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A STUDY ON SYSTEMATIC FUNGAL INFECTIONS IN NEONATES

Kumar Vimal*	PhD Scholar of Microbiology, NIET, Nims University, Rajasthan, Jaipur*Corresponding Author
Dr. Diksha Srivastava	Assistant Professor, Guide, NIET, Nims University, Jaipur
Dr. Sumangala Biswas	Assistant Professor of Microbiology & Co-guide, MGM Medical College, Dimna Road, Mango, Jamshedpur, Jharkhand
Dr. Shyam Lal Murmu	Associate Professor of Paediatrics, MGM Medical College Hospital Jamshedpur

ABSTRACT Fungal infections have emerged as an important cause of late-onset neonatal sepsis in the last two decades. Neonates represent a unique and highly vulnerable patient population. Advances in medical technology have improved the survival and quality of life of newborns, especially those who are extremely premature or with congenital defects. In addition, immunological immaturity and altered skin barrier play some role in the vulnerability of newborns to nosocomial infections. Our objective to determine the epidemiology and clinicopathological features of fungal sepsis in neonates, the organisms involved, clinical features, laboratory diagnosis, involved in the causation of fungal sepsis. Especially in the Indian population Epidemiological data on fungal sepsis in newborns are limited.

KEYWORDS : Fungal infections, Candida, Risk Factors, Laboratory Diagnosis,

Introduction:

Invasive fungal infections are a major problem in the neonatal intensive care unit (NICU). Candida species are as the most common cause of late-onset infections in the neonatal intensive care unit and are responsible for considerable morbidity and mortality [1].

The incidence of invasive Candida infection is inversely proportional to birth weight. The incidence rate in VLBW is 1 to 4 %, ELBW is 2 to 8 % and in incredibly low birth weight (< 750 gm) or gestation < 26 wks is 20.[2] The rate of systemic fungal infection as reported by DeNIS study group from India is 3.5% (only outborn cohort[3]. Among candida infection C. albicans is the most common species (50%) followed by C. parapsilosis (33%), C. glabrata, C. krusei, C. tropicalis and C. pseudotropicalis.

Despite improvement in survival rates of very low birth weight babies, nosocomial infection remains an important contributing factor to neonatal morbidity and mortality. The emergence of fungi as important pathogens is likely to be the result of changes in the neonatal intensive care environment and their interaction with the host.

Objective

To determine the epidemiology and clinicopathological features of fungal sepsis in neonates, the organisms involved, clinical features, laboratory diagnosis, involved in the causation of fungal sepsis.

Risk factors

The factors of fungal infections include prolonged use of antibacterial [> 5 days or 2 or more] and use of medical devices, among other conditions that lead to fungal disease. Complicated gastrointestinal disease, lack of enteral feedings, intralipid use >7 days, use of central venous catheter, endo-tracheal intubation and mechanical ventilation, hyperglycaemia, use of steroids/ H2 blockers. In addition, bio films are frequent on the surface of medical devices, being consider a negative event, since it characterizes greater pathogenicity and antifungal resistance of fungi. [4-5]

Pathogenesis

Candida species are yeast that frequently colonize skin, the gastrointestinal (GI) tract, and the female genitourinary tract.Infants admitted to the NICU are colonized by Candida rapidly after birth, with the GI and respiratory tracts being the most frequent sites during the first two weeks of life. Colonization during this age period may be related to the birthing process; infants delivered vaginally have higher rates of colonization than infants born by Caesarean section and the colonizing Candida species are identical to those isolated from the maternal genitourinary tract in the majority of cases. Colonization of infants >2 weeks of age frequently occurs on the skin and may be related to contact with maternal skin or the hands of health care providers.In particular, health care workers may be the primary source of Candida parapsilosis colonization in the NICU environment.[6-13] Colonization of infants by Candida species is not sufficient for the development of invasive candidiasis , although up to 5–10% of very low birth weight (VLBW; birth weight <1500 g) infants colonized by Candida develop invasive disease.Premature infants are predisposed to invasive candidiasis for several reasons. First, the typical barriers to invasion by Candida species are not fully developed in premature infants. The epidermis of the infant born at <30 weeks gestational age is thin and poorly formed compared with the skin of term infants. Moreover, immaturity of the barrier and immune functions of the GI tract predispose to translocation by Candida. Cellular immunity is also impaired; premature infants have fewer neutrophils and T lymphocytes than term infants, and both groups have altered neutrophil chemotaxis and phagocytosis compared with older children and adults. Finally, virulence factors of the colonizing yeast isolate also appear to be important in determining risk of progression to invasive disease. Bliss et al. observed enhanced virulence characteristics among more than half of Candida isolates from infants with invasive candidiasis. [14-21]

Once Candida species have invaded across mucosal surfaces or entered the bloodstream, they have a predilection for tissue invasion in the central nervous system, kidneys, liver, spleen, heart, and retina. Within the central nervous system, Candida can cause a meningoencephalitits, cerebral abscesses, and ventriculitis with obstructive hydrocephalus. Candida can also infiltrate with or without abscess formation in the liver, spleen, and (most commonly) the kidneys. Finally, endocarditis and endogenous endophthalmitis may result from seeding of the heart valves or eyes during fungemia.[22-24]

Neonatal Candida and other fungal infections

Around the 150 species of Candida, the majority of cases of invasive candidiasis among infants are caused by a relatively small number of species. Candida albicans is generally the most commonly isolated species, accounting for 45–55% of episodes of invasive candidiasis among infants.[25-26]

C. parapsilosis is the most frequent non-albicans Candida species (20–35%), followed by Candida tropicalis (1–6%).[26] Non-albicans species may be responsible for a growing proportion of neonatal candidiasis.[27] Candida krusei and Candida glabrata warrant special consideration given their inherent or potential resistance to

fluconazole.[27] However, these species still account for a relatively small proportion (<5%) of neonatal candidiasis, and no increase in disease caused by these species was observed in recent studies.

C. albicans is also the most pathogenic species of Candida. In a number of studies, mortality associated with invasive candidiasis caused by C. albicans was higher than for disease caused by C. parapsilosis.[30] Moreover, the mortality differences in several of these studies were substantial, the case fatality rates for invasive candidiasis caused by C. albicans and C. parapsilosis were 24% and 3%, respectively.[29]

The incidence of infections caused by other fungi in newborns is not significant, except for Malassezia species, which may occur in epidemic outbreaks [31-33]. Since the 1980s, this genus has been recognized in sepsis and systemic infections in neonates receiving lipidic parenteral nutrition using a central venous catheter. It is believed that lipid supplementation facilitates colonization of catheters that are used to infuse nutrients. Removal of the infected catheter is sufficient to limit infection in most cases.

Most cases of thrush occur in babies under the age of 12 months. In this population, this Malassezia infection rarely remains asymptomatic. Interstitial pneumonia and thrombocytopenia are common clinical manifestations in this group of patients, and the most frequent symptoms in systemic infections are fever and respiratory disease with or without apnea .[34-37]

Other less common symptoms include lethargy, malnutrition, bradycardia and hepatosplenomegaly. However, there are no visible signs of erythema, swelling or pus at the catheter entry site. Skin rash symptoms are also non-specific in children with systemic infection. Interstitial bronchopneumonia can be found in up to 40% of children. [34-37]

Fungal infection by Malassezia is diagnosed by isolating the microorganism from blood collected through a catheter or by culture after removal of the catheter tip. In suspected sepsis by Malassezia, the tip of the catheter should be cultured in a broth enriched with lipids. [34-37]

Standard clinical management for systemic infections by Malassezia is still not well defined, as fungemia by this microorganism is relatively uncommon. However, some authors recommend the use of Amphotericin B to treat these infections. Morrison and Weisdorf found that all patients enrolled in their study were cured without the administration of systemic antifungal therapy. Studies have indicated that the most important factors for therapeutic success against systemic infection are removal of infected catheters and interruption of lipid infusion with or without antifungal. [34-37]

Clinical Presentations

Clinical features range from localized skin infection in newborns to disseminated infection in extremely preterm neonates. The severity depends on factors such as gestation, birth weight, and invasive procedures. It usually presents after 2 weeks of age. The signs and symptoms are similar to those of bacteraemia. Usually in newborns there is a smouldering course. Common features include frequent apnoea, lethargy, GI symptoms (abdominal distension, bloody diarrhea, and gastric aspirates), respiratory distress, increased oxygen requirement, thrombocytopenia, hyperglycaemia, metabolic acidosis, hypotension, and elevated leukocyte count. Thrombocytopenia lacks specificity and sensitivity for diagnosing invasive candidates.

Various organ system involvements and their clinical presentation are Renal - UTI, renal abscess, CNS- Meningitis, ventriculitis, abscess, Gastrointestinal-Peritonitis, spontaneous intestinal perforation, Respiratory-Pneumonia and End organ dissemination like Eye-endophthalmitis, chorioretinitis, Heart-endocarditic and thrombi, Bones and joint-septic arthritis and osteomyelitis.

Laboratory Diagnosis

Culture of fungi from a normally sterile site (blood, urine, CSF, bone or joint, peritoneum, pleural space) is the diagnosis. In case of suspected catheter-related infection, cultures should be obtained from both peripheral venous blood and the indwelling catheter.

1. Blood Culture: Blood culture remains the gold standard . In 90% of the cultures, fungus develops within 72 hours. Monitor cultivation for 10 days to ensure growth of slow growing species. The sensitivity of blood culture varies from 28% to 78% in different studies.

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful technique for the rapid identification of bacteria and fungi by growth on solid media. MALDI-TOF MS uses mass spectrometry to identify bacterial and fungal species based on ribosomal protein patterns, often providing results in less than an hour. Several studies confirm that MALDI-TOF identifies Candida species from solid growth with 90–95% accuracy, effectively reducing the time required for species identification following blood culture positivity.

2. Urine: Urine should be collected by suprapubic aspiration/sterile catheterization for culture and microscopy. Visualize hyphae (true and pseudo) and budding yeast cells under microscopy.Candida UTI defined as $^{3}10^{4}$ CFU of candida species/mL urine.

3. Obtain culture from other sites depending on clinical presentations (CSF, peritoneal fluid etc).

4. Identification of species and susceptibility: Most of the species are susceptible to both fluconazole and amphotericin B except C. glabrata and C. krusei, which are resistant to fluconazole and C.lusitaniae, which is resistant to amphotericin B.

5. The following baseline tests should be done before starting treatment with amphotericin B- Hemoglobin, TLC, ANC, Urea and creatinine , Serum electrolytes, Bilirubin and liver enzymes.

6. End organ diffusion (EOD) screen should be performed in all confirmed bloodstream infections including eye examination for fungal conjunctivitis or retinitis, renal ultrasound for fungal balls, echocardiography and cranial ultrasound/CT/MRI.

7. Newer methods of diagnosis are molecular diagnostic assay using PNA FISH (peptide nucleic acid) yeast traffic light assay.

The Peptide Nucleic Acid Fluorescent In Situ Hybridization (PNA-FISH) Yeast Traffic Light Assay enables the rapid detection of Candida directly from liquid media, including positive blood cultures. This probe uses species-specific fluorescent probes and is able to identify most of the five isolates. Candida species within 90 minutes. When viewed under a fluorescent microscope, green fluorescence is seen in the presence of C. albicans or C. parapsilosis, yellow fluorescence with C. tropicalis, and red fluorescence in the presence of C. glabrata or C. krusei. . For each probe, the sensitivity and specificity are above 90%, and this probe typically identifies blood culture isolates more rapidly than MALDI-TOF because it does not require growth on solid media.

Polymerase chain reaction (PCR) for earlier detection of Candida species from positive blood cultures. Several PCR-based tests are commercially available that can identify yeast species from positive blood cultures with sensitivity and specificity >98%. Furthermore, like the PNA-FISH yeast traffic light assay, PCR does not require development on solid media, and the total time required for this technique is typically <4 h. PCR is also being evaluated for the direct detection of Candida species from whole blood, given its ability to detect small amounts of fungal DNA.

8. Fungal Antigen: There are many fungal antigens that can be found in the blood of patients with invasive candidiasis. These include mannan, a component of the outer cell wall of Candida species, and 1-3-β-D-glucan, which is found in the middle layers of the cell wall. The Platelia candida antigen plus assay is most often used to detect mannan antigen in the blood. Mannan is poorly expressed by C. parapsilosis and the mannan antigen is likely to reduce susceptibility to invasive candidiasis caused by this species.

Treatment planning of neonatal fungal infections

Amphotericin B deoxycholate is the recommended first-line drug for systemic candidiasis, including meningitis. Fluconazole is an option in neonates who are not on fluconazole prophylaxis. The lipid formulation amphotericin B is an alternative, but should be used with caution; it may not be effective in urinary tract infections. In meningitis with insufficient clinical response to amphotericin B therapy, the addition of flucytosine may be considered as rescue therapy. Micafungin is used in the treatment of fungal infections unresponsive or resistant to amphotericin B and fluconazole. There is no evidence to recommend empiric therapy in extremely preterm neonates. [38-39]

Central venous catheter care in neonates with blood stream infection
1. Administer a single dose of antifungal through the 'old' CVC (for diagnostic purposes - do a blood culture from the CVC)
2. Keep a Perimeter Line
3. Remove CVC and send tip for culture
4. Place a new CVC at a site other than the previous one at least 36-48 hours after CVC removal (ideally wait for 3 sterile.

Prevention:

Since colonization of the newborn with subsequent mucocutaneous candidiasis can lead to systemic Candida infection, efforts have been made to eradicate the organism from the skin and gastrointestinal tract of at-risk infants. Clotrimazole vaginal tablets and cream given to pregnant women with vaginal candidiasis have significantly reduced the number of newborns infected with the fungus. Breast feeding has no effect on the incidence of oral thrush and neither does chlorhexidine alcohol spray applied to the nipples of lactating women. The most promising prophylactic measure is probably the use of oral antifungal drugs, namely nystatin and miconazole oral gel, which have been shown to be effective in reducing the incidence of postnatally acquired gastrointestinal fungal colonization. Nevertheless, the success of oral antifungal prophylaxis has not translated into prevention of systemic Candida infection in newborns. As disseminated fungal infections are becoming more frequent in premature neonates, there should be strict control in the selection and use of antibiotics, judicious use of home lines and expedient mechanical ventilation. These should be coupled with strict implementation of infection control surveillance measures in the intensive care nursery and particularly careful hand washing.

Conclusions:

Systemic fungal infection, previously considered a rare complication, is now frequently diagnosed in VLBW infants receiving intensive care. Confirming the diagnosis by laboratory tests is difficult and requires a high index of suspicion. Prompt and aggressive use of antifungal treatment is justified in the clinically septic neonate, especially those with an increased serum concentration of C-reactive protein, who have not shown a satisfactory response to antibiotics. Liposomal amphotericin and other the new generation antifungal drugs appear to be safe, effective, and well-tolerated. With increased awareness, prompt treatment, and improved neonatal intensive care, the outcome of systemic fungal infections in preterm infants should improve.

Although neonatal candidiasis has decreased substantially over the past decade due to better identification of risk factors, this infection remains an obstacle to further reduction of morbidity and mortality associated with extreme prematurity. The diagnosis of invasive candidiasis relies on clinical suspicion and detection of candidemia. Several methods were recently developed that can reduce the length of time required for yeast identification from positive blood cultures. However, better diagnostics that can rapidly identify infants with invasive candidiasis and allow prompt antifungal therapy are still needed.

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