Candida Species Causing Neonatal Septicaemia
- Experience in a Tertiary Care Centre

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ABSTRACT

BACKGROUND

Candida species is one of the most common causes of bloodstream infections among neonates and accounts for 9-13% of such infections. Non-albicans Candida have emerged as important opportunistic pathogens, notably C. tropicalis, C. glabrata, C. parapsilosis and C. krusei. With the introduction of antifungal agents, the causes of Candida infections shifted from an almost complete dominance of Candida albicans to the common involvement of Candida glabrata and other non-albicans Candida species.

METHODS

This prospective, microbiological observational study was conducted in a tertiary care hospital for one and a half years from July 2016 to December 2017. Blood samples of neonates collected into Bactec Peds Plus/F Culture vials of an automated blood culture system (Bactec 9120, Becton Dickinson, USA) from clinically suspected cases of neonatal septicemia were subjected to culture. Detailed clinical history such as presence of respiratory distress, abdominal distension, lethargy, feed intolerance, failure to thrive, poor perfusion, history of convulsions, duration of NICU stay and antibiotic use was taken from the medical records. The Candida species isolated were identified using standard mycological techniques.

RESULTS

Out of the total 250 clinically suspected cases of neonatal septicemia, fungal growth was positive in 30 (12%) cases. Candida albicans accounted for 10% and non albicans Candida accounted for 90% of the fungal isolates. Candida glabrata (73.33%) was the commonest species followed by Candida tropicalis (16.67%) and Candida albicans (10%). Failure to thrive (60%), lethargy (50%) and respiratory distress (30%) were the most common clinical presentations seen, followed by feed intolerance (26.67%), abdominal distension (13.33%) and fever (10%). Among the risk factors observed for neonatal candidemia, low birth weight (76.67%) and prematurity (73.33%) were commonest followed by broad spectrum antibiotic use (66.67%), total parenteral nutrition (53.33%), ventilator support (36.67%) and indwelling catheters (26.67%).

CONCLUSIONS

Non-albicans Candida has emerged as an important pathogen causing neonatal septicemia. Fungal sepsis is more common among preterm, low birth weight infants, those with prolonged antibiotic use and those on total parenteral nutrition. Candida glabrata caused the highest number of cases of fungal septicemia.

KEY WORDS

Neonatal Candidemia, Non-Albicans Candida, Candida Glabrata
SIGNIFICANCE OF Candida Species in Neonatal Intensive Care Unit (NICU) is increasingly being recognized. Candida species are one of the most common causes of bloodstream infections among neonates and accounts for 9-13% of such infections. Historically Candida albicans has been the most frequently isolated species worldwide. Recently non-albicans Candida have emerged as important opportunistic pathogen, notably C. tropicalis, C. glabrata, C. parapsilosis and C. krusei. Candidemia is a significant cause of mortality and morbidity in neonates. Previous studies have suggested that possible risk factors such as very low birth weight (LBW), prematurity, prolonged antibiotic therapy, prolonged use of fat emulsions in total parenteral nutrition (TPN), use of artificial ventilation, presence of indwelling central venous catheterers (CVC), and intensive care unit (ICU) stay have made neonates prone to candidemia. Preterm, very low birth weight (VLBW): ≤1500 g; extremely LBW: ≤1000 g; and critically ill infants are at highest risk of invasive Candida infections. The clinical manifestations are respiratory insufficiency, apnoea, bradycardia, feeding intolerance, temperature instability and abdominal distension. Colonization of skin and gastrointestinal tract is the first step in the pathogenesis of invasive candidiasis. Delay in recognition of Candida infections and in the initiation of appropriate antifungal therapy often leads to significant morbidity and mortality rates among high-risk infants.

The genus Candida encompasses more than 150 species, only a few of which cause disease in humans. With rare exceptions, the human pathogens are Candida albicans, Candida guilliermondii, Candida krusei, Candida parapsilosis, Candida tropicalis, Candida kefyr, Candida lusitaniae, Candida dubliniensis, and Candida glabrata. These organisms are found on inanimate objects, in foods, and on animals, and are normal commensals of humans. They inhabit the gastrointestinal tract (including the mouth and oropharynx), the female genital tract, and the skin in humans with introduction of antifungal agents, the causes of Candida infections shifted from an almost complete dominance of Candida albicans to the common involvement of Candida glabrata and the other species listed above. The NAC species now account for approximately half of all cases of candidemia and hematogenously disseminated candidiasis. Recognition of this change is clinically important, since the various species differ in susceptibility to the newer antifungal agents. In developed countries, where medical therapeutics are commonly used, Candida species are now among the most common nosocomial pathogens. There are multiple risk factors that are responsible for neonatal candidemia like preterm such as low birth weight ≤1500 g and infection through vertical transmission from maternal flora or via horizontal transmission from hands of health care workers. The incidence and associated mortality due to candidemia can be influenced by several factors including characteristic of population at risk, standard of the health care facilities available, distribution of Candida species and prevalence of antifungal resistance. Systemic candidiasis in neonates is increasing in frequency especially since the survival of babies with low birth weight (LBW) has increased. Considering all these facts, the present study was conducted to find out the percentage of isolation of Candida species from clinically suspected cases of neonatal septicaemia; the underlying co-morbid conditions in culture proven cases of candidemia and identify Candida species causing septicaemia in neonates.

METHODS

The study was a cross-sectional microbiological observational study carried out for one and a half year duration from July 2016 to December 2017. The study was conducted after approval from the Institutional Ethics Committee. Informed consent was taken from the parent (of neonate) prior to the patient enrolment.

Blood samples of neonates collected into Bactec Peds plus/F culture vials of an automated blood culture system (Bectec 9120, Becton Dickinson, USA) from clinically suspected cases of neonatal septicemia was subjected to culture. Detailed clinical history such as presence of respiratory distress, abdominal distension, lethargy, feed intolerance, failure to thrive, poor perfusion, history of convulsions, duration of NICU stay and antibiotic use was taken from the medical records. When pathogenic bacteria gain access into the bloodstream, they may cause overwhelming infection without much localization (septicaemia). Candidemia was defined as the presence of at least one positive blood culture containing pure growth of Candida species with supportive clinical features.

Smears were prepared of the samples that flashed positive in the automated blood culture system. The positive samples were subcultured on 5% sheep blood agar plate and were kept for overnight incubation at 37°C. Candidal growth was confirmed by gram staining of the smears prepared from growth on blood agar plate. Any growth indicated was subcultured on Sabouraud dextrose agar with chloramphenicol (0.05%) and cycloheximide and incubated at 37°C and at room temperature. All the Candida isolates were subjected to germ tube test using normal human serum. Colonies were identified up to the species level on the basis of morphology on Corn meal agar (Dalmau method) and sugar assimilation pattern.

Statistical Analysis

For data analysis, frequency and percentages were calculated. The percentage of isolation of Candida species from the total number of clinically suspected cases of neonatal septicemia was calculated. The underlying co-morbid conditions in the culture proven cases of candidemia were enlisted and frequency table was formulated. Candida species causing neonatal septicemia were identified and frequency tables were formulated.

RESULTS

Out of the total 250 clinically suspected cases of neonatal septicemia, growth was seen in 40 (16%) of cases. Fungal growth was positive in 30 (12%) cases, bacterial growth was seen in 10 (4%) cases, 11 (4.4%) showed contamination and 199 (79.6%) did not show any growth. Candida albicans accounted for 10% and non albicans Candida accounted for...
90% of the fungal isolates. *Candida glabrata* (73.33%) was the commonest species followed by *Candida tropicalis* (16.67%) among the fungal isolates. The bacterial growth constituted of *Klebsiella pneumoniae* 4 (1.6%), methicillin resistant *Staphylococcus aureus* (MRSA) 3 (1.2%), *Enterococcus sp.* 1 (0.4%), *Streptococcus sp.* 1 (0.4%) and *Enterobacter sp.* 1 (0.4%). *Candida* sp. were isolated from 30/40 (75%) of positive cases while bacteria were isolated from 10/40 (25%) of positive cases. *Candida glabrata* (73.33%) was the commonest isolate followed by *Candida tropicalis* (16.67%) and *Candida albicans* (10%). The average gestational age was 32 weeks (30-39 weeks) and average birth weight was 1.77±0.615 kg (0.82 – 3 kg). Among the risk factors observed for neonatal candidemia, low birth weight (76.67%) and prematurity (73.33%) were commonest followed by broad spectrum antibiotic use (66.67%), total parenteral nutrition (53.3%), ventilator support (36.67%) and indwelling catheters (26.67%). Failure to thrive (60%), lethargy (50%) and respiratory distress (30%) were the most common clinical presentations seen, followed by feed intolerance (26.67%), abdominal distension (13.33%) and fever (10%).

**Distribution of Candida spp. in NICU**

- *Candida glabrata* 16.67%
- *Candida tropicalis* 73.33%
- *Candida albicans* 10%

**Potential Risk Factors Observed for Candidemia among Fungal Culture Positive Cases (n=30)**

- Low birth weight 66.67%
- Prematurity 36.67%
- Broad spectrum antibiotic use 26.67%
- Total parenteral nutrition 53.3%
- Ventilator support 36.67%
- Indwelling catheters 36.67%

**Various Clinical Presentations Observed in Cases of Neonatal Candidemia (n=30)**

- Failure to thrive 79.6%
- Respiratory distress 76.67%
- Abdominal distension 66.67%
- Lethargy 53.3%
- Feed intolerance 36.67%

**DISCUSSION**

The rate of isolation of *Candida* species from fungal culture positive isolates in our study is 12% which is comparable to the study done by Kumar et al.\(^1\) in 2011, in which the rate of isolation was 14.9%. Various studies have been carried out over the last decade which showed the rate of isolation of *Candida* species as 19.14% (Baradkar et al, 2008),\(^1\) 30.1% (Sardana et al, 2012),\(^3\) 20.39% (Srinivas Rao et al, 2014),\(^4\) 32.6% (Wadile et al, 2015).\(^6\)
Several studies have demonstrated the association of various risk factors with candidemia like low birth weight, preterm delivery, prolonged use of higher antibiotics like third generation cephalosporins and carbapenems, use of intravenous catheters, parenteral nutrition etc.15,16 These risk factors increase the susceptibility to infections because of the immaturity of the immune system and invasive medical equipment needed for improvement of the survival rate of the neonates. LBW, prematurity and use of broad spectrum antibiotics were the major risk factors implicated followed by total parenteral nutrition, ventilator support and indwelling catheters in the present study. This was in consistency with other studies done by Sardana et al, Juyal et al, Srinivas Rao et al and Wadile et al.13,6, 14,16

Failure to thrive (60%), lethargy (50%) and respiratory distress (30%) were the most common clinical presentations seen, followed by feed intolerance (26.67%), abdominal distension (13.33%) and fever (10%). Failure to thrive (18/30) and lethargy (15/30) were consistent clinical findings in our neonatal cases, similar to other studies.17,18,6,19 Respiratory distress (74.55%) followed by failure to thrive and lethargy were most common clinical findings in a study by Sardana et al whereas Juyal et al showed failure to thrive (74.42%) followed by abdominal distension and feed intolerance.13,16

_Candida_ species are an increasingly common cause of neonatal sepsis and are responsible for considerable morbidity and mortality.20,21,17 Modern day neonatal care has definitely improved the survival rate of neonates but also increased the use of multiple invasive medical equipment, which has further enhanced the acquired sepsis among the newborns. Over the last two decades, non-albicans _Candida_ (NAC) are accounting for a large burden of neonatal septicemia.18,22,13 Agarwal and co-authors reported that 76 out of the 90 isolates were NAC. Yadav et al also observed 88.46% of the isolates belonged to non albicans group.20,21 In our study, non-albicans _Candida_ bloodstream infections were commonest (90%). This finding is consistent with other studies where non-albicans _Candida_ spp. predominate in Asia, South Europe, South America and also in the subcontinent of India.17,24

_C. glabrata_ was the commonest species isolated in 73.33% cases of candidemia followed by _C. tropicalis_ (16.67%) and _C. albicans_ (10%) in the present study. Whereas in a study by Yadav et al,20 the commonest species isolated was _C. tropicalis_ (26.92%). The rate of isolation of _Candida glabrata_ (73.33%) in the present study is quite high in comparison to other studies done over the last ten years. According to previous studies the rate of isolation of _C. glabrata_ has been 61.22% (Baradkar et al, 2008),1 19.69% (Kumar et al, 2011),2 39% (Sardana et al, 2012),13 15.50% (Juyal et al, 2014),16 19.23% (Srinivas Rao et al, 2014),14 6.7% (Shrivastava et al, 2015),9 10% (Wadile et al, 2015),6 15.8% (Sil et al, 2017)25 and 33.3% (Pu et al, 2017).26 Non-albicans _Candida_ spp. are of special concern, due to their high virulence and low azole susceptibility characteristics, augmenting the high mortality rates. This rise is suggested to be a result of fluconazole prophylaxis given as a practice in many tertiary care centers.

In the present study, out of the total 250 clinically suspected cases of neonatal septicemia, fungal growth was positive in 12% cases and bacterial growth was seen in 4% cases. The clinical manifestations of neonatal sepsis are indistinguishable in bacterial and fungal septicemia. Thus it is important to identify the etiological agent in order to start appropriate treatment. 4.4% showed contamination which included micrococci and diphertheloids.

Probably infections due to _Candida_ species are endogenous. It has been studied that about 10% of babies in the NICU get colonized in the first week of life and 64% babies get colonized by 4 weeks of hospital stay. The gastrointestinal tract is the first to become colonized though multiple sites may be involved.1,27 There is some evidence showing correlation between fungal colonization and invasive disease in very low birth weight, premature babies.1,27,28 Microorganisms including _Candida_ spp. causing pneumonia acquired during labour and delivery may also act as source of candidemia.29 Various fungal agents colonize hospitalized infants, healthcare workers and visitors. Pathogenic organisms can be transmitted by direct contact or indirectly via contaminated instruments and intravenous fluids.30 This endogenous source, together with other predisposing factors as long-term antibiotics, catheterization, patients on ventilator, respiratory distress syndrome lead to candida septicemia in NICU.1

Historically, _Candida glabrata_ has been considered to be relatively nonpathogenic saprophyte of normal flora of healthy individuals rarely causing serious infections.30 However, following widespread and increased use of immunosuppressive therapy, broad spectrum antibiotic therapy, increased conditions causing compromise of the immune system, the frequency of mucosal as well as systemic infections caused by _Candida glabrata_ has increased significantly.1,13,32,33,30 It is the only species of _Candida_ that does not form pseudohyphae, it is found as blastoconidia (1-4 μm), both as commensal and as pathogenic states. On Sabouraud’s Dextrose agar, it forms glistening smooth, cream colored colonies, indistinguishable from _Candida albicans_ but on Cornmeal agar it does not form pseudohyphae. It assimilates only glucose and trehalose. Karen et al,29 compared _Candida glabrata_ sepsis with other. According to this significant study, _Candida glabrata_ sepsis occurred in infants with higher gestational periods (29.7 weeks as against 26.6 weeks in case of _Candida albicans_) and birth weight (Candida glabrata 1442 g, Candida albicans 931 g), but no such difference was observed in the present study.

The reasons behind the emergence of the species as predominant pathogen could be because of selection of lesser susceptible species due to frequent use of fluconazole as prophylaxis.4 The changing epidemiology of candidaemia, therefore, highlights the need for close monitoring of _Candida_ species distribution and susceptibility in order to optimise therapy and outcome. We should also develop guidelines for empiric therapy based on the epidemiology of India.34 Understanding the mechanisms of innate and acquired resistance may facilitate the development of new targets for antifungal agents. More and more comprehensive studies of its epidemiology, pathogenesis, and resistance are needed to control the infections by _Candida glabrata_ properly.1
CONCLUSIONS

In our study, among the two hundred and fifty (250) clinically suspected cases of neonatal septicemia, 12% were fungal culture positive. Amongst these, Candida glabrata (73.33%) was the commonest pathogen isolated, followed by Candida tropicalis (16.67%) and Candida albicans (10%). Non-albicans Candida has emerged as an important pathogen causing neonatal septicemia. Fungal sepsis is more common among preterm, low birth weight infants, those with prolonged antibiotic use and in those on total parenteral nutrition. Candida glabrata caused the highest number of cases of fungal septicemia.

REFERENCES


