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Case Report

A CASE OF CO-INFECTION OF *ASPERGILLUS, MUCOR* & *RHIZOPUS* IN RENAL TRANSPLANT PATIENT FROM CENTRAL INDIA

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ABSTRACT
A case of invasive sinusitis due to co-infection of aspergillus niger, mucor and rhizopus species in renal transplant patient has been described. Treatment was complicated by renal failure and amphotericin B therapies. Later on he was successfully managed with both amphotericin B and voriconazole.

Keywords: *Aspergillus Niger, Mucor Spp., Rhizopus Spp., Renal Transplantation, Pansinusitis*

INTRODUCTION
Organ transplantation is the most effective means of rehabilitating patients with organ failure of diverse etiologies. Antimicrobial therapy in transplant patients requires more prolonged therapy than in other patient populations because of the increased microbial burden and the continuing need for immunosuppression. As a result, toxicity is usually greater and antimicrobial resistance may be selected. The range of organisms to be considered is not the usual bacteria and viruses, but more unusual pathogens like fungi. The effect of the newly introduced pathogens is particularly threatening in transplant patients and other immune-suppressed hosts (Rubin, 2002). Invasive fungal infections are a significant and often a lethal problem in transplant patients. The high rates of mortality and graft loss owing to fungal infections render early diagnosis and treatment imperative in immuno-suppressed patients (Badiee *et al.,* 2011). Invasive fungal infections (IFI's) occur in upto 20% of recipients of renal transplantation and remain as diagnostic and therapeutic challenge (Hamdi *et al.,* 2014).

CASES
A 32-year-old male farmer was admitted to our super-speciality hospital with the complaints of pain and swelling in his left cheek, 30 days following renal transplantation from his 55-year-old live mother as a donor. Prior to transplantation, he was suffering from a chronic kidney disease and was on dialysis since last one year.

The initial immuno-suppression consisted of tacrolimus (5mg/day) and prednisolone (20 mg/day). The post operative course was uncomplicated. The ureteral stent was removed on post operative day tenth. A kidney biopsy showed no evidence of immediate, acute rejection. A patient was discharged 21 days after transplantation on co-trimoxazole (400mg: 80mg/day), levofloxacin (500 mg/day) and doripenem (500mg/day). His plasma creatinine was stable at 2.04 mg/dl at the time of discharge.

At the time of current admission, the patient suffered from a graft rejection. His temperature was 97.6^{o}F, blood pressure was 140/80 mm Hg and pulse was 100/min. He was awake and oriented. His laboratory testing results included a White Blood Cell (WBC) count of 11,100/cu mm (93% neutrophils and 4% lymphocytes), hemoglobin level of 8.4 gm%, mean cell volume of 93.6 fl and platelet count of 2.43 lacs/cu mm. Biochemistry results included a normal chemistry panel with the exception of blood urea nitrogen level of 53 mg/dl and a creatinine level of 1.63 mg/dl.

Liver function tests were normal.

The centrifuged urine contained 8-10 Red Blood Cells (RBC) and 10-12 pus cells/hpf. The electrocardiogram was within normal limits. Blood, urine and sputum cultures were negative. Other biochemical and hematological parameters were within normal limits. His pain and swelling in the cheeks increased continuously with enormous nasal discharge. Total WBC count rose to 16,300/cu mm and creatinine value fluctuated between 1.63 and 3.74.

Case Report

The sinus x-ray and computed tomography scan revealed the condition of pansinusitis. Nasal endoscopy followed by surgical debridement yielded black colored eschar from the affected site. The material was sent to the Microbiology laboratory for microscopy and culture. Simultaneously, his renal pathology revealed severe acute tissue injury in the form of inflammatory arterial necrosis and acute tubular necrosis indicating graft rejection. He was suspected as a case of antibody mediated graft rejection simultaneously involving invasive fungal pan sinusitis. Clinicians strongly suspected it as the case of mucoromycosis. Treatment was instituted with liposomal Ampotericin B (1 mg/Kg/d). Microbiological investigations revealed the co-infection of *Aspergillus niger, Mucor* and *Rhizopus* species. He was successfully managed by both 200 mg/day amphotericin B and 200 mg/day voriconazole (Badiee *et al.*, 2011; Hamdi *et al.*, 2014; Dunn *et al.*, 2003; Maranes *et al.*, 1996).

DISCUSSION

Fungal infections following solid organ transplantation remain a major cause of morbidity and mortality (Maranes *et al.*, 1996). Invasive fungal infections are most often associated with intravascular invasion or infection of the paranasal sinuses, orbits and brain (Badiee *et al.*, 2011). In our case, the patient got infection in paranasal sinuses.

Currently, there are four diagnostic categories of fungal infections affecting the paranasal sinuses: Acute or fulminant invasive fungal sinusitis, Chronic or indolent fungal sinusitis, Fungus ball (mycetoma) and Allergic fungal sinusitis.

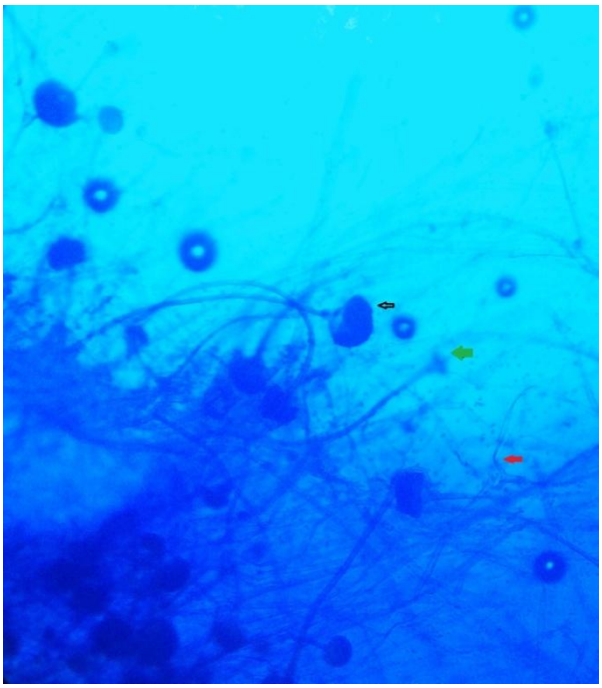

Figure 1: LCB mount (10x) showing conidiophores of *Aspergillus niger,* sporangiophore of *Mucor* spp. and rhizoids of *Rhizopus*

Each type of condition has a distinct presentation and therapy. Patients at highest risk for acute invasive fungal sinusitis includes poorly controlled diabetes, hematologic disorders, Acquired Immuno-deficiency syndrome (AIDS), transplantation, immunosuppressive states following chemotherapy or those with condition that predisposes to metabolic acidosis such as chronic renal failure or diarrhea (Blitzer *et al.*, 1980). Our case was of a patient who was immunosuppressed following transplantation and therefore he was susceptible to opportunistic infections. The unusual pathogens like fungi which are known to participate in these opportunistic infections are not only the traditional organisms such as Candida but

also opportunistic species like *Aspergillus*, *Cryptococcus* and newly emerging fungi like *Fusarium*, *Scendosporium* and *zygomycetes* (Rubin, 2002; Badiee *et al.*, 2011; Maranes *et al.*, 1996). The frequency of invasive aspergillosis in renal transplant varies between 0.4 and 2.4% and those with mucoromycosis are known to be 2% with high case fatality rate of up to 88% (Badiee *et al.*, 2011; Hamdi *et al.*, 2014; Singh, 2003). The offending fungi in our case were *Aspergillus* and members of the class zygomycetes that included *Mucor* and *Rhizopus* species (Rubin, 2002). Direct identification of the etiologic agent by culture or histopathology is the gold standard. In our case, the identification done through direct microscopic examination of nasal discharge in potassium hydroxide (KOH) wet mount revealed characteristic broad, ribbon-like, non-septate, non-pigmented hyphae with wide-angle or right angle branching at irregular intervals. Culture on Sabauraud's Dextrose Agar (SDA) with chloramphenicol and without cycloheximide revealed greyish-white floccose, dense and wooly colonies having hairy appearance within 72 hours of incubation and turning dark brown to black after 10-12 days of incubation at both 25^{o}C and 37^{o}C. Reverse of the slants were creamish white to yellow in colour. Lactophenol Cotton Blue (LCB) preparations of the growth confirmed the presence of non septate hyphae that were irregularly branched at right angles.

The presence of sporangiophores and sporangium containing sporangiospores was also appreciated. Few vegetative, non-septate hyphae also revealed the presence of rhizoids arising beneath the regions where sporangiophores were rising. These findings confirmed the presence of the members of Zygomycetes i.e *Mucor* species and *Rhizopus* species respectively. Apart from these, several septate, dichotomously branched hyphae were seen. Presence of conidiophores ending into biseriate phialides covering the entire vesicle was also appreciated, thereby confirming the presence of *Aspergillus niger*. Exactly, the identical LCB mounts were revealed by the SDA slants incubated at both the temperatures (Chander, 1995) (Figure 1). These saprophytic fungi reproduce and grow in soil, decaying food, grains and plants.

The disease process is usually aggressive. After becoming airborne, their spores settle onto the mucosa of the susceptible host, penetrate into the tissue and causes angio-invasion (Blitzer, 1980). Our patient probably would have acquired the infection during his post operative hospital stay but the signs and symptoms of infection became obvious only after he was discharged. He could only stay at his home for 7 days and had to return back to our hospital with the signs of both pansinusitis and graft rejection. Kidney transplant patients usually exhibit relatively fewer clinical manifestations of infection and few or no findings on conventional radiotherapy.

Laboratory test results reveal nothing abnormal except for elevated creatinine levels, which are characteristic of both a state of chronic rejection and opportunistic infection. In our case also, patient only showed abnormal creatinine levels with no other obvious signs of rejection or infection during his post operative hospital stay.

Most fungal infections occur in the first six months after transplant because of the excessive use of immunosuppressors (Badiee *et al.*, 2011). In our case, graft rejection must be antibody mediated and the invasive fungal infection was due to his immunosuppressive state. Also, rejection occurred within 1 month following transplantation.

The accelerated cause of rejection must be the doses of amphotericin B that was started immediately following his admission with the signs of pansinusitis.

To conclude, fungal infections in renal transplant patients should be managed crucially. Antifungal therapy should be initiated early in suspected patients and should be administered with an appropriate adjustment of immunosuppressive regimen.

Fungal infections pose difficult diagnostic and therapeutic challenges. High rates of mortality and graft loss owing to fungal infection require early diagnosis and treatment to ensure the likelihood of survival in immunosuppressed patients. Treatment with amphotericin B remains the gold standard of systemic antifungal therapy but application of triazole drugs shouldn't be ignored in cases of co-infection. Broad spectrum triazole antifungal agents that show good activity against clinically important yeasts and molds including *Aspergillus* and Mucorales should be preferred in case of co-infection. Therefore, proper management of drug therapy should be ensured in cases of graft rejection.

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