Epidemiology, Clinical Spectrum and Outcomes of Fungal Sepsis in Neonates in Neonatal Intensive Care Unit: A Prospective Observational Study

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ABSTRACT

Introduction: With the advancement in the management of newborn there has been significant improvement in the survival of prematurely delivered newborns. Study aimed to describe the epidemiology of fungal sepsis in neonatal intensive care unit at a tertiary care level. Also to identify risk factors and possible predictors of mortality of fungal sepsis.

Material and Methods: A prospective observational study was done in a tertiary care centre of Northern India during 2014-2015. All neonates who were admitted in neonatal intensive care unit during August 2014 to July 2015 with suspected sepsis. Outcome measures assessed was the incidence of candidemia in our NICU with clinical profiles and associated risk factors.

Results: Of the 526 neonates enrolled with suspected sepsis 83 (15.7%) were candida positive. 53 (63.8%) were *C. krusei,* 22 (26.5%) were *C. albicans* and 5 (6%) were *C. tropicalis*. On multivariate analysis of risk factors of fungal sepsis, very low birth weight babies (OR 2.3, 95% CI 1.4-6.8) and preterm babies (OR 1.9, 95% CI 1.0-5.3) were most significant. Other risk factors were prolonged mechanical ventilation >7 days (OR 5.2, 95% CI 1.4-19.1), prolonged stay in NICU (13±4.7 days) and administration of broad spectrum antibiotics, more than 7 days (OR 4.9, 95% CI 1.9 – 12.7). Very low birth weight (OR 3.9,95% CI 1.3-11.8), need of ventilation (OR 2.9, 95% CI 1.0-8.3) and NEC (OR 0.3, 95% CI 0.1-0.9) were significant risk factors for death.

Conclusion: Fungal sepsis is common infection among neonates in NICU, specially preterm and very low birth weight babies. There is progressive epidemiological shift in candida species from *C. albicans* to non albicans candida species.*C. krusei* is an emerging fungal pathogen.

Keywords: Candidemia, Preterm Neonates, Very Low Birth Weight Babies

INTRODUCTION

Premature newborn have shown considerable risk of systemic infections, either bacterial or fungal which further led to significant morbidity and mortality. The incidence of blood stream infections (BSIs) caused by Candida accounts for about 9-13% of the BSI in neonates[1]. The incidence of neonatal candidiasis has increased over last two decades in NICU[1,2]. The increase in incidence of neonatal candidemia is largely attributed to extensive use of broad spectrum antibiotics and advances in medical field like use of TPN and central lines. Systemic Candida infection, though previously thought to be rare complication, but now seems to occur in 5% of low birth weight babies and even more common in very low birth weight (VLBW) babies who receive prolonged intensive care. About 20% of the babies weighing less than 1000 g develop invasive candidiasis. Mortality rate is also quite high among neonates with disseminated fungal infection, often approaching 50%[3]. The increasing use of prophylactic antifungal agents to prevent *Candida* infections has led to emergence of resistant species

As most of the studies about the epidemiology and risk factors association of the blood stream infection due to candida species are retrospective, so we planned a prospective study to evaluate the epidemiology, risk factors and microbiological parameters associated with fungal sepsis in neonates and identify possible predictors of poor outcomes of fungal sepsis and compare morbidity profile of candidemia and bacterial sepsis in NICU patients.

Primary objective was to determine the incidence of candidemia in our neonatal intensive care unit (NICU) setup. Secondary objectiveswere to demonstrate clinical profile of neonates with fungal sepsis, to evaluate risk factors associated with neonatal candidemia, to determine incidence of various Candida subspecies, to determine complications and predictors of mortality andto compare morbity and mortality rates of i) Candida and bacterial sepsis ii) Albicans and non-albicans candida sepsis

MATERIAL AND METHODS

This study was designed as prospective observational study conducted for a period of one year (August 2014 to July 2015) at a neonatal division of Department of Paediatrics of tertiary care centre in north India.This study was approved from the institutional ethical committee. Informed written consent was obtained from guardian in local language prior to enrolment. All neonates <28 days old, admitted in NICU with clinical features of sepsis, were enrolled.

From total of 526 neonates two simultaneous blood cultures were taken in BactT/Alert pediatric vial (1 ml blood taken from peripheral vein under aseptic precaution) from

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patients at a interval of 15 minutes to minimize chances of contaminations and sent immediately to microbiology laboratory for culture. Culture was performed using standard microbiological techniques and identification of species and antifungal sensitivity was done by VITEK 2 compact system (Biomeriux). Simultaneously urine sampling were done under aseptic condition either by catheterization or suprapubic aspiration and sent to lab for routine, microscopy, culture and sensitivity. In all the patients who show candida growth in their blood culture, lumbar puncture was also performed under asepsis and CSF samples were sent for routine, cytobiochemistry and culture.

A case was defined as an NICU inpatient with 1). Blood culture positive for candida in both the culture or 2). One positive blood culture of pure growth of candida species with clinical features supportive of Candida sepsis or sepsis screen positive. A control was defined as a neonate admitted in NICU whose blood cultures were negative for candidal species. Candida positive in one blood culture with bacterial culture positive in other or single culture without clinical features or without sepsis screen positive were considered contaminants and were included in control. All the demographic data was collected on a pre-designed study proforma.

During the management of the baby all the neonatal risk factors for fungal septicaemia were studied, like need of mechanical ventilation in days, catheterization, any invasive or surgical procedures done, number of days on antibiotics. Clinical signs of sepsis like letharginess, abdominal distension, feed intolerance, respiratory distress were recorded regularly.

STATISTICAL ANALYSIS

Statistical analysis was done using Stata software, Texas, USA. Continuous variables were analyzed using. Categorical variables were analyzed using chi-square/Fisher exact test. P value of <0.05 was considered significant.

RESULTS

Out of 526 neonates who were suspected as clinical sepsis, 252 (47.9%) were culture positive and 83 (15.7%) neonates among them were diagnosed as culture positive candidemia.

Variables	#Cases (n=83)	##Control (n=274)	OR (95% CI)	P Value
*VLBW <1500 g	39 (47)	64 (23.4)	2.9 (1.7 – 4.9)	<0.001
Preterm	60 (72.2)	230 (83.9)	0.5 (0.3 – 0.9)	<0.05
**SGA	8 (9.6)	5 (1.8)	5.7 (1.8 – 18.0)	<0.05
Gender				
Male	46 (55.4)	145 (52.9)	1.1 (0.7 - 1.8)	0.68
Mode of delivery:				
NVD	53 (63.8)	189 (68.9)	0.8 (0.5-1.3)	0.38
LSCS	30 (36.2)	85 (31.1)		
Extramural	58 (69.8)	131 (47.8)	2.5 (1.5 – 4.3)	<0.001
Mean weight on admission (g)	1900.0±870.0	2100.0±580.0		
Mean gestational age (weeks)	33.7±2.5	35.1±2.4		

*VLBW=very low birth weight >1500g, **SGA=Small for Gestational age (Birth weight <10th centile),#Cases=blood culture +ve for Candida,##Control=blood culture –ve, g=grams

Table-1: Baseline characteristics of cases and controls

Variables	*Cases (n=83)	**Control (n=274)	p-value	OR (95% CI)	***Ajusted OR (95% CI)
Preterm	60 (72.2)	230 (83.9)	<0.05	0.5 (0.3 – 0.9)	1.9 (1.0-5.3)
Very low birth weight <1500 g	39 (47)	64 (23.4)	<0.001	2.9 (1.7 – 4.9)	2.3 (1.4-6.8)
Extramural	58 (69.8)	131 (47.8)	<0.001	2.5 (1.5 – 4.3)	-
Broad spectrum antibiotics (>7 days)	78 (93.9)	208 (75.9)	<0.001	4.9 (1.9 – 12.7)	-
Ventilation (> 7 days)	6 (7.2)	4 (1.4)	<0.05	5.2 (1.4 – 19.1)	-
Intubation(> 2times)	31 (37.3)	17 (6.2)	<0.001	9.0 (4.6 - 17.5)	-
Duration of stay Median days (p_{25} –p_{75})	21.4(14-28)	10.5(7-14)	<0.05	5.5 (20 - 22)	-
Maternal factors					
#PROM (>18 hrs)	49 (59.0)	28 (10.2)	<0.001	12.7 (7.0 – 22.8)	4.3 (2.1-8.7)
Fever	50 (60.2)	43 (15.7)	<0.001	8.1 (4.7 - 14.0)	-
UTI	7 (8.4)	29 (10.6)	0.569	0.78 (0.32 - 1.8)	-
##GDM	2 (2.4)	12 (4.4)	0.424	0.54 (0.12 – 2.45)	-

*Cases= Candida +ve, **Control= Culture –ve. #PROM=Premature rupture of membrane (>18 hrs), ##GDM= Gestational diabetes mellitusas per the ADA position statement 2015,OGTT done at 24-26 Weeks of gestation, plasma glucose levels taken at fasting, one and two hrs after 75 gm of anhydrous glucose.***Multivariate analysis adjusted for gestational age, VLBW,intramural/extramural, broad spectrum antibiotics and mechanical ventilation,
Values in bracket are percentage unless specified otherwise

Table-2: Risk factors associated with neonatal candidemia

	Deaths (%)	P value	OR (95% CI)	Adjusted OR# 95% CI
Gestational age <32 weeks (27) ≥32 weeks (56)	 10 10	0.15	2.1 (0.8-5.6)	2.0 (1.0-4.4)
Birth weight <2500 g (64) ≥2500 g (19)	 18 2	0.21	2.7 (0.6-12.6)	-
Birth weight <1500 g (36) >1500 g (47)	 15 5	<0.05	3.9 (1.3-11.8)	1.4 (1.2-4.6)
Need for ventilation Yes (37) No (46)	 14 6	<0.05	2.9 (1.0-8.3)	-
*NEC Present (56) Absent (27)	 8 12	<0.05	0.3 (0.1-0.9)	-
Meningitis (n=64) Present (7) Absent (57)	 4 (57.2) 15 (26.3)	0.26	2.2 (0.6-8.4)	-
Candiduria Yes (38) No (45)	 9 11	0.95	1.0 (0.4-2.6)	2.8 (1.4-5.8)
Species Non albicans (61) Albicans (22)	 17 3	0.28	2.0 (0.5-7.6)	-
#Multivariate analysis adjusted for gestational age, VLBW,intramural/extramural, broad spectrum antibiotics and mechanical ventilation *NEC=necrotizing enterocolitis.				

Table-3: Predictors of mortality

Table 1 depicts baseline characteristics. Mean birth weight was 1900.0±870.0g and mean gestational age was33.7±2.5 weeks (range 27-39 weeks). On univariatre analysis preterm, very low birth weight babies, extramural birth, use of broad spectrum antibiotics and need of ventilation were significant risk factors for developing fungal sepsis. On multivariate analysis using logistic regression preterm and very low birth weight remained significant (Table 2, 3). Fungal sepsis was first suspected at a mean age of 7.4±5.3 days (3-28 days) of life.

Analysis of clinical features revealed respiratory distress (49.5%), feed intolerance (42.0%), abdominal distension (46.7%), shock (68.8%), bleeding (68.2%), and convulsion (31.7%)to be the commonest major morbidities. CSF examination could be done in 64 (73.1%) patients. Of these 7/64 (10.9%) patients had meningitis. Meningitis was diagnosed in 28/130 (21.5%) babies with bacterial sepsis.

Among the total 83 neonates with candidemia, 22 (26.5%) were identified as *C. albicans* while remaining 61 (73.5%) were identified as Non anlbicans candida species (NAC). *C.krusei* was found to be most predominant species with incidence of 86.8% (53/61) among NAC species which was followed by *C.tropicalis*8.1% (5/61). *C. utilis* which is very rare species was been isolated from 3 cases. Overall mortality was 27.1% (20/83) among the neonates with candida positive sepsis out of which 15 were preterm.

Antifungal susceptibility profile was done which showed that C. krusei was found to be resistant to fluconazole and sensitive to rest all other antifungals used in AFS, which could be a reason for such a major predominance of C. krusei species in our NICU attributed to extensive use of fluconazole. Other species were found to be susceptible to all antifungal agents used in AFS.

DISCUSSION

In the present study, the incidence of candidemia in neonates was 9.2% among all admissions in NICU. Candida was isolated from 83/526 (15.7%) of all culture sent. Of the babies with sepsis, candida isolation rate was 32.9%. Non albicans candida accounted for 73% of total candidemia. *C. krusei* was commonest (63.5%) among all candida species. 3 cases of *C.utilis* were isolated, which is very rare subspecies. 11% candidemia babies had meningitis with 57% mortality among them. Overall mortality due to candidemia was 31.7% in our study. Most candida strains were sensitive to all antifungals, except *C. krusei* which was resistant to fluconazole.

In our study the incidence of candidemia in neonates was 9.2% among all admissions over one year. This incidence is quite high when compared to under 1% reported by Femitha et al and Ariff et al[2,4]. This high incidence of candidemia in our study possibly could be due to higher rate of extramural and premature babies admitted in our NICU. Isolation rate of 15.7% observed in our study was comparable to 13.6% reported by Agarwal et al[5]. In their study mean age at the time of investigation was 3.4 days and 38.6% were premature but in our study 67% were premature. In a retrospective analysis of candidemia in neonates Kossoff et al found >11 fold

increase in the the rate of candidemia over last 15 years[6]. In our study a total of 252/526 (47.9%) cases were blood culture positive of all culture sent. Candida isolation in 33% of all culture positive sepsis in our study was comparable to 30% and 34% reported by Pandey et al and Rani et al repectively[7,8]. In above study 28.2% were preterm babies with neonatal septicemia and 16.1% were low birth weight babies. In our study 65% of all canida isolates were non-albicans *Candida.* Earlier studies like that by Ariff et al, who have reported *C. albicans* to be the leading candidal subtype (55%) in neonatal candidemia[2]. However recent studies have reported emergence of non-albicans species. ARTEMIS Antifungal Surveillance study done between June 1997 and December 2007 in 41 countries, has noted a decreasing trend in the isolation of *Candida albicans* from 70.9% to 65%[9]. Similar trend of emergence of non albicans Candida in blood stream infection has also been reported in a number of Indian studies. Femitha et al reported 44.4% incidence of *C glabrata* followed by 25% of *C.albicans,* Rani et al reported 92% incidence of *C tropicalis* and only 4% incidence of *C. albicans* while Gunjan et al reported 85.6% incidence of non-albicans species[4,8,10].

Our study reported *C. krusei* to be the commonest species accounting for total of 63.5% of all candidal isolates. This high incidence of *C. krusei* has never been reported in previous studies. This could be a due to use of fluconazole in empirical treatment of suspected neonatal systemic candidiasis, which might have led to a selection of a candida species innately resistant to it. In western data only three isolates of *C. krusei* were reported by Fredkin et al[11] while Handrick et al reported an outbreak of seven cases[12]. Indian study like Xess et al reported 3.3% incidence of *C. krusei* in north India[13]. Only one Indian study by Gunjan et al reported 38% incidence of *C. krusei* in central India[10]. 3 cases of *C. utilis*, a very rare species, were isolated in our study. Amarela et al reported 3 neonates with *C. utilis* infection. No other published data is available for this candida species. *C. utilis* has been associated with the use of artificial ventilation, central venous catheter and any surgical procedures done at the time of birth[14]. In our study all 3 neonates with C. utilis were on mechanical ventilation and had umbilical lines.

The incidence of neonatal candidemia in very low birth weight babies in our study was 26% which is quite higher than 2.6 to 3.1% reported in previous studies[6,15–17]. Femitha et al also reported only 3.1% incidence in VLBW babies from India[4]. The reason for low incidence of candidemia in above study was routine use of fluconazole prophylaxis in VLBW babies in NICU while we were not using fluconazole prophyalxis in our NICU. However incidence in our study is somewhat similar to 25% reported by Parikh et al[18]. Even higher incidence of 47% of candidemia in VLBW had been reported by Ariff et al.[2]

Mean age of onset of detection of fungal infection in our study was 7.4±5.3 days. This was comparable to 8.3±5.8 days reported by Femitha et al and 8 days (5-167 days) by Fernandez et al[4,19]. Previous studies have reported mean age of detection from 15 to 33 days[4]. Thrombocytopenia can be specific marker of fungal sepsis in NICU. In our study 66/83 (80%) developed thrombocytopenia (<150000/mm3) which is comparable to Guida et al which reported nearly 85% incidence of thrombocytopenia in patients with invasive fungal sepsis[20]. While Ariff et al reported, 60% patients had low platelet count in their study[2].

CSF analysis could be done in 64/83 patients in our study and seven cases (11%) were diagnosed with meningitis which was much lower than 21% observed in bacterial sepsis (21%). A 10-year retrospective review of 106 cases of systemic candidiasis in neonates by Fernandez et al had reported 21% incidence of candida meningitis[19] which is quite higher than our study. This could be due to more number of premature babies (median gestational age 26 weeks) in their study. Kavuncuoğlu et al also reported candida infection was the responsible for only 5% cases of meningitis and Gram-positive bacteria and Gram-negative were responsible for 51% and 44% of meningitis[21]. Candidemia is generally associated with high moratility.

In our study mortality rate of 31.7% was observed in neonates with candida sepsis. 6-22% of mortality has been reported due to candida sepsis. Feja et al reported 22% crude mortality though *C. albicans* incidence was higher in their study. Agarwal et al reported a much higher mortality of 52.6% in their study[5,17]. The reason for high mortality in our study could be high incidence of *C. krusei*which is usually resistant to fluconazole, our first line antifungal drug. In our study mortality in VLBW was 41% which is comparable the reported incidence of 44% as described by Ariff et al.[2]

The strengths of our study are its prospective design, large sample size, inclusion of neonates in both early and late neonatal period and doing double blood cultures to rule out contaminants

Taking blood culture from two sites demands physician and nurse time, costs money, increases baby handling. Duration of study was short in our study, just one year, on the basis of which we cannot comment on changing trend of epidemiology as most of the other studies are 3-4 year studies.

What is already known?

- Candida has become important nosocomial pathogen over the last 2 decades in intensive care units.
- Non-albicans candida species has emerged as most frequent pathogens in neonatal intensive care patients.
- This increase in incidence of neonatal candidemia is largely attributed to extensive use of broad spectrum antibiotics and advances in medical field.

What this study adds?

- In our study *C. krusei* came to be most predominant Non Albicans Candida species which could be due to rampant use of fluconazole.
- A very rare species *C.utilis* was isolated from 3 cases in our NICU which is usually associated with central venous catheter use and mechanical ventilation.
- Very low birth weight and mechanical ventilation were significant risk factors and important predictor of

mortality in neonates with fungal sepsis in NICU.

CONCLUSION

Candida sepsis is a major cause of neonatal sepsis. Our study shows that nonalbicans *Candida* has emerged as a major cause of neonatal candidemia and is becoming an important cause of neonatal morbidity and mortality. Among the non albicans Candida, *C. krusei* was commonest species, which has innate resistance to fluconazole. Very low birth weight (<1500g), use of broad spectrum antibiotics and mechanical ventilation were found to significant risk factors as well as important predictors of mortality in fungal sepsis in neonates.

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