ASSOCIATION OF DIFFERENT TYPES OF MILK FEEDING WITH BLOOD CULTURE POSITIVE NEONATAL SEPSIS

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ABSTRACT

Objective: To ascertain and compare microbial growth pattern in blood culture of septic neonates who were either totally breast or formula fed.

Study Design: Cross sectional study.

Place and Duration of Study: The Children's Hospital Lahore, Pakistan from Feb 2012 to Dec 2012.

Methodology: All clinically septic neonates, who were either exclusively breast fed or formula fed, were enrolled in the study. They were divided into two groups and studied for the type of organisms grown on blood culture. Group-A were breast fed and group-B were formula fed. Neonates who were blood culture negative or had growth of multiple organisms or had incomplete data or who died / left against medical advice before completing the required data or babies receiving milk feeding from multiple sources or no feeding at all were excluded. BACTEC technique was used for obtaining bacterial growth. SPSS version 19 was used for statistical analysis.

Results: A total of 380 clinically septic neonates were enrolled. Each group consisted of 190 subjects. Incidence of culture positive sepsis in breast fed and in formula fed was 6.7% and 15.7% respectively (p-value = 0.0001). Overall, gram-negative organisms constituted the majority (61·1%). Thirty seven percent cultures grew coagulase negative Staphylococcus (CoNS) followed by Klebsiella spp (23.4%). In group A, gram-negative and gram-positive organisms were equally distributed whilst in group-B, gram-negative organisms were three times more frequent than gram-positive organisms. Predominant pattern of organisms was also different in the two groups. In group-A, CoNS was predominant while in group-B, Klebsiella spp. was most frequent.

Conclusion: Culture positive sepsis is more than two times greater in formula fed babies and is caused predominantly by gram-negative organisms whilst in breast fed babies, CoNS is the commonest organism.

Keywords: Feeding pattern, Micro-organisms, Neonatal sepsis.

INTRODUCTION

Neonatal sepsis is characterized by bacteremia and clinical symptoms caused by micro-organisms and their toxic products[1]. Sepsis that occurs within first 72 hours is called early onset and is acquired mainly from pathogens of maternal genital tract, whereas late onset sepsis (after 72 hours of birth) has environmental origin, either from the community or hospital[2].

According to a report published in 2012 by Child Health Epidemiology Reference Group of WHO and UNICEF,[3] 7.6 million children under 5 years of age died in 2010, 64% (4.879 million) were due to infection and out of these 40.3% (3.072 million) were neonates. Ninety eight percent of these deaths occurred in developing countries. In Pakistan, out of a total of 284149 neonatal deaths, 20% were due to infections[4].

In Asia, including Pakistan, gram-negative organisms like E. Coli and Klebsiella spp,[3,5,6] have been found to be the leading cause of neonatal infections followed by gram-positive organisms that include Staphylococcus aureus,[7] Staphylococcus epidermidis, Listeria, Clostridia and group B streptococcus (GBS)[8].

Morbidity and mortality associated with neonatal sepsis depends upon multiple factors including preterm delivery, asphyxia at birth,[9] feeding pattern and type of feed[10]. While prematurity and asphyxia increase the risk, breast milk feeding is known to be protective against infections[11]. A comparative study done in

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Baguio General Hospital in Philippines showed that those neonates who were fed animal or formula milk were 18 times more likely to get infection than those receiving breast milk[12].

Different factors in breast milk are known to reduce the risk of infection in neonates. These include breast milk antibodies, lactoferrin, α-lactalbumin, lysozymes, immunoglobulin (sIgA, IgG, IgM), and certain factors e.g. casein, free fatty acids, cytokines (TNF-α, IL-1, IL-6, IL-8, IL-12, IFN-γ, nucleotides, enzymes, hormones and growth factors, etc. that induce infant's immune system to mature more quickly[13]. Although many studies have shown reduced mortality and morbidity in newborns fed on mother's milk, but none have differentiated etiological types of organisms grown on blood culture in neonates associated with breast and formula feeding. Present study was designed to document and compare the microbial growth pattern in septic neonates exclusively breast or formula fed.

PATIENTS AND METHODS

A prospective, observational, descriptive cross sectional study was carried out at the Neonatology Department of the Children's Hospital and Institute of Child Health, Lahore, Pakistan from February 2012 to December 2012.

Clinically septic neonates, who were either exclusively breast fed or formula fed, were included in the study. The cohort was divided into two groups based on type of feeding: group-A consisted of septic exclusively breast fed neonates and group-B of septic exclusive formula fed neonates. Septic profile comprising of complete blood count, CRP, urinalysis and blood culture was sent to laboratory for analysis. Blood cultures using BACTEC technique were incubated and checked daily for growth of pathogenic microorganisms for 7 days.

Neonates who were blood culture negative or had growth of multiple organisms or had incomplete data or who died/left against medical advice before the required data could be completed or babies receiving milk feeding from multiple sources or no feeding at all were excluded from the study.

Sample size was calculated on the basis of the fact that neonatal sepsis constituted 20% of admissions in the neonatal department. Sample size was calculated using the formula: $n = N\ x/((N-1)\ E2 + x)$, where N is population size; E is the margin of error; x is assumed value i.e. 20 in this study. Sample size with 95% confidence interval at 0.05 level was calculated to be 380. A total of 4015 neonates were included to complete the required sample size: 2805 in group-A and 1210 in group-B. Cases meeting the exclusion criteria were 2615 and 1020 in groups-A and B respectively.

Demographic data including age, gender, duration of stay, mode of delivery, type of feeding, and microbial growth patterns were entered into a predesigned proforma. Frequency and microbial growth pattern in neonatal sepsis was studied in relation to type of feeding. The results were analyzed by using SPSS version 19.

Qualitative data is presented as mean ± SD, while quantitative data is presented in frequency or percentages. Statistical test between dependent and independent variables was done using Chi-square test ($\chi 2$). Where the numbers in a cell was less than five, Fisher's exact test was used. A *p*-value ≤ 0·05 was considered statistically significant.

This research received no grant from any funding agency or from public, commercial or not-for-profit sectors. The study was approved by the IRB/Ethical Committee of the Children's Hospital & the Institute of Child Health, Lahore, Pakistan. Informed written consent was taken from every parent or guardian.

RESULTS

A total of 380 clinically septic neonates were enrolled in this study. Each group consisted of 190 subjects. The incidence of culture positive neonatal sepsis in group-A was calculated to be 6·7% whilst in group-B it was 15.7% (*p* value = 0·0001).

Both groups were comparable in their demographic data as shown in table-1.

No specific dominance of any type of organism was found in group-A as both gram-positive and gram negative organisms had equal distribution (51.6% versus 48.4%) but in group-B, gram-negative organisms were three times more common than gram-positive organisms (73.7% versus 26.3%).

Thirty seven percent of all the 380 positive cultures grew coagulase negative staphylococcus (CoNS), hence making it the commonest organism found in neonatal sepsis. This was followed by Klebsiella spp. (23.4%),

Table-1: Comparison of demographic data.

Characteristics	Group-A n=190	Group-B n=190	*p*-value
Age at admission (days) mean ± SD	7·62 ± 6.3	7·73 ± 6.7	0.15
Male	111 (58·4%)	119 (62.6%)	0.462
Female	79 (41·6%)	71 (37.4%)	0.462
Weight at admission (kg) mean ± SD	2·351 ± 0.504	2.424 ± 0.567	0.137
Caesarian section	86 (45·3%)	94 (49.5%)	0.472
Spontaneous vaginal delivery	100 (52·6%)	88 (46.3%)	0.259
Outlet forceps delivery	4 (2·10%)	8 (4.2%)	0.379

Table-2: Distribution of organisms based on gram staining.

Gram staining	Group-A n = 190	Group-B n = 190	Total	*p*-value	Odds ratio
Gram +ve	98 (51·6%)	50 (26·3%)	148 (38·9%)	0·0001	2·98
Gram –ve	92 (48·4%)	140 (73·7%)	232 (61·1%)		
Total	190 (100%)	190 (100%)	380 (100%)		

Table-3: Distribution of micro organisms in group-A and group-B.

Gram staining	Pathogenic bacteria	Group-A n=190	Group-B n=190	Total	*p*-value
Gram positive organisms (n = 148)	Coagulase negative Staphylococcus	93 (48%)	49(25·7%)	142 (37·3%)	0·001
	Group D streptococcus	5 (2·6%)	1(0·005%)	6 (0·01%)	0·215
Gram negative organisms (n = 232)	Klebsiella spp·	34 (17·8%)	55 (29%)	89 (23·4%)	0·015
	Pseudomonas aeruginosa	11 (5·7%)	35 (18·4%)	46 (12·1%)	0·002
	Enterobacteriaceae	12 (6·3%)	22 (11·5%)	34 (8·9%)	0·104
	E· coli	10 (5·2%)	9 (4·7%)	19 (0·05%)	1·000
	Providencia stuarti	5 (2·6%)	5 (2·6%)	10 (0·02%)	1·000
	Acinetobacter baumannii	10 (5·2%)	9 (4·7%)	19 (0·05%)	1·000
	Citrobacter	5 (2·6%)	2 (1%)	7 (0·01%)	0·448
	Pantoes	3 (0·01%)	2 (0·01%)	5 (0·01%)	0·3717
	Morganella morganii	0	1 (0·001%)	1 (0·01%)	1·000
	Stenotrophomonasmaltophilia	1 (0·001%)	0	1 (0·01%)	1·000
	Serratia marcescens	1 (0·001%)	0	1 (0·01%)	1·000

Pseudomonas aerogenosa (12.1%) and Enterobacteriaceae (8.9%). However, the predominant pattern varied between groups. In group-A, CoNS was the predominant organism (48%) followed by Klebsiella spp. (17·8%) and Enterobacteriaceae (6.3%), while in group-B, Klebsiella spp. led the list (29%) followed by CoNS (25.7%) and Pseudomonas aerogenosa (18·4%). The detailed results of distribution of microorganisms in group-A and B are given in table-3.

DISCUSSION

Sepsis is one of the leading causes of neonatal morbidity and mortality[14]. Causative organisms vary from place to place and their frequency differs in different hospitals and even in the same hospital at different times[15,16]. Neonates are generally more susceptible to infections compared to adults due to a number of factors, including an inadequately developed immune system[17]. Despite improvements in diagnosis and management of neonatal sepsis in recent years, sepsis still remains a major cause of neonatal morbidity and mortality especially in developing countries. Developing countries share 98% of neonatal deaths world over. These countries have an overall neonatal mortality rate of 33/1000 live births while neonatal mortality resulting from all causes of neonatal sepsis is about 36%[18]. In comparison, developed countries have neonatal mortality around 5/1000 live-births[17].

Human breast milk contains a variety of factors with anti-infection potential, such as immunoglobulins (especially secretory IgA), oligosaccharides and glycoproteins with anti-adhesive capacity, and cytokines. Secretory IgA antibodies are produced by lymphocytes that have migrated from the mother's gut to the mammary glands. These secretory IgA antibodies are mainly directed against the mother's previous and recent gut micro-flora and are transferred to the neonate via breast milk. Thus breastfeeding modulates the early exposure of the neonatal intestinal mucosa to microbes and limits bacterial translocation through the gut mucosa. It is also possible that nonpathogenic maternal bacteria, transmitted via breast milk, colonize the preterm infant gut, thus inhibiting colonization of other pathogenic bacteria[19] Trans-placentally obtained maternal IgG antibodies also contribute in protection of the neonate against micro-organisms[20]. These may be major reasons why breastfeeding protects against neonatal infections. These advantages are not obtained from formula milk feeding.

However, some of the newly developed formula milks contain probiotics and/or prebiotics that claim to have beneficial role in immune modulation, though more studies are required to establish their role. Patel et al studied the impact of early human milk received during first 28 days of life by very low birth weight septic neonates and concluded that every 10 milliliters of human milk per kilogram decreased the odds of sepsis by almost 20 percent[21]. Our study also showed that the incidence of culture positive neonatal sepsis in formula fed babies was significantly higher than breast fed babies (15.7% vs 6.7%).

Breast milk has additional protective role in preventing some specific types of bacteria. Presence of lysozymes, white blood cells and lactoferrin has been shown to encourage the growth of healthy Lactobacilli and reduce the growth of E. coli and other gram-negative pathogenic bacteria[22]. Lactoferrin is a multi-functional protein that has bacteriostatic, bactericidal[23], antiviral, antifungal and immune modulatory effects. These benefits are not expected in formula fed babies where gram-negative organisms are likely to predominate because of absence of lactoferrin. The results of our study are in conformity to this where Gram-negative septicemia was found to be significantly higher in formula fed septic neonates.

Different microbial culture studies of septic neonates conducted at various centers in Asia and Africa have shown Klebsiella, Pseudomonas, Enterobacteriaceae and E. coli as predominant

gram-negative organisms and Staphylococcus aureus as the commonest gram-positive organism[16,19,22]. In our study although similar results were obtained as regards gram-negative organisms (Klebsiella, Pseudomonas spp. and Enterobacteriaceae) but CoNS was found to be the commonest gram-positive organism which is in contrast to Staphylococcus aureus detected as predominant gram-positive organism in these studies. Although standard precautions were observed, high percentage of CoNS in our study may be related to possible contamination from skin during blood sampling. We did not use two culture technique as mentioned in other studies[24,25]. Huge number of admissions, overcrowding and extensive invasive procedures conducted in our unit may also be reasons for the higher incidence of CoNS in blood culture in our set up. The difference in results from other studies may also be because of their small sample size, cultures taken from blood as well as from other areas including skin,[26] eyes,[27] and umbilical cord,[28] and later age group of neonates (average 6.5 days versus 16 days) in other studies[29].

CONCLUSION

Gram-negative septicemia is higher in formula fed septic neonates with Klebsiella as the most frequent organism, while in breast fed babies, although infections are significantly less than formula fed neonates, but CoNS is the commonest pathogen isolated.

What is already known on this topic "What this study adds"

In Pakistan, out of a total of 284149 neonatal deaths, 20% were because of infections[7]. In Asia, gram-negative organisms[3,8,9] have been found to be the leading cause of neonatal infections followed by gram-positive organisms, the neonates who are fed animal or formula milk are 18 times more likely to get infection than those receiving breast milk[13].

Although many studies have shown reduced mortality and morbidity in newborns fed on mother's milk as opposed to formula milk, but have not compared the spectrum of etiological microorganisms grown on blood culture in septic neonates. Present study is the first to address this topic in this region. The knowledge acquired from this study shall greatly help in understanding the distribution of microorganisms in septic neonates on different types of milk feeds and thus shall help in narrowing down the choice of empirical antibiotic therapy.

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