Role Of Plasma Fibrinogen In Diagnosis And Prediction Of Short Term Outcome Of Neonatal Seps

By

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LIST OF ABBREVIATION USED

CSF : Cerebro spinal fluid

ESR : Erythrocyte sedimentation rate

EOS : Early onset sepsis

LOS : Late onset sepsis

nRBC **:** Nucleated red blood cells

CRP : C Reactive Protein

IV : Intravenous

DIC : Disseminated intravascular coagulation

SLE : Sysytemic Lupus Erythematosis

EDTA : Ethelene Diamine Tetra Acetic acid

PT : Prothrombin time

aPTT : activated partial thrombin time

PCT : Procalcitonin

ROC : Receiver Operator Curve

ABSTRCT

BACKGROUND:

Neonatal septicemia is characterized by systemic response to bacterial infection documented by positive blood culture in first 4 weeks of life. Neonatal septicemia is one of the leading cause of neonatal mortality and morbidity[1,2,3]. It is a clinical syndrome characterized by signs and symptoms of infection with or without bacteremia in the first month of life. Early diagnosis of neonatal sepsis is difficult clinically as various other disorders affecting newborn mimic it.

Blood culture is considered to be the gold standard diagnostic modality for neonatal sepsis, but the culture reports would be available only after 48-72 hours.[3,4] Since early detection and treatment can reduce morbidity and mortality, there is a need for markers of sepsis like hematological parameters and acute phase reactants.

Plasma fibrinogen is one such acute phase reactant. Fibrinogen also known as factor I of coagulation pathway, is produced in liver as a high molecular weight glycoprotein and plays an important role in haemostasis as a coagulation factor[2]. Thrombin splits off fibrinopeptides A and B from fibrinogen to form fibrin monomers which polymerize subsequently. Fibrin is stabilized to form a dense aggregate by covalent cross-linkage catalyzed by activated factor VIII[2].

Decrease in fibrinogen level is observed in disseminated intravascular coagulation (DIC) where it is consumed in the coagulation process. Fibrinogen levels are increased in inflammation where it acts as positive acute phase reactant[2].

OBJECTIVES:

To study the relation between plasma level of fibrinogen and neonatal sepsis and its outcome.

MATERIALS AND METHODS:

A prospective case control hospital based study was carried out on neonates fulfilling the inclusion and exclusion criteria admitted in NICU, department of Pediatrics, referred to the Department of Pathology in BLDE (Deemed to be University) Shri B.M. Patil Medical College Hospital & Research Center, Vijayapura.

Study period: 1st December 2017 to 30th June 2019.

Blood samples were collected in Citrated, plain and EDTA anticoagulated vacutainers for Plasma fibrinogen, blood culture and analysis of hematologic parameters respectively.

RESULTS:

48 suspected cases of neonatal sepsis and 42 cases of neonatal jaundice were included in study group and control group respectively. Plasma fibrinogen levels were found to be significantly elevated in study group than in control group ($p< 0.050$). It had sensitivity and specificity of 80% and 72.8% respectively for diagnosis of neonatal sepsis at cut off value of 305.5 mg/l.

CONCLUSION:

In our study, we found that plasma fibrinogen act as an acute phase reactant and can be used as an immediate marker for detection of early onset neonatal sepsis.

Plasma fibrinogen is a simple, rapid and cost effective test.

It could guide the clinicians in instituting early treatment and adopting aggressive treatment whenever applicable, thus reducing the neonatal morbidity and mortality.

KEYWORDS: Sepsis, Acute phase reactant, plasma fibrinogen.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

"Neonatal sepsis is a clinical syndrome resulting from the pathophysiological effect of severe bacterial infection in the first month of life." [1] Neonatal sepsis is the most common cause of morbidity and second most common primary cause of neonatal mortality next to perinatal asphyxia. Neonatal mortality rate was 25.3 per 1000 live births as per National Neonatal Perinatal Database (NNPD) 2002-2003 Report.

Since neonatal sepsis is a preventable cause of mortality, early detection of onset of sepsis is helpful for treatment and also in preventing complications. Neonatal sepsis symptoms and signs includes refusal to feed, fever, excessive cry, hypoglycemia, hypothermia, tachycardia, tachypnea, convulsions. These are not specific to neonatal sepsis and also seen in various other conditions, which pose difficulty in diagnosis. [2, 3, 4]

Blood culture, CSF, urine or other body fluids culture are gold standard methods for diagnosis of sepsis but they need at least 48 to 72 hours for reporting.[4, 5] This delays crucial periods during which antibiotics should be administered for treatment. Also there are few false negative blood culture cases present which need to be diagnosed.

This necessitates other alternate methods for diagnosis or other indicators for sepsis which requires less time and hence reducing the time to start the treatment.

There are few other method which are used to support the diagnosis like, leukocyte indices, absolute neutrophil count, toxic granules, nRBC's, Acute phase reactants like micro ESR, C-reactive protein, procalcitonin.

Plasma fibrinogen is also one such an acute phase reactant. Mean value of plasma fibrinogen level in healthy preterm and full term neonates aged upto 30days range from 2.43 to 2.54g/L and 2.70 to 2.83g/L respectively[2,6,7]. It is seen to increase several fold in inflammatory conditions as a positive acute phase reactant.[2] It is produced in the liver as one of the coagulation pathway agent, which is needed for haemostasis. This feature is used for prediction of neonatal sepsis in this study.

This study was undertaken to study the relation between plasma levels of fibrinogen and neonatal sepsis and it's outcomes like prediction of complications in neonatal sepsis.

AIMS AND OBJECTIVES

To study the relation between plasma level of fibrinogen and neonatal sepsis and its outcome.

REVIEW OF LITERATURE

"Neonatal sepsis is defined as a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in first month of life"[8]. Neonatal sepsis can be early onset or late onset neonatal sepsis depending on the age at onset of sepsis. Early onset neonatal sepsis is said when newborn presents with sepsis within 72 hours of birth and late onset of neonatal sepsis when newborn develops sepsis 72 hours after the birth.[9]

According to National Neonatal Perinatal Database (NNPD) 2002-2003 Report, most common organism reported in blood culture was Klebsiella pneumonia (30.1%), Staphylococcus aureus (16.2%), E coli (13%) and Pseudomonas species (9.3%).

According to National Neonatal Forum of India neonatal sepsis is divided into **Clinical sepsis** and **Culture positive sepsis**.[5,9] As described in Table 1

Table1	**Clinical sepsis**	**Culture positive sepsis**.
	Neonate having signs and symptoms of sepsis clinically, along with one of the following criteria	Neonate having signs and symptoms of sepsis clinically, along with one of the following criteria
1)	Existence of predisposing factors- maternal fever, foul smelling liquor or prolonged rupture of membranes for >24hrs	Isolation of pathogen from blood or CSF or urine or abscess.
2)	Positive septic screen- presence of two of the four parameters, TLC (<5000/mm), band to	Pathological evidence of sepsis on autopsy.

	total polymorphonuclear cell ratio of >0.2, ANC < 1800/mm, CRP>1mg/dl & micro ESR > 10mm/hr	

Early onset neonatal sepsis is mainly caused by antenatal or perinatal infections through mother. Following are the risk factors associated with it; [2,8,10]

- Premature rupture of membranes
- Low birth weight or prematurity
- Repeated per vaginal examination during labor
- Prolonged labor after rupture of membrane (>24 hrs including 1st and 2nd stage of labor)
- Complicated deliveries (forceps delivery or vacuum assisted delivery)
- Perinatal asphyxia
- meconium stained amniotic fluid
- Maternal infection within 2 weeks prior to the delivery.[8]

Late onset neonatal sepsis is caused either by nosocomial or community based infection. Following are the risk factors associated with it;[2,7,8,9,10]

- Low birth weight or prematurity

- Poor care of the cord
- Unnecessary prolonged stay at the hospital
- Invasive procedures
- Bottle feed

PATHOGENESIS OF SEPSIS:

Fetus is protected by passive immunity provided by maternal antibodies and intact amniotic membrane, which acts as a barrier for most of the pathogens during in utero period. Few pathogens like Treponema pallidum and Listeria monocytogenes can invade through placental barrier causing stillbirths or congenital anomalies.[11,12]

Perinatal infections are caused by ascending infection from maternal genital tract or through aspiration of the infected amniotic fluid. Transplacental infection or infection through surroundings after delivery can also lead to neonatal sepsis. [11, 12]

The skin and mucosal epithelial layers act as first line defense same as adults and pediatric age group. Once pathogen pass these barriers innate immunity comprised of dendritic cells, monocytes, neutrophils, Natural Killer cells and mast cells play an important role in preventing infection. If it fails then the Adaptive immunity takes up the role.

"The immune system of the both full term and preterm neonates' exhibit physiological immunodeficiency."[13] This results in increased susceptibility of newborns to sepsis than adults and younger children. In neonates primary mechanism of distinguishing of various antigens is depended on Antigen Presenting Cells (APC's) like dendritic cells. Lymphocytes are stimulated by proinflammatory cytokines, so lymphocytic response to sepsis is more favored by cytokines and other alarm signals. Hence mature virgin neonatal cells can be immunized or tolerated depending on environment or the type of APC's.[13]

Neonatal T lymphocytes differentiates from adults in the way that, there is a high CD_4 and CD_8 ratio, Neonatal T lymphocytes are hard to stimulate in comparison to adults, resulting in poorer response. Neutrophils in bone marrow are easily exhausted and function also deteriorates in the presence of persistence of infection. Compliment system function is also not in par with adults.[13]

Signs and symptoms of neonatal sepsis:[8]

- Lethargy
- Refusal to feed
- Excessive cry
- Fever or hypothermia
- Hyperglycemia
- Tachycardia or bradycardia
- Respiratory distress or tachypnea
- Convulsions
- Hypotonia and absence of reflexes
- Metabolic acidosis
- Shock/ hypoperfusion
- Skin changes, petechae, hemorrhage, pustules
- Discharge from umbilicus
- Diarrhea/ vomiting.

INVESTIGATIONS: Since above mentioned signs and symptoms are not specific and can be subtle sometimes, so various investigations are carried out to support diagnosis. Following are the investigations done in neonatal sepsis:

Blood culture: Culture and sensitivity to antibiotic from blood and body fluids is still the gold standard method for diagnosis of neonatal sepsis.[2,5] Sample for culture and sensitivity should be collected before starting antibiotic administration. It takes at least 48 to 72 hours for reporting.[2,5] In few cases false negative reports may come, can be due to antibiotic administration of mother in perinatal period or infection can be transient or sufficient blood sample was not inoculated.[14] While if sample is taken from contaminated catheter or IV canal then false positive results will come.

Septic screen: Sepsis screen is defined as when two out of these following indirect parameters are positive; total leucocyte count <5000/cm, band form to total neutrophil ratio >0.2, micro-ESR >15mm at the end of 1st hour and C-reactive protein > 0.8/100ml.[3,5] Since septic screen comprises haematological parameters, it provides results in a short duration and is also a better option for predicting sepsis for primary care centers, where only basic haematological investigations are done and blood culture facility is not available.[5]

Bandita Das *et al*[5] evaluated the validity of hematological parameters in neonatal septicemia. Criteria used by them were components of septic screen. They concluded that septic screen is good tool to diagnose neonatal sepsis, the best single test was CRP and best combination was positive CRP with leucopenia.

Hematological parameters:

- Leucocyte count- Total counts more than 30,000/µl and less than 5000/ µl are considered as abnormal. [14]

  In a study conducted on 60 neonatal sepsis cases by Das B *et al*[5] found out that leucopenia (<5000 cell/cumm) was present in 54.5% of proven cases of sepsis and 37% of clinically suspected cases of sepsis.

  In a study done by Zawar MP *et al*[15]observed sensitivity and specificity of 82% and 70% respectively. Leucopenia was present in 83% of culture positive cases.

  Bhat RY *et al*[16] in their study observed that total leucocyte count of < 5000 and > 20000 in 29.7% of cases. It showed significantly more positivity among the symptomatic than the asymptomatic neonates.

  Buch *et al*[17] considered total count of <5000 cells/cumm or >20,000 cells/cumm as abnormal in their study. They observed 50.77% sensitivity and 63.4% specificity. They concluded that total leucocyte count alone is not significant in the diagnosis of neonatal septicemia.

- Platelet count- normal value is 1.5lac/ µl. Decreased platelet counts are associated with late onset sepsis. Mechanism causing thrombocytopenia in sepsis can be decreased production due to bone marrow infection, increased destruction due to endothelial injury or sepsis related complications like DIC causing consumption of platelet.[18]

- nRBC's- Presence of nRBCs in peripheral blood in neonates was reported by Lipmann in 1924. For neonates nRBCs levels of more than 10/100WBCs considered as increased level. It increased in conditions which induce erythropoietin production like hypoxia, anemia and chorioamnionitis due to various infections like TORCH infection. [9,19,20].

- In a study conducted on 60 neonates by Abhishek MG *et al*[9] found that nRBCs were reliable parameter in diagnosing neonatal sepsis.

Erythrocyte sedimentation rate: "The erythrocyte sedimentation rate measures rate of settling (sedimentation) of erythroblasts in anticoagulated whole blood."(21) The normal range for ESR in neonates is day of life plus 3mm/hr up to 15mm/hr.[22]

Mechanism of ESR is red blood cells tends to forms rouleaux, which is heavier than individual red blood cells and tend to settle at the bottom due to gravity. The length of the plasma on top is measured in millimeters, which corresponds to ESR. Various conditions which affect ESR are red cell shape and size, red cell mass, plasma composition, fibrinogen, acute phase reactants, technical factors. Mechanism of increase in ESR in acute inflammation is due to increased levels of fibrinogen that neutralizes the negative charges in the red blood cells facilitating increased rouleaux formation.[21,23, 24]

Factors influencing ESR are enlisted below (**Table 2)**

Factors increasing ESR	Factors decreasing ESR
Physiological, (old age, pregnancy)	Newborns [24]
Hematological (anemia, macrocytes)	Microcytosis, polycythemia, marked-leucocytosis
Elevated fibrinogen or other plasma proteins	Low fibrinogen
Technical factors like high temperature, tilting of ESR tube	Technical factors like vibration of tube during test

Micro-ESR: is performed by using capillary blood in anticoagulant coated capillary tube. As it uses minimal amount of blood it is apt for neonates and pediatric patients.

Causes of increased ESR:

Infections– pyogenic arthritis, tuberculosis, osteomyelitis, acute rheumatic fever, acute hepatitis.
Inflammatory conditions – rheumatoid arthritis, SLE, Temporal arteritis, acute infarctions, malignancy, paraproteinemias and others. (anemia, ruptured ectopic pregnancy, renal disease).

Das B *et al*[5] found out that micro ESR was ≥ 15mm at first hour in 60% of proven cases of sepsis and 37% of clinically suspected cases of sepsis.

ACUTE PHASE REACTANTS:

- **C reactive protein –** It is discovered in 1930 by Tillet and Francis and it reacts with C-polysaccharide of capsule of pneumococci hence named as C- reactive protein. It is a β-globulin, weighing around 115 to 140 kD. Levels of CRP increase at least by 25% in various inflammatory conditions, infection and neoplastic conditions. It also supportive in stimulating complement activation and macrophage phagocytosis.[21,23,24,26,27].

- The normal serum level is 0-10μg/ml. It takes 6 to 8 hours to increase following any exposure to infection or any inflammation. The levels above 8μg/ml indicate an inflammatory response. It has a half life of nineteen hours and during acute phase response it increases by 1000 folds.[28]

  Uses of CRP:

  1) Detection of infections especially in neonatal bacterial sepsis meningitis and also postoperative infection,

  2) Inflammatory conditions like Myocardial infarction,

  3) Severity assessment in inflammatory conditions like rheumatoid arthritis,

  4) Screening for organic diseases. [21]

In a study conducted by Mondal *et al*[1] on 62 cases over a period of 1 year found out that CRP is the single best marker for diagnosis of sepsis between 24 to 48 hours of onset.

Vandana *et al*[3] found out that CRP has 90% sensitivity and 50% specificity in prediction of neonatal sepsis.

- **Serum procalcitonin-** It is produced as precursor of calcitonin in thyroid gland. It is seen to be increased in neonates with sever bacterial sepsis, fungal or parasitic infection, vascular and respiratory distress syndromes.

  In a study conducted by Jean Guibourdenche *et al*[4] on 136 neonates, they found that procalcitonin had a good negative predictive value in excluding infection shortly after birth.

  In a study conducted by Mondal *et al*[1] on 62 cases over a period of 1 year found out that procalcitonin is a useful marker for diagnosis of Early onset neonatal sepsis.

- **PLASMA FIBRINOGEN-**

  It is also known as factor 1 of coagulation pathway, needed for final step of clot formation. It is normally produced in liver along with other coagulating factors and in megakaryocytes. It is a glycoprotein molecule weighing 340kD, made up of two sets of tripeptide units of α, β and γ chains. These are linked at N-terminal region by disulfide bonds. This N-terminal is projecting out segment from central domain of the three domains of fibrinogen.[2,27,29]

"The N-terminal region of α-α^1, β-β^1 subunits are highly negatively charged and through charge-charge separation, prevent aggregation of fibrinogen. Thrombin cleaves these N-terminal peptides and allows the resulting fibrin molecules to produce soft clot." (27,29) This fibrin is then stabilized to form a hard clot by activated factor XIII, which induces formation of isopeptide linkage between the S-carboxylamide of glutamine residues and the ε amino acid of lysine residues of two fibrin molecules and individual fibrin molecule are interconnected by covalent linkage in hard clot. [2,27,29]

Fibrinogen also acts as a positive acute phase reactant, besides playing a major role in coagulation pathway to maintain haemostasis.[2,23,24,25,29,30] Functions of fibrinogen are:

1) Final step of clot formation in coagulation.

2) Contributes to the viscosity of blood.

3) Acts as a positive phase reactant.

4) Facilitates ESR by neutralizing negative charges and increasing rouleaux formation, leading to increased ESR in inflammatory condition.

Increased levels of Fibrinogen is seen in[25,31]:

- Inflammatory conditions
- Pregnancy
- In women using oral contraceptive pills

- Infections
- And following trauma.

Decreased levels of Fibrinogen is seen in[25,31]:

- Acquired disease like; disseminated intravascular coagulopathy,

  Primary and secondary fibrinolysis

- Congenital diseases like; Dysfibrinogenemia, Hereditary afibrinogenemia.
- Pancreatic and severe hepatic dysfunction.

"Mean value of plasma fibrinogen level in healthy preterm and full term neonates aged up to 30days range from 2.43 to 2.54g/L and 2.70 to 2.83g/L respectively"(2,6,7).

In a study conducted by Piyali Mitra *et al*[2] on 65 cases of neonatal sepsis with 75 cases of neonatal jaundice taken as controls found out that plasma fibrinogen is significantly elevated in neonatal sepsis compared to those without sepsis where it acts as acute phase reactant in early phase of infection. They also found out that in sepsis cases which developed complications showed low plasma levels of fibrinogen but PT and APTT were prolonged where as in cases which did not develop complications, plasma levels of fibrinogen was elevated but PT and APTT were within normal range.

In a study conducted by Guibourdenche *et al*[4] on 120 neonatal sepsis cases have found out that plasma fibrinogen is significantly increased in neonatal sepsis.

MATERIAL AND METHODS

Source of data:

Neonatal sepsis cases and hyperbilirubinemia cases as controls were collected for case control study, from hospital NICU, in department of pediatrics, BLDEU Shri B .M. Patil Medical College, Hospital and Research Centre, Vijayapura. Blood samples of both group cases, which fulfill inclusion and exclusion criteria, were received at department of pathology for investigation in BLDEU Shri B .M. Patil Medical College, Hospital and Research Centre, Vijayapura.

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Study period: 1st December 2017 to 30th June 2019.

Methods of collection of Data:

Clinically suspected cases of neonatal sepsis and hyperbilirubinemia, which were admitted in Neonatal Intensive Care Unit (NICU) were taken as cases and controls for this Case Control study.

Data of the both study group like clinical history, antenatal history, clinical examination and demographic data were collected.

Blood samples were collected in plain, EDTA and Citrate anticoagulated vacutainers from umbilical cord blood or arterial or venous blood for blood culture, CRP, analysis of hematologic parameters and coagulation profile consisting PT, aPTT and plasma fibrinogen respectively.

For blood culture, sample from plain vacutainer was used. Sample blood was first inoculated in glucose broth and incubated for 48 hours and then streaked into Mc conkey agar for further growth.

Also blood from plain sample was used for estimation of ESR.

Blood samples collected in EDTA anticoagulated vacutainers were run in quantitative haematology analyzer Sysmex XN-1000 to obtain various hematologic parameters. Peripheral smears were prepared, stained using Leishman's stain.

Blood samples collected in Citrate anticoagulated vacutainers were used for estimation of PT, aPTT and plasma fibrinogen. Sample was centrifuged for 15minutes at 1500 rpm to obtain plasma and was run on 'Automated coagulometer ACL Elite Pro' to estimate PT and aPTT values.

Plasma fibrinogen was estimated by automated Erba Mannheim ECL 105 coagulometer by using 'Erba Thrombin reagent for fibrinogen determination' which is made up of approximately 100 NIH units/ml bovine thrombin with stabilizers and erba owrens veronal buffer.

Sample collection and storage: 2 ml of blood was collected in Citrate anticoagulated vacutainers, plasma separated by centrifuging sample at 1500 x g for 15 minutes. Testing was done within four hours of sample collection. In case of delay sample is stored at -20^{o} **C.**

Principle : Clauss method, a simple method for quantitative determination of fibrinogen by measuring the clotting time of dilute plasma after addition of thrombin. This clot time is proportional to the fibrinogen levels.

Procedure:

1) Working reagent is prepared by reconstituting each vial of Erba thrombin reagent with 5ml of purified water and is stable upto 1 month at -20^{o} **C.**
2) 10µl of plasma is pipetted into a reaction tube, added with 90 µl and intubated at 37 ^{o} **C** for 2 minutes.
3) Then 50 µl of working reagent (thrombin reagent) solution was added.
4) Readings are taken.

Sample Size:

With mean +/- Standard Deviation of fibrinogen level in cases & control 347.82 +/- 131.2 and 279.32 +/- 48.51[2] at 90% power considered in the study, sample size (n) calculated is 36 per group.

$$n = \frac{(Z\alpha + Z\beta)^2 \times SD^2}{d^2}$$

Zα –Z value at α level

Zβ –Z value at β level

SD – common standard deviation

d- Difference between two parameters.

Total sample size = 36+36 = 72.

STATISTICAL ANALYSIS:

The data obtained were recorded in a Microsoft Excel sheet, and statistical analysis was performed using statistical package for the social sciences (Version 17). Results are presented as drawings, Mean±SD, counts and percentages.

Optimal cut off values of Fibrinogen and CRP for were defined by Youdens index. Sensitivity and specificity of these variables were performed using ROC curves.

Categorical variables were compared using chi square test, quantitative variables were compared using Independent t test & Mann Whitney U test. For all tests, significant value was achieved at $p<0.05$. All statistical tests performed were two tailed.

Inclusion criteria:

- Neonatal cases with early signs of sepsis.
- Neonates born to mothers with premature rupture of membranes and fever.

Exclusion criteria:

- Neonates with congenital anomalies.

RESULTS

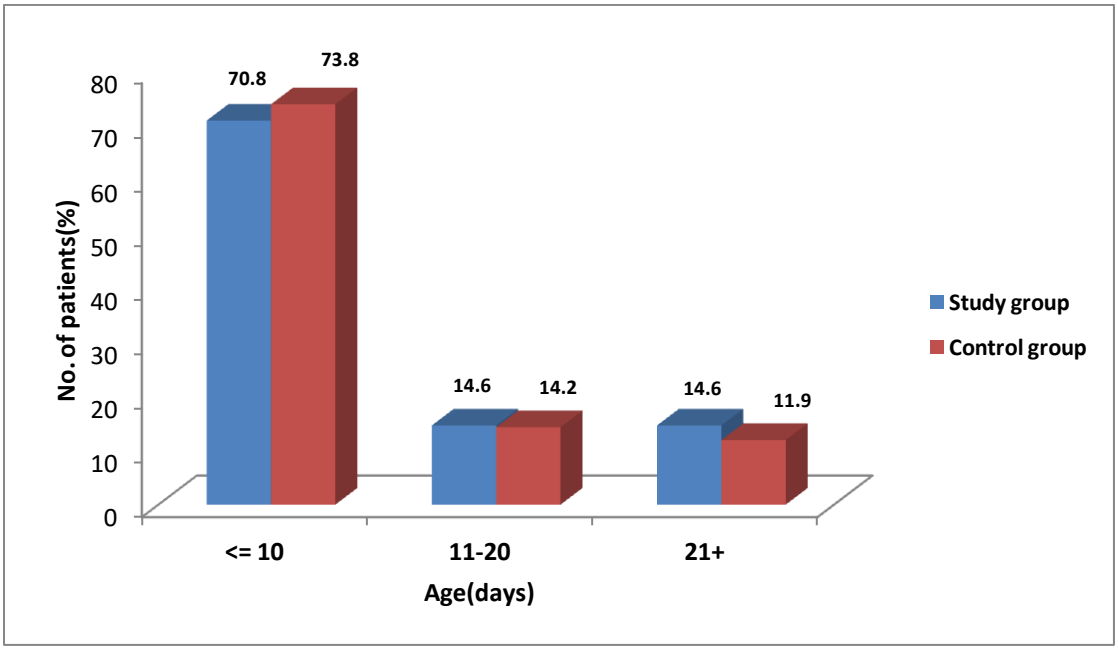
A total of 90 neonates were included, 48 in the study group and 42 neonates in the control group, with an age range of 1- 30 days who presented with signs and symptoms of sepsis to BLDE (Deemed to be University) Shri B.M. Patil Medical College Hospital & Research Center, Vijayapura in between December 2017 to June 2019.

In this study among 48 suspected neonatal sepsis cases 34 (70.8%) were less than 10 days, 7(14.6) were between 11 to 20 days and 7(14.6) were between 21 to 30 days. Maximum cases of neonatal sepsis are seen in early neonatal period of less than 10 days.

TABLE 3: COMPARISON OF AGE (IN DAYS) OF PATIENTS BETWEEN STUDY & CONTROL GROUP

Age(Days)	Study group		Control group	
	No. of patients	Percentage	No. of patients	Parentage Percentage
<= 10	34	70.8	31	73.81
11 - 20	7	14.6	6	14.281
21+	7	14.6	5	11.901
Total	48	100.0	42	100.0

FIGURE 1: COMPARISON OF AGE (IN DAYS) OF PATIENTS BETWEEN STUDY & CONTROL GROUP

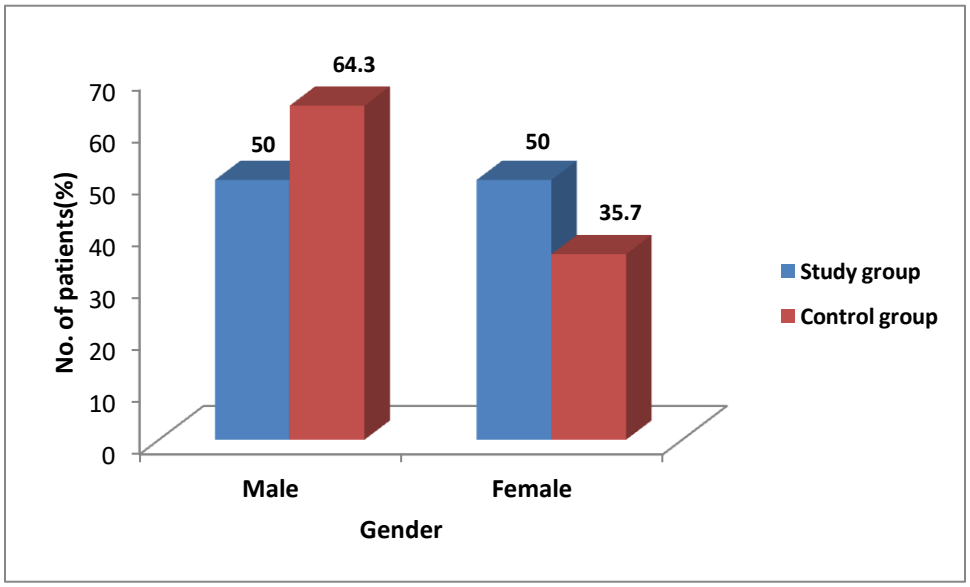


Among study group 24 (50%) were male and 24(50%) were female neonates and among control group 27(64.3) were male and 15(35.7%) were female neonates which are statistically matched.

TABLE 4: COMPARISON OF GENDER OF PATIENTS BETWEEN STUDY & CONTROL GROUP

Gender	Study group		Control group	
	No. of patients	Percentage	No. of patients	Parentage Percentage
Male	24	50	27	64.3
Female	24	50	15	35.7
Total	48	100.0	42	100

FIGURE 2 : COMPARISON OF GENDER OF PATIENTS BETWEEN STUDY & CONTROL GROUP



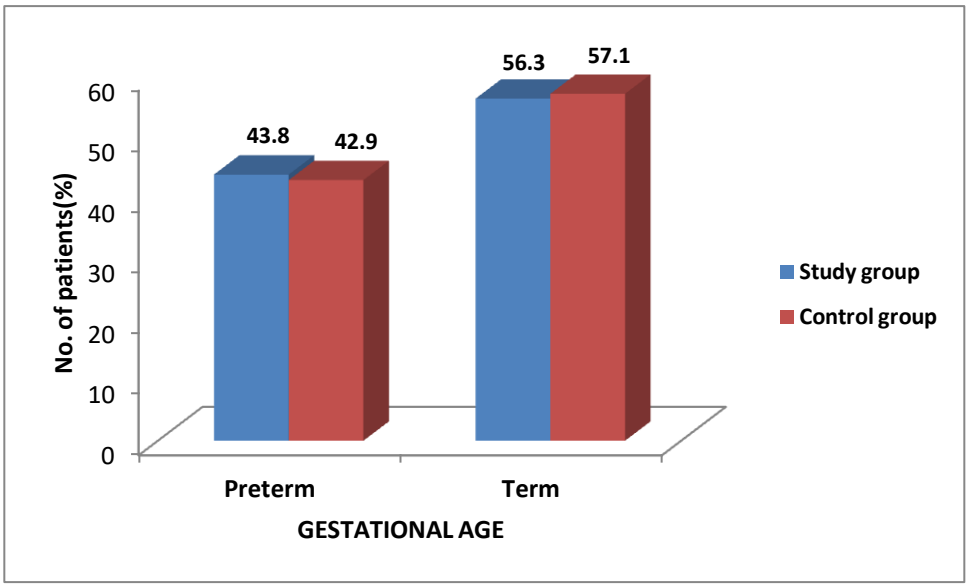
DISTRIBUTION OF CASES ACCORDING TO GESTATIONAL AGE

Among 48 cases of study group, 21(43.7%) of the neonates were preterm and the remaining 27(56.3%) were term neonates and among 42 cases of control group 18(42.9%) of the neonates were preterm and the remaining 24(57.1%) were term neonates

TABLE 5: COMPARISON OF GESTATIONAL AGE OF PATIENTS BETWEEN STUDY & CONTROL GROUP.

GESTATIONAL AGE	Study group		Control group	
	No. of patients	Percentage	No. of patients	Percentage
Preterm	21	43.7	18	42.9
Term	27	56.3	24	57.1
Total	48	100.0	42	100.0

FIGURE 3: COMPARISON OF GESTATIONAL AGE OF PATIENTS BETWEEN STUDY & CONTROL GROUP.



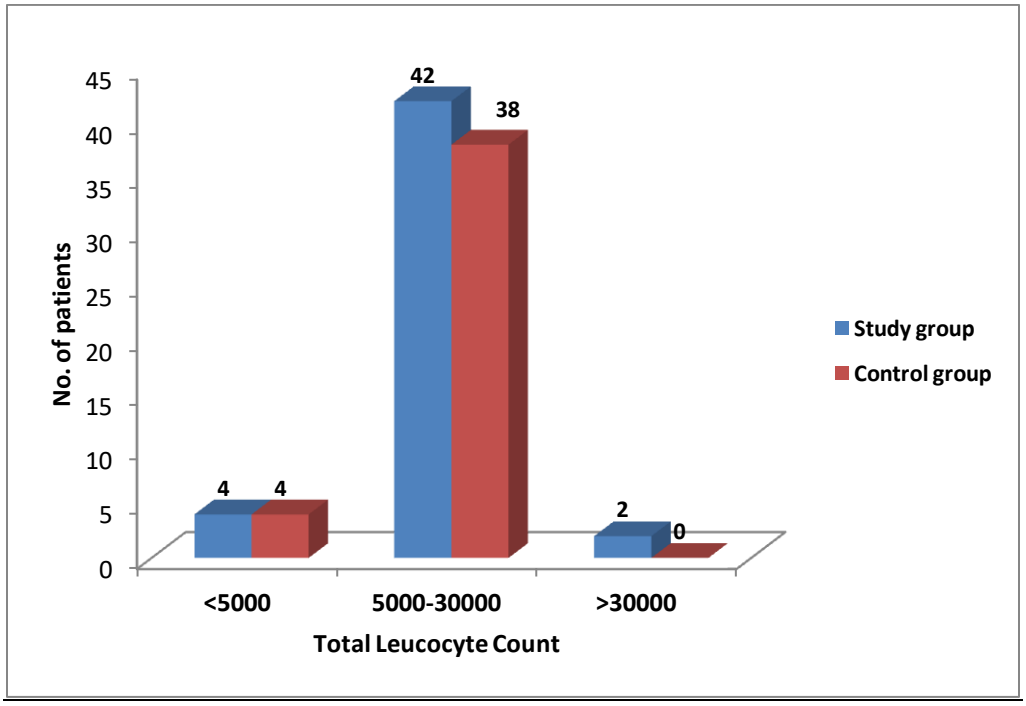
COMPARISON OF TOTAL LEUCOCYTES COUNT OF PATIENTS BETWEEN STUDY & CONTROL GROUP

In the current study, among 48 cases from study group 4 (8.34%) cases presented with total leucocyte count of <5000 cells/cumm, 42(87.50%) cases presented with total leucocyte count between5000 to 30000 cells/cumm, 2(4.16) cases presented with total leucocyte count above 30000 cells/cumm. In control group among 42, cases 4(9.52) cases presented with total leucocyte count of <5000 cells/cumm, and remaining 38(90.48%) cases presented with total leucocyte count between5000 to 30000 cells/cumm and none cases had total leucocyte count above 30000 cells/cumm.

TABLE 6:COMPARISON OF TOTAL LEUCOCYTES COUNT OF PATIENTS BETWEEN STUDY & CONTROL GROUP

Total Leucocytes Count(cells/cumm)	Study group		Control group	
	No. of patients	Percentage	No. of patients	Percentage
<5000	4	8.34	4	9.52
5000-30000	42	87.50	38	90.48
>30000	2	4.16	0	0
Total	48	100	42	100.0

FIGURE 4: COMPARISON OF TOTAL LEUCOCYTES COUNT OF PATIENTS BETWEEN STUDY & CONTROL GROUP



In this study mean total leucocyte count was 14193 ±SD 7208 for study group and 10861 ±SD 4274, which was higher statistically significant with p value of 0.008.

TABLE 7: COMPARISON OF MEAN VALUES OF TOTAL LEUCOCYTE COUNT BETWEEN STUDY GROUP AND CONTROL GROUP

Variables	**Study group(n=48)**		**Control group(n=42)**		**Mann Whitney U test/Independent t test**	**P value**
	Mean	**±SD**	**Mean**	**±SD**		
Total leucocyte Count	14193	7208	10861	4274	**t=**2.705	P=0.008 HS

HS = Highly significant

FIGURE 5 : COMPARISON OF MEAN VALUES OF TOTAL LEUCOCYTE COUNT BETWEEN STUDY GROUP AND CONTROL GROUP

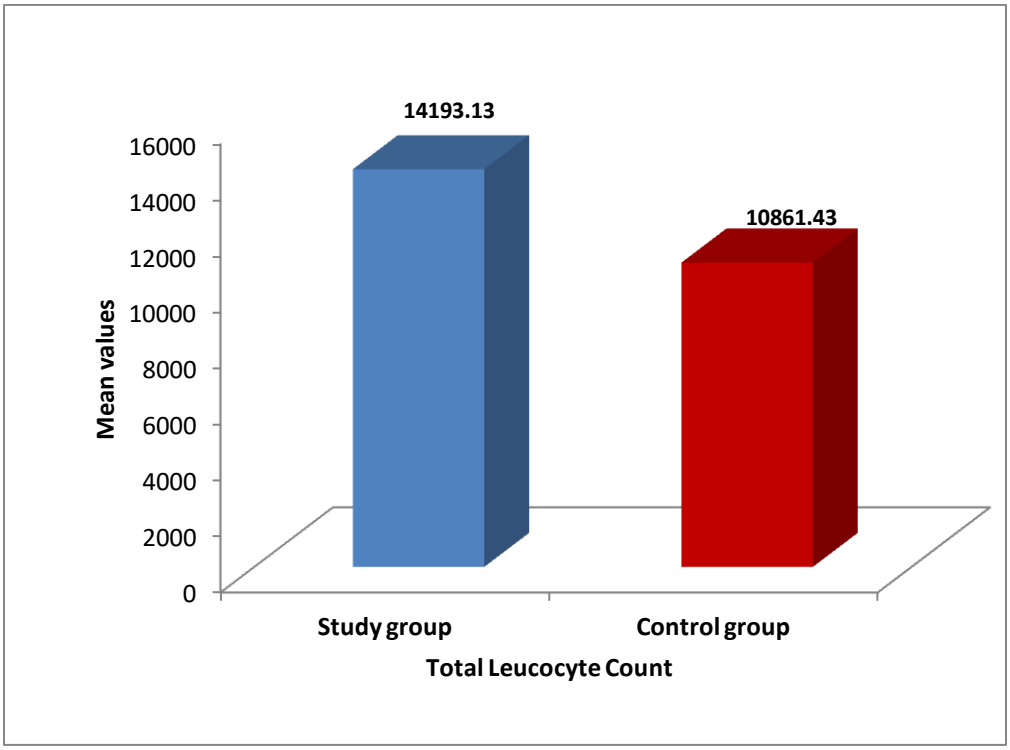
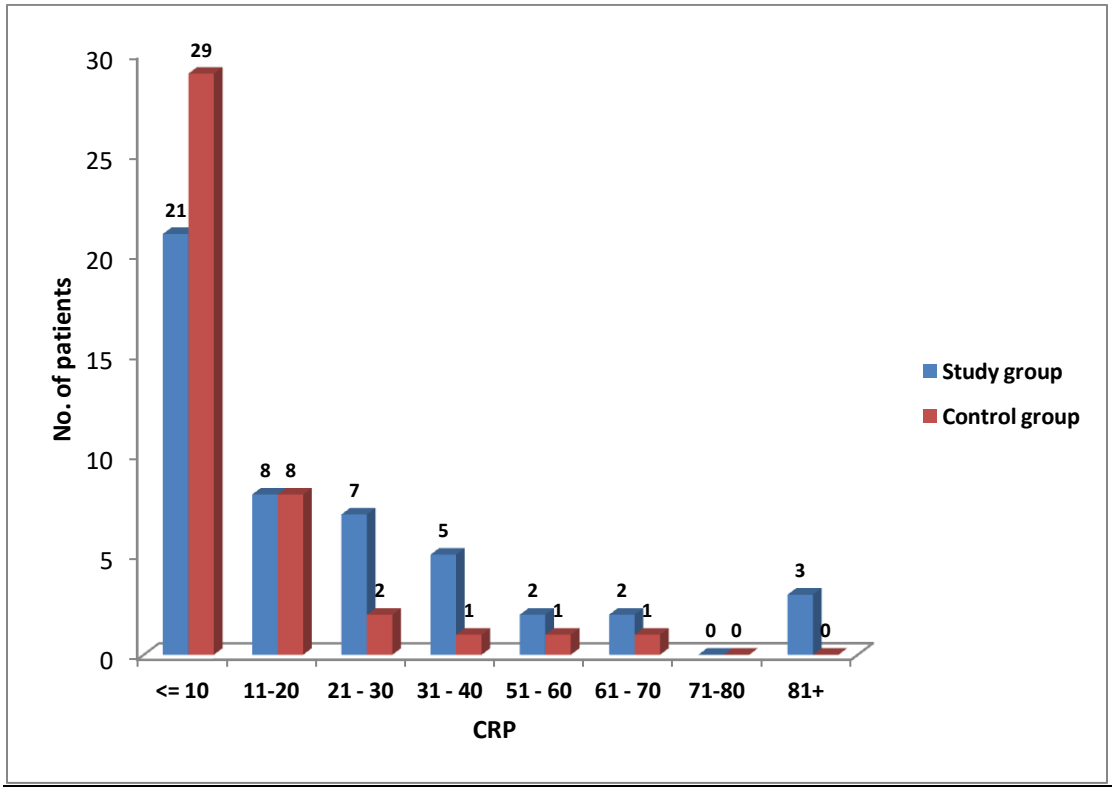


TABLE 8: COMPARISON OF DISTRIBUTION OF CRP OF PATIENTS BETWEEN STUDY & CONTROL GROUP

CRP	Study group		Control group	
	No. of patients	Percentage	No. of patients	Percentage
<= 10	21	43.8	29	69.0
11 - 20	8	16.7	8	19.0
21 - 30	7	14.6	2	4.8
31 - 40	5	10.4	1	2.4
51 - 60	2	4.2	1	2.4
61 - 70	2	4.2	1	2.4
71-80	0	0	0	0
>80	3	6.3	0	0
Total	48	100.0	42	100.0

FIGURE 6 : COMPARISON OF CRP OF PATIENTS BETWEEN STUDY & CONTROL GROUP



In this study, mean value of CRP was 22.92 ±SD 24.45 for study group and 10.86 ±SD 13.69, which was higher and statistically significant with p value of 0.005.

TABLE 9: COMPARISON OF CRP OF PATIENTS BETWEEN STUDY & CONTROL GROUP

Variables	**Study group(n=48)**		**Control group(n=42)**		**Mann Whitney U test/Independent t test**	**P value**
	Mean	**±SD**	**Mean**	**±SD**		
CRP	22.92	24.45	10.86	13.69	**U**=660.50	P=0.005

HS- Highly significant

FIGURE 7: COMPARISON OF CRP OF PATIENTS BETWEEN STUDY & CONTROL GROUP

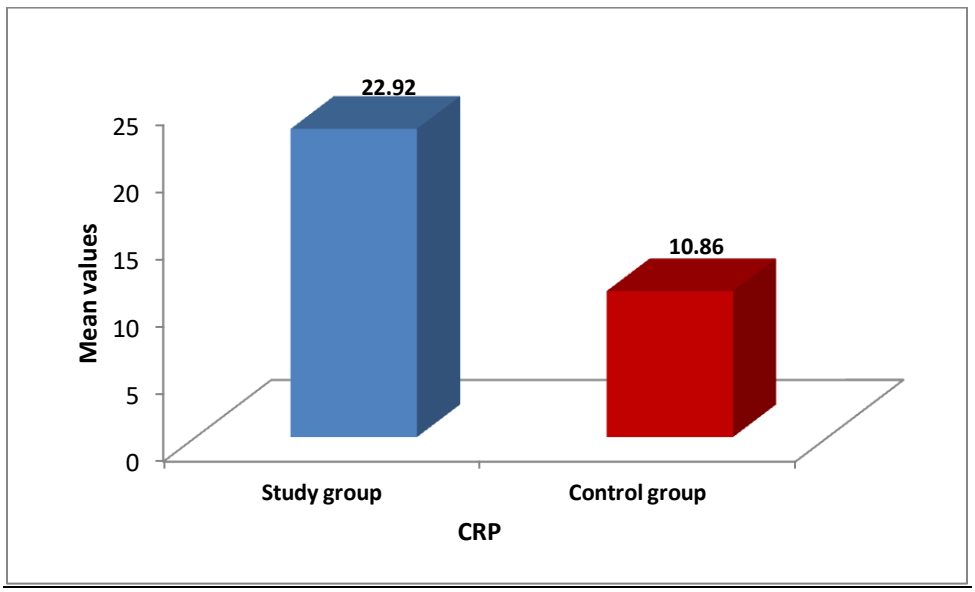
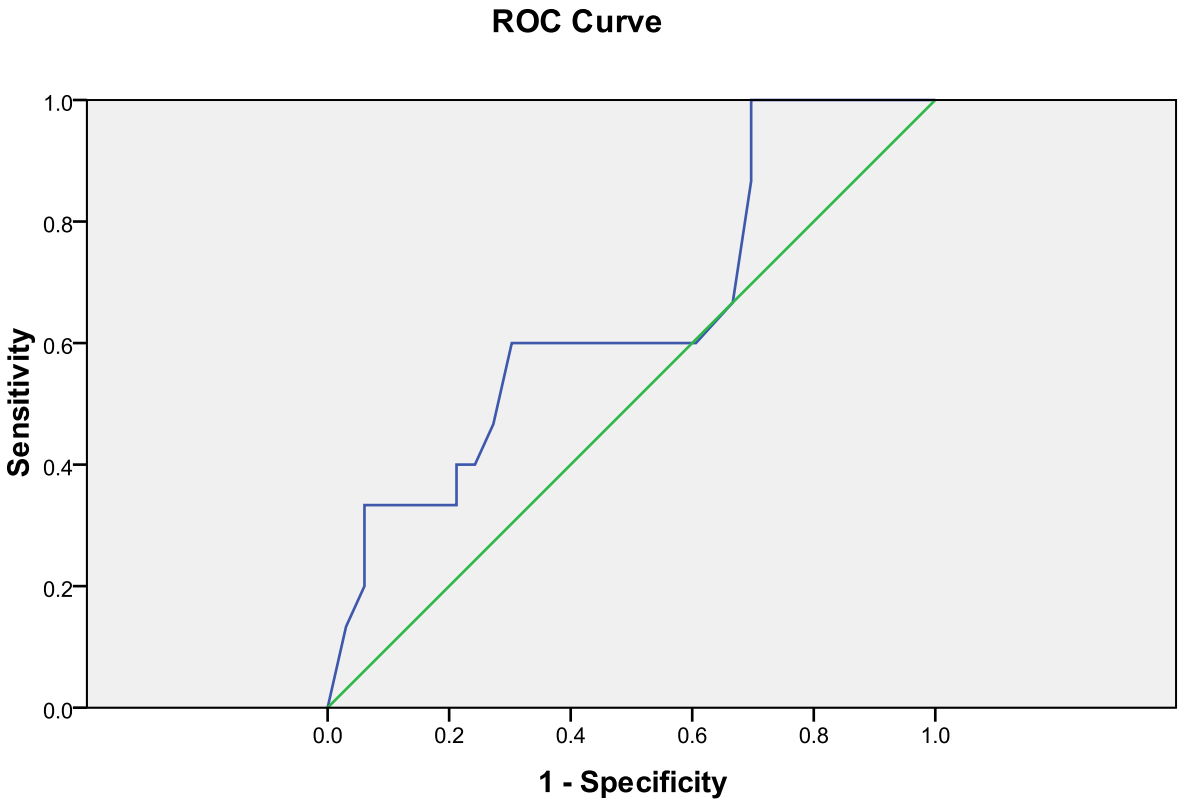


FIGURE 8: ROC CURVE OF CRP IN DIAGNOSIS OF NEONATAL SEPSIS WITH ALL CASES IN STUDY GROUP



Optimal cut-off value of CRP

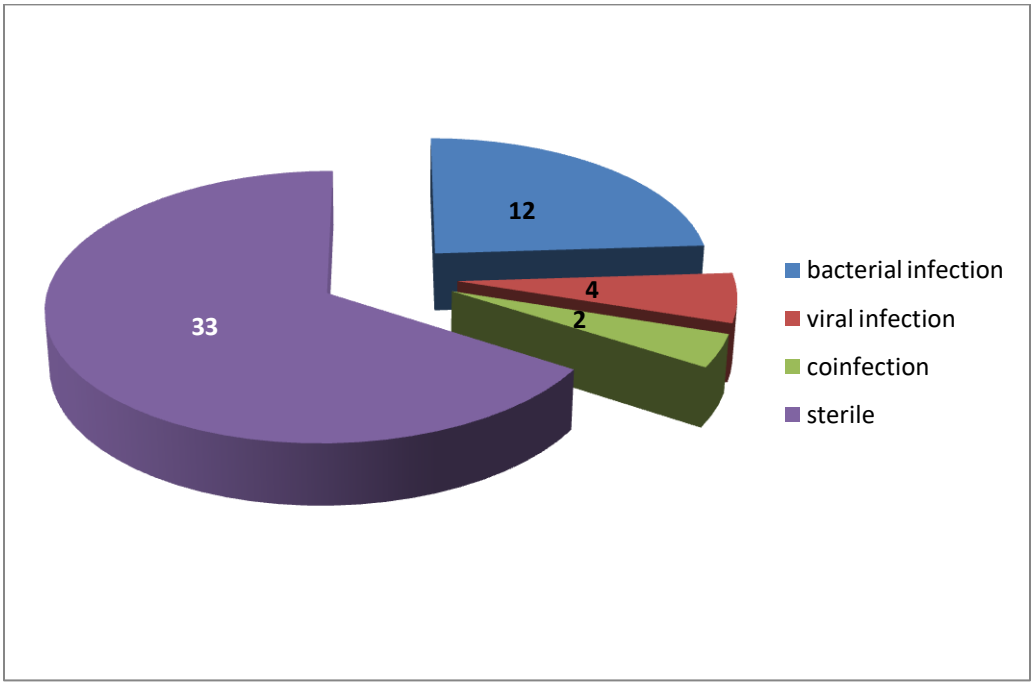
In the ROC Curve for Blood culture, the AUROC of CRP, is 64.5% (95% of Confidence Interval=47 % to 82%) and the optimal cut-off value is 6.5mg/l Using this cut-off value, the Sensitivity and specificity are 60% and 70 % resp.

DISTRIBUTION OF CASES ACCORDING TO BLOOD CULTURE

In this study we found out that among 48 suspected cases of neonatal sepsis, blood culture was positive for 12 cases and positive for viral infection in 6 cases and 2 case having co-infection of both bacteria and virus. Remaining 33 cases were sterile for blood culture and body fluid culture.

All 42 cases from control group were reported as sterile for blood culture and body fluid culture.

FIGURE 9: DISTRIBUTION OF CASES ACCORDING TO BLOOD CULTURE



DISTRIBUTION ORGANISMS ISOLATED :

In present study out of 12 culture positive cases, 3 (25%) cases were of E. coli, 2(16.66%) cases each were of Klebsiella pneumonia, Staphylococcus and Coagulase negative Staphylococcus. 1(8.34%) cases each of Staphylococcus Aureus, Staphylococcus pneumoniae and Streptococci.

Among these 1 case of CON Staphylococcus with CMV positive was obtained from CSF culture.

1 case was coinfection with Staphylococcus pneumoniae, CMV, Rubella & Toxoplasma.

Cases for positive for viral infection: 2 cases were CMV positive, 2 cases were positive for Herpes IgG and 1 case was positive for CMV and Rubella.

Table 10 : Cases positive for viral infection:

CMV	2
CMV WITH RUBELLA	1
HERPES	2
CMV, RUBELLA & TOXOPLASMA	1

TABLE 11: DISTRIBUTION OF CASES ACCORDING TO ORGANISMS ISOLATED ON BLOOD CULTURE

BLOOD CULTURE	STUDY GROUP	
	NO. OF PATIENTS	PERCENTAGE (%)
ESCHERICHIA COLI	3	25
KLEBSIELLA PNEUMONIA	2	16.66
STAPHYLOCOCCUS	2	16.66
CON STAPHYLOCOCCUS	2	16.66
STREPTOCOCCI	1	8.34
STAPHYLOCOCCUS AUREUS	1	8.34
GRAM POSITIVE STAPHYLOCOCCUS PNEUMONIAE	1	8.34

FIGURE 10: DISTRIBUTION OF CASES ACCORDING TO ORGANISMS ISOLATED

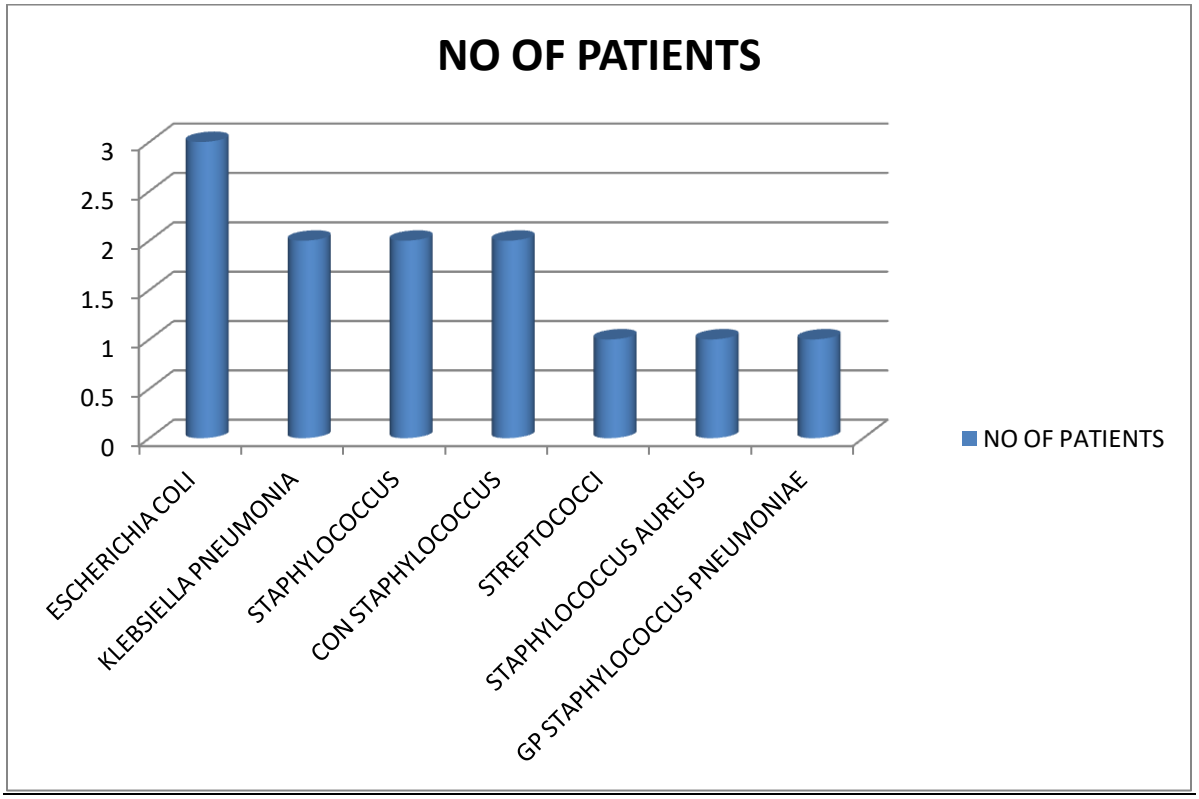
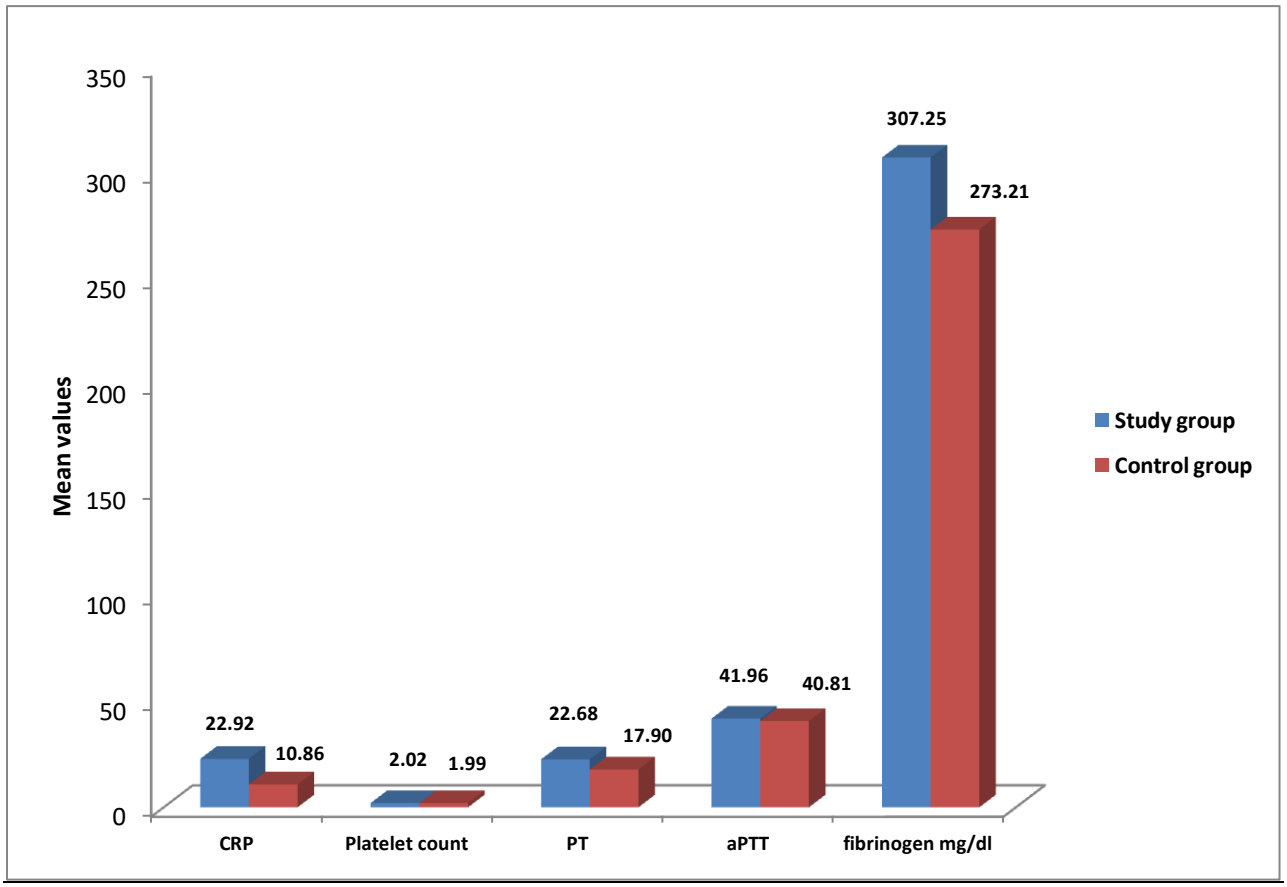


TABLE 12 : COMPARISON OF VARIABLES BETWEEN STUDY AND CONTROL GROUP

Variables	**Study group(n=48)**		**Control group(n=42)**		**Mann Whitney U test/Independent t test**	**P value**
	Mean	**±SD**	**Mean**	**±SD**		
Age(Days)	8.46	9.142	6.07	2.16	U=716.0	P=.0.1429 NS
Total leucocyte Count	14193.13	7208.278	10861.43	4274.709	**t=**2.705	P=0.008 HS
ESR	7.79	9.804	6.29	5.591	t=0.671	P=0.505 NS
CRP	22.92	24.450	10.86	13.693	**U**=660.50	P=0.005 HS
Platelet count	2.02	1.862	1.99	.845	**U**=839.00	P=0.169 NS
PT	22.677	24.1926	17.902	18.8428	**U**=763.500	P=0.048 NS
aPTT	41.96	19.169	40.81	15.141	**U**=940.00	P=0.582 NS
fibrinogen mg/dl	307.25	90.015	273.21	80.884	**U**=689.50	P=0.010 HS
NS; Not significant HS: Highly significant						

FIGURE 11: COMPARISON OF VARIABLES BETWEEN STUDY AND CONTROL GROUP



In this study mean plasma fibrinogen was 307.25 ±SD 90.015 for study group and 273.21 ±SD 80.884, which was higher statistically significant with p value of 0.010.

TABLE 13: COMPARISON OF PLASMA FIBRINOGEN OF PATIENTS BETWEEN STUDY & CONTROL GROUP

Variables	Study group(n=48)		Control group(n=42)		Mann Whitney U test/Independent t test	P value
	Mean	±SD	Mean	±SD		
plasma fibrinogen	307.25	90.015	273.21	80.884	U=689.50	P=0.010

HS - Highly significant

FIGURE 12: COMPARISON OF PLASMA FIBRINOGEN OF PATIENTS BETWEEN STUDY & CONTROL GROUP

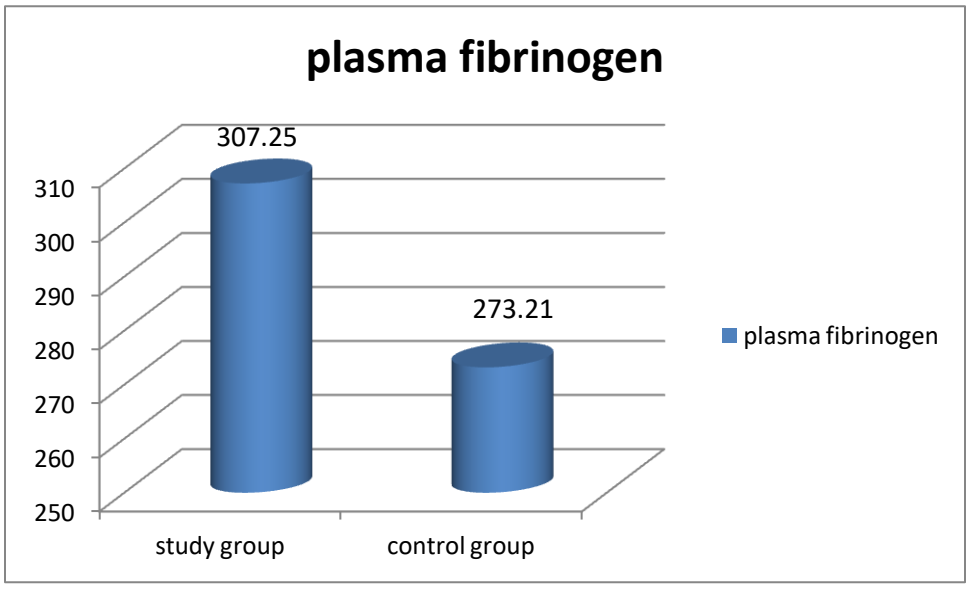
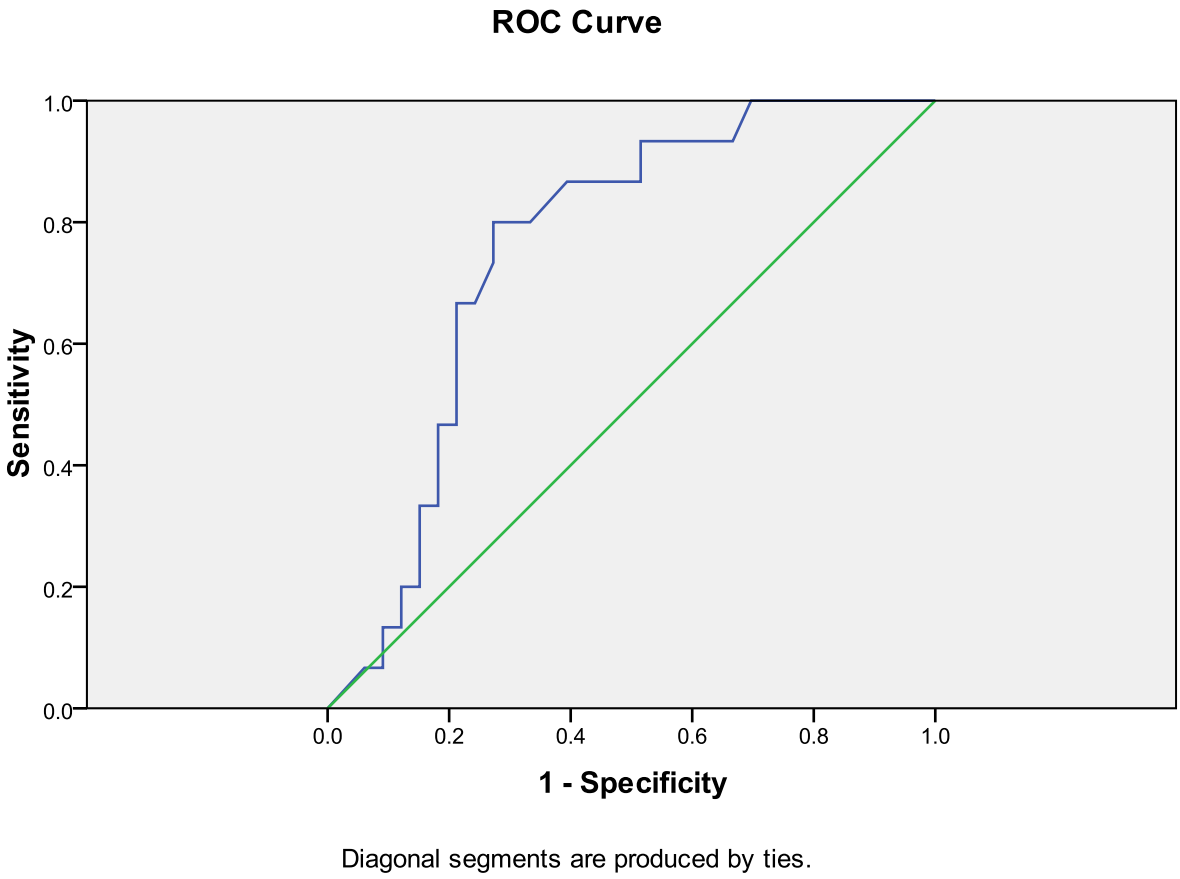


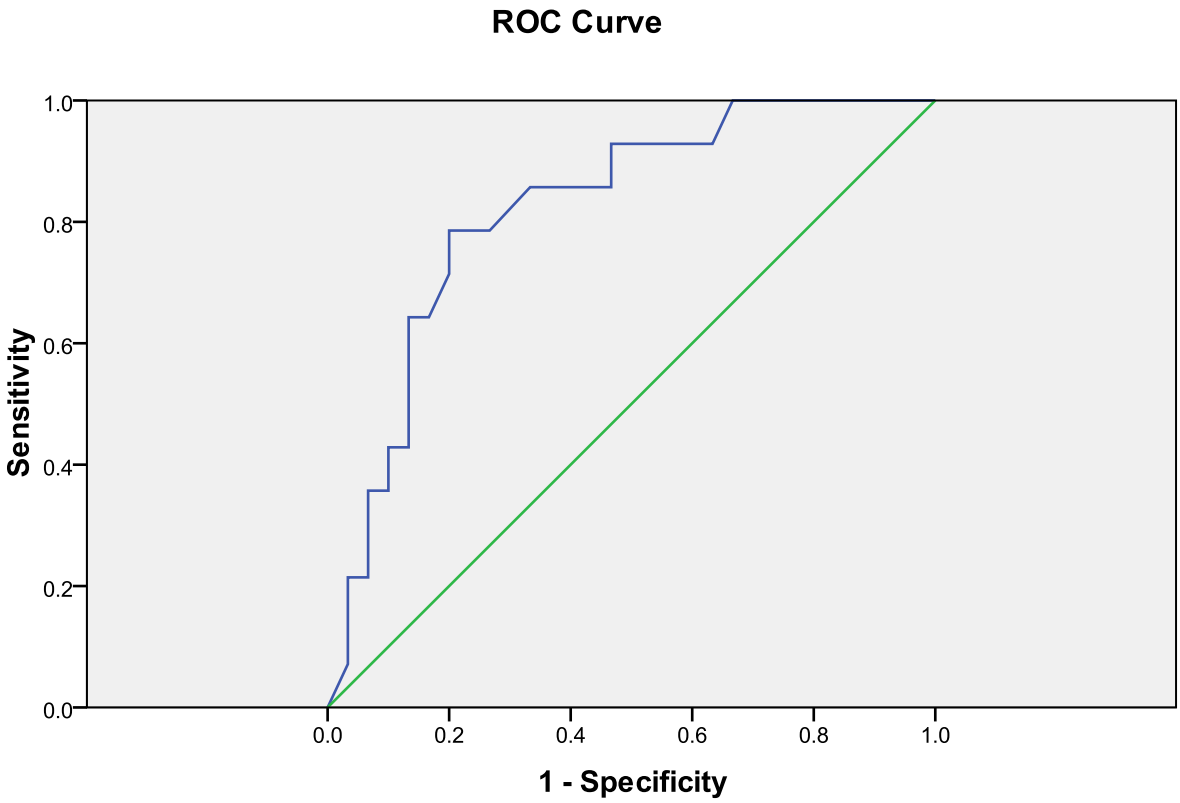
FIGURE 13: ROC CURVE OF PLASMA FIBRINOGEN IN DIAGNOSIS OF NEONATAL SEPSIS WITH ALL CASES IN STUDY GROUP



Optimal cut-off value of Fibrinogen

In the ROC Curve for Blood culture, the AUROC of Fibrinogen (without complicated cases), is 76% (95% of Confidence Interval=62 % to 90%) and the optimal cut-off value is 305.5. Using this cut-off value, the Sensitivity and specificity are 80% and 72.8 % resp.

FIGURE 14: ROC CURVE OF PLASMA FIBRINOGEN IN DIAGNOSIS OF NEONATAL SEPSIS WITH ALL CASES IN STUDY GROUP



In the ROC Curve for Blood culture, the AUROC of Fibrinogen (cases including complicated cases) is 82% (95% of Confidence Interval=69%-95%) and the optimal cut-off value is 305.5 Using this cut-off value, the Sensitivity and specificity are 78.5% and 80% respectively.

DISCUSSION

Neonatal sepsis is the most common cause of morbidity and second most common primary cause of neonatal mortality next to perinatal asphyxia. With early diagnosis and proper treatment it can be prevented and neonatal mortality rate due to neonatal sepsis can be reduced to a considerable amount.

Neonatal sepsis mimic's various other neonatal diseases and conditions, making it difficult to diagnose clinically. Blood culture or other body fluid like CSF culture are gold standard for diagnosis but requires 48 to 72 hours to produce results [4, 5]. This delays treatment leading to loss of precious time and leading to complications and death. Hence there is a need of laboratory tests which are rapid and easy.

Plasma fibrinogen is normally present in blood as a factor of coagulation system and helps in haemostasis. But also acts as positive acute phase reactant in cases of inflammation and infection.

The current study was undertaken to evaluate the efficacy of plasma fibrinogen in the diagnosis of neonatal sepsis and its outcome as in complications. Along with it other haematological indices and CRP are also evaluated for their efficacy.

Age distribution in neonatal sepsis:

In current study 34 (70.8%) cases of neonatal sepsis were less than 10 days, 7(14.6%) cases each were between 11 to 20days and 21 to 30 days. In this study we found out that neonatal sepsis is more common in first week of life.

This finding was consistent with study conducted by Vandana G *et al*[3], who found out that 76 cases among 94 neonatal sepsis cases were within first week of life.

Sex distribution in neonatal sepsis:

In current study it was found that male and female neonates were equal in number in study group and control group had a little male predominance. This difference between study group and control group were statistically insignificant.

Gestational age analysis in neonatal sepsis:

In current study it was found that, both in study group and control group term cases were more than preterm cases. It was found out that neonatal sepsis was more common with term neonates.

In this study we found out that early onset neonatal sepsis is more common in term neonates. This finding was consistent with study conducted by Vandana G *et al*[3], J N Mishra *et al*[32] and Vesikari *et al.*[33]

Organisms isolated in neonatal sepsis:

In present study the most common organism isolated was E coli followed by Klebsiella pneumonia, staphylococcus and coagulase negative staphylococcus. Similar findings were seen in Cortese F *et al*[34], Manroe *et al*[35] Chandna A *et al*[36] and Renolder B *et al*[37]. Most common viral infection was CMV.

TABLE NO 14: COMPARISON OF MOST COMMON ORGANISMS ISOLATED AMONG VARIOUS STUDIES

Studies	Most common organism isolated
Cortese F *et al*[34]	E.coli
Chandna A *et al*[36]	E.coli
Renolder B *et al*[37]	E.coli
Manroe *et al*[35]	E.coli
Das B *et al*[5]	E.coli
Hasan Sobaih B *et al*[38]	Staphylococcus epidermidis
Present study	E.coli

TOTAL WBC COUNT IN NEONATAL SEPSIS:

In this study mean total leucocyte count was 14193 ±SD 7208 for study group and 10861 ±SD 4274, which was higher than control group having statistical significance with p value of 0.008.

As per study done by Ahirrao BM *et al*[39]. Total WBC count has lower sensitivity but higher specificity in detecting neonatal sepsis. It is because of wide normal range of WBC count between 5000-34000cells/cumm.

In this study total leucocyte count of less than 5000 cells/cumm ie; leucopenia cases and total leucocytes count more than 30000 cells /cumm ie; leucocytosis are considered as indicator for sepsis.

In a study conducted by Das B *et al*[5] they found out that 54.5% of pod proven neonatal sepsis cases had leucopenia.

CRP IN NEONATAL SEPSIS:

In current study we found out that CRP was significantly elevated in study group than control group. Mean CRP level was 22.9 ±SD 24.450 for study group and 10.86 ±SD 13.693, which was higher than control group having statistical significance with p value of 0.005.

In a study conducted by Mondal *et al*[1] found out that CRP is most sensitive and is single best marker for detecting early onset Neonatal sepsis.

Stephan E *et al*[40] found that CRP levels were most important parameter in guiding the duration of treatment with antibiotics of neonates with suspected sepsis.

In current study after comparing proven neonatal sepsis cases with our control population, on analysis of ROC curves we got cut off value of 6.5mg/l with sensitivity and specificity of 60% and 70%.

Guibourdenche J *et al*[4] found that cut off value of 7.5mg/l for neonatal sepsis on analysis of ROC curves for CRP.

Khursid *et al*[41] observed CRP had sensitivity and specificity of 66% and 48%.

In study conducted by Das B *et al*[5] got cut off value of 6 mg/l for neonatal sepsis.

PLASMA FIBRINOGEN IN NEONATAL SEPSIS:

In current study we evaluated role of plasma fibrinogen levels on neonatal sepsis and its outcome and we found out that plasma fibrinogen levels were significantly elevated in study group containing suspected cases of neonatal sepsis compared to control group. In this study mean plasma fibrinogen was 307.25 ±SD 90.015 for study group and 273.21 ±SD 80.884, which was higher statistically significant with p value of 0.010.

It was also observed that 4 cases which develop complications showed lower level of plasma fibrinogen but PT and APTT were prolonged where as in cases which did not develop complications, plasma levels of fibrinogen was elevated but PT and APTT were within normal range.

In current study after comparing proven neonatal sepsis cases with our control population, on analysis of ROC curves we got cut off value of 305.5mg/dl with sensitivity and specificity of 80% and 72.8%, which was comparable to study conducted by Mitra P *et al.*[2]

TABLE NO 15: COMPARISON OF STUDIES FOR PLASMA FIBRINOGEN

Plasma fibrinogen	Present study	Mitra P *et al*[2]
Cut off value	305.50	301.90
sensitivity	80%	70.8%
Specifictiy	72.8%	82.7%

According to study conducted by Guibourdenche J *et al*[4] concluded that Plasma fibrinogen acts as an acute phase reactant. They got cut off value of 300mg/dl for Plasma fibrinogen in diagnosing neonatal sepsis which was comparable to present study.

CONCLUSION

Plasma fibrinogen is normally present in blood as a factor of coagulation system but will be increased in concentration in any infection or inflammation and acts as positive acute phase reactant.

It is a rapid, simple and cost effective test, can be used along with other hematological parameters & biochemical markers like CRP in diagnosis of neonatal sepsis.

The present study concluded that plasma fibrinogen is not only immediate reliable marker in diagnosing neonatal sepsis but also aids in predicting the complications thus improving the diagnostic efficacy. So this complimentary test also helps the clinicians to start the treatment as early as possible & thereby reducing neonatal mortality, bringing a significant impact in the neonatal health care.

SUMMURY

In the present study 90 neonates were evaluated with an age range of 1-30 days from 1st December, 2017 to 30th June, 2019 in Pathology department in BLDE (Deemed to be university), Shri. B. M. Patil Medical College, Hospital and Research Centre, Vijayapura.

These 90 cases were further divided into study group and control group. Study group involved 48 cases who presented with signs and symptoms of neonatal sepsis and control group involved 42 neonates who presented with hyperbilirubinemia.

Among a total of 48 study cases, 34 were less than 10 day old, 7cases each were between 11 to 20days old and21 to 30 days old.

In present study among 48 neonatal sepsis cases 50 were male and 50 were females. Male: Female ratio was 1:1.

Among 48 neonatal sepsis cases 27 were term neonates and 21 were preterm neonates. Gestation age was not found to be affecting plasma fibrinogen levels in neonatal sepsis.

Among 48 suspected neonatal cases of sepsis, 12 were culture proven cases of sepsis, 6 cases were positive for viral infection and 2 cases had both bacterial and viral co-infection and 33 were sterile and were diagnosed as clinically suspected cases of neonatal sepsis.

E. coli was the most common organism isolated followed by Klebsiella pneumonia.

Elevated CRP, total leucocyte count of < 5000 or >30000cell/cumm and elevated plasma fibrinogen were statistically significant parameters with a p-value of <0.05.

At a cut off value of ≥ 305.5 for plasma fibrinogen, the sensitivity and specificity was 80% and 72.8% respectively.

REFERANCES

1. Mondal S K, Nag D N, Bandyopadhyay R, Chakraborty D, Sinha S K. Neonatal sepsis: Role of a battery of immunohematological tests in early diagnosis. Int J Appl Basic Med Res. 2012 Jan-June 2(1):43-7.
2. Mitra P, Guha D, Nag S S,Mondal B C, Dasgupta S. Role of Plasma Fibrinogen in Diagnosis and Prediction of Short Term Outcome of Neonatal Sepsis. Indian J Hematol Blood Transfus. 2017 Apr-June 33(2):195-9.
3. G Vandana, S LokeshRaoMagar, Praveen, Kavithadevi, Sandhya Rani, Sandhya Anil. Haematological profile in neonatal sepsis. IOSR Journal of Dental and Medical sciences. 2017 Apr; Vol 16(4):11-17.doi:10.9790/0853-1604091117.
4. Guibourdenche J, Bedu A,Petzold L, Marchand M, Mariani-Kurdjian P, Hurtaud-Roux M F, AujardY, Porquet D. Biochemical markers of neonatal sepsis: value of procalcitonin in the emergency setting. Ann ClinBiochem 2002; 39: 130- 5.
5. Das B, Das A, Saikia M. A study of haematological parameters in neonatal septicemia. Indian Journal of Basic and Applied medical Research. 2016 March; vol- 5(2): 491-500.
6. Andrew M, Paes B, Milner R, Johanson M, Mitchell L, Tollefsen DM, Powers P (1987). Development of the human coagulation system in the full term infant. Blood.1987 july 70(1):165-72.
7. Andrew M, Paes B, Milner R, Johanson M, Mitchell L, Tollefsen DM, Castle V, Powers P (1988). Development of the human coagulation system in the healthy preterm infant. Blood. 1988 nov 72(5): 1651-57.
8. Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the newborn. Indian Journal of Paediatrics 2008;75(3):261-6.

9. AbhishekMG,Sanjay M. Diagnostic efficacy of Nucleated Red Cell count in the early diagnosis of neonatal sepsis. Indian Journal of Pathology and Oncology 2015;2(4):182-5.
10. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed.2006;91:F208-12
11. Chiesa C, Panero A, Rossi N, Stegagno M, De Giusti M, Osborn JF, et al. Reliability of Procalcitonin Concentrations for the Diagnosis of Sepsis in Critically Ill Neonates. Clin Infect Dis. 1998;26(3):664–72.
12. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem. 2004;50: 279-87.
13. Cant A J, Gennery A R. Immunodeficiency. Rennie and Robertson's Textbook of Neonatology 5th Ed: Chruchill Livingstone Elssevier 2012; p994-1011
14. Haque KN. Neonatal Sepsis in the Very Low Birth Weight Preterm Infants : Part 1: Review of Patho-physiology. 2010;3:1–10.
15. Zawar MP, Tambekar RG, Deshpande NM, Gadgil PA, Kalekar SM. Early diagnosis of neonatal septicemia by sepsis screen. Indian J Pathol Microbiol. 2003; 46(4):610-12.
16. Bhat RY, Lewis LE, Vandana KE. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India.Italian Journal Of Pediatrics. 2011;32:37
17. Buch AC, Srivastava V, Kumar H and Jadhav PS. Evaluation of haematological profile in early diagnosis of clinically suspected cases of neonatal sepsis. International Journal of Basic and Applied Medical Sciences. 2011;1(1):1-6

18. Agarwal AM, Rodgers GM. Miscellaneous causes of thrombocytopenia. In Greer JP, Arber DA, Glader B, List AF, Means RT, Paraskevas S, Rodgers GM (eds) Wintrobe's Clinical Hematology. 13th Ed. Philadelphia. Williams and Wilkins.2014:1097-105.
19. Hermansen MC. Nucleated red blood cells in the fetus and newborn. Arch Dis Child Fetal Neonatal Ed. 2001;84(3):F211–5.
20. Constantino BT, Cogionis B. Nucleated RBCs - Significance in the peripheral blood film. Lab Med. 2000;31(4):223–9.
21. Kwatalkar SM, Erytrocyte Sedimentation Rate, Essentials of Clinical Parhology; 1st Ed, JP; 2010; 215-9.
22. Diwakar KK. Revised look at Micro erythrocyte sedimentation rate in neonates. Indian Pediatrics 1999;36:703-5
23. Pal GK. Textbook of Medical Physiology; 3rd Ed, JP Medicals Ltd; 2016;54, 64,108,131
24. Oaei-Bimpong A, Burthem J, Supplementary Techniques Including Blood Parasites Diagnosis, Dacie and Lweis Practical haematology; 12th Ed, Elsevier; 2017;p 63-111
25. Kumar V, Abbas KA, Aster CJ, Diseases of the immune system, Robbins and Cotran: Pathologic Basis of Disease; 9th Ed. Elsevier; 2015: p 99-106.
26. Vasudevan PM, Sreekumari S, Vaidyanathan K. Textbook of biochemistry 7th Ed: JP Medicals; 2013: p 383-6
27. Devlin TM, textbook of Biochemistry with clinical correlation; 7th Ed. Wiley-Liss;2006: p987-8

28. Horns KM. Neoteric physiologic and immunologic methods for assessing early onset neonatal sepsis. J Perinat Neonat Nurs. 2000;13(4):50-66

29. Laffan M A, Manning R A, Investigation of haemostasis, Dacie and Lweis Practical haematology; 12th Ed, Elsevier; 2017; p 366-409.

30. Bos M H A, Veer C, Reitsma P H. Molecular Biology and Biochemistry of the Coagulation Factors and Pathways of Hemostasis, Williams Hematology, 9th Ed, McGraw Hill; 2016; 1915-48.

31. Brummel-Ziedins K, Orfeo T, Jenny N S, Everse S J, Mann K G, Blood Coagulation and fibrinolysis,Wintrobe's Clinical Hematology; 13th Ed, Lippincott Williams and Willkins Publishers; 2003; p 428-97.

32. Mishra J N, Rai MG, Chakraborthy S, Prasad S. Study of neonatal septicemia. Indian pediatrics 1985; 22; p 281-5

33. Vesikari T, Janas M, Gronroos P, Tuppurainen N, Nitin Shah, Dinesh Chirla, Mamta Manglani, Immuno-hematology of neonatal sepsis. Recent advances in the management of hematological disorgders of childhood. National workshop 1985;60: p 922-7

34. Cortese F, Scicchitano P, Gesualdo M, Filaninno A, De Giorgi E, Schettini F, et al. Early and Late Infections in Newborns: Where Do We Stand? A Review. Pediatr Neonatol. 2016;57(4):265–73.

35. Manroe BL, Rosenfeld CR, Browne R, Weinberg AG. The differential leucocyte count assessment and outcome of early-onset neonatal Group B streptococcal disease. *J Pediatr* 1977; 91: 632- 7

36. Chandna A, Rao MN, Srinivas, Shyamala S. Rapid diagnostic tests in neonatal septicemia. Indian J Pediatr.1988;55:947-53

37. Renolder B, Hofer N, Resch B. Early-Onset Neonatal Sepsis: Group B Streptococcal Compared to E. coli Disease. J Neonatal Biol.2015; 04:78-92.

38. Hasan Sobaih B. Early and Late Onset Neonatal Sepsis in Very Low Birth Weight Infants in a Tertiary Center in Saudi Arabia. J Neonatal Biol. 2014; 03(05):8–11.

39. Ahirrao B et al. Diagnostic utility of Hematological Scoring System (HSS) with clinicopathological and bacteriological evaluation in early diagnosis of neonatal sepsis. Annals of pathol and laboratory medicine.2017;4(6):721-6

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

41. Khurshid S, Sultan Mustafa. Rapid Identification of neonatal sepsis. Journal of Pakistan Medical Association 2000;46(3); p1-4

42. NNPD Network (2005) National Neonatal Perinatal Database 2002-2003. NNPD Nodal Center at Department of Pediatrics, AIMMS, New Delhi. http://newbornhocc.org/pdf/HRRC-Report_2002-03.pdf. accessed on 20 Aug 2019

43. Tripathi S, Malik GK. Neonatal sepsis: past, present and future; a review. Internet Journal of MedicalUpdate 2010; 5(2):45-54.

44. Peters M, Ten Cate JW, Jansen E, Breederveld C. Coagulation and fibrinolytic factors in the first week of life in healthy infants. J Pediatr1985; 106: 292- 5

45. Yentis SM, Soni N, Sheldon J. C Reactive protein as an indicator of resolution of sepsis in Intensive Care Medicine.1995;21:602-605

46. Setal BC, Viren V, Bimal BC. C-reactive protein (crp) in early diagnosis of Neonatal septicaemia. National journal of medical research. Volume 2(3);2012:276-78

47. Khassawneh M, Hayajneh WA, Kofahi H, et al. Diagnostic markers for neonatal sepsis:comparing C-reactive protein, interleukin-6 and immunoglobulin M. Scand J Immunol.2007;65(2):171-5

48. Misra RN et al. Role of sepsis screen in the diagnosis of neonatal sepsis. Medical J of Dr.D Y Patil Vidyapeeth.2013;6(3):254-57

49. Cetinkaya M, Ozkan H, Köksal N, et al. Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants. J Perinatol. 2009;29(3):225-31.

50. Russell G A B et al. Receiver operator characteristic curves for comparison of serial neutrophil band forms and CRP in neonates at risk of infection. Arch Dis Child.1992; 67:808-12

51. Sucilathangam G et al. Early diagnostic markers for neonatal sepsis: Comparing Procalcitonin (PCT) and C-reactive Protein (CRP). Journal of Clinical and Diagnostic Research.2012;6(4):627-31

52. Narasimha A, Kumar MLH. Significance of haematological scoring system in early diagnosis of neonatal sepsis. Indian J Haematol Blood Transfus 2011;27(1):14-7.

53. Gerdes JS. Clinicopathologic approach to the diagnosis of neonatal sepsis. *Clin Perinatol* 1991; **18**: 361- 81

54. Gendrel D, Bohuon C. Procalcitonin as a marker of bacterial infection. *Pediatr Infect Dis J* 2000; **19**: 679- 88

55. Rifai N, Tracy RP, Ridker PM. Clinical ef.cacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem* 1999; **45**: 2136- 41

56. Radulova P. Neonatal infections. Diagnostic markers of infection. Akush Ginekol. 2010;49:42–51

57. Varsha, Rusia U, Sikka M, Faridi MM, Madon N. Validity of hematologic parameters in identification of early and late onset neonatal infection. Indian J Pathol Microbiol. 2003;46:565–8.

58. Schultz DR, Arnold PI (1990) Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, alpha 1-acid glycoprotein and Fibrinogen. Semin Arthritis Rheum 20: 129-47

59. Kilicarslan A, Uysal A, Roach EC(2013) Acute phase reactants. Acta Med 2:2-7

60. Kale A, Bonde V. Neonatal Sepsis: An Update. Iranian Journal of Neonatology. 2013; 4(4):39-51.

ANNEXURE-I

INFORMED CONSENT FOR PARTICIPATION IN

DISSERTATION/RESEARCH

TITLE OF THE PROJECT : ROLE OF PLASMA FIBRINOGEN IN DIAGNOSIS AND PREDICTION OF SHORT TERM OUTCOME OF NEONATAL SEPSIS.

RISK AND DISCOMFORTS:

I understand that, there are risks involved in the procedures performed like continued pain at the procedure site, infection.

BENEFITS:

I understand that my participation in the study will help to know the diagnosis of diseases.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of the hospital. If the data is used for publications the identity of patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time to_____ **.**

The Department of Pathology is available to answer my questions or concerns.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw from the study at any time.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

I have read and fully understood this consent form. Therefore I agree to participate in the present study.

_____ _____

Participant / Guardian Date:

_____ _____

Signature of Witness Date:

I have explained the patient the purpose of the study, the procedure required and possible risk and benefit to the best of my ability in the vernacular language.

_____ _____

Investigator / P.G. Date:

_____ _____

Witness to Signature Date

ANNEXURE-II

PROFORMA:

NAME **:**OP/IP No. **:**

AGE **:**

SEX **:** D.O.A **:** D.O.D **:**

RESIDENCE **:**

Presenting Complaints:

Past history:

Perinatal history:term/ preterm

Birth weight-

Maternal history :

Family history :

Treatment history :

General physical examination:

Head to toe examination-

VITALS: PR: RR:

BP: TEMPERATURE: WEIGHT:

SYSTEMIC EXAMINATION:

Cardiovascular system:

Respiratory system:

Per Abdomen:

Central nervous system:

Clinical Diagnosis:

INVESTIGATIONS:

1.Haematological investigations:

Parameters	
WBC	
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
PLATELETS	
LYMPHOCYTES(%)	
NEUTROPHILS(%)	
EOSINOPHILS(%)	
MONOCYTES(%)	
BASOPHILS(%)	
RDW	
PDW	
MPV	

P-LCR	
nRBC	

Peripheral Smear Examination:

RBC:

WBC:

Platelets:

IMPRESSION:

Blood Culture Reports:

Plasma fibrinogen levels:

PT:

aPTT:

DIAGNOSIS:

ANNEXURE-IV

KEY TO MASTER CHART

Sex : M - Male, F - Female

Qns : quantity not sufficient

N : Neutrophil

L : Lypmhocyte

E : eosinophil

M : Monocyte

B : Basophil

E : 1- Normal, 2- Abnormal

M : 1- Normal, 2- Abnormal

B : 1- Normal, 2- Abnormal