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Review Article

Is there any association between maternal risk factors and early neonatal sepsis?

Usha Christopher* and C. Adlin Rose

Department of Obstetrics & Gynaecology, Dr SMCSI Medical College & Hospital, Karakonam – 695504, Kerala, India

***Correspondence Info:**
Dr. Usha Christopher
Associate Professor
Department of Obstetrics & Gynaecology
Dr SMCSI Medical College & Hospital,
Karakonam – 695504, Kerala, India.
E-mail: ushawilson@gmail.com

Abstract

This study was done to investigate the association between maternal factors and early neonatal sepsis. 250 babies with alleged risk factors for sepsis were followed up prospectively to determine the development of early neonatal sepsis. The factors analysed were prelabour rupture of membranes, intrapartum fever, foul smelling liquor, more than three vaginal examinations after rupture of membranes and untreated urinary tract infection. Neonatal sepsis was diagnosed if there was positive blood culture or if there were signs of sepsis along with positive laboratory parameters. Newborns that did not fulfill the criteria for culture proven or probable neonatal sepsis were considered negative for sepsis. Comparison of the different variables was done by Student's t-test, Chi-square test and Fisher's exact test. This study failed to show any statistically significant association between maternal factors conventionally associated with risk of sepsis and early neonatal sepsis. However three or more vaginal examinations after rupture of membranes come nearer to significance in its association with early neonatal sepsis.

Keywords: Maternal, neonatal sepsis, prelabour rupture of membranes

1. Introduction

Septicaemia in the newborn is a leading cause of perinatal mortality, next only to perinatal asphyxia. Early onset neonatal sepsis, infection occurring in the first 48 hours of life has an incidence of 1 - 10 per 1000 live births [1]. Damage by infective agents during this period of rapid growth may leave lasting effects on the ultimate size and function of various organs in survivors. Sepsis neonatorum can be devastating and surviving infants can have significant neurologic sequelae as a consequence of central nervous system involvement, septic shock, or hypoxemia secondary to severe parenchymal lung disease or persistent pulmonary hypertension.

Early onset septicaemia is caused mostly by organisms prevalent in the genital tract. This occurs either due to ascending infection following rupture of membranes or during the passage of the fetus through an infected birth canal. A ten-fold increase in neonatal infection has been noted in prelabour rupture of membranes (PROM) compared with those without prelabour rupture of membranes [2].

Earliest signs of sepsis are often minimal and similar to those observed in many non-infectious processes. A “baby just not looking well” may provide the only evidence that infection is present. Findings that are more prominent are respiratory distress, apnoea, lethargy, hypothermia or hyperthermia, jaundice, vomiting, abdominal distension and skin manifestations including petechiae and sclerema.

Many infants remain relatively asymptomatic until the infection is well established, often resulting in delayed therapy and a high morbidity rate. Because clinical signs of bacterial infection are non-specific in newborn infants, the common approach is to initiate antibiotic therapy in all infants with clinically suspected bacterial infection and to discontinue treatment if blood culture remains sterile and clinical signs disappear. Therefore, most infants appear to receive antibiotics unnecessarily when the information is analyzed retrospectively.

The development of multiple drug resistant bacteria makes the restriction of antibiotic therapy to truly infected patients essential. The difficulties in accurately identifying the septic neonate have prompted evaluation of many adjunctive tests that may indicate infection, but

which do not identify the organism. Because of the severity of the disease, it is most important for an adjunctive test not to miss any case (i.e. 100% sensitivity) and to rule out sepsis convincingly when in fact the disease is not present (i.e. a high negative predictive accuracy).

Early recognition of neonatal sepsis is vital to the outcome of this serious illness. Isolations of microorganisms from blood and cerebro-spinal fluid still remain the most specific and valid method of diagnosing bacterial sepsis. Unfortunately, culture results are usually available only after 48 hours. In our country, microbiology facilities are limited. More over many babies would have received prior antibiotics. A number of indirect markers e.g., white blood cell count, absolute neutrophil count, immature:total neutrophil ratio, C - reactive protein (CRP) have been used to diagnose neonatal sepsis.

This study was done to evaluate the association between different maternal factors and early neonatal sepsis. Identification of independent risk factors for early onset sepsis may help to identify those newborn babies who may benefit from maternal antibiotic prophylaxis before birth. Effective management of relevant aspects of labour and delivery may minimise subsequent neonatal infection and allow a reduction in the usage of antibiotics in the neonatal period.

2. Material and methods

2.1 Aim

To investigate the association between maternal factors and early neonatal sepsis.

2.2 Methodology

This prospective study was conducted to investigate the association between maternal factors and early neonatal sepsis.

2.3 Population

Babies born with alleged risk factors for sepsis were included in the study. These alleged risk factors included:

1) Prelabour rupture of membranes – rupture of membranes at least one hour before onset of labour pain
2) Mothers having had three or more vaginal examinations after rupture of membranes
3) Intrapartum fever – oral temperature $> 38^0$ C
4) Foul smelling liquor
5) Untreated or partially treated maternal urinary tract infection in the antenatal period.

Babies born at gestational age less than 28 weeks, weighing less than 1000 grams or with lethal congenital anomalies were excluded from the study.

Cord blood was collected and sent for total and differential white cell counts and C-reactive protein. Infants were assessed at birth and followed up by the neonatologists. Blood cultures were sent at 2 - 4 hrs of age for newborn babies with alleged risk factors according to the protocol of the Neonatology Department. C- reactive protein, total and differential white cell counts were repeated in the peripheral blood for all these babies after 24 hrs. All babies were observed for signs of sepsis for 48 hours. Mothers were also followed up for any evidence of puerperal endometritis. Other maternal and perinatal data were collected from case records.

Total white cell counts were done on a Neubauer counting chamber. Differential counts were done on peripheral blood smears stained with Leishman stain. Absolute neutrophil counts and immature: total neutrophil ratios were calculated from white cell counts. C – reactive protein estimation was done by latex agglutination method. C-reactive protein ≥ 6 mg / L was considered as positive.

For blood culture, a minimum of 1 – 2 ml of blood was inoculated into each of two blood culture bottles, one containing Biphasic Mackonkey medium (BPMM) and the other Brain Heart Infusion (BHI) broth. BPMM is specific for growing gram negative organisms. BHI broth is a highly enriched medium. After inoculation the bottles were transported to the microbiology laboratory immediately, where they were incubated for 48 hours at 37º C. If there was no growth by 48 hours, the bottles were held for up to 14 days and checked for growth.

2.4 Criteria for neonatal sepsis:

Proven neonatal sepsis was diagnosed if there was positive blood culture. Probable neonatal sepsis in the absence of a positive blood culture was diagnosed, if there were one or more signs of sepsis along with two or more positive laboratory parameters.

The signs of neonatal sepsis include:

1) Lethargy
2) Poor feeding
3) Temperature changes ($<36^0$ C or $> 37.8^0$ C) for more than one hour (checked in axilla)
4) Jaundice (severe unconjugated / conjugated)
5) Apnoea
6) Any respiratory distress (> 6 – 12 hrs age)
7) Poor peripheral perfusion (capillary filling time > 5 sec)
8) Tachycardia > 160 / min
9) Gastrointestinal manifestations (vomiting, diarrhoea, ileus)
10) Skin manifestations (petechiae, paronychia, omphalitis)
11) Central nervous system manifestations (high pitched cry, bulging fontanelle, seizures)
12) Bleeding diathesis

The laboratory parameters were:

1) Total white cell count < 5000 / mm^3 or > 20000 / mm^3
2) Absolute neutrophil count < 1500 / mm^3
3) Immature: total neutrophil ratio > 0.2
4) Platelets < 150000 / mm^3
5) Evidence of infection on blood picture (Toxic granules, cytoplasmic vacuolation)

Newborns who did not fulfill the criteria for culture proven or probable neonatal sepsis were considered negative for sepsis. Antibiotic therapy was given according to the present guidelines of the Neonatology Department. If blood culture was positive, antibiotics were given for 14 days. If blood culture was negative, antibiotics were discontinued after 3 –5 days.

2.5 Statistical Methods

Data were entered in Microsoft Excel and statistical analyses were done using SPSSPC+ software. Comparison of the different variables was done by Student's t-test, Chi-square test and Fisher's exact test. Data were analysed to see the association between each maternal risk factor and early neonatal sepsis.

3. Results

Two hundred and fifty consecutive babies who satisfied the entry criteria were included in the study. The distributions of various maternal factors are given in tables-1 & 2.

Table 1: Age

Age (Years)	Frequency
≤ 20	33
21 - 25	119
26 - 30	78
> 30	20

About 50% of women in the study were between 21 and 25 years (Table-1). The mean age of the study population was 24.85 years. Most women in the study population were nulliparous.

Table 2: Gestational age at delivery

Gestational age (weeks)	28 -32	33 - 36	37 - 40	>40
Number	8	24	198	20
Cumulative frequency	3.2%	12.8%	92%	100%

Of the 250 women, 12.8% were delivered before 37 weeks (Table-2). The median gestational age at delivery was 39 weeks. Only 8% were delivered beyond 40 weeks.

Most babies (68%) weighed between 2500 and 3499 grams at birth. The mean birth weight was 2938 grams.

Table 3: Alleged maternal risk factors

S. No	Alleged maternal risk factors	No. of babies
1	Prelabour rupture of membranes (PROM)	177
2	Three or more vaginal examinations after rupture of membranes	114
3	Intrapartum fever	22
4	Foul smelling liquor	6
5	Untreated or partially treated urinary tract infection in antenatal period	0

The commonest risk factor for sepsis was prelabour rupture of membranes followed by number of vaginal examinations after rupture of membranes (Table 3). Of the 250 women, 186 had one risk factor, 60 had two risk factors, 3 had three risk factors and 1 had four risk factors.

Among the 250 babies, only two had positive blood cultures. Group B streptococci were grown in one culture while coagulase negative staphylococci were grown in the other. The alleged risk factors for sepsis in these babies were prelabour rupture of membranes and 4 vaginal examinations after rupture of membranes in the first case, and intrapartum fever (38.3°C) and 3 vaginal examinations after rupture of membranes in the second case.

Table 4: Alleged risk factors for proven sepsis

	Risk factors for sepsis
1st baby	Prelabour rupture of membranes: 4 vaginal examinations after rupture of membranes
2nd baby	Intrapartum fever: 3 vaginal examinations after rupture of membranes

Five babies had probable sepsis according to the criteria used. The alleged risk factors for sepsis in these babies are listed below.

Table 5: Alleged risk factors for probable sepsis

	Risk factors for sepsis
1st baby	3 vaginal examinations after rupture of membranes
2nd baby	Prelabour rupture of membranes
3rd baby	Prelabour rupture of membranes, 5 vaginal examinations after rupture of membranes
4th baby	3 vaginal examinations after rupture of membranes
5th baby	3 vaginal examinations after rupture of membranes

The association between different maternal factors and early neonatal sepsis (proven + probable) was analysed in Table 6.

Table 6: Alleged maternal risk factors and early neonatal sepsis

Variables		Sepsis*		P-Value	RR(95% CI)
		Yes	No		
PROM	Yes	3	174	0.099	0.31(0.07,1.35)
	No	4	69		
Duration of ROM	≥24 hours	2	73	0.93	0.93(0.19,4.7)
	<24 hours	5	170		
Duration of labour	≥12 hours	5	98	0.09	3.57(0.71,18.04)
	<12 hours	2	145		
PV before ROM	≥3	1	9	0.16	4.0(0.53,30.16)
	0 - 2	6	234		
PV after ROM	≥3	6	108	0.05	7.16(0.87,58.59)
	0 - 2	1	135		
Total no. of PVS	≥3	6	113	0.55	6.61(0.81,54.07)
	0 -2	1	130		
Fever	Yes	1	21	0.60	1.73(0.22,13.71)
	No	6	222		
Foul smelling liquor	Yes	0	6	0.84	
	No	7	237		

*proven + probable sepsis

Alleged risk factors like prelabour rupture of membranes, duration of rupture of membranes and number of vaginal examinations before rupture of membranes were not statistically significant for sepsis. Intrapartum fever and foul smelling liquor were found to have no significant association with early neonatal sepsis. However three or more vaginal examinations after rupture of membranes come nearer to significance in its association with early neonatal sepsis.

4. Discussion

Early onset septicaemia when defined as onset of infection during first 48 hours of life is caused mostly by organisms prevalent in the genital tract. These infections are typically acquired in the intrapartum or early neonatal period from vertical transmission of pathogenic bacteria from the maternal genital tract. The fetus is well protected in utero from infections by the maternal immune system and by the placenta and membranes.

Risk Factors

Several maternal and fetal factors have been associated with breaches in these barriers, which lead to neonatal sepsis.

Maternal factors that predispose to neonatal infections are:

1) Prelabour rupture of membranes
2) Prolonged labour
3) Infected birth canal
4) Chorioamnionitis
5) Multiple vaginal examinations

Fetal factors that increase the possibilities of neonatal infections are:

1) Prematurity
2) Low birth weight
3) Birth asphyxia

Infections may be transmitted to the newborn by

1) Ascending pathway

Ascent of vaginal or cervical organisms into the amniotic cavity is the most frequent route by which a fetus is infected. Infection in the neonate may result from fetal aspiration or swallowing of organisms that have proliferated in the amniotic fluid. Vertically transmitted infections are clinically recognizable within 48 hours after birth often earlier in preterm babies.

2) Direct contact

During labour, the fetus comes into direct contact with the organisms in the cervix and vagina.

3) Transplacental

Transplacental transmission of bacterial infections is rare and this requires maternal bacteremia. This route is characteristic of vertically transmitted non-bacterial infections.

4.1 Maternal Risk Factors

Among infants born to women with prelabour rupture of membranes at term, clinical chorioamnionitis and maternal colonization of group B streptococci are the most important predictors of subsequent neonatal infection[3]. Soper *et al*[4] observed that the duration of ruptured membranes, number of vaginal examinations, duration of labour and use of internal monitors were independently associated with intra-amniotic infection[5]. These peripartum risk factors for early neonatal sepsis have been reported mainly by investigators using univariate analysis. Few studies have used multivariate analysis, a technique that assesses the simultaneous effects of multiple independent risk factors on outcome variables [6,7]. The accurate identification of independent risk factors for neonatal sepsis could allow for intrapartum

management directed towards minimizing risks and aiding in neonatal evaluation and management of infants at risk.

4.2 Intra-Amniotic Infection

Clinically evident intrauterine infections during the latter half of pregnancy develop in 1 to 10% of pregnancies and lead to increased maternal morbidity as well as perinatal mortality and morbidity. In general, the diagnosis is based on the presence of fever and other signs and symptoms, such as maternal or fetal tachycardia, uterine tenderness, foul odour of the amniotic fluid and maternal leukocytosis. Although not invariably present, rupture of the membranes (ROM) or labour also occurs in most cases. A number of terms have been applied to this infection including chorioamnionitis, intrapartum infection, amniotic fluid infection and intra-amniotic infection (IAI).

4.3 Pathogenesis

Before labour and rupture of membranes, amniotic fluid is nearly always sterile. With the onset of labour or with rupture of membranes, bacteria from the lower genital tract usually enter the amniotic cavity. This ascending route is the most common pathway for development of intra-amniotic infection[8].

Occasional instances of documented intra-amniotic infection in the absence of rupture of membranes or of labour, support a presumed haematogenous or transplacental route of infection. Fulminant intra-amniotic infection without labour and without rupture of membranes may be caused by Listeria monocytogenes[9]. Other virulent organisms, such as group A streptococci have also been the cause of transplacental infection[10]. Intra-amniotic infection may develop less commonly as a consequence of obstetric procedures such as cervical cerclage[11], diagnostic amniocentesis, cordocentesis or intrauterine transfusion.

Two large studies [8,12] of risk factors for intra-amniotic infection identified certain characteristics of labour as major risks. These features were low parity, increased number of vaginal examinations in labour as well as increased duration of labour, prelabour rupture of membranes and internal fetal monitoring. Risk factors for intra-amniotic infection have been stratified for term and preterm pregnancies[13]. For patients at term with intra-amniotic infection, these investigators observed by logistic regression analysis, that the independent risk factors were rupture of membranes greater than 12 hours (OR 5.81, 95% CI 5.12, 6.59), internal fetal monitoring (OR 2.01, 95% CI 1.68, 2.41), and having had more than four vaginal examinations in labour (OR 3.07, 95% CI 2.53, 3.73). For preterm pregnancies, these three risk factors were again identified as being independently associated with intra-amniotic infection, but with differing odds ratios. Specifically, in the preterm pregnancies for rupture of membranes longer than 12 hours, the odds ratio was 2.49 (95% CI 1.77, 3.50); for internal fetal monitoring, the odds ratio was 1.42 (95% CI 0.99, 2.04); and for having had more than four examinations, the odds ratio was 1.59 (95% CI 1.11, 2.27).

Subsequently a multivariate analysis demonstrated quantitatively the importance of chorioamnionitis in neonatal sepsis[14]. The odds ratio for neonatal sepsis accompanying clinical chorioamnionitis was 26 (95% CI 14.1, 47.9), whereas for preterm delivery, rupture of membranes longer than 12 hours, endometritis and group B streptococcal colonization, the odds ratios were all less than 5.

4.4 Diagnosis

In general, the most common clinical and laboratory criteria for diagnosis of intra-amniotic infection are fever, leukocytosis and ruptured membranes; fetal tachycardia and maternal tachycardia are noted in variable proportions of cases [15,16]. Foul-smelling amniotic fluid and uterine tenderness, although are more-specific signs, occur in a minority of cases. Bacteraemia occurs in only 10% of cases or less. Other causes of fever in the parturient include concurrent infection of the urinary tract or other organ systems and perhaps dehydration. The differential diagnosis of fetal tachycardia includes prematurity, medications, arrhythmias and hypoxia, while maternal tachycardia may be associated with drugs, hypotension, dehydration and anxiety.

4.5 Subclinical genital infection

Infants born before the thirty-seventh week of gestation account for approximately 6% of births but 80% of all perinatal deaths [17]. In most cases, the underlying cause of preterm labour is not evident. Evidence from many sources points to a relationship between preterm birth and genitourinary tract infections [18,19]. In addition to the association between symptomatic urinary tract infection and preterm birth, infection of the genital tract either clinical or subclinical has been implicated as a cause of preterm birth or of low birth weight infants. Evidence supporting this relationship includes the following observations:

1) The incidence of histologic chorioamnionitis is increased after preterm birth.

2) The incidence of clinical infection is increased after preterm birth in both mother and neonate.

3) Some lower genital tract microbes or infections are associated with an increased risk of preterm birth.

4) There are biochemical mechanisms linking prematurity and infection.

5) Infection and inflammation cause cytokine release and prostaglandin production.

6) Bacteria and bacterial products induce preterm delivery in animal models.

7) Amniotic fluid cultures are positive in some patients with preterm labour.
8) Some trials have shown a decrease in number of preterm births with antibiotic treatment.

Maternal genital tract colonization with group B streptococci may lead to neonatal sepsis, especially when birth occurs prematurely or when the membranes have been ruptured for prolonged intervals. In addition, Regan[20] and co-workers have found an association between colonisation of the cervix with these organisms and premature birth. These investigators noted delivery at less than 32 weeks in 1.8% of the total population but in 5.4% of women colonised with group B streptococci ($p<0.005$). Prelabour rupture of membranes also occurred significantly more often in the colonised group (15.3% versus 8.1%, $P<0.005$). Of the studies evaluating the association between group B streptococcal genital colonisation and preterm labour or delivery, no association was found in many[19]. In majority of the studies of prelabour rupture of membranes, group B streptococcal genital colonisation was associated with preterm prelabour rupture of membranes. In contrast to the conflicting data regarding group B streptococcal genital colonisation, group B streptococcal bacteriuria has been consistently associated with preterm delivery. Treatment of this bacteriuria has resulted in a marked reduction in prematurity (37.5% in placebo versus 5.4% in treated group)[21,22].

Current recommendation of Centres for Disease Control and Prevention (CDC) for treatment of group B streptococcal infection/colonisation in pregnancy is intrapartum treatment only. Group B streptococcal bacteriuria is an exception and should be treated antepartum. For intrapartum treatment, the Centres for Disease Control recommend adoption of either a risk factors-based approach or universal screening. With the risk factors-based approach, intrapartum intravenous penicillin G or ampicillin (clindamycin in the penicillin allergic patient) should be given if the women has any of the following: fever in labour irrespective of gestational age, preterm labour, preterm prelabour rupture of membranes, prolonged rupture of membranes (>18 hours), history of either group B streptococcal bacteriuria or a previously affected neonate. The universal screening approach involves screening all women at 35 to 37 weeks gestation with proper collection and culture techniques, followed by intrapartum treatment of all women with positive cultures[23].

4.6 Prelabour rupture of membranes

Prelabour rupture of membranes is a common but poorly understood problem. Because there is little understanding of its etiology, management has been largely empirical and obstetricians have been sharply divided about what constitutes the best approach to care. In several reports, the incidence of prelabour rupture of membranes has ranged from 4 to 7% of total deliveries [24]. Preterm prelabour rupture of membranes related to preterm birth occurs in approximately 1% of all pregnancies [25,26] and in 30% of all preterm births[27].

4.7 Etiology

Several clinical variables have been associated with prelabour rupture of membranes [25,28], including cervical incompetence, cervical operations and lacerations, multiple pregnancies, polyhydramnios, antepartum haemorrhage and heavy smoking. However in most instances, none of these clinical variables is present. In addition to being a possible cause of preterm labour, subclinical infection may be a cause of prelabour rupture of membranes. Acute inflammation of the placental membranes is twice as common when membranes rupture 4 hours before labour than when they rupture after the onset of labour, which suggests that this "infection" may be the cause of prelabour rupture of membranes[29].

Patients with prelabour rupture of membranes before term or with prolonged rupture of membranes are more likely to have anaerobes in the endocervical cultures than are women without prelabour rupture of membranes at term[30]. These observations may be interpreted as showing that subclinical anaerobic infection leads to prelabour rupture of membranes. The risk of neonatal sepsis increases with duration of membrane rupture in a linear fashion during the first 36 hours, independently of labor duration [55,56].

4.8 Complications

Table 5 summarises maternal and perinatal outcomes observed in studies reporting on more than 100 infants.

Table 5: Maternal and perinatal complications after prelabour rupture of membranes

Complication	Rate (%)
Perinatal mortality	
Term	0-2.5
All preterm	2-43
1000-1500 g	29
1501-2500 g	7
RDS- all preterm	10-42
Infection	
Chorioamnionitis	4-33
Endometritis	3-29
Neonatal sepsis (culture proven)	0-7
Neonatal sepsis overall (including clinically diagnosed sepsis)	3-28

RDS = Respiratory distress syndrome

4.9 Diagnosis of Infection

Non-invasive procedures such as measuring the level of maternal serum C-reactive protein and amniotic fluid volume have also been suggested as predictors of infection. Several groups have evaluated C-reactive

protein as such a predictor [31-34]. An elevated level of C-reactive protein in serum from patients with prelabour rupture of membranes has a modest positive predictive value for histologic chorioamnionitis (40 to 96%), but its predictive value for clinically evident infection is poor (10 to 45%). The value of a normal level of C-reactive protein for predicting absence of clinical chorioamnionitis is better (80 to 97%). In view of the low predictive value of a positive test, it does not appear wise to attempt delivery solely on the basis of an elevated C-reactive protein level.

Women who have prelabour rupture of membranes with oligohydramnios appear to be at increased risk for clinically evident infection, but the positive predictive value is modest (33 to 47%). Gonik[35] and co-workers noted that chorioamnionitis developed in 8(47%) of 17 patients with no pocket of amniotic fluid larger than 1 cm on ultrasound examination, whereas chorioamnionitis developed in 3 (14%) of 22 patients with adequate pockets (i.e., >1x1cm) ($p<0.05$). To improve the predictability of these tests, Vintzileos[36] and colleagues used a biophysical profile that included amniotic fluid volume, fetal movement and tone, fetal respiration, and a non stress test. However, positive predictive value of the biophysical profile has been variable (31 to 60% for clinical chorioamnionitis and 31 to 47% for neonatal sepsis)[37].

The bacteria that cause perinatal infections are part of the normal vaginal flora. Of the numerous strains of bacteria, those most frequently involved in ascending infection are group B streptococci, *E. coli* and other Gram negative bacilli. In the west, early onset septicaemia are mostly caused by group B streptococci and *E. coli*, while in India most cases are due to *E. coli*, *Klebsiella* and Enterobacter species [38].

Many infants with sepsis are symptomatic at birth. However, others remain relatively asymptomatic until the infection is well established, often resulting in delayed therapy and a high morbidity rate. Because clinical signs of bacterial infection are non-specific in newborn infants, the common approach is to initiate antibiotic therapy in all infants with clinically suspected bacterial infection and to discontinue treatment if blood culture remains sterile and clinical signs disappear. Therefore, most infants appear to receive antibiotic unnecessarily. The development of multiple drug resistant bacteria makes the restriction of antibiotic therapy to truly infected patients essential.

The signs suggestive of neonatal sepsis are[39]:

1) Lethargy
2) Poor feeding
3) Temperature changes ($<36^0$ C or $>37.8^0$ C) for more than one hour
4) Jaundice (severe unconjugated / conjugated)
5) Apnoea
6) Any respiratory distress (> 6 – 12 hrs age)
7) Poor peripheral perfusion (capillary filling time > 5 sec)
8) Tachycardia > 160 / min
9) Gastrointestinal manifestations (vomiting, diarrhoea, ileus)
10) Skin manifestations (petechiae, paronychia, omphalitis)
11) Central nervous system manifestations (high pitched cry, bulging fontanelle, seizures)
12) Bleeding diathesis

The difficulties in accurately identifying the septic neonate have prompted evaluation of many adjunctive tests [40] that may indicate infection, but which do not identify the organism. They are

1) White blood cell (WBC) count: Reduced counts (less than 5000 / mm^3) and elevated counts (more than 20000 / mm^3) are indicators of sepsis.
2) Absolute neutrophil count of $>1500/mm^3$ in the first 48 hours is more sensitive than leukocyte count.
3) Band form count of > 5% is suggestive of infection and more than 20% is definitive of infection.
4) Immature: total neutrophil ratio <0.2 has a good negative predictive value.
5) Morphological changes in neutrophils such as toxic granulation, cytoplasmic vacuolisation and Dohle bodies are also suggestive of infection.
6) Acute phase reactants:

a) C-reactive protein: C-reactive protein is an acute phase protein produced exclusively in the liver. Within 6 hours of an acute inflammatory challenge the C-reactive protein level starts to rise. The C-reactive protein level increases dramatically following microbial infections and this can be particularly useful for the diagnosis and monitoring of bacterial septicaemia in neonates.
b) Micro erythrocyte sedimentation rate (ESR) >10mm/hour
c) Fibrinogen
d) Haptoglobulin
e) Alpha 1 acid glycoprotein

7) Counter immune electrophoresis: It is a method of rapid detection of antigen.
8) Leukocyte enzyme activity (Nitrobluetetrazolium dye test): Phagocytosing neutrophils ingest this dye and reduce it to purple formazan. Hence, there is increased number of Nitrobluetetrazolium positive cells in infection.
9) Limulus lysate assay: It is a rapid diagnostic test for detection of endotoxin released by gram negative bacteria.
10) Other newer methods include

a) Fibronectin - level are decreased in infection.
b) Cytokine concentration - cytokines like that of tumour necrosis factor-α, interleukin-6, interleukin-8 are endogenous mediators of infection.

For many years, the white blood count was felt to be of little value for aid in the diagnosis of neonatal sepsis[41]. However the work of Manroe *et al*[42] has increased the utility of this test through the addition of absolute neutrophil count and immature: total neutrophil ratio. These workers observed a 100% negative predictive value if the total counts, absolute neutrophil count and immature: total neutrophil ratio (I/T) were all normal. In subsequent studies however these indices identified only 94% of septic patients [43,44].

Further efforts to improve the diagnostic accuracy of the white blood cell count have demonstrated that the presence of neutrophil vacuolization or toxic granulation is probably as good an indicator of sepsis as some of the white blood cell indices. Rodwell[45] *et al* developed a seven point hematological scoring system based on the white blood cell count, total and immature neutrophil counts and ratios, degenerative changes in neutrophils and thrombocytopenia. This approach appears to be useful in that 96% sensitivity and 99% negative predictive value were obtained. However the pitfalls of the blood count and failure to identify all septic infants make this score as a useful but not a definitive test.

4.10 C-Reactive Protein

C - reactive protein (CRP) is a rapidly responsive acute phase reactant synthesised by the liver within 6-8 hours of an inflammatory stimulus [46]. Since the protein is produced by the fetus and the neonate and does not cross the placental barrier, it can be used for the early detection of neonatal sepsis.

Though C - reactive protein levels are possibly helpful in combination with other tests as part of a "sepsis screen", when used alone as an initial test for infection, even if the most favourable results are assumed, approximately 10% of cases will be missed and 5% of healthy infants will be over diagnosed[40]. Nevertheless, determination of serial C - reactive protein levels does appear to be of value in excluding serious infections [47,48]. Despite the large number of infants whose assays are normal at the onset of invasive bacterial disease, rising C - reactive protein levels are usually apparent within a day and levels peak at 2 to 3 days and remain elevated until infection is controlled and resolution of the inflammatory process begins[49,50]. Several more recent studies document that serial determination of C - reactive protein levels over 1 to 3 days after onset of possible neonatal bacterial infection yields diagnostic sensitivity of 75% to 98%, specificity of 90% and most notably negative predictive value of 99%[51,52].

Culture proven sepsis is most unlikely if the C - reactive protein does not rise within 24-48 hour of the onset of the illness. The combination of a normal C - reactive protein and negative culture at 48 hours is a generally safe basis for stopping antibiotic therapy that was started on clinical grounds [53,54].

This study was done to find out the association between different maternal factors and early neonatal sepsis. Previous studies have proved that chorioamnionitis, preterm delivery, group B streptococcal colonisation and prolonged duration of internal monitoring are independent risk factors for neonatal sepsis [14]. Risk factors like duration of ruptured membranes, number of vaginal examinations were significantly associated with neonatal sepsis [13]. It is prudent to identify predisposing factors and develop management schemes to alter the effects of these factors on morbidity and potential mortality.

This study analysed 250 women with risk factors for early neonatal sepsis. Of the five risk factors that were studied specifically, none of them were found to have a significant association with early neonatal sepsis. The factors analysed were prelabour rupture of membranes, intrapartum fever, foul smelling liquor, three or more vaginal examinations after rupture of membranes and untreated urinary tract infection. However three or more vaginal examinations after rupture of membranes come nearer to significance in its association with early neonatal sepsis.

In earlier studies, logistic regression analysis had identified these factors to be independently associated with infection[12]. The reasons for the negative results in this study could be because of small sample size. Moreover all babies born to women with risk factors were on prophylactic antibiotics from the first day of birth. Blood culture does not correctly identify all cases of neonatal infection. Antibiotic treatment may alter the true number of positive neonatal infection. So most of the babies with probable sepsis could have been missed. Another reason could be that antibiotics might have masked the changes in other laboratory parameters that were used in the diagnosis of sepsis.

5. Conclusions

The aim of the study was to find out the association between different maternal factors and early neonatal sepsis. Unlike the other studies, this study failed to show any statistically significant association between maternal factors conventionally associated with risk of sepsis and early neonatal sepsis. However three or more vaginal examinations after rupture of membranes come nearer to significance in its association with early neonatal sepsis.

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