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Predictors of positive blood culture and death among neonates with suspected neonatal sepsis in Gondar University Hospital, Northwest Ethiopia.

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ABSTRACT

Neonatal sepsis is a common cause of neonatal morbidity and mortality in developing countries. It is a serious bacterial infection of blood with a clinical syndrome characterized by systemic signs of infection and bacteremia in the first month of life. Factors associated with positive blood culture and perinatal deaths among neonates are rarely described in developing countries. This study aimed to identify the predictors of positive blood culture due to neonatal sepsis and to identify predictors of death among neonates suspected to have sepsis at Gondar University Hospital neonatal unit, Gondar, northwest Ethiopia. A prospective cross sectional study was conducted among neonates admitted at Gondar University Hospital neonatal unit between July 2011 and June 2012. Standard data collection form was used to collect all socio-demographic data and clinical characteristics of neonates. Three milliliter of venous blood sample was collected aseptically and inoculated in to Trypton soya blood culture medium for 2-14 days aerobically. Turbidity or growth was checked every 3 days. Bacterial isolates were identified following standard procedures and anti bacterial susceptibility test was done following agar disc diffusion method. The neonates were followed for their outcome until their discharge from the hospital. A total of 181 neonates (99 male and 82 female) admitted to neonatal unit with clinical features of sepsis were studied during the study period. One hundred twenty two (67.4%) of them were of early onset and 59 (32.6%) with late onset neonatal sepsis based on clinical parameters. Out of the clinically suspected cases there were 39 (32%) and 19 (32.2%) culture proven early and late onset neonatal sepsis cases respectively. Predictors of positive blood culture in both early and late onset neonatal sepsis were failure to suck, fast breathing, lethargy, seizure, respiratory distress, meconium stained liquor, premature rupture of the membrane and low birth weight. Forty four percent of gram negatives were resistant to third generation cephalosporins while 41.2% of the isolated Staphylococcus aureus were found to be methicilin resistant (MRSA). Factors that predicted deaths were positive blood culture ($p = 0.002$), infection with methicilin resistant Staphylococcus aureus ($p = 0.008$) and gram negative sepsis ($p = 0.004$). Our finding suggests that failure to suck, meconium stained liquor, premature rupture of membrane, lethargy, seizure and fast breathing are significantly associated with positive blood culture in both early and late onset neonatal sepsis. Mortality rate in our setting was much more significantly associated with positive blood culture and multidrug resistant gram negative bacteria.

Keywords: Death, Neonatal sepsis, Predictors**,** Positive blood culture

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INTRODUCTION

Neonatal sepsis is a serious bacterial infection of blood with a clinical syndrome characterized by systemic signs of infection and bacteremia in the first month of life [1]. The disease is an important cause of morbidity and mortality in neonates especially in developing countries where identification of the bacteria and treatment is often unsatisfactory [2]. It is the single most important cause of neonatal death in the developing countries, accounting for over half of the deaths [3]. If diagnosed early and treated aggressively with antibiotics and good supportive care, it is possible to save most cases of neonatal sepsis [4].

Hospital based incidence of neonatal sepsis is high and this high incidence is related to the prevalence of predisposing factors and lack of basic amenities for optimal hygiene [5-9]. However, the factors associated with early onset (0-7 days) and late onset (7–28 days) sepsis and perinatal death among infants are rarely described in developing countries [2-7]. The disease remains an important cause of morbidity and mortality in Ethiopia [6, 11].

Epidemiological data from developing countries shows differences in the incidence, risk factors, pattern and antimicrobial sensitivities of bacteria and mortality from that of developed countries [12, 13]. Blood culture to isolate the bacteria remains the gold standard for definitive diagnosis of septicemia [14] but the results of blood culture takes hours to days, thus necessitating initial empirical treatment of suspected cases [14, 15]. Knowledge of predictors of positive blood culture and death as well as anti-microbial susceptibility pattern of common bacteria in a given area is essential in guiding local empirical choice of antibiotics [16].

This study was, therefore, conducted to determine the prevalence of neonatal sepsis, predictors of positive blood culture and factors associated with deaths due to the disease at Gondar University Hospital, neonatal unit, which can provide essential information to formulate a policy for management of neonatal sepsis.

MATERIALS AND METHODS

Study area and population

A Hospital based prospective cross sectional study was conducted among neonates admitted at Gondar University Hospital, neonatal unit between July 2011 and June 2012. Gondar University Hospital is located in Gondar town, 727 km north from the capital city of Ethiopia, Addis Ababa. It is one of the oldest health institutions in Ethiopia rendering primary and referral health services to over five million people in northwest Ethiopia.

Neonates (0-28 days) with clinical sign and symptoms of sepsis at the time of admission or who developed sepsis during their hospital stay were included in this study. Neonates who started antibiotic therapy before blood culture was taken were excluded [14]. Neonatal sepsis was categorized according to its time of onset as early-onset sepsis (0-7 days) and late-onset sepsis (8-28 days).

Sample collection and examination

After getting written consent from the guardians, a standard structured data collection form [15] was designed to obtain socio demographic data and other relevant information such as maternal fever, premature rupture of membranes (PROM), weight of the baby, gestational age, temperature of the infant, respiration rate, vomiting, pulse rate, seizure, cardiovascular system, perinatal asphyxia, diffused intravascular coagulation, place of delivery, mode of delivery, presence of organomegaly in infant, jaundice, umbilical redness, meconium staining, reduced movement and inability to feed. Appropriate treatment was initiated after collection of blood and neonates were followed until discharge or death; hospital stay to discharge or deaths was recorded in days. Then after, 3ml of blood was collected from neonates aseptically and inoculated into trypton soya broth and incubated at 37^{o}C for 2-14 days aerobically, checking for turbidity or growth every 3 days. Sub-cultures were made on blood agar, chocolate agar and McConkey agar after 24 hours. Bacterial growths obtained were identified by the standard bacteriological techniques [38]. Susceptibilities to common antibiotics were determined by Kirby-Bauer disc-diffusion method. The susceptibility of the bacterial isolates were tested against amoxicillin (30mg), ampicillin (10 mg), ceftriaxone (30 mg), chloramphenicol (30 mg), norfloxacin (30 mg), penicillin (30 mg), ciprofloxacin (5 mg), tetracycline(30 mg), methicillin (5mg), trimethoprim-sulfamethoxazole (5mg), and vancomycin (30 mg). The zones of inhibition were measured and compared with National Committee for Clinical Laboratory Standards (NCCLS) guidelines (39, 40). *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) susceptible to all antimicrobial agents were used

as a control strains. Isolates showing an intermediate level of susceptibility were classified as resistant and multiple drug resistance was defined as resistance to two or more drugs from different category.

Data analysis

Data was analyzed using SPSS for windows version 16.0. Statistical test between dependent and independent variables was done using Chi-squared test ($\chi 2$). Where the numbers in a cell was less than five, a Fisher's exact test was used. P-values ≤ 0.05 were considered statistically significant. All variables with association using univariate analysis were subjected to multivariate analysis. Separate multivariable analyses were carried out to identify risk factors associated with early and late onset sepsis. Organisms causing, and outcomes of cases with early and late onset sepsis were also compared.

Ethical issues

Written informed consent was obtained from mothers/caretakers of neonates after explaining the purpose and objective of the study. The results were communicated to the treating physician for immediate and appropriate treatment. Ethical clearance was obtained from the Institutional Review Board of University of Gondar.

RESULTS

A total of 181 clinically suspected neonatal sepsis cases were included in this study. Ninety nine (54.7%) of them were males and 82 (45.3%) were females with a male to female ratio of 1.2:1 (Table 1). The mean age of the neonates was 4.1 days with standard deviation of 5.4 days. The mean duration of hospital stay was 5.6 days with a standard deviation of 4 days.

Out of the total cases, 148 (81.8%) of them were of early onset and 33 (18.2%) with late onset neonatal sepsis based on clinical parameters (Table1). From these clinically suspected cases there were 48 (32.4%) and 10 (30.3%) culture proven early and late onset neonatal sepsis cases respectively. The predominant bacterial groups were gram negatives, 36 (62.1%), as compared to gram positives. More than 70% of *Staphylococcus aureus* and 73% of *Klebsiella ozaenae* were isolated from neonates delivered in the hospital. Forty four percent of gram negatives were resistant to third generation cephalosporins and 41.2% of isolated *Staphylococcus aureus* were found to be methicilin resistant (MRSA).

Percentage of neonates with early onset sepsis born at home was 27.7% and that of neonates with late onset sepsis born at home was 33.3%. Among neonates with early onset sepsis those delivered at home had higher positive blood culture rate than those delivered at hospitals ($p = 0.031$) (Table 1). No significant difference in positive blood culture results was observed between early and late onset sepsis and there was no significant association between place of birth and positive blood culture in late onset sepsis. Clinical characteristics which were found to be significantly associated with positive blood culture on both univariate and multivariate analysis in early and late onset sepsis were failure to suck, fast breathing, lethargy, seizure and respiratory distress (Table 2). Increase in temperature (0.053) and PNA (0.038) were only found to be a predictor of positive blood culture in late onset sepsis whereas jaundice (0.012) and diffused intravascular coagulation (0.044) were significantly predictors in early onset sepsis (Table 2).

Perinatal factors like meconium stained liquor, PROM and low birth weight were strongly associated with positive blood culture in both early and late onset sepsis ($p \leq 0.05$). Mode of delivery, gestational age and febrile maternal illness were not found to influence rate of positive blood culture in both early and late onset neonatal sepsis (Table 1).

Twenty four point one percent of neonates with positive blood culture died compared to only 7.3% of those with negative blood culture ($p = 0.002$). Overall the mortality rate was 8.84%. Gram negative sepsis had higher mortality than gram positive sepsis ($p = 0.004$). Increased mortality was also seen with sepsis due to MRSA isolates ($p= 0.008$) (Table 3). Mortality in early onset sepsis was 13(8.7%) compared to 3(9.1%) in late onset sepsis ($p = 0.955$). The outcome of neonates with sepsis caused by sensitive isolates was relatively good, 82.5% of neonates with sensitive isolates improved after 3 days of treatment compared to 38.8% of those with resistant isolates ($p = 0.0001$). Overall a total of 34(85%) neonates with sensitive isolates survived compared to only 8 (44.4%) of those with resistant isolates ($p= 0.0001$) (Table 4).

DISCUSSION

Factors associated with neonatal sepsis, an important cause of child mortality, are poorly described in Africa. As the disease remains a major cause of morbidity and mortality in this region, the etiology, risk factors and outcome of this problem need to be understood [16, 17, 18]. This study, which is the first of its kind in northwest Ethiopia, showed strong predictors of positive blood culture and death that could help improve the empirical treatment where culture and sensitivity testing might not be available.

Results of this prospective study indicate that neonatal sepsis was confirmed in about 32.1% of the 181 neonates with a provisional diagnosis of the disease. These findings are consistent with reports from other tertiary hospitals in Ethiopia [6] and other developing countries [17, 19, 20, 21]. This prevalence however contrasts with the findings of Haque et al [22] in Riyadh, Saudi Arabia and of Ako - Nai et al [23] in Ile - Ife, Nigeria which reported prevalence of 15% and 55% respectively. The latter study had a relatively small number of children. This study result is also higher than those reported in developed countries [24]. The variations between developed countries and developing countries are due to high quality of life and hospital services in developed countries [24].

In the present study clinical characteristics such as failure to suck, fast breathing, lethargy and seizure were found to be significantly associated with positive blood culture ($p < 0.05$) in both early and late onset neonatal sepsis (Table 2); similar findings were observed in other studies [16, 25]. Increase in body temperature and PNA were found to be a predictor of positive blood culture in late onset sepsis whereas jaundice and diffused intravascular coagulation were significant predictors in early onset sepsis (Table 2). This result is comparable to a study done in Tanzania by Kyange et al [18].

Table 1: Background characteristics and positive blood culture among neonates with early and late onset neonatal sepsis, University of Gondar Hospital, Gondar, Ethiopia

Parameter (N)		Early onset(≤7days)			Late onset(>7days)		
		N	Culture positive	P=value	N	Culture positive	P=value
SEX	Male (99)	81	28	0.542	18	5	0.730
	Female (82)	67	20		15	5	
Mode of delivery	SVD (113)	91	26	0.205	22	8	0.284
	C/S (68)	57	22		11	2	
Gestation al age	28-32 (23)	20	7	0.794	3	1	0.695
	33-36 (59)	45	16		14	6	
	37-41 (99)	83	25		16	3	
Delivery place	Home (52)	41	20	0.031	11	3	0.789
	Hospital (129)	107	32		22	7	
Febrile maternal illness	Yes (29)	25	9	0.676	4	2	0.361
	No (152)	123	39		29	8	
Meconium stain	Yes (50)	38	23	0.004	12	10	0.012
	No (131)	110	37		21	8	
PROM	Yes (75)	65	37	0.005	10	7	0.062
	No (106)	83	28		23	8	
PPROM	Yes (25)	23	12	0.028	2	0	0.336
	No (156)	125	36		31	10	
Birth Weight (g)	1000-1500 (68)	56	26	0.050	12	8	0.033
	1501-5000 (113)	92	28		21	6	

PROM: Premature rupture of membrane, PPROM: Prolonged rupture of membrane

In this study, perinatal factors which were found to strongly predict positive blood culture in both early and late onset disease were meconium stained liquor, PROM and low gestational birth weight (Table 1). Other studies also reported similar results [18, 25, 26]. Delivery place was only found to be predictor of positive blood culture in early onset sepsis ($p = 0.031$). This can be explained by the relatively non aseptic deliveries at home that can lead to early infection. Place of delivery had no influence on positive blood culture among neonates with late onset sepsis in this study ($p=0.789$).

In contrast to studies in developed countries [27], gram negative bacteria constituted majority of the isolates in our study. *Klebsiella ozaenae* was the commonest gram negative isolate recovered in the present study. It is known that Gram negative organisms are dominant flora in pregnant females increasing the probability of these organisms

gaining access to nurseries and causing infection [28]. *Staphylococcus aureus* was the most frequent gram positive isolate in this study while Maryam [29] and Roy [30] have reported *Staphylococcus epidermidis* as the most common gram positive organism. In this study about 44% of gram negatives were resistant to third generation cephalosporins and 41.2% of isolated *Staphylococcus aureus* were found to be MRSA. Similarly high rates of MRSA were observed in a study conducted in Tanzania [18]. The antimicrobial susceptibility pattern differs in different studies done in Ethiopia [6] as well as other studies [31-33].

Table 2 Clinical characteristics and positive blood culture among early onset and late onset neonatal sepsis University of Gondar Hospital, Gondar, Ethiopia

Parameter(N)	Early onset sepsis(≤7days)			Late onset sepsis(>7days)		
	N	Culture positive (%)	P value	N	Culture positive (%)	P value
Temperature						
< 36°C (109)	94	35	0.100	15	2	0.053
> 37.5°C (72)	54	13		18	8	
Pulse rate						
100- 145b/m (149)	127	41	0.923	22	6	0.592
146-180b/m (32)	21	7		11	4	
Jaundice						
Yes (12)	10	7	0.012	2	1	0.532
No (169)	138	43		31	9	
Failure to suck						
Yes (70)	55	34	0.001	15	11	0.001
No (111)	93	31		18	3	
Fast breathing						
Yes (43)	32	19	0.003	11	8	0.006
No (138)	116	36		22	5	
Vomiting						
Yes (27)	20	6	0.803	7	3	0.416
No (154)	128	42		26	7	
Seizure						
Yes (9)	8	6	0.013	5	4	0.028
No (172)	140	45		28	8	
Change in mentation						
Yes (5)	4	2	0.447	1	0	0.503
No (176)	144	46		32	10	
Respiratory distress						
Yes (41)	29	16	0.025	12	9	0.041
No (140)	119	39		21	8	
CVS						
Yes (5)	5	2	0.713	0	0	1
No (176)	143	46		33	10	
Hepatosplenomegally						
Yes (2)	2	1	0.593	0	1	0.116
No (179)	146	47		33	9	
Lethargy						
Yes (156)	126	75	0.016	30	22	0.010
No (25)	22	7		3	0	
PNA						
Yes (58)	46	17	0.430	12	1	0.038
No (123)	102	31		21	9	
DIC						
Yes (19)	16	9	0.044	3	2	0.199
No (162)	132	41		30	9	

Factors which were significantly found to predict deaths in this study were being blood culture positive, isolation of gram negative bacteria and MRSA (Table 3). Other studies have also shown that gram negative sepsis is associated with severe sepsis and increased mortality [18, 34, 35]. In this study, as in other few studies, no significant difference was observed in mortality among neonates with early or late onset sepsis [16, 18, 35, 36]. Majority of neonates with multi-drug resistant organisms died within 3 days of initiation of antimicrobials. Relatively good survival was demonstrated in neonates with sensitive organisms; more than 80% of them improved after 3 days of treatment. Similar studies elsewhere have also found comparable result [18, 35, 37]. In this study the mortality rate

among neonates with sepsis was 8.84% which is similar to that observed in other studies in East Africa region [16, 17, 18]; this can be explained by relative similar management practices and similar hospital services.

Neonatal sepsis in our setting is higher and this finding suggests that failure to suck, meconium stained liquor, PROM, lethargy, seizure and fast breathing are significantly associated with positive blood culture in both early and late onset neonatal sepsis. Mortality rate in our setting was significantly associated with positive blood culture and multidrug resistant gram negative bacteria. Knowledge of predictors of positive blood culture and death as well as anti-microbial susceptibility pattern of common bacteria in a given area is essential in guiding local empirical choice of antibiotics and decreasing morbidity and mortality associated with neonatal sepsis.

Table 3 Factors associated with increased neonatal deaths among neonates with neonatal sepsis, University of Gondar Hospital, Gondar, Ethiopia

Parameter	N	Death (%)	P value
Culture			
Positive	58	14 (24.1%)	0.002
Negative	123	9 (7.3%)	
Gram reaction			
Negative	36	22 (61.1%)	0.004
Positive	22	5 (22.7%)	
MRSA			
Positive	7	6 (85.7%)	0.008
Negative	10	2 (20%)	

Table 4 Outcome of neonates with positive blood culture in relation to duration of treatment and sensitivity pattern, University of Gondar Hospital, Gondar, Ethiopia

Duration of treatment	Improved/Discharged N (%)	Died N (%)	P = Value
1 day			
Sensitive (40)	40 (100)	0 (0.0)	p = 0.0001
Resistant (18)	12 (66.7)	6 (33.3)	
2 days			
Sensitive (40)	36 (90)	4 (10)	P = 0.0001
Resistant (18)	8 (44.4)	10 (55.5)	
3 days			
Sensitive (40)	33 (82.5)	7 (17.5)	p = 0.0001
Resistant (18)	7 (38.8)	11 (61.2)	
> 3 days			
Sensitive (40)	34 (85)	6 (15)	P = 0.0001
Resistant (18)	8 (44.4)	10 (55.6)	

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