ORIGINAL ARTICLE

Risk factors and outcome analysis of gram-positive and gram-negative neonatal sepsis: A case-control study

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ABSTRACT

Background: In developing countries, neonatal sepsis is responsible for 30-50% of the total neonatal deaths. The aim of this study was to investigate the host related, clinical practice related and environment related risk factors for neonatal gram-positive sepsis (GPS) and gram-negative sepsis (GNS) and their effect on outcome.
Methods: We conducted a case-control study including 50 neonates with Blood Stream Infections (BSI) caused by both susceptible and resistant bacteria and 50 controls without BSI.
Results: Applying Chi square test of significance and running Logistic regression analysis it was observed that neonates with low birth weight, premature rupture of membranes, congenital anomalies (host related factors) exposure to broad spectrum antibiotics and steroids, caesarean section (clinical practice related factors), expressed milk/formula feeding, mechanical ventilation, and delay in enteral feeding (environmental factors) were the independent risk factors for blood stream infections (BSI) (*p value <0.05*). BSI caused by both gram-positive and gram-negative organisms increase mortality compared to the controls. The mortality rate tabulated for neonates with GPS was observed to be 45.9% and 28.6% for neonates with GNS. The odds of neonates with GPS and GNS to die are 3.55 and 6.29 times more respectively, than neonates not having sepsis.

Conclusion: Morbidity and mortality due to BSI can be prevented by controlling the risk factors at an early stage.

KEY WORDS
risk factor, outcome analysis, neonatal sepsis

INTRODUCTION

Neonatal sepsis is responsible for 30-50% of the total neonatal deaths in developing countries (1). The reported incidence of neonatal sepsis varies from 7.1 (2) to 38 (3) per 1000 live births in Asia. Many pre- and intra-partum obstetric complications are associated with an increased risk of infection in the newborn. Sophisticated equipment used for respiratory and nutritional support combined with invasive techniques provide extensive opportunities for relatively non-virulent pathogens to establish infection and to invade the host (4).

Thus, identifying the risk and prognostic factors prevailing in the different geographical contexts has become a crucial issue for optimizing neonatal care. As there is scarce information on these topics, this study was carried out with the aim of analysing the risk factors independently associated with Gram-positive Sepsis (GPS) and Gram-negative Sepsis (GNS) in neonates and to compare the clinical outcomes of such patients with that of the controls.

METHODS
Settings
This study was carried out at a tertiary care hospital in North India. The hospital is a 2,000-bed hospital providing dedicated medical services in all major specialties, including intensive care, medical and surgical care.

Study design

This was a case-control study, with a 1:1 allocation. Blood stream infection (BSI) in a neonate was confirmed as per the Centers for Disease Control and Prevention (CDC) criteria (5). Neonates (age 0-28 days) meeting these criteria were included in the study as "case patients", with an equal number of controls without BSI. Control patients had to be a neonate, admitted in the same ward on the same day (+/- 2 days) as that of the case patients. The time when the first positive blood culture was obtained was chosen as the time of inclusion into the study. The study design was approved by the Institutional Ethics Committee.

Data collection

Health records of the case and control patients were used to collect the data. The details included patient characteristics, clinical condition, practice related data, environment related data and investigation data. Outcomes of interest included length of hospital stay (LOS), post infection length of stay and in-hospital mortality.

Definitions

GPS was defined as retrieval of gram-positive organism from at least one blood culture specimen. GNS was defined as isolation of gram-negative organism from at least one blood culture specimen. Mixed cultures having both gram-positive and gram-negative organisms were not included in the study, though mixed growth of either gram-positive or gram-negative organism was included. All positive blood culture specimens were categorized as true or contaminant based upon the clinical history, physical finding, clinical course and response to treatment. Total LOS was defined as the period between admission and discharge. Post infectious LOS was defined as period between enrolment in the study and discharge. In-hospital mortality was defined as any death occurring in the hospital due to sepsis.

Microbiological methods

All microbiological specimens were processed at the Microbiology Laboratory of the tertiary care centre. Blood culture and identification was performed as per standard microbiological techniques. Antimicrobial susceptibility test was performed according to Clinical and Laboratory Standards Institute guidelines (6).

Statistical analysis

The demographic and the clinical profile of the cases and controls were compared using descriptive tables. Risk factor analysis was performed for both cases and controls. Statistical analysis was performed separately for GNS and GPS. Significant parameters were evaluated using Pearson's Chi-Square and for cases with expected count less than five Fisher's exact test was used. Logistic regression was used as confirmatory test for statistically significant parameters. Further, odds ratio (OR), probability and confidence interval at 95% significance have been tabulated, individually and overall, to depict the association between the risk factors and neonatal sepsis. To aid the tabulation of OR, a value if 0.25 (according to recent simulation) was added to cases with cell frequencies as null. An unpaired t-test was used to compare the means of LOS for GPS and GNS. To summarize the outcome analysis, logistic regression was conducted, keeping mortality rate as a dependent variable and presence of sepsis as an explanatory variable and mortality rate of sepsis related cases was projected. All analysis were performed using SPSS statistics version 19.0 software.

RESULTS

Study population and patient characteristics

As is seen in Table 1, cases were matched to control at a ratio of 1:1 and the demographic and patient characteristics were compared. In our study the age range of the patients at the time of presentation with GPS is 1-25 days while that of GNS is 1-8 days.

Microbiological characteristics

Among the 50 cases, 48% were GPS while 52% were GNS. Among the gram-positive organisms, most commonly isolated bacteria were *Staphylococcus aureus* (18%) and coagulase negative bacteria (18%) (Confirmed on repeat isolation). Only three isolates were of *Streptococcus* and *Enterococcus* each. Most of the organisms implicated in GNS were *Klebsiella* (20%) and *E.Coli* (14%). *Acinetobacter* caused sepsis in one patient and four cases were of *Pseudomonas* septicaemia. Four cases had sepsis due to mixed flora which were all gram-negative.

Risk factors for acquisition of GPS and GNS

Table 2 presents host related, practice related and environmental risk factors associated with neonatal sepsis analysed independently for their association with GPS and GNS in neonates by appropriate statistical methods.

Clinical outcome analysis of patients with GPS and GNS

Table 3a, describes the mean LOS for cases which was higher than controls. The post infectious LOS was less in GNS cases. The factors discussed earlier are independent risk factors for causing Gram-positive Sepsis which thus led to more neonatal deaths. On running logistic regression analysis the projected mortality rate was 45.9% for neonates with GPS and 28.6% for neonates with GNS (Table 3b). The odds ratio depicts the mortality risk for cases to be 3.55 times more than controls for patients with GPS and 6.29 times higher for cases than controls for patients having GNS.

DISCUSSION

Based upon its transmission, sepsis could be classified as, early onset sepsis occurring due to vertical transmission and being mainly caused by gram-negative bacteria; while the late onset sepsis would occur by horizontal transmission and would be principally associated with gram-positive bacteria acquired after delivery (7) a finding corroborated by this study.

In the present study *Klebsiella sp*. and *Staphylococcus*

sp. were most commonly isolated. Leal et al also had *Staphylococcus* as their most frequently isolated species[8]. In a study carried out in Overall, gram-negative organisms are mainly represented by *Klebsiella, E.coli, Pseudomonas and Salmonella* (10,11). Of the gram-positive organisms, S. *aureus* (9,10,12,13), Coagulase negative *Staphylococci, S.pneumoniae* and *S.pyogenes* (14), are most commonly isolated.

Similar to our study previous studies have identified prematurity, low birth weight, premature rupture of membranes, maternal pyrexia, poor intra- and post-partum hygiene, invasive medical procedures, and hospital stay (15-17) as the risk factors for neonatal sepsis. Though no such association was seen in our study, few other studies describe a strong association between prematurity and neonatal sepsis. Multivariate analysis of risk factors for proven neonatal sepsis by Yancey MK et al, has demonstrated a statistically significant association with decreasing gestational age (18). At birth, an infant's immune system remains immature. Some protection is provided by maternal antibodies (IgG) crossing the placenta. This process is less complete in the premature baby, especially if markedly premature. Not only this, preterm infants are more likely to require invasive procedures, such as umbilical catheterization and intubation.

Whilst infections can occur in utero, birth represents an abrupt transition from a highly protected environment to exposure to a vast array of new pathogens ex utero. Parturition also places the baby in direct contact with maternal blood or genital secretions and infections may result, especially if there was prolonged or early rupture of membranes.

Hence, PROM is associated with 1% increase in the incidence of neonatal sepsis; however, when chorioamnionitis accompanies the rupture of membranes, the incidence of neonatal infection is quadrupled (19). In our study PROM increased by 40.76 times, the chances of development of GPS. PROM may occur in response to an untreated Urinary tract infection (UTI) or birth canal infection which are themselves independent risk factors for neonatal sepsis.

Congenital malformations predisposed the infant 159.9 times to the development of GPS, as has been noticed in the present study. For example, congenital lung anomalies may cause aspiration pneumonitis predisposing the neonate to sepsis. Similarly congenital malformations requiring surgical treatment form an independent risk factor for development of sepsis (20).

Neonatal fungal infection has almost exclusively been described in the very low birth weight baby. In general larger babies are almost never affected unless are on prolonged intravenous feeding, for example because of gut pathology or congenital malformation (21). In the present study low birth weight babies had 12.26 times more chances of development of GNS. Also, the study reveals that 69.7% neonates with low birth weight developed gram-negative sepsis.

Studies have consistently shown that duration of antibiotic use, particularly broad spectrum antibiotics, is a major risk factor for neonatal fungal infection (22). Here prior antibiotic administration predisposed the child to develop GPS (OR 33.2) and as well as GNS (OR 5.33). This may be because the prior antibiotic administration may be due to some other coexisting risk factor such as congenital malformations, surgery or PROM.

Similarly prior steroid use was associated with GNS with an OR of 90.26, as steroid is usually administered in case of premature delivery which itself is an independent risk factor for development of neonatal sepsis.

Though delivery due to caesarean section may protect the

TABLE I: Demographic and clinical characteristics of study population (n=100)				
Characteristics	**Patients with gram positive bacteremia**		**Patients with gram negative bacteremia**	
	Cases (n=24)	Controls (n=24)	Cases (n=26)	Controls (n=26)
Sex *n* (%)				
Male	18 (66.6)	12 (50)	15 (57.7)	17 (65.4)
Female	6 (33.3)	12 (50)	11 (42.3)	9 (34.6)
Diagnosis at Admission *n* (%)				
Medical	19 (79.2)	24 (100)	20 (76.9)	22 (84.6)
Surgical	5 (20.8)	0 (0)	6 (23.1)	4 (15.4)
Fever/hypothermia n (%)	11 (45.8)	2 (8.33)	17 (65.4)	4 (15.4)
Respiratory Rate >60/min, n (%)	19 (79.1)	16 (66.67)	10 (38.5)	14 (53.8)
Total Leucocyte Count <5000 or >15000, n (%)	6 (25)	4 (16.7)	13 (50)	12 (46.1)
Immature WBCs <20 %, n (%)	10 (41.7)	2 (8.33)	11 (42.3)	11 (42.3)
Lethargic n (%)	14 (58.3)	4 (16.7)	9 (34.6)	6 (23.1)
Age at admission (age in hours)	16 (>72)	5 (>72)	24 (≤72)	26 (≤72)

Table 2: Risk factor analysis for gram positive sepsis in neonates										
	GPS						**GNS**				
Characteristics	**Cases (n=24)**	**Control (n=24)**	***P* value**	**Odds Ratio**	**Probability**	**CI (95%)**	**Cases (n=26)**	**Control (n=26)**	***P* value**	**Odds Ratio**	**Prob-ability**	**CI (95%)**
Host Factors												
Low Birth Weight	8	16	0.021				23	10	<0.001	12.26	69.7%	2.908-51.76
Prematurity	5	14	0.008				13	10	0.402			
Maternal Infection	0	2	0.489				1	3	0.698			
PROM	7	0	0.009	40.76	undefined		3	0	0.235			
Meconium staining	1	6	0.097				8	5	0.337			
Congenital anomalies	15	0	<0.001	159.9	undefined		8	13	0.158			
Resuscitation	8	2	0.380				16	14	0.779			
Practice related												
Prior antibiotic use	6	0	0.022	33.2	undefined		16	6	0.005	5.333	27.3%	1.595-17.829
Prior steroid use	2	8	0.033				12	0	<0.001	90.26		
Caesarean section	5	8	0.330				12	2	0.002	10.28	14.3%	2.004-52.749
Previous surgery	2	0	0.489				6	4	0.482			
Environment related												
Mechanical ventilation	20	8	<0.001	10.0	28.6%	2.545-39.293	25	7	<0.001	67.8	21.8%	7.682-599.415
Venous catheter	20	18	0.477				26	18	0.004			
Expressed/ formula feed	20	12	0.014	4.99	37.5%	1.311-19.074	26	15	<0.001			
Delayed enteral feed	3	4	0.683				23	9	<0.001	14.48	28.05%	3.4-61.689

TABLE 3a: Clinical outcome of patients with sepsis caused by Gram Positive (n=24) and Gram Negative (n=26) organisms						
	GPS	**Controls**	***P-value***	**GNS**	**Controls**	***P-value***
Length of stay (LOS)(mean±SD)	12.75±10.16	10.88±5.4	0.43	12.46±8.24	9.85±7.34	0.23
Post infection LOS (mean±SD)	11.96±10.01	10.46±5.57	0.52	8.46±5.32	9.77±7.39	0.46
Mortality	13	6	0.038	13	6	0.002

Unpaired t test was applied.

TABLE 3b: Mortality in patients with GPS and GNS		
Mortality	**Odds Ratio**	**Probability**
Gram Positive Sepsis	3.55	45.9%
Gram Negative Sepsis	6.29	28.6%

child's exposure to the microflora of the birth canal during delivery; nevertheless, some data suggest an association between caesarean section and increased neonatal respiratory morbidity and lacerations (23). Not only this, the indications of caesarean section may themselves be independent risk factors contributing to neonatal sepsis. In our study the odds for developing GNS in neonates with caesarean section were 10.28 (OR). A total of 14.3% neonates born by caesarean section developed GNS.

It was observed that an invasive procedure such as mechanical ventilation was associated with 10 times increased risk of GPS and 67.8 times increased risk of GNS (with probability values 28.6% and 21.8% respectively). Central venous catheters, peripheral arterial and venous cannulas, hyperalimentation infusions and assisted ventilation provide enormous opportunities for relatively non-virulent pathogens to establish infection and to invade the host.

Neonates on expressed and formula feed had more chances of acquiring GPS (OR 4.99 probability 37.5%). GNS was 14.48 times more common in neonates with delayed enteral feeding (probability 28.05%) as seen in our study. In a study conducted by Ashraf RN et al (24) a highly significant odds ratio of 18 was obtained for formula fed neonates, showing that even partial breastfeeding protects against sepsis. Our findings indicate, however, that early breast milk may have a direct anti-infective action and may stimulate neonatal immune function as well as decreasing the ingestion of infectious pathogens. Close contact between the infant-mother dyad and stimulation of the entero-mammary mucosa-associated lymphoid tissue system may also contribute (25). Delayed enteral feed also increased the risk of GNS in the present study. The risk of death as a result of infectious causes increased with increasing delay in initiation of breastfeeding (26).

A further aim of the present study was to evaluate clinical outcome variables of neonatal sepsis caused by gram positive and gram negative bacteria. As expected the mean LOS and post infectious LOS, is more in GPS as compared to controls. The lower mean post infectious LOS for GNS may be due to the earlier mortality in these cases. The in-hospital significantly greater mortality rate of the neonates with sepsis is in line with other studies (8,27).

This case-control study provides a preliminary evidence on which further larger trials may be planned in order to provide higher experimental evidence.

CONCLUSION

To define effective strategies to prevent neonatal sepsis risk-factor analysis should be done and possible sources of infections should be defined. Proper ante natal check-ups and monitoring play an important role in improving the outcome of pregnancy. Exclusive early breastfeeding practices and rationale antibiotic use should be practiced. Minimizing invasive procedures has shown an impact in reducing nosocomial infections. Fewer venepunctures and intravenous catheters minimize the risk for infections (2). Skin preparation before procedures has been shown to be effective, but studies are needed on the

exact procedures and antiseptics to be used in newborn. The importance of appropriate sterilization procedures for the equipment used in neonatal units also needs to be emphasized.

Studies looking at early discharge policies for low risk new born as a means of reducing infection need to be undertaken. In summary longer hospitalization periods and increased mortality caused by neonatal sepsis, suggest the need to control influencing risk factors in such patients at an early stage. Not only this, the presence of the particular set of risk factors can help in deciding the empirical antibiotic therapy and there by prevent delay in starting appropriate treatment.

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