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***Candida albicans*-associated sepsis in a preterm neonatal rhesus macaque (*Macaca mulatta*)**

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Abstract

Invasive *Candida* infections (ICI) have been associated with neurodevelopmental impairment or death in human pre-term neonates. Candidiasis in nonhuman primates is seen mostly in immunosuppressed animals and ICI is not commonly reported. Here, we report a case of *Candida albicans*-associated ICI in a pre-term neonatal rhesus macaque.

Keywords

Candida albicans; fungal sepsis; nonhuman primate; continuous positive airway pressure

1 INTRODUCTION

It is common practice in the human neonatal intensive care unit to use continuous positive airway pressure (CPAP) in premature infants born at less than 37 gestational weeks exhibiting signs of respiratory distress syndrome. However, prematurity alone, even without signs of clinical respiratory disease, is a risk factor for decreased lung function and increased wheezing and asthma in the first few years of life.¹ Current studies are investigating the ability of prophylactic CPAP administration to improve lung growth and function in premature rhesus macaque (*Macaca mulatta*) infants without signs of respiratory distress. We report a case of *Candida albicans*-associated sepsis in a premature neonatal rhesus macaque undergoing CPAP administration.

2 SUBJECT AND METHODS

All animals were born and housed at the Oregon National Primate Research Center, an AAALAC International-accredited facility, and experimental methods approved by the Institutional Animal Care and Use Committee. Animal care was in accordance with the Public Health Services Policy on Humane Care and Use of Laboratory Animals.

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Declaration of conflicting interests

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As part of a CPAP protocol, a male rhesus macaque was delivered via cesarean section at gestational age (GA) 140; full term is 165 gestational days. The neonate was 387 g (normal range 394.6 ± 30.8 g). He was resuscitated following the guidelines of the Neonatal Resuscitation Program,² which includes establishing functional residual capacity with positive end expiratory pressures. Oxygen supplementation was administered as needed to maintain adequate oxygen saturation measured via pulse oximetry. A dual-lumen peripherally inserted central catheter (PICC) (Medtronic, Minneapolis, MN) was introduced through the right saphenous vein and advanced into the caudal vena cava. Intravenous (IV) fluid support (125 mL/kg/day) with Lactated Ringer's solution (Hospira, Inc., Lake Forest, IL) plus 10% dextrose was initiated on the day of delivery and transitioned to total parenteral nutrition at a rate of 125 mL/kg/day on postnatal day (PND) 1. Additionally, prophylactic cefepime (Qilu Pharmaceutical Co., LTD, Jinan China) was given on PND 0–2 (50 mg/kg every 12 hours intramuscularly). The neonate began daily sedation on PND 1 with IV ketamine (Henry Schein Brand pharmaceuticals, Dublin, OH) boluses (2.5–5.0 mg/kg as needed) and clonidine (Mylan Institutional LLC, Rockford, IL) (1.5 mcg/kg/hr constant rate infusion) to allow for 12 hours of CPAP administration via nasal prongs. GA-140 infants received either treatment with nasal CPAP or sham, beginning 24 hours post-delivery. Both groups received 5 liters per minute of humidified room air flow, through custom-made modified Hudson™ nasal prongs. Infants allocated to the CPAP arm of the study received a distending pressure of 5 cm of water via a bubble delivery system (Babi-Plus™ CPAP Jar, Fisher & Paykel, Irvine, CA). Infants allocated to the sham study arm received no distending pressure (0 cm water) through an identical apparatus. All injectable fluids and medications were free of benzyl alcohol preservative.

3 RESULTS

Early PND 3, the neonate exhibited tonic-clonic seizure activity lasting less than one minute with oxygen desaturation ($\text{SpO}_2 \sim 30\%$) and bradycardia (80–100 beats per minute). Flow by oxygen was administered and the neonate's vitals quickly recovered upon seizure cessation. A subsequent seizure occurred 35 minutes later and midazolam (Hospira, Inc.) (0.1 mg/kg IV) was administered. No further seizure activity was observed and the neonate was not sedated for CPAP administration that day. The morning of PND 4, the fluid pump began intermittently alarming for distal occlusion of both PICC lumen; fluid lines were flushed with saline and eventually replaced until both PICC lumens flushed with minimal manual pressure. Based on the increased manipulation of the PICC line, cefepime was restarted for two days. Sedated CPAP resumed PND 4–7 without complications. Upon recovery from sedation on PND 7, the animal had several tonic-clonic seizures that were initially managed with IV midazolam. However, seizures eventually becoming refractory to midazolam. Due to clinical decline, the neonate was submitted for euthanasia and post-mortem examination on PND 8.

Gross post-mortem examination revealed generalized edema of the body wall subcutis, periarticular connective tissues, epididymides, and serosal surfaces of the gastrointestinal tract. The brain was edematous with mild meningeal hemorrhage over the left caudal parietal and left rostral occipital lobes. The renal cortices were pale tan. Petechia were present within the lung parenchyma and gastric mucosa. There was mild ecchymosis of the left rectus

femoris muscle. There was mild hemorrhage within the skin and subcutis at the PICC site. The PICC line was intact and extended from the right saphenous vein through the femoral vein into the caudal vena cava. No blood clots or hemorrhages were noted along the PICC line or in the surrounding vasculature and connective tissues.

Microscopically, there was marked neutrophilic meningoencephalitis (Figure 1a) and rarefaction of the cerebral parenchyma. Blood vessels in the cerebrum, lung, and kidney exhibited severe necrotizing vasculitis (Figure 1b) with multifocal intravascular fibrin thrombi. Intravascular fibrin thrombi were also present in the heart, and hemorrhage was present in the adrenal cortex and urinary bladder mucosa. Renal collecting ducts were dilated and filled with neutrophils, necrotic debris, and fungal yeasts (Figure 1c). Periodic acid-Schiff (PAS) and Gomori's methenamine-silver (GMS) staining demonstrated large numbers of regularly-septate, 5 um-wide, non-parallel walled, fungal pseudohyphae with terminal buds, morphologically consistent with *Candida* spp (Figure 2a–c).

Complete blood cell count collected prior to euthanasia showed a normal white blood cell and platelet counts and a mild regenerative anemia. The serum chemistry panel was unremarkable. Anaerobic culture swabs collected at time of post-mortem examination yielded few colonies of yeast from the meninges and bone marrow, Bone marrow culture grew one additional colony of alpha-hemolytic *Streptococcus* spp. The cecal culture was negative. To determine the *Candida* species noted on histology, paraffin-embedded formalin fixed brain tissue was sent to the Washington Animal Disease Diagnostic Laboratory for PCR assay and was positive for *Candida albicans*.

4 DISCUSSION

Clinical history of seizures, together with post-mortem findings of multiorgan edema, hemorrhage, necrotizing vasculitis, fibrin thrombi, and intravascular fungal pseudohyphae, are consistent with disseminated vascular coagulopathy and sepsis due to fungal infection. Invasive *Candida* infections (ICI) have been associated with neurodevelopmental impairment or death in human pre-term neonates.^{3–5} Predisposing factors for ICI in human neonates include low birthweight, prematurity, male gender, presence of a central catheter, and antibiotic therapy.^{6–8} In nonhuman primates (NHP), candidiasis is the most frequent fungal infection reported, especially in immunosuppressed animals with either experimental retroviral infections or irradiation.⁹ Antibiotic therapy, history of recent importation, younger age, and concurrent infection with other non-viral pathogens, particularly *Mycobacterium* spp. or parasitic organisms, also increase the risk of candidiasis.^{9–11} While NHPs with candidiasis generally present with gross pseudomembrane formation along alimentary tract mucosa and microscopic intralesional fungal elements,⁹ ICI is much less commonly recognized with only rare cases described in the literature.^{9,12–13}

To the authors' knowledge, this is the first report of fungal sepsis in a neonatal rhesus macaque. As *Candida* spp. are common environmental contaminants, fungal sepsis should be considered a differential for neonatal seizures. Additionally, prophylactic therapy protocols in premature NHP neonates may wish to add an antifungal drug to the antibiotic regimen.

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Figure 1:
Cerebral neutrophilic encephalitis (A), pulmonary necrotizing vasculitis (B), and renal neutrophilic tubulitis with intralesional yeast (C) in a preterm rhesus macaque.

Figure 2:
Periodic acid-Schiff staining of brain (A), lung (B), and kidney (C) demonstrates large numbers of *Candida albicans* pseudophyphae.