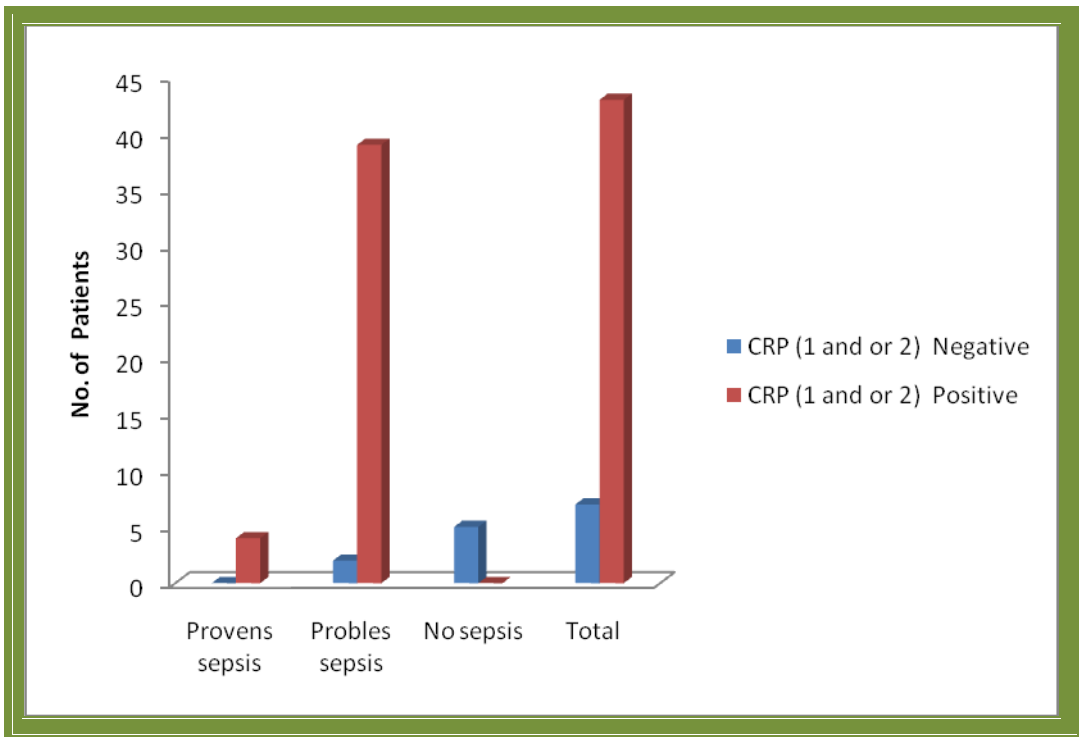

Fig 5. Bar diagram showing Serial CRP Measurements and type of sepsis

COMPARATIVE ANALYSIS

Relationship between the CRP level with the type and onset of sepsis is analyzed.

The usefulness of CRP level to predict or to exclude different types of sepsis is assessed by examining the relationship of CRP level with the positivity of blood, Urine or CSF culture.

In comparison to the single CRP 1 measurements, the serial measurement of CRP1 and 2 showed increase in sensitivity, PPV, NPV of infection in all the occasions.

CRP and body fluid culture

On comparative analysis of CRP and culture of the body fluid, serial measurement of CRP 1 and 2 showed increase in sensitivity from 25.0% to 100%, NPV from 90% to 100% and PPV was increased from 5.0% to 9.3% but decrease in specificity from 58.69% to 15.21%.

Table 6. Comparative analysis between CRP and culture findings.

		Culture +ve	Culture -ve	Sensitivity	Specificity	PPV	NPV
CRP 1	+ve	1	19	25%	58.69	5	90
	-ve	3	27				
CRP 1 and or 2	+ve	4	39	100%	15.21	9.3	100
	-ve	0	7				

On comparative analysis of CRP and culture of the body fluid, serial measurement of CRP shows increased sensitivity, NPV and PPV but decreased specificity.

CRP and onset/type of sepsis

Table 7. Comparison of CRP with the culture findings in early onset proven sepsis.

Early onset sepsis (n=22)							
		Proven sepsis	Probable/ No sepsis	Sensitivity	Specificity	PPV	NPV
CRP 1	+ve	1	8	50%	60	11	92
	-ve	1	12				
CRP 1 and or 2	+ve	2	17	100%	15	10.5	100
	-ve	0	2				

In early onset proven sepsis, serial monitoring of CRP shows increased in sensitivity and NPV, and decreased specificity. PPV almost remained same

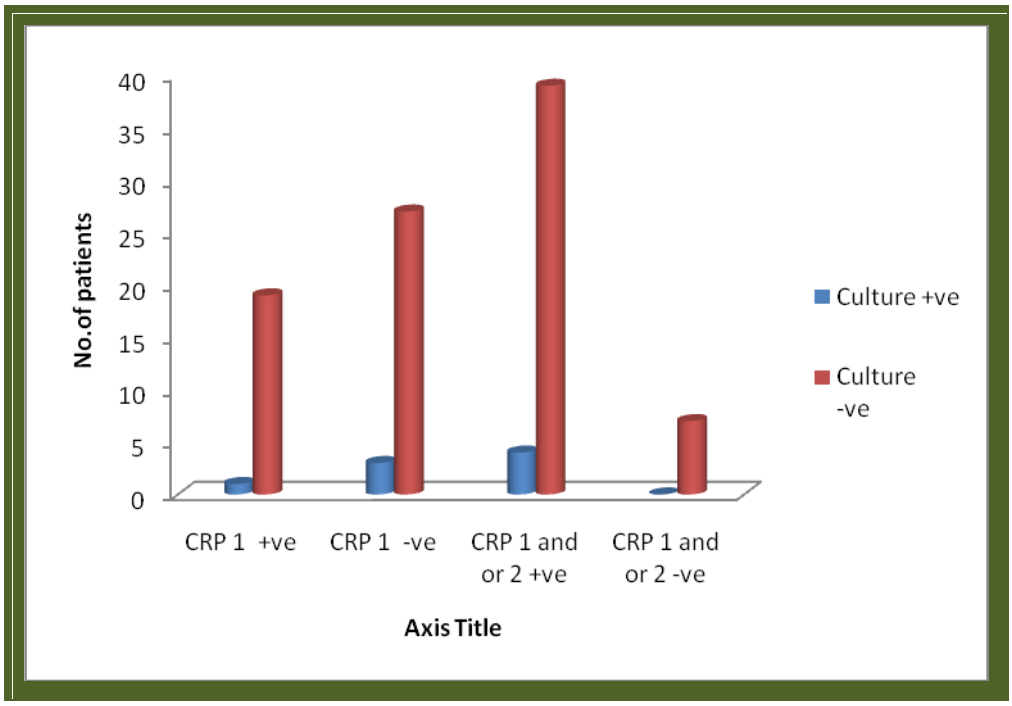

Fig 6. Bar diagram showing Comparative analysis between CRP and culture findings.

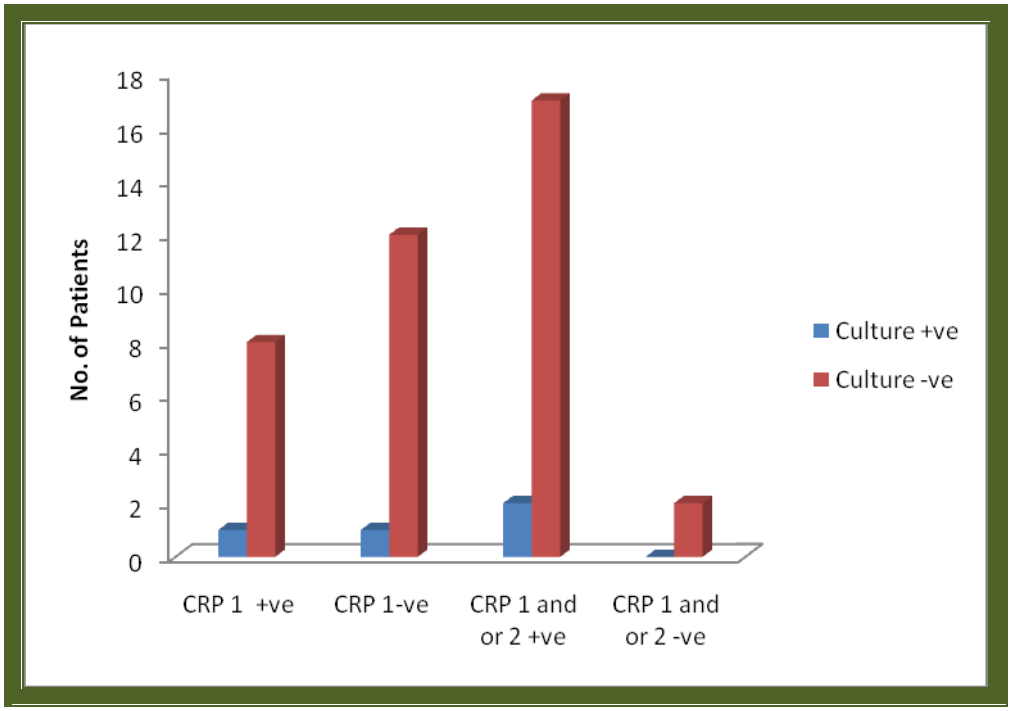

Fig 7. Bar diagram showing Comparison of CRP with the culture findings in early onset proven sepsis.

Table 8. Comparison of CRP with culture findings in late onset proven sepsis.

Late onset sepsis (n=28)							
		Proven sepsis	Probable/ no sepsis	Sensitivity	Specificity	PPV	NPV
CRP 1	+ve	0	11	0%	57.69	0	88.2
	-ve	2	15				
CRP 1 and or 2	+ve	2	22	100%	15.5	8.3	100
	-ve	0	4				

In late onset proven sepsis, serial monitoring of CRP shows increased sensitivity, PPV and NPV, and decreased specificity.

Table 9. Comparison of CRP with culture findings in early onset probable sepsis.

Early onset sepsis (n=22)							
		Probable	Proven/ No sepsis	Sensitivity	Specificity	PPV	NPV
CRP 1	+ve	8	1	44.40%	75	88.8	23
	-ve	10	3				
CRP 1 and or 2	+ve	17	2	94.00%	50	89.4	66.6
	-ve	1	2				

In early onset probable sepsis, serial monitoring of CRP shows increased sensitivity PPV and NPV, and decreased specificity.

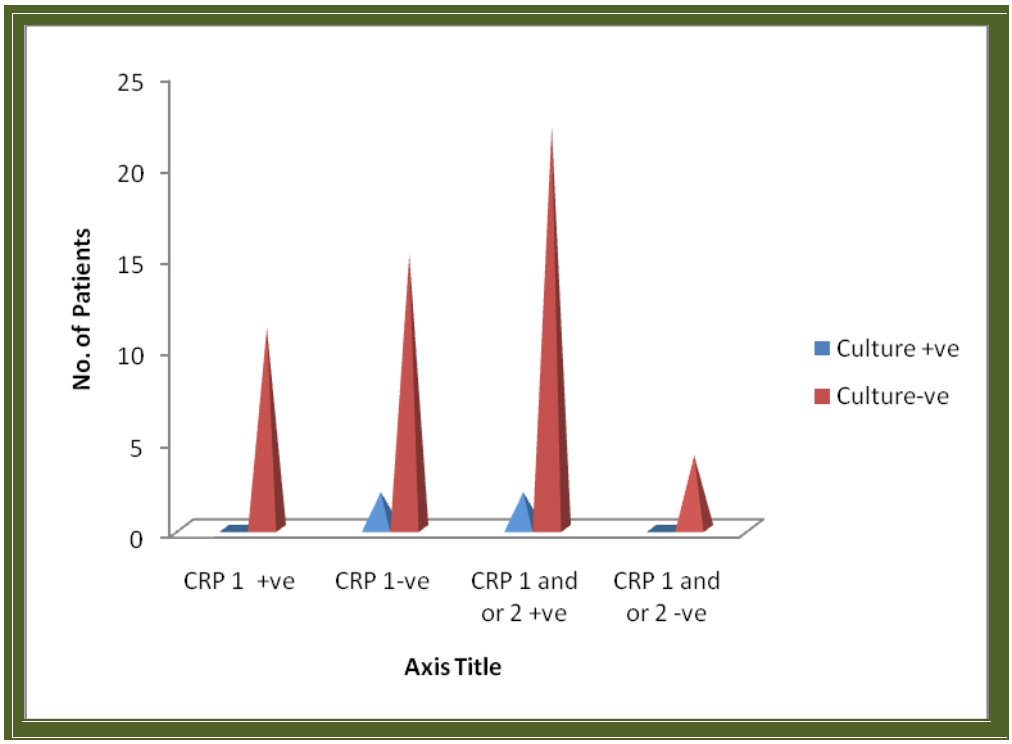

Fig 8. Bar diagram showing Comparison of CRP with culture findings in late onset proven sepsis.

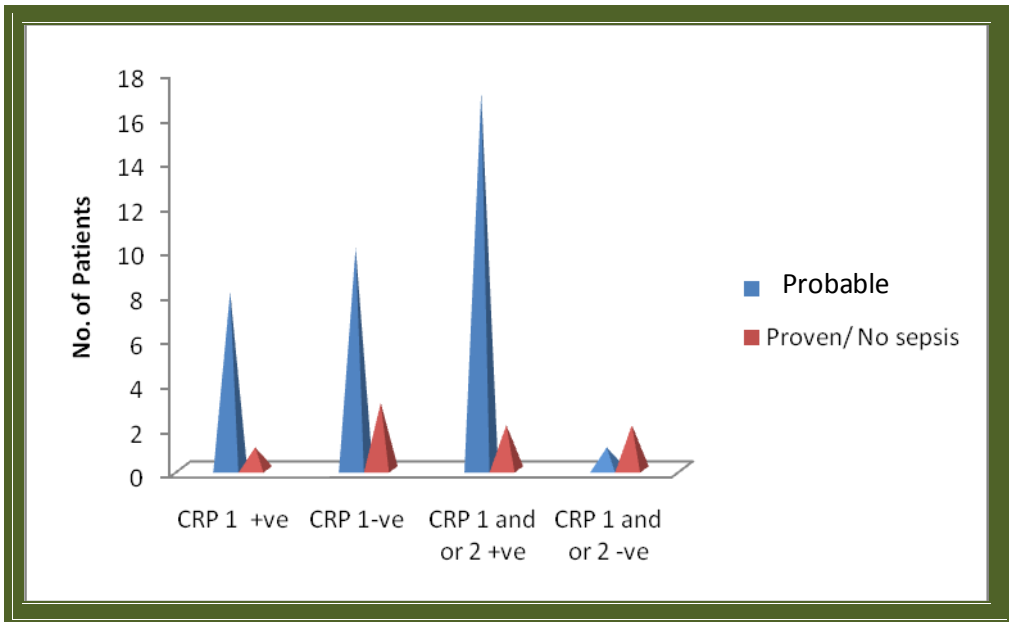

Fig 9. Bar diagram showing Comparison of CRP with culture findings in early onset probable sepsis.

Table 10. Comparison of CRP with culture findings in late onset probable sepsis:

Late onset sepsis (n=28)							
		Probable	Proven / No sepsis	Sensitivity	Specificity	PPV	NPV
CRP 1	+ve	11	0	47.8%	100	100	29.4
	-ve	12	5				
CRP 1 and or 2	+ve	22	2	95.6%	60	91.66	75
	-ve	1	3				

In late onset probable sepsis, serial monitoring of CRP shows increased sensitivity and NPV, and decreased specificity and PPV.

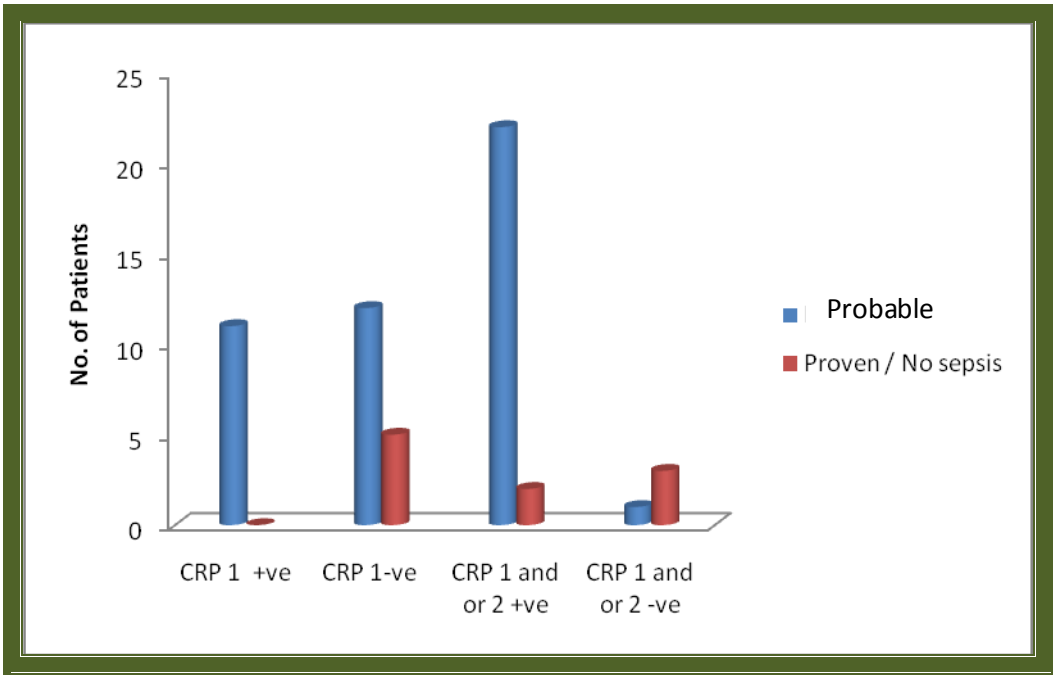

Fig 10. Bar diagram showing Comparison of CRP with culture findings in late onset probable sepsis.

There is increase in sensitivity, PPV and NPV with decrease in specificity in CRP 1 and 2 measurement compared to CRP 1 measurements alone, both in early and late onset proven and probable sepsis.

DISCUSSION

Of the 50 neonates included in the present study,31(62%) are male and 19(38%) are female with ratio of 1.6:1 which is similar to the study conducted in TUTH with male: female ratio of 1.8:1.[31] Majority of the study population are LOS (56%) with equal sex distribution. EOS is found in 44% with male predominance(77%). Sholl B J and Kliegman R.M have reported approximately two fold higher incidence of sepsis in male than female suggesting the possibility of a sex linked factor in host susceptibility.[11]

Majority of the study group had probable sepsis (82%). Only 8% had proven sepsis and 10% had no sepsis. . Among those suspected of early onset sepsis (EOS), only 2 had proven sepsis and 18 had probable sepsis and 2 were proven to have no evidence of sepsis on clinical examination and investigation. Similarly among 28 neonates suspected of Late onset sepsis (LOS) 2 had proven sepsis and 23 had probable sepsis and 3 had no evidence of sepsis. Proven sepsis is more in early onset sepsis 2(9%) in comparison with late onset sepsis 2(7.14%). The higher percentage in early onset sepsis is probably due to direct admission to our neonatal unit without prior antibiotic therapy.

In contrast to other studies number of proven cases were less as shown below. This could be probably due to prior antibiotic therapy as ours being tertiary hospital, many cases were referred with prior antibiotic therapy.

	Present study	Kaiser et al [49]	Pourcyrous et al	Parikh et al
Proven sepsis(%)	8	15.6	27	47

In the present study statistical analysis between positive serial CRP measurement and age of onset of sepsis shows no significant relationship ($p>0.05$)

In the present study among the culture positive cases, there is equal incidence of Gram positive and Gram negative organisms. These findings are in contrast with the study carried out in Chennai by Karthikeyan et al,with staphylococcus aureus being the most common pathogen 61.5%, Klebsiella 21.9% and E. coli being 13.5%.[35] Siegel JD has reported group B Streptococci as the most common pathogen in the United States followed by CONS.[32] The Janssens R, reported the group B Streptococci and E. coli accounted for 60-70% of neonatal infection.[29] The equal incidence of Gram positive and Gram negative organisms in our study may be because of difference of place and microbial flora.

In the present study CONS and NFGNB are isolated from blood culture in 4.5% each of the early onset sepsis. Staphylococcus is isolated from urine in 3.5% of late onset sepsis only. The organisms isolated from CSF is Pseudomonas aeruginosa in 3.5% of late onset sepsis. This is a referred case of LOS from private hospital where treatment was given for 3 days and hence nosocombial infection is suspected.

In the present study, 1(5%) case of proven sepsis and 19(95%) cases of probable sepsis is present among CRP1 positive cases with significant correlation($p<0.05$).While 4(9.3%) cases of proven sepsis and 30(90.6%) cases of probable sepsis is present among CRP1 and 2 positive cases with highly significant correlation ($P<0.001$), which is consistent with the study of Benitz et al indicating that serial CRP monitoring had clinical utility in diagnosis of neonatal sepsis.[10]

Further comparative analysis of CRP and culture of the body fluid showed that serial measurement of CRP 1 and 2 showed increase in sensitivity from 25.0% to 100%, NPV from 90% to 100% and PPV was increased from 5.0% to 9.3% but decrease in specificity from 58.69% to 15.21%.

The sensitivity, specificity, positive predictive value and negative predictive value in the present study is shown in the table with comparison to similar study carried out by Benitz et al[10].

Comparison of present study with the similar study carried out by Benitz et. al.

	Present study		**Benitz et. al[10]**	
	CRP 1	**CRP 1 and 2**	**CRP 1**	**CRP 1 and 2**
Early onset sepsis				
Number	*22*		*982*	
Proven sepsis				
Sensitivity	50.0%	100%	78.9%	88.9%
Specificity	60.0%	15.0%	78.4%	73.8%
PPV	11.0%	10.5%	6.7%	6.0%
NPV	92.0%	100%	99.3%	99.7%
Probable sepsis				
Sensitivity	44.4%	94.0%	92.9%	97.6%
Specificity	75.0%	50.0%	83.9%	79.3%
PPV	88.8%	89.4%	35.4%	30.6%
NPV	23.0%	66.6%	99.2%	99.7%
Late onset sepsis				
Number	*28*		*150*	
Proven sepsis				
Sensitivity	0%	100%	84.4%	96.4%
Specificity	57.69%	15.3%	74.6%	71.8%
PPV	0%	8.3%	47.4%	45.0%
NPV	88.23%	100%	94.6%	98.8%
Probable sepsis				
Sensitivity	47.8%	95.6%	85.0%	94.4%
Specificity	100%	60.0%	79.1%	76.1%
PPV	100%	91.66%	59.6%	56.7%
NPV	29.4%	75.0%	93.5%	97.6%

It is found that the sensitivity of CRP1 was substantially higher, but maximum sensitivities are achieved by combination of CRP1 and 2. The sensitivity of CRP1 ranged between 0-50%, specificity 60-100%, negative predictive value between 23-92% and likelihood ratio 0.93 to 1.68 in present study. The sensitivity of CRP1 and 2 ranged between 94-100%, specificity 10-60%, negative predictive value 66.6-100% and likelihood ratio 1.35 to 2.38. The statistical analysis showed CRP1 and 2 had significantly high correlation compared to the single measurement of CRP1 only with both the proven and probable sepsis of the early as well as the late onset group ($P<0.05$).

These findings are consistent with the findings of several other authors; Hengst, found the sensitivity between 78.9%-98%, specificity between 84%-97% and negative predictive value of 99% in detecting sepsis[12]. Baptista et al also reported the sensitivity of 91%, specificity of 93%[55]. Similarly, Nuntnarumit et al reported sensitivity of even 100%, specificity 94%, positive predictive value 91.6% and negative predictive value 100% and concluded that predictive value of CRP could be enhanced by serial rather than a single measurement[57].

Stephan S et al indicated that serial C reactive protein levels is a useful marker for guiding duration of antibiotic therapy in suspected neonatal infection[65]. Burke reported that CRP measurement correctly identified 99 of 100 cases not requiring further antibiotic therapy and thus concluded that safe and practical approach of CRP in a suspected neonatal sepsis[66].

CONCLUSION

Neonatal sepsis is one of the leading causes of neonatal morbidity and mortality especially in developing countries including India. It requires rapid and accurate diagnosis as well as timely treatment for the improved outcome. As the culture positivity rates are low and takes time for growth, serial CRP levels can be used in the diagnostic evaluation of neonates with suspected infection. Further serial CRP levels are more sensitive than single CRP measurement.

SUMMARY

This study is conducted in the Neonatal unit, Department of Pediatrics, R. L. Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Medical College (SDUMC), Kolar, to find out the significance of serial C reactive protein levels in neonatal sepsis. This study revealed the following findings:

1. Early onset neonatal sepsis is higher in male (77.2%) as compared to female 5(22.8%), whereas male: female ratio is equal in late onset sepsis. Overall M: F ratio is 1.6:1.

2. Prevalence of late onset sepsis is higher (56%) in comparison to the early onset sepsis (44%).

3. Majority of the cases are probable sepsis (82%) and remaining are proven sepsis 4(8%) and no sepsis 5 (10%).

4. CRP measurement is positive in 86% cases. Among them CRP1 is positive in 46% and subsequently CRP2 became positive in remaining 54% cases.

5. 5% cases of proven sepsis and 95% cases of probable sepsis is present among CRP1 positive cases with significant correlation($p<0.05$).While 9.3% cases of proven sepsis and 90.6% cases of probable sepsis is present among CRP1 and 2 positive cases with highly significant correlation ($P<0.001$)

6. The serial CRP (1 and 2) measurement showed higher sensitivity and negative predictive value compared to CRP1 alone, in both early as well as late proven and probable sepsis.

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ANNEXURES: PROFORMA

Study No.

Details of Mother:

IP No. ……..

Name …… Age ……. yrs.

Husband's Name …….

Address …… Tel No. Off. …….. Res.……..

Date and time of admission ……..……

LMP …../…./…. EDD ……/…./…. Gestation by date …… wks.

Gravida 1) Primi. 2) Multi.

Blood grouping / Rh typing …….

H/O Diabetes Mellitus Y/N

No. of ANC visits 1) No visit 2) < 4 visits. 3) > 4 visits.

PROM 1) <18 hrs 2) 18-24 hrs. 3) > 24 hrs. 4) > 48 hrs.

Antibiotics if any, specify …….. Duration

Fever Temp. ≥ 38oC within 24 hrs or delivery Yes / No.

Foul smelling liquor Yes / No.

Chorioamnionitis Yes / No.

Details of labour:

Mode of delivery:

i) ND

ii) Instrumental a) LSCS

b) Vacuum

c) Forceps

If assisted indication ……

Amniotic fluid colour:

i) Clear

ii) Mild meconium stained

iii) Moderate meconium stained

iv) Thick meconium stained□

Details of Baby:

IP No. ……

Date of birth …….. Time ……. Sex ……

Birth weight i) <1.5 kg ii) 1.5-2.459 kg iii) 2.5-4 kg iv) > 4kg

AFD / SFD / LFD.

Apgar score 1 min …… 5 min ……

Resuscitation if any a) Bag and mask …… Duration …….

b) Intubation …….. Duration …….

c) Medication Y / N if Yes, specify……

Clinical Signs and Symptoms:

A) Symptoms a) Refusal to feed Y / N

b) Vomiting Y / N

c) Lethargy Y / N

d) Abdominal distension Y / N

e) Fever Y / N

B) General signs a) Lethargy Y / N

b) Temperature …… oC

Hypothermia Y / N

c) Cyanosis Y / N

d) Icterus Y / N

e) Pallor Y / N

f) Skin Normal Y / N

If No i) Petechiae Y / N

ii) Pustules Y / N

g) Apnoea Y / N

h) Tachypnoea Y / N

Others

C) Respiratory a) Resp. rate …….. / min

b) Grunting Y / N

c) Nasal flaring Y / N

d) Chest retraction Y / N

e) Auscultation Added sounds Y / N

If yes specify ……...

D) CVS a) Heart rate i) < 100/min ii) > 100/min

b) Cap. refill time i) < 2 sec ii) > 2 sec

c) Heart sound S1 S2 Normal Y / N

If no, specify …….

d) Murmur Y / N

If yes, specify ……

E) P / A a) Umbilical discharge/redness Y / N

b) Abdominal girth …… cm

c) Liver …… cm

d) Spleen …….. cm

e) BS Present / Absent

F) CNS a) Neonatal Reflexes:

i) Moro's Absent / Complete / Incomplete

ii) Sucking Absent / Poor / Good

iii) Grasp Absent / Present

b) OFC …….. cm

c) Fontanelle Flat / Bulged

G) Congenital Anomalies Y / N

If yes specify ……..

Investigations:

1) Blood i) TC a) <5000/cumm b) >20000/cumm

ii) DC N ….. % L ….. % E ….. % M ….. % Bands ….. %

iii) Hb ……. gm/dl

iv) Platelets …….. /cu mm

v) Blood sugar …….. mmol/L or …….. mg/dl.

vi) Blood culture and sensitivity

a) Sterile in 96 hrs.

b) Organism …… Sensitive to ……

2) Chest X-ray …….

3) Urine C/S a) Sterile

b) Organism ……. Sensitive to ……..

4) CSF i) TC ……. /cu mm

ii)DC N …….. % L …….. %

iii) Sugar …….. mmol/L

iv) Culture and sensitivity

a) Sterile

b) Organism ……. Sensitive to ……

5) Serum C-Reactive protein level

1st ……. mg%

2nd ……. mg%

3rd …….. mg%

6) Others

Diagnosis …….

Treatment ……

Duration of Hospital stay ……...

Outcome ……...

KEY TO MASTER CHART

B/O	Baby of
C/S	culture and sensitivity
ROF	Refusal of feeds
CON	Convulsions
TACP	Hurried respiration
DIA	Diarrhea
ICT	Icterus
VOM	Vomiting
UM.DIS	Umbilical discharge
CONS	Coagulase negative Staphylococcus
P.Aeru	Pseudomonas Aeruginosa
NFGNB	Non Fermenting Gram Negative Bacilli
MSSA	Methicillin Sensitive Staphylococcus Aureus
EOS	Early onset sepsis
LOS	Late onset sepsis
WBC	White blood cells (X 10^6 cells/ cu mm)