PREVALENCE, RISK FACTORS AND OUTCOMES OF NEONATAL INVASIVE FUNGAL INFECTION IN SOUTHERN THAILAND (1989-2017)

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Abstract: Prevalence, risk factors and outcomes of invasive fungal infection (IFI) in neonates were examined in a retrospective matched case-control study conducted in a tertiary hospital in southern Thailand from 1989 to 2017. Among inborn, incidence of IFI among total, very low birthweight (VLBW) and extremely low birthweight (ELBW) neonates was 0.4, 17.3 and 30.2 per 1,000 live births, respectively, while among neonatal intensive care unit admissions, incidence of IFI was 0.8, 2.0 and 3.4%, respectively. Applying a multivariate analysis, outborn neonates (adjusted odds ratio: adjusted OR) = 7.17, 95% confidence interval (CI): 2.56-20.05, *p*-value <0.001), history of cefoperazone plus sulbactam treatment (adjusted OR = 3.94, 95% CI: 1.22-12.72, *p*-value = 0.021), use of invasive mechanical ventilation (adjusted OR = 2.79, 95% CI: 1.36-5.73, *p*-value = 0.005), and total parenteral nutrition (adjusted OR = 6.58, 95% CI: 2.28-19.01, *p*-value < 0.001) are significant risk factors of IFI. Fungemia and *Candida albicans* was the most common source and species of IFI respectively, and mortality rate and daily cost are significantly higher in neonates with IFI (*p*-value <0.050). These findings will be useful to attending physicians in preventing neonates from contracting invasive fungal infection.

Keywords: *Candida albicans*, invasive fungal infection, neonatal sepsis, newborn, Thailand

INTRODUCTION

Invasive fungal infection (IFI) has a poor prognosis among neonates, children and adults as well as high mortality and morbidity globally (Kullberg and Arendrup, 2015; Thatrimontrichai *et al*, 2019b). Prevalence of IFI is variable and age-specific, particularly among low birthweight (LBW) and preterm infants (Fu *et al*, 2017; Cleminson *et al*, 2015; Austin *et al*, 2015). Among preterm neonates, presence of central vascular catheters, invasive mechanical ventilation, total parenteral nutrition, recent surgery, and administration of broad-spectrum antibiotic therapy constitute the major risk factors for IFI (Hsieh *et al*, 2012; Kullberg and Arendrup, 2015). Preterm and ill

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full-term neonates are more susceptible to sepsis from bacteria and fungi due to poor immune system and long term hospitalization. Oral and systemic antifungal prophylaxis can prevent IFI in very low birthweight (VLBW: BW <1,500 g) and extremely low birthweight (ELBW: BW <1,000 g) neonates in neonatal intensive care unit (NICU) (Cleminson *et al*, 2015; Austin *et al*, 2015).

However, there is very little information regarding risk factors, types of organisms and outcomes in neonatal IFI, especially in developing countries. Knowledge of local epidemiology and fungal ecology is essential and needed as a guide for appropriate antifungal prophylaxis. Here, we identified prevalence, risk factors and outcomes of IFI in neonates in southern Thailand during 1989 to 2017.

MATERIALS AND METHODS

Study site and subjects

The investigation, a retrospective matched case-control study, was conducted at the NICU of Songklanagarind Hospital, Songkhla, Thailand. NICU is a level IV, 15-bed single room in a university-affiliated teaching hospital at Prince of Songkla University, which is the referral center (for inborn and outborn neonates) covering 14 provinces of southern Thailand.

Control group was selected from a neonate population with culture-negative sepsis, matched by month and year with culture-positive cases admitted to NICU. Gender and gestational age were later matched. Matched cases with closest birthweights were selected. Medical records of the cases were retrieved from Songklanagarind Hospital clinical microbiological laboratory case files, which were reviewed to ensure all eligible subjects were identified. The medical records of all neonates with IFI admitted to NICU from 1 January 1989 to 31 December 2017 were included in the study. Exclusion criterion was incomplete or missing medical record data. Data collected of neonatal patients consisted of baseline characteristics, underlying diseases, clinical manifestations, antimicrobials used, and outcomes. Prevalence of IFI represented all cases during 1989-2017, and of VLBW and ELBW neonates during 2004-2017 as data from 1989 to 2004 were unreliable as regards numbers of VLBW and ELBW neonates.

The study was approved by the Ethics Committee Board of the Faculty of Medicine, Prince of Songkla University (REC. 59-269-01-1). As data were anonymous, prior written consent was not required.

Fungi isolation and identification

IFI is defined as a positive blood, cerebrospinal fluid, urine or peritoneal fluid culture with fungal infection. Blood cultures were performed using BACTEC Peds Plus/F culture vials (BD, Frankin Lakes, NJ). Gram-positive cultures were sub-cultured on chocolate, blood and MacConkey agar (BD, Frankin Lakes, NJ), respectively. Yeast pathogens were sub-cultured on Sabouraud dextrose agar (BD, Frankin Lakes, NJ). Germ tube test was carried out for a presumptive identification of *Candida albicans*. Yeast isolates were processed for identification up to species level by an assimilation test.

Evaluation criteria

Risk factors in case or control group were identified from birth until detection of positive culture (in case group) or negative culture (control group). A negative culture is defined as one that is negative for both fungus and bacteria. Previous antibiotic

exposure is defined as having received intravenous antibiotic treatment for at least 72 hours in the preceding 28 days before obtaining culture. Data also were gathered regarding previous surgery, invasive mechanical ventilation, total parenteral nutrition, and central line use within 7 days prior to or on the date of culture for both case and control groups.

Statistical analysis

An R program was used to develop a database of categorical and continuous variables (R Foundation for Statistical Computing, Vienna, Austria, 2019). Sample size was calculated based on a previous prevalence (1.4%) of neonatal IFI in China (Fu *et al*, 2017). Using a formula for an infinite population proportion (proportion = 0.014, error = 0.0028, and alpha = 0.05) (Fu *et al*, 2017), the sample size was estimated to be 6,764. Categorical variables are reported as frequency and percentage, and compared using χ^2 test or Fisher's exact test. Continuous (nonparametric) variables are presented as median and interquartile range (IQR) and compared using Mann-Whitney U-test. *p*-values were 2-tailed and a *p*-value <0.050 is considered statistically significant. Univariate and multivariate analyses were performed, and independent variables with *p*-value <0.200 in univariate analysis were subjected to a forward stepwise multiple logistic regression analysis. The model with lowest Akaike information criteria is judged as the most parsimonious model. Adjusted odds ratios (adjusted ORs) and 95% confidence intervals (CIs) were computed for variables independently associated with IFI versus control groups.

RESULTS

From 1989 to 2017, prevalence of IFI in inborn neonates and total NICU admissions was 0.4/1,000 live births and 0.8% respectively, and from 2004 to 2017, that of IFI inborn neonate, and total NICU admission of VLBW and ELBW neonates was 17.3/1,000 VLBW live births and 2.0% respectively and 30.2/1,000 ELBW live births and 3.4% respectively (Table 1). Fungemia and *Candida albicans* was the most common source and species of IFI, respectively (Table 1). Median duration prior to detection of neonatal IFI is not significantly different from hospitalization stay of case-matched controls.

Comparison of baseline characteristics and risk factors between IFI and case-matched control groups revealed highly significant differences (*p*-value <0.001) in outborn number, presence of congenital gastrointestinal tract pathology, prior treatment with cefoperazone plus sulbactam, and having within seven days prior to culture testing surgery, invasive mechanical ventilation and total parenteral nutrition (Table 2). Multivariate analysis with the lowest Akaike information criteria model confirmed (except for presence of congenital gastrointestinal tract pathology) outborn neonates, history of cefoperazone plus sulbactam use, invasive mechanical ventilation and total parenteral nutrition are significant risk factors of IFI compared to case-matched controls (Table 3). Types of fungal organisms (*C. albicans* = 46% and *C. parapsilosis* = 36%) during 1989-2004 survey are not significantly different from those (*C. albicans* = 58% and *C. parapsilosis* = 32%) during 2005-2017 survey.

As regards outcomes, mortality (overall and of VLBW) and daily cost during hospitalization of the IFI group are significantly higher than case-matched control group (Table 4).

Table 1
Numbers of inborns, total neonatal intensive care unit (NICU) admissions and invasive fungal infections (IFI) among very (VLBW) and extremely low birthweight (ELBW) neonates at Songklanagarind Hospital, Songkhla Province, Thailand (1 January 1989 - 31 December 2017).

Neonate	Number of inborn neonates	Number of NICU admissions
Total[a]	82,070	10,686
IFI[a]	34	83
VLBW[b]	867	1,145
VLBW with IFI[b]	15	23
ELBW[b]	265	356
ELBW with IFI[b]	8	12

Type of specimen	Number of inborn neonates			Number of NICU admissions		
	Total number[a]	Number from VLBW neonates[a]	Number from ELBW neonates[a]	Total number[a]	Number from VLBW neonates[a]	Number from ELBW neonates[a]
Blood	18	11	5	44	22	10
Cerebrospinal fluid	2	1	1	4	3	2
Urine	13	7	4	29	10	4
Peritoneal fluid	1	1	1	6	1	1

Type of fungus	Number of inborn neonates			Number of NICU admissions		
	Total number[a]	Number from VLBW neonates[a]	Number from ELBW neonates[a]	Total number[a]	Number from VLBW neonates[a]	Number from ELBW neonates[a]
Candida albicans	18	10	6	44	20	8
C. parapsilosis	13	9	5	28	15	9
C. tropicalis	2	1	0	8	1	0
C. krusei	0	0	0	1	0	0
Candida spp.	1	0	0	1	0	0
Trichosporon beigelii	0	0	0	1	0	0
Total	34	20	11	83	36	17

[a]Data from 1989 to 2017; [b]Data from 2004 to 2017; ELBW: <1.0 kg; VLBW: 1.0-1.499 kg.

Table 2
Characteristics and risk factors of invasive fungal infection (IFI) among neonates at Songklanagarind Hospital, Songkhla Province, Thailand (1 January 1989 - 31 December 2017).

Characteristics and risk factors	IFI Number (%) (n = 83)	Case-matched control Number (%) (n = 83)	p-value*
Birthweight (g) [median (interquartile range)]	1,855 (1,027-2,780)	1,805 (1,090-2,754)	0.924
Weight on the date of taking culture (g) (mean ± SD)	2,124 ± 986	2,288 ± 875	0.259
Birthweight compared with gestational age (GA)			0.316
Small for GA	11 (14)	18 (22)	
Appropriate for GA	71 (85)	64 (77)	
Large for GA	1 (1)	1 (1)	
Vaginal delivery	54 (65)	33 (40)	0.002
Outborn neonate	49 (59)	14 (17)	<0.001
Apgar score <8 at 5 minutes	24 (29)	19 (23)	0.479
Underlying disease before taking culture			
Neurologic sequelae, congenital or acquired	7 (8)	3 (4)	0.328
Respiratory disease	30 (36)	32 (39)	0.873
Cardiovascular disease	31 (37)	14 (17)	0.005
Congenital gastrointestinal tract pathology	29 (35)	9 (11)	<0.001
Congenital genitourinary tract pathology	6 (7)	1 (1)	0.117
Respiratory disease before taking culture [a]			
Respiratory distress syndrome	26 (31)	28 (34)	0.868
Meconium aspiration syndrome	2 (2)	4 (5)	0.682
Persistent pulmonary hypertension of the newborn	3 (3)	0 (0)	0.245
Previous antibiotic exposure	74 (89)	52 (63)	<0.001
Cefotaxime, ceftazidime	29 (35)	15 (18)	0.022
Cefoperazone plus sulbactam	20 (24)	2 (2)	<0.001
Carbapenem	27 (32)	12 (14)	0.010
Aminoglycoside	53 (64)	50 (60)	0.749
Previous surgery[b]	29 (35)	5 (6)	<0.001
Invasive mechanical ventilation[b]	66 (79)	27 (32)	<0.001
Total parenteral nutrition[b]	56 (67)	16 (19)	<0.001
Central line use[b]	58 (58)	27 (32)	0.002

*Significance at p <0.050; [a]One IFI case with a combination of meconium aspiration syndrome and persistent pulmonary hypertension of the newborn; [b]Within seven days prior to or on date of taking culture.

Table 3
Univariate and multivariate analyses of risk factors for invasive fungal infection (IFI) among neonates at Songklanagarind Hospital, Songkhla Province, Thailand (1 January 1989 - 31 December 2017).

Risk factor	IFI versus case-matched control group			
	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Vaginal delivery	2.82 (1.44-5.56)	0.002	1.82 (0.72-4.57)	0.201
Outborn neonate	7.10 (3.28-15.77)	<0.001	7.17 (2.56-20.05)	<0.001
Underlying disease before taking culture				
Cardiovascular disease	2.94 (1.35-6.58)	0.005	1.26 (0.45-3.50)	0.683
Congenital gastrointestinal tract pathology	4.42 (1.83-11.41)	<0.001	1.18 (0.35-4.01)	0.799
Congenital genitourinary tract pathology	6.39 (0.74-296.99)	0.120		
Previous antibiotic exposure				
Cefotaxime, ceftazidime	2.43 (1.13-5.39)	0.02	0.96 (0.45-2.03)	0.938
Cefoperazone plus sulbactam	12.86 (2.91-116.20)	<0.001	3.94 (1.22-12.72)	0.021
Carbapenem	2.85 (1.26-6.73)	0.01	0.91 (0.42-2.00)	0.830
Previous surgery	8.38 (2.92-29.14)	<0.001	2.42 (0.90-6.48)	0.080
Invasive mechanical ventilation	8.05 (3.78-17.38)	<0.001	2.79 (1.36-5.73)	0.005
Total parenteral nutrition	8.69 (4.04-18.97)	<0.001	6.58 (2.28-19.01)	<0.001
Central line use	2.84 (1.44-5.63)	0.002	0.57 (0.20-1.64)	0.295

Adjusted OR: adjusted odds ratio; CI: confidence interval; OR: odds ratio.

Table 4
Outcomes of neonates with invasive fungal infection (IFI) at Songklanagarind Hospital, Songkhla Province, Thailand (1 January 1989 - 31 December 2017).

Outcome	IFI Number (%)[b]	Case-matched control Number (%)[b]	*p*-value[a]
Mortality (*n* = 83)	26 (31)	9 (10.8)	0.002
very low birthweight (*n* = 36)	12 (33)	4 (11.1)	0.040
extremely low birthweight (*n* = 17)	5 (29)	4 (23.5)	1.000
Daily hospital cost (USD) [median (interquartile range)]	199 (158-305)	73 (50-113)	<0.001

[a]Significance at $p < 0.050$; [b]Otherwise indicated

DISCUSSION

An understanding of the risk factors of IFI is important to neonatologists and pediatricians when considering empirical antifungal therapy (Thatrimontrichai, 2014). In the present study, the major risk factors for IFI were being outborn neonates, history of cefoperazone plus sulbactam use, invasive mechanical ventilation, and total parenteral nutrition. The two latter risk factors as well as broad-spectrum antibiotic use have been observed in previous studies (Rowen *et al*, 1994; Cotten *et al*, 2006; Benjamin *et al*, 2010; Hsieh *et al*, 2012; Yu *et al*, 2013; Kullberg and Arendrup, 2015; Manzoni *et al*, 2015; Lovero *et al*, 2016). As Hospital is a referral center, outborn neonates are more ill than inborn neonates, and may experience prolonged intubation, parenteral nutrition, and broad-spectrum antibiotic therapy, thereby at greater risk of sepsis, antifungal therapy should be considered until culture tests show no IFI. In previous studies, gastrointestinal anomaly and surgery are risk factors (Shetty *et al*, 2005; Neu *et al*, 2009; Yu *et al*, 2013). However, in the present study these risk factors were not significant.

Prevalence of overall IFI (1.4%) was lower than that reported in China (Fu *et al*, 2017), IFI prevalence in VLBW and ELBW neonate groups given prophylactic systemic antifungal agents is 6.3 and 3.0%, respectively (Cleminson *et al*, 2015), while that in VLBW and ELBW groups receiving prophylactic oral/topical antifungal agents is 4.4 and 3.8% respectively (Austin *et al*, 2015). From the present study, prophylactic oral or systemic antifungal therapy are probably not needed as the prevalence of IFI in VLBW and ELBW neonates lower than the treatment groups reported in the literature. However, case fatality rate of VLBW neonates with IFI in the present study (13-18%) was higher than in previous reports (Austin *et al*, 2015; Cleminson *et al*, 2015). *C. albicans* infection is associated with a high mortality rate (Roilides *et al*, 2004) and this organism was predominant in the present study although *C. parapsilosis* was shown to be more predominant in previous studies (Roilides *et al*, 2004; Neu *et al*, 2009; Ballot *et al*, 2013; Lovero *et al*, 2016; Caggiano *et al*, 2017). Prevention of IFI should be early extubation, early enteral feeding and application of antibiotic stewardship programs in areas with presence of high multidrug resistant microorganisms (Thatrimontrichai *et al*, 2013; Thatrimontrichai *et al*, 2016; Thatrimontrichai, 2017; Thatrimontrichai *et al*, 2018; Thatrimontrichai *et al*, 2019a; Thatrimontrichai *et al*, 2019c; Thatrimontrichai *et al*, 2019d).

The study had several limitations. Firstly, we lacked data on patient-day, diagnostic tests (*eg* serum detection of β-d-glucan and *Candida* PCR assay), antifungal susceptibility due to inadequate antifungal therapy, and major extra daily cost. Secondly, as prevalence of neonatal IFI was very low, it was not possible to meet the calculated sample size. Thirdly, data were retrieved from a single center and may not represent conditions in other hospitals (*ie* external validity).

In conclusion, the survey covering a period of 29 years shows neonatal invasive fungal infection has a low prevalence but high mortality and daily cost. Outborn, broad-spectrum antibiotic use, invasive mechanical ventilation, and total parenteral nutrition were the risk factors for IFI, and *C. albicans* was the predominant causal organism. These findings should be of assistance in

developing preventive measures against invasive fungal infection of neonates in Thailand.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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