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Short Communication

Bacterial Culture of Neonatal Sepsis

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Abstract

Neonatal bacterial sepsis is one of the major cause of morbidity and mortality in neonates. This retrospective study was performed to determine the incidence of bacterial sepsis with focus on Gram negative organisms in neonates admitted at Beheshti Hospital in Kashan, during a 3-yr period, from September 2002 to September 2005. Blood culture was performed on all neonates with risk factors or signs of suggestive sepsis. Blood samples were cultured using brain heart infusion (BHI) broth according to standard method. From the 1680 neonates 36% had positive blood culture for *Pseudomans aeruginosa*, 20.7% for Coagulase negative *Staphylococci,* and *17%* for *Klebsiella* spp. Gram-negative organisms accounted for 72.1% of all positive cultures. The overall mortality rate was 19.8% (22 /111) of whom 63.6% (14 /22) were preterm. *Pseudomona aeruginosa* and *Klebsiella* spp. showed a high degree of resistance to commonly used antibiotics (ampicillin, gentamicin) as well as third generation cephalosporins. Continued local surveillance studies are urged to monitor emerging antimicrobial resistance and to guide interventions to minimize its occurrence.

Key words: *Neonatal sepsis, Gram negative bacteria, Antibacterial resistance, Iran*

Introduction

Neonatal bacterial sepsis (NBS) remains as an important cause of mortality and morbidity among infants. Its incidence varies with geographical area and may change in the same area with time. NBS has been classified as either early onset (0-7 d of age) or late onset (7-28 d of age) (1). The incidence of NBS varies from 0.3 to 3/ 1000 live birth (LB) in Europe (2), 1 to 4/1000 LB in North America (3), 1.4 /1000 LB in a hospital study in Jamaica (4), 8.9 /1000 LB in Guadeloupe (5) and 10/1000 LB at the San Fernando general hospital in South Trinidad (6).

In most of developing countries, gram-negative bacilli remain the major cause of neonatal sepsis (7, 8). These organisms have developed increased drug resistance over the last two decades (7, 9) and management of patients is becoming a major problem.

The objective of this study is to investigate the causative organisms of neonatal sepsis with a focus on gram-negative organisms (GNOs) in Beheshti Hospital in Kashan, Iran.

Materials and Methods

A total of 1680 neonates with clinical diagnosis of septicemia who admitted at the Beheshti Hospital, Kashan, Iran through September 2002 to September 2005 were included in this study. Kashan is located in the center of Iran with a population about 400000 and there are about 5000 deliveries annually. Beheshti Hospital is a 400 bed tertiary teaching hospital with a 20-bed neonatal unit.

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Blood cultures were performed routinely on all neonates with clinical signs suggestive of sepsis (poor feeding, respiratory distress, fever, and hypothermia) or whose mothers had a history of prolonged rupture of membranes (≥ 24 h), maternal fever, and premature labor. Blood was cultured using brain heart infusion (BHI) broth according to standard methods. Subcultures were performed on days 1, 2, 3, 5, 7 and 10. The isolates were identified by standard biochemical tests. Antibacterial resistance pattern of the isolates was studied by Kirby- Bauer disc diffusion technique. Susceptibility of the isolates were done and interpreted according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations. The antibiotic concentration per disk was as followa: amikacin (30µg), ampicillin (10µg), ceftizoxime (30µg), ceftriaxone (30µg), cephalexin (30µg), and gentamicin (10µg), manufactured by Padtan Teb.

Results

There were 1680 neonates admitted during the study period, of whom 111(6.6 per cent) had proven sepsis confirmed by positive blood culture (Table 1).

A compelete list of causative microorganisms of neonatal sepsis according to birth weight is shown in Table 2. Gram-negative organisms (GNOs) contributed 72.1% of the total number of neonates with proven sepsis. *Pseudomonas aeruginosa* topping the list followed by coagulase negative *Staphylococci* (CoNS*)* and *Klebsiella* spp., respectively. Approximately 65% of all neonates with sepsis had birth weight < 2500 gr. The overall mortality rate was 19.8% (22 out of 111). Episodes of early onset sepsis revealed a higher percentage of mortality than those of late onset (21/86 vs. 1/25) (24.5% vs. 4%). The outcome of sepsis were also depends on the causative microorganisms (Table 3). Overall, the case mortality rate of episodes caused by GNOs especially *Pseudomonas* was higher than that of episodes caused by gram-positive organisms (91% vs. 9%). In our study the lowest mortality rate was due to CoNS (0%). The mortality rate was influenced by gestational age but gender has no effect.

In Table 4 there is a focus on sensitivity pattern of the gram negative organisms to various antibiotics. Considerable resistance rates too traditionally and more potent antibiotics were observed.

Table 1: Categorization of sepsis cases

		Full - term babies			Preterm babies		
Category	**Total n (%) Of neonates peresenting**	**n (%) of neonates**	**n (%) of females**	**n (%) of males**	**n (%) of neonates**	**n (%) of females**	**n (%) of males**
Early-onset sepsis	86 (77.5)	44(71)	7(41.2)	37(82.2)	42(85.7)	13(86.7)	29(85.3)
Late-onset sepsis	25 (22.5)	18(29)	10(58.8)	8(17.8)	7(14.3)	2(13.3)	5(14.7)
Total	111	62	17	45	49	15	34

Table 2: Number of microorganisms isolated according to birth weight

Microorganism \ Birth weight	<1500 gr n (%)	1501-2500 gr n (%)	>2500 gr n (%)	Total n (%)
Pseudomonas spp	12(35.3)	16(42.1)	12(30.8)	40(36)
Coagulase negative Staph	9(26.5)	6(15.8)	8(20.5)	23(20.7)
Klebsiella spp.	4(11.8)	8(21.1)	7(17.9)	19(17.1)
Enterobacter cloacae	3(8.8)	4(10.5)	4(10.3)	11(10)
Citerobacter	2 (5.9)	3(7.9)	2(5.1)	7(6.3)
Staphylococcus aureus	1(2.9)	1(2.6)	5(12.8)	7(6.3)
Escherichia coli	2 (5.9)	0(0)	1(2.6)	3(2.7)
Enterococcus faecalis	1 (2.9)	0(0)	0(0)	1(0.9)
Total	34	38	39	111

Table 3: Characteristics of mortality in neonatal sepsis according to causative organisms

	Pseudomonas Spp.	*Klebsiella*	*Citerobacter*	*Staph. aureus*	*Enterobacter*	*Ecoli*	Total
Onset of sepsis							
Early	11	5	2	1	1	1	21
Late	-	-	-	1	-	-	1
Gestational Age							
Term	5	2	-	1	-	-	8
Preterm	6	3	2	1	1	1	14
Sex							
Male	7	4	1	2	1	1	16
Female	4	1	1	-	-	-	6
Mode of delivery							
NVD	8	4	1	1	1	-	15
C/S	3	1	1	1	-	1	7

Table 4: Pattern of resistance to various antibiotics

Antibiotic		*Pseudomonas* sp.	*Klebsiella* sp.	*Enterobacter*	*Citerobacter*	*E.coli*
Ampicillin	S	20	0	20	0	0
	R	80	100	80	100	100
Gentamicin	S	53	25	40	50	11
	R	47	75	60	50	89
Amikacin	S	70	56	33	50	100
	R	30	44	67	50	0
Cephalexin	S	17	25	25	0	0
	R	83	75	75	100	100
Ceftriaxone	S	15	35	83	50	40
	R	85	65	17	50	60
Ceftizoxime	S	14	0	---	50	---
	R	86	100		50	

Values are percentages, S: sensitive, R: resistant

Discussion

The overall sepsis rate of 6.6 per cent of all admissions in our study fall within the range quoted in the literature (10) and by some researchers who gave a figure of 7.4 per 100 neonatal intensive care unit admissions (11).

There were 86 (77.5%) cases of early and 25 (22.5%) of late onset sepsis. We found that early onset sepsis was more common than late onset disease which is compatible with the reports from the other developing countries (3, 12), but in contrast with a report from Bangladesh that late onset disease was more common (13).

The causative organisms in our study were similar to findings from India, where GNO were responsible for 67.2% of their cases. *Pseudomonas aeruginosa* was the most common organism (38.3%), followed by *Klebsiella* spp. (30.4%) and *Escherichia coli* (15.6%) (9). Similar patterns have been reported in Trinidad (6) and Nigeria (14), with *Pseudomonas aeruginosa* contributing 26%, *Klebsiella pneumonia* 14%, *Escherichia coli* 7% and *Enterobacter aerogenes* 5%. Another study from Bangladesh revealed GNO were responsible for almost 73% of episodes of neonatal sepsis with *Escherichia coli* as the most common cause (30%) followed by *Klebsiella spp* (23%) (13).

The mortality rate in the present study was 19.8% which demonstrate the major impact of preterm delivery and low birth weight on mortality due to invasive infection. Various mortality figures have been reported from other Asian (9, 15-17) and European centers (2, 18, 19) ranging from 14 to 32% and 15 to 33%, respectively. Mortality was also dependent on the age at onset of symptoms and the causative microorganisms.

In our study mortality rate in early onset sepsis was significantly higher than late onset (6.1 times). This finding is compatible with the data from other studies (16, 17, 19-21).

Pseudomonas aeruginosa was the major pathogen with the highest mortality rate in this study (11 out of 40 patients died). In a study (22) *P. aeruginosa* and *Klebsiella pneumoniae* had highest mortality rate (71% and 59% respectively), while the least mortality rate was observed in Group B *Streptococcus* whereas in our study the least mortality rate was due to CoNS (0%). Similar results reported from Panama (17).

Drug resistance in causative organisms of sepsis is a rapidly emerging issue. Our study revealed a very high degree of resistance of GNO not only to commonly used antibiotics, but also predominantly to broad spectrum cephalosporins. These findings were compatible with other studies (9, 23). On the contrary studies from Sydney Neonatal Infection Surveillance (24) have mentioned that all the GNO were suceptible to gentamicin and third generation cephalosporins.

Although positive blood culture is the gold standard in the diagnosis of neonatal sepsis, but in the absence of proof of sepsis many clinicians and even some neonatologists felt obliged to continue antibiotics for a full 0f 10 d course. If the infant is not infected he or she is being subjected to unnecessary treatment. There is also danger of removing useful or susceptible organisms and encouraging resistant ones. If this occurs we shall reach a stage to go on to use more expensive antibiotics, or we have no more chance to use new drug combinations.

Strict infection control in neonatal units, hand washing combined with judicious policy for antibiotic therapy are the main solution to this problem. It will be important, however, to continue surveillance of neonatal sepsis in order to follow closely changes in trends and risk factors, to obtain information for empiric antibiotic therapy and to react rapidly in case of major changes in susceptibility patterns and occurrence of outbreaks.

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