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Original Article

Paediatrics Section

Association and Outcome of Intracranial Haemorrhage in Newborn with Fungal Sepsis- A Prospective Cohort Study

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ABSTRACT

Introduction: Neonatal sepsis is a leading cause of mortality and morbidity. In spite of using appropriate antibiotics, those who are bacterial culture-negative, still succumb to fungal infection. Fungal sepsis is common in the Neonatal Intensive Care Unit (NICU), especially with invasive procedures and prolonged empirical use of antibiotics. The incidence of fungal infection varies widely across centres, likely due to differences in practice related to modifiable risk factors such as exposure to empiric antibiotics and length of parenteral nutrition. Neonates are at high risk for acquiring infections due to their specific Central Nervous System (CNS) structure as well as functionally immature immune system, causing CNS infection or Intracranial Haemorrhage (ICH) due to sepsis induced coagulopathy. Intracranial haemorrhage in neonates often results in devastating neurodevelopmental outcomes and also results in significant mortality in the neonatal period.

Aim: To find the association and outcome of ICH in newborn with fungal sepsis.

Materials and Methods: This study was a prospective cohort study conducted at the Department of Paediatrics, Government Kilpauk Medical College, Chennai, Tamil Nadu, India, on neonates admitted in the NICU during the period January 2018 to December 2020. Neonates with a diagnosis of fungal sepsis were identified from blood cultures. They were also subjected to Complete Blood Count (CBC), Capillary Refill Time (CRT), Prothrombin Time (PT), activated Partial Thromboplastin clotting time (aPTT) and cranial ultrasound. Chi-square analysis for descriptive data and Cox Proportional Hazard Regression for survival and non survival neonates and Kaplan-Meier curve analysis were done.

Results: Out of total 80 neonates, nine had ICH, 21 neonates had Intraventricular Haemorrhage (IVH) and no haemorrahge in 50 neonates. More the gestational age, lesser were the chances of ICH and the difference was statistically significant ($p<0.001$). A total of 50 babies died, majority (42%) were in the gestational age between 37-40 weeks. All the babies diagnosed with *C. albicans* sepsis succumbed to infection.

Conclusion: The present study highlights the fact that the lesser the gestational age, the more the chances of ICH. A close monitoring of the coagulation profile PT and aPTT will help us to identify the babies more prone to ICH. Expert cranial ultrasound will pick up the haemorrhage early. Timely treatment measures instituted will help in preventing mortality due to bleeding manifestions in fungal sepsis. The Cox regression analysis reveals that both PT and aPTT and ICH are the associated risk factors for non survival in fungal sepsis neonates.

Keywords: Antibiotics, *Candida albicans*, Gestational age, Intraventricular haemorrhage, Neonatal infection

INTRODUCTION

Neonatal sepsis poses a challenge for every paediatrician. It is the leading cause of mortality and morbidity. With advances in the field of Neonatology, the doctors can save most newborn babies with sepsis by using appropriate antibiotics. In spite of appropriate antibiotics, those who are bacterial culture negative, still succumb to fungal infection [1]. Fungal sepsis has been found to be a major cause of mortality in the Neonatal Intensive Care Unit (NICU), especially with invasive procedures and prolonged empirical use of antibiotics. Infection with fungal species is associated with significant morbidity and mortality in infants. The incidence of fungal infection varies widely across centres, likely due to differences in practice related to modifiable risk factors, such as exposure to empirical antibiotics and length of parenteral nutrition [2]. Neonatal fungal infections especially candidiasis is associated with 20% mortality, and 50% of survivors have severe neurodevelopmental impairment. End-organ damage in the Central Nervous System (CNS) is more common in neonatal fungal sepsis [2].

According to the national neonatal-perinatal database, 2002-03 report over 1 million newborn infants died every year in the neonatal period (first 4 weeks of life) in India, amounting to the highest-burden of newborn deaths for any country in the world [1]. Neonatal sepsis presents as a multi-spectrum of symptoms and signs. They can be subtle or stormy, varying from diminished spontaneous activity, poor sucking, temperature instability, jitteriness, seizures, irritability, vomiting, respiratory distress, jaundice, feeding intolerance, apnoea and increased need for ventilatory support, hypoglycaemia as well as hyperglycaemia and perfusion abnormalities [2]. Most fungal sepsis presents as late-onset sepsis in newborn infants, often after the second and third week of life. Although persistent thrombocytopenia leads to the suspicion fungal sepsis, it is not consistently present in every case. If fungal sepsis is not recognised early, it can lead to meningitis, sepsis induced coagulopathy causing ICH. The degree of CNS involvement is directly proportional to the mortality rate [2]. Although bloodstream infection due to *Candida* species in the NICU is less frequent than that due to Gram positive or Gram negative bacteria, it has higher morbidity and mortality rates [3].

The incidence of candidiasis in Extremely Low Birth Weight (ELBW) infants is approximately 10%. This varies as much as 20 fold between various centres. Neonatal candidaemia is associated with 20% mortality

and 50% of survivors have a severe neurodevelopmental impairment [4], either due to sepsis or fragile vascularity of intracranial vessels. The babies who survive candida septicaemia have a high incidence of complications. Severe CNS sequelae, such as hydrocephalus, mental retardation and aqueductal stenosis are known to occur due to ICH.

Diagnosis of neonatal candidaemia has to have a high index of suspicion. A thorough evaluation is a must when blood culture is positive for candida. The blood culture is the gold standard for the detection of candidiasis, even though its sensitivity is very poor. A positive blood culture should never be considered a contaminant [5]. Only 37% of infants with proven candida meningitis had positive blood cultures for candida. In addition to this, normal Cerebrospinal Fluid (CSF) parameters are present in half of the infants with candida meningitis [5]. The doctors must also perform urine culture, CSF analysis and CSF culture, ophthalmological examination, echocardiogram, renal ultrasound and even skeletal survey, if necessary [5].

Non albicans candida species were predominant in Asia with proportions ranging from 25-92% with a median of 75% [6]. *Candida albicans* was known to contribute to 60% of candidaemia reported till recently. However, many studies show that non albicans candida such as *C. glabrata, C. krusei, C. parapsilosis,* and *C. tropicalis* were found to account for the majority of fungal infections. This is a major change [7]. Very Low Birth Weight (VLBW) infants are known to be at high risk of candidaemia because of more aggressive and invasive therapies such as indwelling central lines, mechanical ventilation, parenteral hyperalimentation and longer hospital stay [8,9].

Earlier studies have shown that candida has emerged as a major cause of neonatal sepsis and that it leads to morbidity and mortality. Studies have also highlighted the predominant organism isolated mostly candida non albicans species. Previous study states that once fungal sepsis is suspected, a coagulation profile done early with a cranial Ultrasonography (USG) will pick up early onset of ICH. Timely medical measures instituted will prevent the high rate of mortality [2]. Hence, this study aimed to provide association of various factors like gestational age, birth weight, platelet count, Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), and cranial ultrasound with ICH and outcome of ICH in newborns with fungal sepsis.

MATERIALS AND METHODS

A prospective cohort study was conducted at the Department of Paediatrics, Government Kilpauk Medical College (tertiary care centre), Chennai, Tamil Nadu, India, during January 2018 to December 2020. Ethical Committee approval was duly obtained (letter No-02A-2017.14/11/2017) and consent from the parents was also obtained. Neonates with a diagnosis of fungal sepsis were identified from blood cultures. All yeast or mold species isolated from blood cultures were cultured on Sabouraud's dextrose agar and species were identified by the CHROMagar method.

Inclusion criteria: All neonates, intramural and extramural admitted to NICU with culture-proven fungal sepsis and those who had been administered an injection of vitamin K at birth were included in the study.

Exclusion criteria: Those neonates with birth asphyxia, vacuum delivery, Meconium Aspiration Syndrome (MAS), cases of outlet forceps delivery, known family history of bleeding diathesis, history of maternal anticoagulants or idiopathic thrombocytopenic purpura, not injected with vitamin K at birth, bacterial culture-positive neonates were excluded from the study.

Procedure

A total of 80 babies were included in the study. All neonates were subjected to Complete Blood Count (CBC), C-Reactive Protein (CRP), PT, aPTT, cranial ultrasound, and blood culture. The neonates were classified into three groups:

- Early preterm: <32 weeks gestational age
- Neonates: 32-36 weeks gestational age
- Term neonates: 37-40 weeks gestational age

STATISTICAL ANALYSIS

The data were analysed using the Statistical Package for the Social Sciences (SPSS) and MedCalc software. Chi-square analysis for descriptive data and Cox proportional hazard regression for survival and non survival neonates and Kaplan Meier-curve analysis were performed.

RESULTS

Out of total 80 neonates, nine neonates had ICH, 21 neonates had Intraventricular Haemorrhage (IVH) and 50 neonates had no haemorrhage. [Table/Fig-1] shows the association of ICH/IVH concerning different variables like the gestational score of the mother, the gestational weight of the baby, sex of the baby, birth weight, platelet count, coagulation profile, and blood culture. Lesser the gestational age, the more the incidence of ICH. However, the present study observed that even term babies were not exempt from such complications due to fungal sepsis.

Variables	Intracerebral haemorrhage (n, %)	Intraventricular haemorrhage (n, %)	No haemorrhage (n, %)	p-value
Gravida (maternal factor)				
Primigravida	1 (2.9)	10 (29.4)	23 (67.6)	>0.05
Multigravida	8 (17.4)	11 (23.9)	27 (58.7)	
Gestational age (weeks)				
<32	0	11 (61.1)	7 (38.9)	<0.001
32-36	6 (23.1)	10 (38.5)	10 (38.5)	
37-40	3 (8.3)	0	33 (91.7)	
Gender of the baby				
Female	6 (13.0)	12 (26.1)	28 (60.9)	>0.05
Male	3 (8.8)	9 (26.5)	22 (64.7)	
Birth weight (kg)				
<1	0	4 (100)	0	<0.001
1-1.49	1 (6.3)	7 (43.8)	8 (50)	
1.5-<2.5	1 (2.9)	10 (28.6)	24 (68.6)	
2.5 and above	7 (28.0)	0	18 (72)	
Platelet count (/µL)				
<150000	8 (10.8)	21 (28.4)	45 (60.8)	>0.05
≥150000	1 (16.7)	0	5 (83.3)	
Coagulation factor				
Abnormal (If PT>70 seconds and aPTT>35 seconds)	8 (15.7)	18 (35.3)	25 (49)	<0.05
Normal	1 (3.4)	3 (10.3)	25 (86.2)	
Blood culture				
Candida glabrata	1 (5.9)	3 (17.6)	13 (76.5)	

Candida krusei	3 (13.6)	6 (27.3)	13 (59)	>0.05
Candida parapsilosis	4 (21)	4 (21)	11 (57.9)	
Candida tropicalis	1 (7.1)	3 (21.4)	10 (71.4)	
Candida albicans	0	5 (62.5)	3 (37.5)	
Outcome				
Survived	0	4 (13.3)	26 (86.7)	<0.05
Died	9 (18)	17 (34)	24 (48)	

[Table/Fig-1]: Associated parameters with respect to haemorrhage. p<0.05 was considered as statistically significant

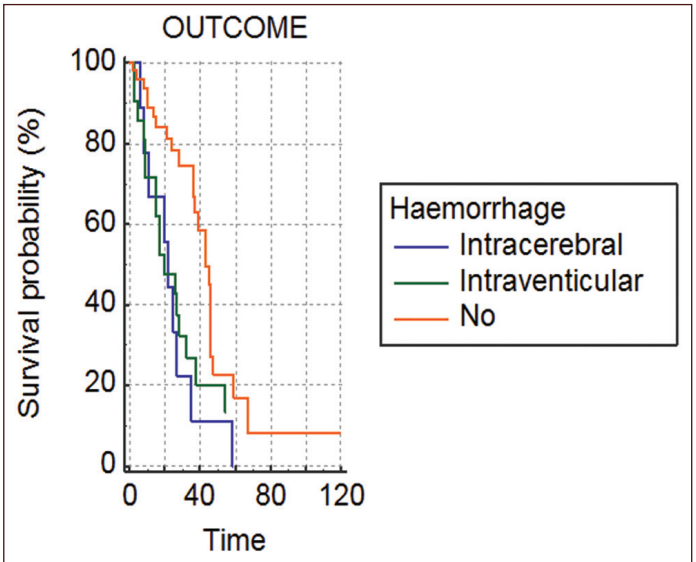
On analysis of the results of blood culture, non albicans candida, were associated with a higher incidence of ICH [Table/Fig-2]. *C. krusei* was the most common organism isolated, followed by *C. parapsilosis, C. glabrata and C. tropicalis*. *Candida albicans* was isolated in only eight babies. The mortality due to candida non albicans species is much more than that due to *C. albicans* fungaemia. A total of 50 babies died and only 30 survived. However, the most alarming fact is that 42% of term babies also succumbed to fungal sepsis. Of the 50 babies who died, 48 (96%) had abnormal PT and aPTT. This was found to be statistically significant (p<0.05).

Variables	Died (N=50) n (%)	Survied (N=30), n (%)	p-value
Gravida (maternal factor)			
Primigravida	20 (40)	14 (46.7)	>0.05
Multigravida	30 (60)	16 (53.3)	
Gestational age (weeks)			
<32	13 (26)	5 (16.7)	<0.001
32-36	16 (32)	10 (33.3)	
37-40	21 (42)	15 (50)	
Gender of the baby			
Female	30 (60)	16 (53.3)	>0.05
Male	20 (40)	14 (46.7)	
Birth weight (kg)			
<1	4 (8)	0	<0.001
1-1.49	9 (18)	7 (23.3)	
1.5-<2.5	19 (38)	16 (53.33)	
≥2.5	18 (36)	7 (23.3)	
Platelet count (/µL)			
<150000	48 (96)	26 (86.7)	>0.05
≥150000	2 (4)	4 (13.3)	
Coagulation factor			
Abnormal (If PT>70 second and aPTT>35 seconds)	48 (96)	3 (10)	<0.05
Normal	2 (4)	27 (90)	
Blood culture			
Candida glabrata (n=17)	10 (58.8)	7 (41.2)	>0.05
Candid krusei (n=22)	15 (68.2)	7 (31.8)	
Candida parapsilosis (n=19)	9 (47.4)	10 (52.6)	
Candida tropicalis (n=14)	8 (57.1)	6 (42.9)	
Candida albicans (n=8)	8 (100)	0	

[Table/Fig-2]: Associated parameters with respect to survival and death. PT: Prothrombin time; aPTT: Activated partial thromboplastin time; p <0.05 was considered as statistically significant

Blood culture revealed that there was a mortality of 10 (58.8%) in *C. glabrata* sepsis,15 (68.2%) in *C. krusei* sepsis, 9 (47.4%) in *C. parapsilosis* sepsis, 8 (57.1%) in *C. tropicalis* whereas,100% mortality occurred in those babies with *C. albicans* sepsis.

[Table/Fig-3,4] infer the difference between the median survival time of ICH/IVH and no haemorrhage. If there was a haemorrhage, the median survival time was lesser (p=0.004). The hazard ratio for PT and aPTT abnormal was 12.85 times more contributing to non survival than normal [Table/Fig-3]. Receiver Operating Characteristic (ROC) curve analysis (Area Under Curve=0.29) also confirmed the above two parameters were, good predictors for non survival of the neonates. The Cox regression analysis revealed that both PT and aPTT and IVH were the associated risk factors for non survival fungal sepsis in neonates [Table/Fig-5].



[Table/Fig-3]: Kaplan-Meier curve for outcome survival/Non survival with respect to intracerebral/intraventricular/no haemorrhage.

Factor	Number of events[a]		Number censored[b]		Total sample size
	N	%	N	%	
ICH	9	100.00	0	0.00	9
IVH	17	80.9	4	19	21
No haemorrhage	24	48	26	52	50
Overall	50	62.5	30	37.5	80

Mean and median survival

Factor	Mean	SE	95% CI for the mean	Median	95% CI for the median
ICH	23.556	5.341	13.086 to 34.025	22.000	6.000 to 35.000
IVH	25.054	3.990	17.234 to 32.873	20.000	9.000 to 32.000
No haemorrhage	45.090	5.740	33.840 to 56.340	43.000	36.000 to 46.000
Overall	36.761	3.998	28.925 to 44.596	35.000	26.000 to 43.000

Comparison of survival curves (Logrank test)	
Chi-square value	10.8559
Degrees of freedom	2
p-value	0.0044

[Table/Fig-4]: Mean and median survival curve.
[a]OUTCOME=1
[b]OUTCOME=0

Parameter	Findings
Survival time	Hospital stay days
Endpoint	Outcome
Method	Stepwise
Enter variable if p-value	0.05
Remove variable if p-value	0.1
Overall model fit	
Null model -2 Log Likelihood	344.598
Full model -2 Log Likelihood	313.599
Chi-square value	30.998
Degrees of freedom	2
p-value	0.0001

Coefficients and standard errors

Covariate	b	SE	Wald	p-value	Exp(b)	95% CI of Exp(b)
Abnormal PT and aPTT	2.5289	0.7234	12. 2216	0.0005	12.5392	3.0376 to 51.7627
Intrave-ntricular haemor-rhage (n=2)	0.6182	0.3125	3. 9132	0.0479	1.8555	1.0057 to 3.4235

ROC curve analysis	
Area under the ROC curve (AUC) (C-index)	0.929
Standard error	0.0357
95% Confidence interval	0.849 to 0.974

[Table/Fig-5]: Cox proportional-hazards regression model.

DISCUSSION

In the present study, 74 out of 80 babies had thrombocytopaenia (platelet count <1, 50,000). Thrombocytopaenia can be a specific marker of fungal sepsis. This result is similar to a study done by Farhana T et al., in which, 22 babies out of 30 (73.3%) had thrombocytopaenia [8].

Amongst the 29 neonates with thrombocytopaenia, eight babies (10.8%) had ICH while 21 babies (28.4%) had IVH. Only five babies with *C. albicans* growth had IVH. Of babies with fungal culture positive sepsis, 14.28% with *C. albicans* sepsis had IVH. A total of 85.7% of babies with non albican candida culture positive sepsis had ICH/IVH. This observation is in contrast to the study by Silva R et al., who reported that neonates with *C. albicans* Body Substance Isolation (BSI), 41.4% had IVH and that neonates with non albican candida blood stream infections 41.5% had IVH [10].

In the present study, *C. glabrata* was found in 21.25%. *C. krusei* was found in 27.5%. *C. paraspilosis* was found in 23.75%. *C. tropicalis* in 17.5%, and *C. albicans* in 10%. In other studies, for example, in a study by Yunus M et al., it was found that 63.8% had *C. krusei,* 26.5% *C. albicans* and 6% *C. tropicalis* [11]. In another study by Ballot DE et al., *C. parapsilosis* (54.2%) was isolated in the majority of cases, followed *C. albicans* (27.1%) [12].

In the present study, it was found that term babies had higher mortality when compared to late preterm and early preterm babies. According to the birth weight, 18 babies >2.5 kg succumbed, 19 babies in the 1.5-2.5 kg group died. Nine babies in the weight category of 1-1.49 kg and four babies in the <1 kg group died. Mortality was found to be higher in 1.5-2.5 kg group and >2.5 kg group while the earlier studies showed that mortality due to fungal sepsis was very high in the very preterm and VLBW babies also impaired neurodevelopmental outcome [13]. Hence, the present study was unique in its observation that term babies were also affected in addition to the late preterm babies and early preterm babies.

Limitation(s)

Small sample size was the major limitation of the study.

CONCLUSION(S)

Gestational age classification, birth weight classification, PT and aPTT parameters with respect to survivors/non survivors were statistically significant invariably. The babies with *C. albicans* sepsis succumbed to infection. By close anticipation and monitoring of the occurrence of haemorrhage among NICU fungal sepsis neonates, chances of survival can be increased. Lesser of the gestational age, greater the chances of ICH/IVH. A close monitoring of the coagulation profile PT, aPTT will help us to identify the babies, more prone for ICH/IVH. Expert cranial ultrasound will pick up the haemorrhage early. Timely treatment measures instituted will help in preventing mortality due to bleeding manifestations in fungal sepsis. Further studies with a large number of samples are recommended for generalising the outcome.

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