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CASE STUDY

DIAGNOSTIC CHALLENGES IN A RARE CASE OF INVASIVE FUNGAL INFECTION

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ABSTRACT

IFIs are 'presence of fungal elements either as molds or yeast in deep tissue biopsy or needle aspirates that is confirmed on culture and histopathological examination. Invasive fungal infections (IFI) have significantly increased due to advances in medical care in the 'at risk' immune compromised population. Diagnosis of IFIs is extremely challenging, because current diagnostic methods are not sufficiently sensitive or specific and results are often available too late to be clinically useful. Here we report a case of Invasive Mucormycosis, successfully managed using multidisciplinary approach. Three months old male child, presented with persisting fever unresponsive to ususal line of management. Child was severely malnourished with bilateral pneumonia with right sided hemiparesis and convulsions. Baby had excoriated skin lesions around nasal alae, shrunken left eye, large palatal perforation, oral thrush, nasal crusting. MRI brain showed large left parietal lobe abscess. Ophthalmological examination revealed left eye exposure keratitis with optic atrophy and old central retinal artery occlusion (CRAO). Child was given antifungal in the form of Liposomal Amphotericin B in addition to Antibiotics, IV fluids and Anticonvulsants. Brain abscess was drained and palatal perforation was debrided. Pus culture from nasal tissue grew aseptate filamentous fungal elements of Mucor. Lymphocyte subset assay suggested subnormal CD 19, CD3/CD4 and NK cell levels. Child responded well to Liposomal amphotericin B. On discharge child was vitally stable, had gained weight, healed skin lesions with no respiratory complaints. Was advised physiotherapy for residual paralysis and plastic surgery for residual palatal perforation. Malnutrition in the child, altered immunological status of the child, relatively nonspecific, multi systemic symptoms and relatively limited sensitivity and specificity of diganostic tests proved challenging in the management of this case.

INTRODUCTION

Invasive Fungal Infections (IFIs) are defined by invasive fungal infections cooperative group (IFICG) as 'presence of fungal elements either as molds or yeast in deep tissue biopsy or needle aspirates that is confirmed on culture and histopathological examination'(Ascioglu *et al*., 2002). Invasive fungal infections (IFI) have significantly increased due to advances in medical care in the 'at risk' immunocompromised population. Fungal species are widely distributed in soil, plant debris and other organic substrates, and make up approximately 7 per cent (611,000 species) of all eukaryotic species on earth, although only about 600 species are human pathogens. From being uncommon during the earlier part of the 20th century when the world was plagued with bacterial epidemics, fungi have evolved as a major global health problem. Major risk factors for IFI include neutropenia <500 neutrophils/ml for more than 10 days, haematological malignancies, bone marrow transplantation, prolonged (>4 wk) treatment with corticosteroids; prolonged (>7 days) stays in intensive care, chemotherapy, HIV infection, invasive medical procedures and the newer immune suppressive agents. Other risk factors are malnutrition, solid organ transplantation, severe burns or prolonged stays in intensive care (>21 days), systemic corticosteroids for >7 days and major surgery. There are also reports of the presence of infection in immunocompetent patients without signs or symptoms of conditions associated with immunocompromised status (Rüping *et al.,* 2008).

Diagnosis of IFIs is extremely challenging, because current diagnostic methods are not sufficiently sensitive or specific and results are often available too late to be clinically useful. Newer diagnostic markers and techniques are available but there are demerits like non-availability, non- affordability and less familiarity about them among clinicians.

Case presentation

Three months old male child 2nd by order of birth, born of 3rd degree consanguineous marriage presented with persisting fever responding to neither first line antibiotics nor to the higher, broad spectrum antibiotics, bilateral pneumonia with right sided hemiparesis and convulsions. On examination baby was severely malnourished, drowsy, pale, febrile, tachycardiac, tachypneaic, pulses were feeble and blood pressure was low. Baby had excoriated skin lesions around nasal alae, shrunken left eye, large palatal perforation, oral thrush, nasal crustingand bilateral conductive sounds on chest auscultation with right sided hemiparesis and ipsilateral exaggerated reflexes with left sided facial palsy. Before being referred to us, child had received several intravenous antibiotics and blood transfusion twice. Nasal swab culture showed growth of pseudomonas sensitive to Cefotaxim, Ceftriaxone, Ciprofloxacin, Ceftazi dimeand blood culture sensitivity revealed Staph aureus sensitive to Gentamicin, Tetracycline Erythromycin, Linezolid. MRI brain showed large left parietal lobe abscess, 8*4*5 cms causing midline shift with few adjacent sub-centimetric

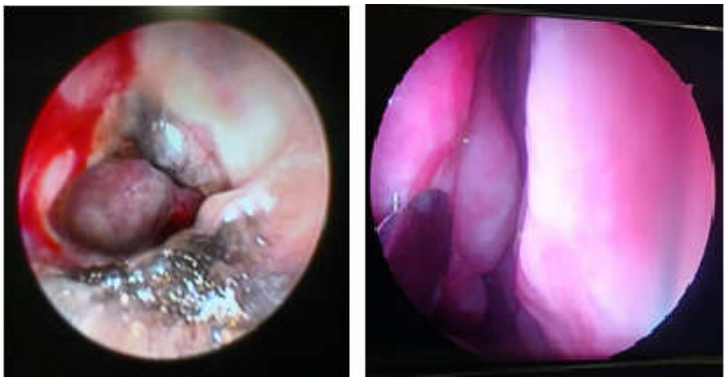

Figure 1. Endoscopic (Left) and preoperative (Right) images of fungal crusts of palatal perforation

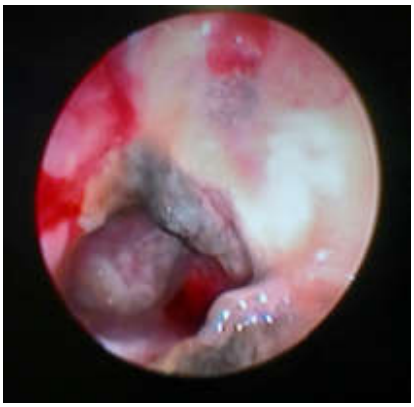

Figure 2. Post operative image of perforation after removal of fungal crusts

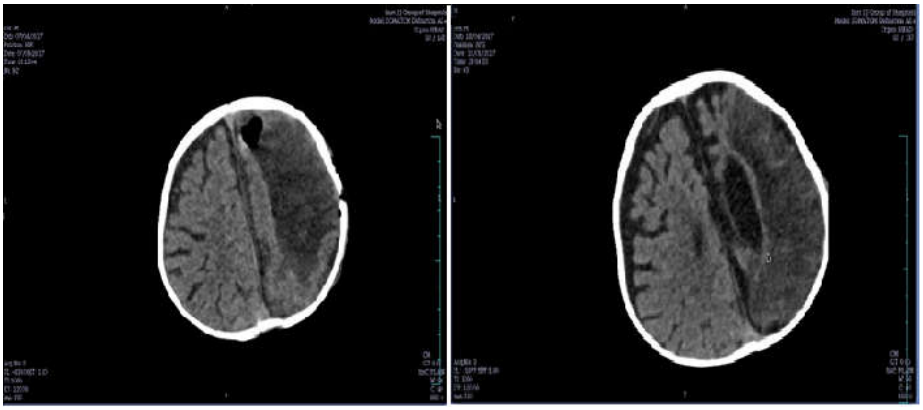

Figure 3. Pre operative (Left) and Post operative (Right) computer assisted tomography image of child showing large abscess and few nodular lesions

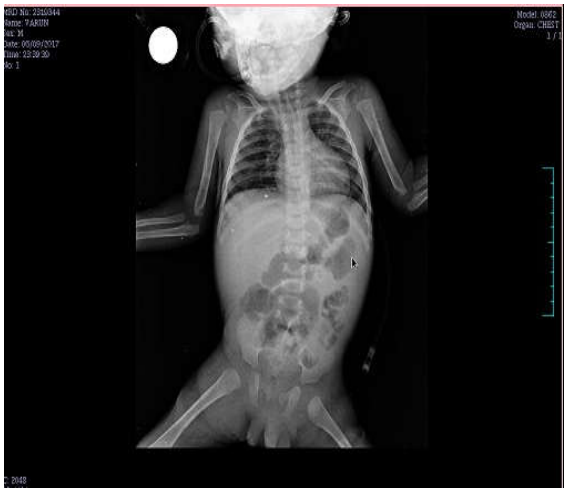

Figure 4. Radiograph of baby presented with chronic LRTI

enhancing nodules, also in posterior fossa. Child was given IV fluids, oxygen by nasal prongs, Intravenous Injection (Inj.) Vancomycin, Intravenous Piperacillin-Tazobactum, Inj. Metronidazole, Inj. Liposomal Amphotericin B with inotropic support and Inj. Levetiracetam to control convulsions. Child was anemic, Complete blood count test showed hemoglobin-6.5 gm%, Total leucocyte count- 7300/cu.mm (N- 56% ; L-34%), platelet count- 2.67 L/cu.mm, blood culture grew no organisms. Both child and mother were seronegative for HIV. IgM Dengue was negative and platelets were adequate and liver function test, prothrombin time-INR, renal function test were normal. Cerebrospinal fluid (CSF) analysis revealed no nucleated cells, CSF-protein 45 mg/dl, CSF-sugar 30 mg/dl. Burr hole tapping of brain abscess was done. Pus KOH mounting s/o fungal elements. Pusculture and Blood culture were negative. Debridement of necrotic palatal perforation was done. Biopsy of the sample revealed chronic osteomyelitis of palate. KOH mounting and special staining techniques revealed no fungal elements. Pus culture revealed Proteus vulgaris sensitive to Ceftazidime. Pus culture from nasal tissue grewaseptate filamentous fungal elements of Mucor. Biopsy of nasal crusting revealed infected keratinous cyst. Ophthal mological examination revealed left eye exposure keratitis with optic atrophy and old central retinal artery occlusion (CRAO). Child also developed left Pseudomonas culture positive Acute SuppurativeOtitis Media (ASOM) and was treated accordingly. Further lymphocyte subset assay and immunoglobulin assay were done to rule out immunod efeciency. Lymphocyte subset assay suggested subnormal CD 19, CD3/CD4 and NK cell levels. Nitroblue Tetrazolium Test (NBT) and Dihydrorhodamine (DHR) were done and results were normal. Immunoglobulin assay was inconclusive. Genetic test for SCID and JAK-STAT mutations were advised to rule out genetic disorders of immune regulation.Child responded well to Liposomal amphotericin B. On discharge child was vitally stable, had gained weight, healed skin lesions with no respiratory complaints with residual right sided hemiparesis for which physiotherapy was advised and for residual palatal perforation plastic surgery was advised.

DISCUSSION

Fungi are saprophytic microorganisms which have evolved mechanisms to survive in the mammalian hosts. Most of the fungal infections have been accidental and systemic fungal infections are a rarity that may result in high mortality. Infection can be transmitted by the inhalation of spores (Aspergillosis, Cryptococcosis, Histoplasmosis), percutaneous inoculation in cutaneous and subcutaneous infections (Dermatophytosis, Madura Foot), penetration into the mucosa by commensal organisms such as **Candida albicans**, and the ingestion of a toxin in contaminated food or drink (gastrointestinal disease). Infections may be mild and only superficial or cutaneous (e.g**.** dermatophytosis and **Tineaversicolor**) or may cause life-threatening, systemic illness (e.g**.** candidiasis, aspergillosis and mucormycosis (Ostrosky-Zeichner *et al*., 2012). Among the fungi that have potential to cause IFI's include Yeasts (*Candidaspp*, *Cryptococcusspp*) and moulds (*Aspergillusspp*, *Fusariumspp*, *Scedosporiumprolificans*, *Mucor*, *Rhizopus* and *Rhizomucor Absidia*). IFIs are also caused by dimorphic fungi incuding *Histoplasmacapsulatum*, *Coccidioides Immitis*, *Blastomyces dermatitidis*, *Paracoccidioidesspp*, *Sporothrixspp* and *Penicilliummarneffii*). Among these fungi *Candidaspp*, *Cryptococcusspp*, *Aspergillusspp*, *Mucor* and *Rhizopus* are either saprophytes in soil and environment or can be present as commensally in human as well as animals. In systemic fungal infections the outcome of the disease depends more on the host factors rather than the fungal virulence. Immune response to fungal infections is a complex subject where in fungi invading goes unrecognised by the immune system and that invasive fungal infections can result in severe inflammatory reactions resulting in morbidity and mortality. This could be attributed to the immunosuppression either because of infections (HIV), malignancies, metabolic disorders like diabetes, inadequate treatment of superficial fungal infections, transplant patients, immunological defeciency and genetic predisposition. Laboratory methods for the diagnosis of IFIs include the conventional mycological techniques, biochemical (finding fungal products), Immunological and molecular methods (Ramana *et al*., 2013). Wet microscopy of clinical samples using different concentrations of potassium hydroxide (KOH) reveal fungal elements. Gram's stain, Giemsa stain, India ink and fluorescent staining with calcofluor white increase the chances of finding fungal elements by microscopy. Immunohistochemistry and immunofluorescence increase the sensitivity and specificity of microscopy. Sabouraud's Dextrose Agar (SDA). Chromogenic media, Czapec-Dox media, Corn Meal Agar (CMA), Muller Hinton agar (MHA) and RPMI 1640 are common culture media used for growth and identification. Antibodies (Specific Anti-*Candida*, Anti-*Aspergillus*) and Antigen of fungi in general (mannan, galactomannan) and specific fungal antigens (Cryptococcal Ag) are useful immunological methods for diagnosing IFIs.

Detection of fungal metabolites including enolase, arabinitol, creatinine and β-(1-3) D-glucan helps in species and genus specific diagnosis. PCR (conventional, nested-PCR, real-time PCR), Microarray, Nucleic acid sequence-based amplification (NASBA) and pyrosequensing are the molecular methods available for typing and confirmation of strains. Rapid method Matrix Assisted Laser Desorption/Ionization (MALDI-TOF) for detection of common fungi responsible for IFIs has been reported recently. There are many serological methods for the diagnosis of fungal infections, and the results of these tests become available sooner than culture. It should be noted that antibody assays are often negative in immunosuppressed patients. The detection of fungal cell wall markers in serum has been reported for galactomannan (GM), (1, 3)-beta-D-glucan (BDG) and mannan. Galactomannan is relatively specific for **Aspergillus** species, and can be detected in urine, bronchoalveolar lavage fluid cerebrospinal fluid and other specimens with enzyme immunoassay. Various sensitivity rates from 30 to 100 per cent, and similarly wide-ranging specificities from 38 to 98 per cent have been reported for GM. Factors that limit the specificity of this test are immune reactivity with other fungi such as **Penicillium** spp. and **Paecilomyces** spp., false positive results with antibacterial agents such as beta-lactam antibiotics, particularly piperacillin–tazobactam and amoxycillin with or without clavulanate, and dietary GM in pasta, cereals and milk. 1,3-Beta-D-glucan (BDG) is present in the cell wall of most pathogenic fungi, including **Fusarium**, **Candida**, **Aspergillus** and **Trichosporon**, and is not species-specific or genus-specific for each organism. However, **Mucorales**, **C. neoformans** and **Blastomycesdermatitidis** contain relatively small amounts of cell wall BDG; therefore, these assays may not be completely reliable in patients infected with these organisms. Sensitivities of 55 to 100 per cent, specificities of

71 to 93 per cent. False-positive BDG findings occur in the patients with fungal colonization or mucositis who have received empirical antifungal therapy. The combined use of a BDG assay (Glucatell) and a GM enzyme immunoassay (Platelia **Aspergillus**) improves the specificity of diagnosis. Mannan is mainly found as a characteristic cell wall component in yeasts. The detection of circulating **Candida** mannan and anti-mannan antibodies has been used as a diagnostic marker for invasive candidiasis or candidemia caused by the most pathogenic species of **Candida** in adult patients with neutropenia and after myeloablative chemotherapy. The overall sensitivity of mannan antigen detection in patients with candidemia has been reported to be between 69 and 90.9 per cent, and specificity between 89 and 46.2 per cent compared to culture as the gold standard. In neonates, this test has yielded promising results particularly for ruling out candidiasis, considering its high negative predictive value of 98 per cent. The polymerase chain reaction (PCR) assay may serve as a powerful non-culture method for the diagnosis of systemic fungal infection in high-risk patients. Qualitative methods are sensitive in detecting fungal DNA in human blood samples, tissues, bronchoalveolar lavage and other body fluids. Molecular methods, which are rapid and can yield results within 6 h, have revolutionized the diagnosis of fungal infections because these enable diagnosis during the incubation period and early stage of infection, and prior to bone marrow transplantation. The diagnosis of infection based on molecular and serologic techniques can provide powerful tools for the early diagnosis of IFI. Because the fungi are common in the environment and opportunistic fungi in immunocompromised patients can cause high morbidity and mortality, the interpretation of positive or negative results with different laboratory methods is difficult for clinicians, so more than one method should be used for early diagnosis. Delay in the identification of a fungal infection and specific species often enables the infections to progress to a point where subsequent antifungal therapy is ineffective or the patient dies before an appropriate regimen can be given.

Conclusion

Diagnosis of fungal infections is challenging. Clinical symptoms of IFIs are often non-specific and therefore are of limited use by themselves to make an early diagnosis. Histopathological identification of fungal pathogens and fungal culture are mainstay of diagnosis. Although microscopic diagnosis provides rapid detection and generic diagnosis of fungal infection, sensitivity and specificity are limited. Culture based techniques are typically required for identification of genus and particular pathogen but not always cultures grow the fungus. Patient populations at highest risk for IFIs are also those at high risk for complications associated with invasive biopsies, limiting the utility of histopathology. Additional surrogate markers of fungal infections are needed in medical mycology to further improve diagnosis and treatment and hence outcomes, for patients with IFIs. As standard diagnostic techniques are lacking in a number of respects and there is need for other surrogates with better sensitivity and specificity that can enable early diagnosis in a practical way that is easily accessible to medical laboratories and clinicians.

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