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A STUDY OF UMBILICAL CORD BLOOD HAEMATOLOGICAL PARAMETERS IN HIGH RISK PREGNANT WOMEN IN PREDICTING EARLY ONSET NEONATAL SEPSIS

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Abstract:

Background:

This study aimed to evaluate cord blood haematological parameters in pregnant mothers with high risk factors in predicting early onset neonatal sepsis.

Methods: This is hospital based study done at Department of Obstetrics and Gynaecology, Siddhartha Medical College, Vijayawada**.**All examinations were done 24 hours after birth. Neonatal sepsis (NS) was characterized as a positive blood culture with symptoms of infection. All smears were examined by the pathologist blinded to the clinical findings. Platelet count & WBC, Differential leucocyte count ,Nucleated RBC (for correction of leucocyte count),Neutrophil morphology for assessing degenerative changes.e A complete blood count, included TLC, platelet count, total neutrophil count (TNC). Absolute neutrophil counts (ANC) were calculated from the observed values.

Results: Out of 100 high risk cases of neonatal septicaemia 56 culture positive cases 39 (69.64%) were males and 17 (30.36%) were females.Among the culture positive cases Klebsiella were the commonest isolates i.e, Klebsiella 23(41.07%), Staphylococci 12(21.43%), Pseudomonas6(10.71%), Enterobacter 5(8.93%), E.coli 4(7.14), Providencia 4(7.14%)And Acinetobacter 2(3.57%). 14. Haematological scoring system at score ≥2 was reliable since it had sensitivity & NPV of 100% but specificity and PPV of 54.7.A score of Haematological score of ≥5 had specificity and PPV of 100%. With increase in the score, the specificity and Positive predictive value increased. Among the different screening parameters assessed, total neutrophil count had highest sensitivity and Negative predictive value. Immature neutrophil count had highest Specificity and Positive predictive value.

Conclusion:Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. Gram negative organisms are the predominant causative agents in neonatal septicemia with Klebsiella pneumonia leading the list. Blood culture is considered “Gold standard” for the diagnosis of septicemia in neonates. A hematological score of 0 and 1 suggested sepsis was unlikely, 2-3 – sepsis possible and score 4 and 5 indicated very likely. The study found that the higher the score the greater the certainty that sepsis occurs.

Key words : Neonatal Sepsis, High Risk Pregnant Women, Umbilical Cord Blood, Neonatal Septicemia

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INTRODUCTION

In developing countries Neonatal sepsis is a most common & high risk factor for morbidity and mortality of neonates [1]. As per NNPD 2002-2003, neonatal sepsis incidence in India was 30/ 1000 live births [2]. Common cause of neonatal mortality is sepsis and accounts for 30- 50% of total deaths of neonates every year in the developing countries [3]. It is estimated that about 20% of all the neonates develop sepsis & approximately 1 percent die due to sepsis and its associated causes[3].Neonatal Septicemia is divided into 2 categories based on onset as early (0-72 hrs. of life) and late (>72 hours) [4]. Incidence of EOS was 8-10 / 1000 live births & it constituted 55.4% of overall sepsis [1] .CFR (Case fatality rate) is more in EOS(Early onset sepsis)when compared to LOS(late onset sepsis).

Due to immune system prematurity, it is accepted as a syndrome, showing metabolic & haemodynamic derangements brought about by infection. The clinical manifestation of sepsis in newborns is non-specific; hence sepsis diagnosis only by clinical findings is difficult [5].Thus, sepsis diagnosis depends on various laboratory investigations. The normal practice is, after clinical sepsis development,, by venipuncture the blood sample is collected from the newborns and sent for hematological investigations and blood culture. This can cause pain to the newborns. If this process were used, babies would not need to have blood drawn and would experience less pain and antibiotics can be started earlier to avoid neonatal morbidity. After delivery, well infants at risk for sepsis are removed from their family, thus interrupting the bonding process. Often an infant will be admitted into a triage bed for evaluation. If the venipuncture is unsuccessful, it may need to be repeated multiple times [6]. Often it is difficult to obtain an adequate blood volume from a newborn which may cause a delay in bacterial growth or interpretation may not be easy [7]. The volume of blood obtainable from the cord is usually more than adequate.

The cord extends from the fetal umbilicus to the fetal surface of placenta. It is the connecting link between growing fetus & the placenta through which the fetal blood flows to and from the placenta. The umbilical cord is the life line fetus as it supplies water, nutrients and oxygen to the growing fetus.

The usage of cord blood would allow the entire evaluation to be performed in the labor or delivery room. The specimen would be attained at the earliest possible time, allowing rapid institution of antibiotic therapy. The method is non invasive and nontraumatic and may be performed by a less skilled member of the health care team, and an adequate blood volume could be easily obtained (7).

AIM: To study cord blood haematological parameters in pregnant mothers with high risk factors in predicting early onset neonatal sepsis.

OBJECTIVES:

To study cord blood haematological parameters (Haematological Scoring System) for detection of EOS
To study cord blood cultures for diagnosis of neonatal septicemia as it is gold standard investigation.
To assess neonates who developed neonatal sepsis even after maternal antibiotic therapy
To initiate early appropriate antibiotic therapy in high risk neonates and to avoid complication
To avoid unnecessary antibiotic usage in low risk neonates

MATERIALS AND METHODS:

DATA SOURCE: This is hospital based study done at Department of Obstetrics and Gynaecology, Siddhartha Medical College, Vijayawada **.**

STUDY POPULATION: All pregnant women who are satisfying inclusion criteria in OBGY Department, Department of Obstetrics and Gynaecology, Siddhartha Medical College, Vijayawada.

STUDY TYPE: Prospective Observational study

SAMPLE SIZE: A total of 100 pregnant women

INCLUSION CRITERIA

Maternal fever (>380c)
Prelabour rupture of membranes (PROM) > 18 hours
After ROM more than 3 vaginal examination
Foul-smelling liquor

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MSL (Meconium stained liquor)
Urinary tract infection <2 weeks before delivery
Instrumental delivery & prolonged delivery

EXCLUSION CRITERIA

Neonates with gestation < 28 weeks
Newborns with weight at birth <1000 gm(very low birth weight)
Newborns with congenital abnormalities
Fetal deaths & still births

METHODOLOGY

Approval from the Institutional scientific and Ethical committee of Department of Obstetrics and Gynaecology, Siddhartha Medical College, Vijayawada was obtained. The antenatal women were given counselling & informed consent taken from them for investigation and enrolment into the study. If at any point of time antenatal woman observed to have parameters in the exclusion criteria then that antenatal woman was excluded in study.

The samples of cord blood are collected in Vacutainer tubes containing EDTA. Peripheral blood smears after staining with Leishman stain examination done with oil immersion light microscopy with magnification of X100. The tests for sepsis includes hematological parameters with HSS & the blood culture. With Beckman Coulter TLC obtained and correction done for nucleated RBC. DLC obtained from peripheral smear after staining with Leishman stain. All smears were examined by the pathologist for

a) Platelet count & WBC
b) Differential leucocyte count
c) Nucleated RBC (for correction of leucocyte count)
d) Neutrophil morphology for assessing degenerative changes

A complete blood count, included TLC, platelet count, total neutrophil count (TNC). Absolute neutrophil counts (ANC) were calculated from the observed values.

FIGURE 5. HEMATOLOGICAL SCORING SYSTEM

TABLE No:1 INTERPRETATION OF SCORE

SCORE	INTERPRETATION
≤ 2	Unlikely Septicemia
3 / 4	Possible Septicemia
≥ 5	Very likely Septicemia

Under strict aseptic precautions, 2 ml of blood from umbilical cord collected for blood culture, since it was gold standard to diagnose sepsis and was done with Bactec technique. On next subsequent days Subcultures were done and Considered blood culture negative if no organism was detected for 5 days from inoculation.

STATISTICAL ANALYSIS:

Blood culture taken as a gold standard for sepsis diagnosis. Complete blood counts observations are compared with blood culture to obtain no.of TP (True positives), FP (False positives), TN (True negatives) & FN (False negatives) cases. Sensitivity, Specificity, PPV (Positive predictive Value) & NPV (Negative predictive Value) calculated from above results.

OBSERVATIONS AND RESULTS

This prospective observational study is carried in Obstetrics and Gynaecology department, Department of Obstetrics and Gynaecology, Siddhartha Medical College, Vijayawada. Blood samples are collected from 100 cases of greater risk pregnant mother for neonatal sepsis. Among 100 patients, 56 patients obtained blood culture positive. Ultrasound has another major advantage in that it reduces certain complications (vascular, pleural and intraneural puncture) by viewing the path of the needle directly.

Despite the accurate targeting provided by ultrasound, anesthetists continue to confirm correct positioning of the needle by electrical stimulation.

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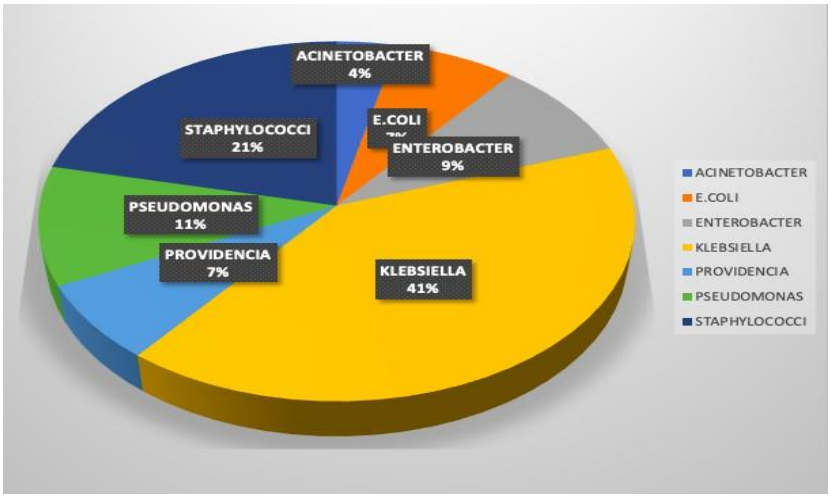
Table 2: Maternal and neonatal parameters

Maternal and neonatal parameters		No.of cases	Positive culture cases	Negative culture cases
Gender	Males	60(60)	39(69.64)	21(47.72)
	Females	40(40)	17 (30.36)	23(52.27)
Weight (Kgs)	LBW (<2.5 Kgs)	34(34)	17(30.36)	17(38.64)
	Normal weight(≥ 2.5 Kgs)	66(66)	39(69.64)	27(61.36)
Age of Gestation (Week)	Preterm(<37 Weeks)	41(41)	22(39.29)	19(43.18)
	Term (37 to 41 Week)	59(59)	34(60.71)	25(56.81)
Delivery mode	Vaginal Spontaneous	61(61)	39(69.64)	22(50)
	Forceps assisted	7(7)	5(8.93)	2(4.55)
	Caesarean section	32(32)	12(21.43)	20(45.45)
Risk factors	PROM / PPROM >18 hrs	48(48)	24(42.86)	24(54.55)
	MSL	25(25)	17(30.36)	8(18.18)
	Fever	4(4)	2(3.570)	2(4.550
	UTI	12(12)	6(10.71)	6(13.64)
	Foul smelling liquor	4(4)	2(3.57)	2(4.55)
	Instrumental delivery	7(7)	5(8.93)	2(4.55)

FIGURE NO :1 CASES ANALYSIS BASED ON ISOLATED ORGANISMS



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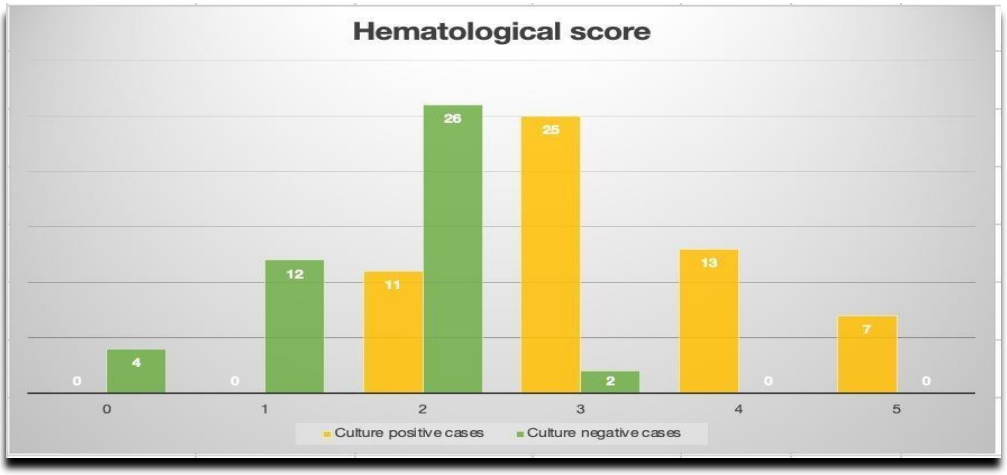
HAEMATOLOGICAL PARAMETERS		Culture Cases Positive	Culture Cases Negative	Sensitivity	Sensitivity	Positive predictive value	Negative predictive value
Total Leucocyte Count	<5,000/µl>25,000	33	10	58.9%	77.2%	76.7%	59.6%
	Normal	23	34				
Total Neutrophil Count	<1800/µl/>5400µl	51	24	91.07%	45.45%	68%	80%
	1800-5400	5	20				
Immature Neutrophil Count	>600	6	0	10.71%	100%	100%	46.80%
	≤600	50	44				
Immature:Total Neutrophil Ratio	>0.12	5	1	9%	97.7%	83.3%	47.25%
	≤0.12	51	43				
Immature:Mature Neutrophil ratio	≥0.3	6	2	10.71%	95.45%	75%	45.65%
	<0.3	50	42				
Morphological changes in neutrophils	Present	16	3	28.57%	93.18%	84.21%	50.61%
	Absent	40	41				
Platelet count profile	≤1,50,000/cumm	28	20	50%	54.54%	68.33%	46.15%
	>1,50,000/cumm	28	24				

FIGURE NO :2 HAEMATOLOGICAL SCORE PROFILE



Of the 100 Cases studied, 60(60%) are male & 40(40%) are females.
Out of 56 positive blood culture cases, 39(69.64%) are males & 17(30.36%) are females
Females are less affected than males with ratio of 1:1.5. 34(34%) were having birth weight<2.5 kgs, 66(66%) cases were of having >2.5 kgs. Twenty three(50%) cases from each category showed culture positivity.
Out of 56 positive culture new-borns, 17(30.36%) were LBW and 39(69.64%) were normal weight at birth. 41(41%) were preterm neonates, 59(59%) were term neonate. In positive culture new-borns, 22 (39.29%) were preterm, 34 (60.71%) were term. Higher positive culture cases are seen in term neonates. The proportion of positive culture cases were greater in new-borns delivered through vaginal delivery constitutes 39(69.64%) of cases. 5(8.93%) delivered by forceps assisted delivery and 12(21.43%) delivered through caesarean section. Majority of culture positive cases had risk factors such as PROM/PPROM >18 hrs 24(42.86%) and MSL 17(30.36%). Other

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conditions such as maternal fever 2(3.57%), UTI 6(10.71%), Foul smelling liquor 2(3.57%), instrumental delivery constituting about 5(8.93%) of culture positive cases. The common organism identified is Klebsiella pneumoniae (41.07%), Staphylococci (21.43%),Pseudomonas(10.71%),Enterobacter(8.93%), E.coli (7.14), Providencia (7.14%) and Acinetobacter (3.57%) were isolated.

DISCUSSION

Neonatal sepsis is a greater cause of morbidity and mortality in India. In developing countries it accounts for 15% of neonatal deaths contributing as a greater cause of neonatal mortality. Early diagnosis can prevent life threatening complications. The early symptoms & signs are nonspecific & confused with no infective causes. Hence there is a need for an investigation that should be cost effective, performed easy with early results.

Currently, many investigators assessed few highly specific & sensitive markers of inflammation to diagnosis neonatal sepsis.. However, the above markers were specific and sensitive, but expensive & sophisticated. so not practical for developing countries,

In present study, the aim is to evaluate the efficacy of various blood counts in predicting EOS. Here, comparision between results of my study with studies of various authors done. • The positive culture ratio greater in Males when compared to females in current study, with a ratio of 1.5:1These results can be comparable with results done by Vinod Kumar [9]., Shah et al[10], Tallur et al [11], Jaswal et al.[12]

The male dominance in neonatal sepsis can be linked with X-linked immunoregulatory gene factor which results in susceptibility of host to sepsis in males. In current study, positive culture patients are higher among normal birth weight neonates. In contrast Tallur et al[11], Roy et al[14], Ahmed et al[13] shows positive culture neonates higher among LBW babies .LBW babies relatively immune deficient, which predispose them to infections. Moreover, these babies likely to be subjected to different interventional procedures leading to nosocomial infections ,these changes most likely reflect variations in the characteristics of the population and the prevalence of the predisposing variables within them. In current study, the distribution of positive culture cases were greater in term neonates. Observations of my study were compared to observations done by Tallur et al [11]In contrast to our study, Ahmed et al[13] , Viswanathan et al[5] and Martono [6], K N Mishra [3] found higher culture positive rates in preterm. These changes most likely reflect variations in the characteristics of the population and the prevalence of the predisposing variables within them.

In current study, the more number of positive culture cases noted in vaginal delivery The observations of our study is compared with studies done by Tallur et al [11], Shah et al [10], Ahmed et al[13]The greater rates of neonatal sepsis through delivered by vaginal neonates may be because of colonization on the surface of new born with the organisms of vaginal canal in vaginal delivery. The greater distribution of positive culture cases observed in PROM / PPROM (>18 hrs) and Meconium stained liquor Among maternal risk factors, current study is compared with the study Shah et al [10], Vinod Kumar et al [9] , Roy et al [14] Neonatal septicaemia risk increases 10 fold over the base line once the duration after rupture is more than 18 hrs

According to Martano [16] study there was significant increase in the sepsis by 2.5% in new borns with MSL.

LBW & Preterm neonates were more susceptible to infections since they have immature immune regulatory mechanisms and undergo more interventional procedures.

In the present study, among 100 cases, culture positivity noted in 56 cases yielding positivity result of 556%. It is compared with work made by Sriram et al [17] and Roy et al [14]

In contrast, Culture positivity was low among work done by Ahmed et al[15]and Tallur et al[11] where as it was very low in study by Ramesh Bhat et al[18]

In current study, Klebsiella (41.07%) is predominant organism, Staphylococcus aureus (21.43%) Same results were obtained by Sriram et al[17], Tallur et al[11]and Roy et al [14]

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Observations done by Shrestha et al [19] and Ahmed et al [13] showed different isolate reflecting geographical differences in causative organism of sepsis.

Sen (Sensitivity), Spe (specificity), PPV and NPV of total WBC count in current study are 58.9%, 77.2%, 76.7%, 59.6% respectively .TLC has less sensitivity & negative predictive value but, high PPV & specificity same as results obtained by Ghosh et al [22], Buch et al[21]and Narashima et al[23]
Variations in the timing of blood sample, the degree of illness, or other factors such as the age of the new borns, the criteria of diagnosis used, and the test's decreased sensitivity beyond the 1st week of birth might be responsible for the variations in this parameter's results between the various studies.
Sen (Sensitivity), Spe (specificity), PPV and NPV results of total PMN count in our study were 91.07%, 45.45%, 68%, 80% respectively. Total PMN count has more sensitivity & NPV result but, less PPV &
specificity value same as result obtained by Khair et al .The variations in observations for this parameter as shown by various studies Could be because of different reference ranges, timing of blood sample as the count differs in hourly basis in neonates, gestational age, site of sampling, stress factor, any prior steroidal therapy to neonate and maternal factors. Sensitivity, specificity, PPV & NPV results of total PMN count in current study are 10.71%, 100%, 100%, 46.80% respectively. Total PMN count has a greater PPV & specificity but low sensitivity and NPV value same as results obtained by the Ghosh et al [22] The variations in this parameter's results as shown by various studies might be because of different reference ranges, timing of blood sample as the count differs in hourly basis in neonates, gestational age, site of sampling, stress factor, any prior steroidal therapy to neonate and maternal factors. Sensitivity, Specificity, PPV & NPV results of I:T ratio in current study are 9%, 97.7%, 83.3% and 47.25% respectively. I:T Ratio has less sensitivity, has high specificity ,PPV, NPV
The variations in this parameter's results as shown by various studies might be because of different reference ranges, timing of blood sample as the count differs in hourly basis in neonates, gestational age, site of sampling(as capillary specimens have relatively more neutrophils than arterial or venous), degree of illness, Neonate age. Specificity , sensitivity ,PPV & NPV of total immature PMN count in current study are 95.45%, 10.71%, 75%, 45.65% respectively.
Total PMN count has more specificity, PPV, low sensitivity & NPV same as results obtained by the Misquith . et al [25]
The variations in this parameter's results as shown by various studies might be because of different reference ranges, timing of blood sample as the count differs in hourly basis in neonates, gestational age, site of sampling (as capillary specimens have relatively more neutrophils than arterial or venous), degree of illness, neonate age. Specificity, sensitivity, PPV & NPV of degenerative changes in neutrophils in current study were 93.18%, 28.57%, 84.21%% and 50.61% respectively.
Degenerative changes in neutrophil has less sensitivity, high specificity PPV & NPV same as observation made by Ghosh et al [22].
Specificity, sensitivity, PPV & NPV of platelet count in current study are 54.54%, 50%, 58.33%, 46.15% respectively.
Total platelet count has less sensitivity, specificity, PPV & NPV, Same as results obtained by K N Mishra et al[3]The variations in various other observations might be because of error in timing of sampling collection since neonates usually show low count in 7 days of birth & increase after 1 week, sampling method , maternal and neonatal factor influence.
HEMATOLOGICAL SCORING SYSTEM:
In our study, Score of ≥ 2 was reliable indicator for sepsis with acceptable sensitivity specificity. Hence score ≤ 2 can be considered as unlikely sepsis.
As the score increased the Specificity increased, denoting certainty of sepsis.
Similar observations were made by Ghosh et al [22], Narashima et al[23], Makkar et al [24], Khair et al. [8]

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CONCLUSION: Neonatal septicemia is still a leading cause of mortality and morbidity in developing countries like India. It is more common among males. It was also found to be more commonly among vaginal delivered neonates. Gram negative organisms are the predominant causative agents in neonatal septicemia with Klebsiella pneumonia leading the list. Sepsis screening included blood culture and haematological parameters (TLC, ANC, Total immature WBC Count, I:T ratio, I:M ratio, platelet count and degenerative changes in neutrophils)Blood culture is considered "Gold standard" for the diagnosis of septicemia in neonates. Among the different screening parameters assessed, total neutrophil count had highest sensitivity and Negative predictive value. Immature neutrophil count had highest Specificity and Positive predictive value .Total immature count and were reliable tests in diagnosis of neonatal sepsis with acceptable sensitivity and specificity. A haematological score of 0 and 1 suggested sepsis was unlikely, 2-3 – sepsis possible and score 4 and 5 indicated very likely. The study found that the higher the score the greater the certainty that sepsis occurs.

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