Rhino-orbital Zygomycosis Secondary to Rhizopus Oryzae in a Renal Transplant Recipient Successfully Treated with Liposomal Amphotericin B

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Rhino-orbital zygomycosis is usually an aggressive, fulminant and, at times, fatal disease that requires urgent medical and surgical treatment. We report a case of rhino-orbital zygomycosis caused by Rhizopus oryzae that developed in a 41-year-old male renal transplant recipient. He was diagnosed in the early post transplant period after anti-rejection therapy. The infection was successfully managed with liposomal amphotericin B and functional endoscopic sinus surgery. *(Chang Gung Med J 2008;31:407-11)*

Key words: renal transplant, zygomycosis, Rhizopus oryzae, liposomal amphotericin B

Zygomycosis is a devastating fungal infection, recognized recently as an increasing clinical disease entity that can be a fatal infection even in hosts with greater immunocompetency. Zygomycosis develops most frequently in immunocompromised patients of hematological malignancies, diabetic ketoacidosis and solid organ transplant recipients.(1) Renal transplant recipients may have zygomycosis in a variety of locations, such as pulmonary, gastrointestinal and cutaneous, and rhino-cerebral area.

Understanding the spectrum of zygomycosis of the paranasal system is complicated by the fact that many published cases either do not include detailed histopathological findings, or cultural results. Fungal infections represent a serious complication after organ transplantation. Early diagnosis and aggressive treatment are crucial. In the present paper, we describe in detail a case of rhino-orbital zygomycosis in a renal transplant recipient successfully treated with liposomal amphotericin B. This case stresses early clinical suspicion and microbiological diagnosis in planning treatment.

CASE REPORT

A 41-year-old Qatari male patient had type 2 diabetes mellitus, hypertension, end stage renal failure (ESRF) with status post renal transplant (living-unrelated donor in June 2005 with 3/6 HLA match), and hyperlipidemia. Initial immunosuppressive therapy consisted of tacrolimus 1 mg per os twice daily and prednisolone 10 mg per os once daily. The patient was admitted through Accident and Emergency with deterioration in his kidney function. On examination, the patient appeared well-built, with temperature 36.8°C, blood pressure 150/85 mmHg, pulse 87 beats/min, no abnormalities detected in chest and cardiovascular system, and abdomen transplanted kidney in situ. Serum biochemistry revealed a urea of 20 mmol/l (normal range 1.7-8.3 mmol/l), creatinine 213 mmol/l (normal range 53-124 umol/l), white blood count 8.4 (4-11) and blood

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sugar 8.4 mmol/l. He was found to have an obstruction of the transplanted kidney and a double j stent was inserted. He improved, and the level of urea and creatinine dropped to 9 mmol/l and 125 mmol/l, which was similar to his post-renal transplant baseline for urea and creatinine. During hospitalization (after one week) the patient developed a fever for which a septic work up was undertaken to look for a focus and he was prescribed meropenem. However, he deteriorated rapidly and had septic shock. After eight days of treatment in the medical ward, the patient began to exhibit recurrent blackish nasal discharge, facial edema and periorbital swelling. Later, he developed a bilateral proptosis, which was more remarkable in the left eye, with edematous, congested conjunctiva and ophthalmoplegia. However, the eye fundus and vision were normal. Preliminary diagnosis of orbital cellulitis was made and urgent computed tomography (CT) was requested. CT showed opacification of ethmoidal and maxillary sinuses. Soft tissue infiltration of the medial aspect of the left orbit was seen displacing the globe laterally and outwards (Figs. 1 and 2). No clear collection or bony erosion was noticed, and the brain was intact. The patient was transferred to the intensive care unit (ICU) for further management. Examination by an otolaryngologist revealed the presence of black necrotic tissue (eschar) in the left and right nasal cavities. A preliminary nasal swab culture revealed the growth of *Candida tropicalis*, a zygomycete, and negative bacterial culture. Based on the mycological and radiological findings, diagnosis was highly suggestive of an invasive fungal infection. Within 24 hours, the patient underwent urgent functional endoscopic sinus surgery with endoscopic debridement of the eschar, and tissues were removed from the maxillary, ethmoidal and sphenoidal sinus bilaterally, as well as orbital decompression to remove the lamina paprecia septal cartilage. The removed material was sent for fungal culture and histopathological study. Both studies confirmed the diagnosis of a fungal infection consistent with zygomycosis. The next day, intravenous liposomal amphotericin B at 5 mg/kg/day was started along with saline nasal irrigation (total received dose 13.2 g). Within ten days, the patient's orbital cellulitis and proptosis had almost resolved. Treatment was continued for six weeks. At six months following the last antifungal treatment, the patient exhibited no evidence of further infection, with marked clinical and mycological improvements, and no adverse events.

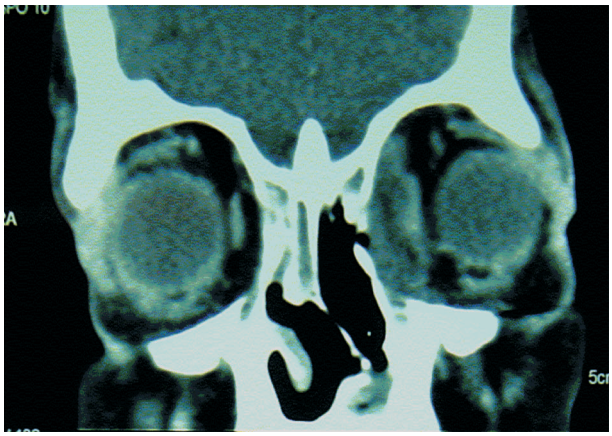

Fig. 1 Computed tomography coronal scan of orbits and ethmoid sinuses showing total opacification of the sinuses with a localized hypodense lesion in the left orbit.

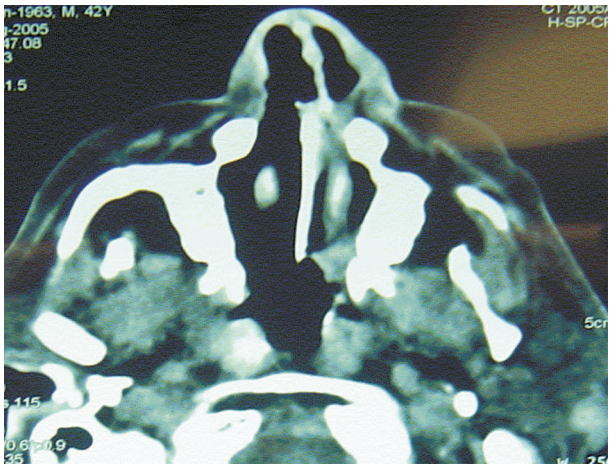

Fig. 2 Computed tomography axial scan of ethmoid sinuses showing a partial opacification of the left ethmoid sinus.

Fungal culturing

The necrotic tissue specimens obtained from the maxillary sinuses by endoscopic surgery were examined for evidence of fungal invasion. The tissue was cut into small pieces in sterilized saline and then ground for 30 seconds in a sealed plastic bag placed in a Stomacher(r) Lab Blender (model No. 80). A drop of the homogenate containing the small pieces of tissue was viewed in 30% potassium hydroxide solution and visualized under light microscopy at a magnification of 400. Branched, nonseptate, broad

hyphae were evident in this preparation. The remainder of the homogenate was cultured onto two sets of three media: Sabouraud dextrose agar (SDA) plus 40 U/ml of streptomycin and 20 U/ml of penicillin (solubilizing and dispersing agent + solvent porcine penicillin) (SDA + SP), SDA lacking antibiotics, and brain-heart infusion plus 40 U/ml of streptomycin and 20 U/ml of penicillin. One set of plates was incubated at room temperature and the other at 37°C. Fungal colonies appeared within 48 h. Examination of the fungus revealed a pure growth of *Rhizopus oryzae* Went and Prinsen Geerlings (the species most frequently recovered in zygomycosis). Identification of the fungal isolate was based on the colonial morphology and microscopic picture. The fungus was further subcultured on SDA + SP slants, stored in screw-capped tubes and preserved in cryotube beads (Mast Diagnostics). Professor J Guarro, Mycology unit, Rovira i Virgili University, Reus, Spain, confirmed the identification of the fungus. All investigations were performed under safety conditions (class II) in order to minimize contamination by airborne organisms.

Histopathology

Tissue sections of the specimen were stained for fungal elements and other histopathological changes. In addition to the routine hematoxylin and eosin (H&E) staining, the tissue sections were stained with periodic acid-Schiff (PAS) and Grocott-Gomori methenamine-silver stain (GMS) to visualize the tissue and fungal etiology. A careful microscopic examination of the debrided nasal tissue after GMS staining demonstrated widespread tissue necrosis that was heavily infiltrated by broad, ribbon-like, nonseptate or barely septate hyphae, which were haphazardly branched or branched at right angles. Histology of the tissue also revealed that the fungal hyphae had invaded the blood vessels, a characteristic feature of zygomycosis (Fig. 3).

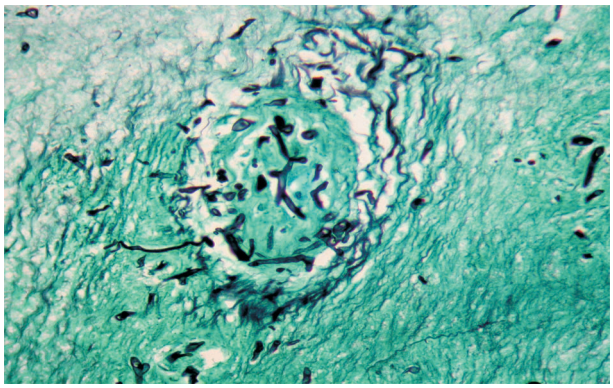

Fig. 3 Cut section of a blood vessel showing the black stained hyphae with angioinvasion (Grocott-Gomori methenamine-silver stain, original magnification x400).

DISCUSSION

Zygomycosis can manifest as one of six different clinical syndromes: it appears in rhinocerebral, pulmonary, gastrointestinal, central nervous system, subcutaneous and disseminated forms. Rhinocerebral zygomycosis (RCZ) is the most common of the six forms and it is subdivided into three subtypes: rhino-maxillary, rhino-orbital and rhino-orbitocerebral. The classification of RCZ has no effect on patient care, however, because the mainstays of therapy are similar regardless of the site of extension. The keys to management are reversal of the underlying cause of immunocompromise, be it diabetic ketoacidosis or neutropenia, and appropriate antifungal therapy and surgical debridement of the involved tissues. The disease is caused by a group of fungi belonging to the class zygomycetes. The fungi are ubiquitous, and often present in soil and decaying organic material. Despite their widespread distribution, disease manifestations are generally restricted to severely immunocompromised patients.[1,2] The most common infections caused by zygomycetes include those caused by members of the genera *Rhizopus, Mucor, Cunninghamella, Apophysomyces* and *Absidia,* with *R. oryzae* being the species most frequently isolated from patients and the sinus being the most common site of infection in the studied cases of zygomycosis.[1] The infection originates in the paranasal sinuses following inspiration of fungal spores and may evolve rapidly extending to neighboring tissue. The manifestation of the disease may reflect the sequential involvement of the nose, sinuses, eyes and brain. Symptoms may include nasal congestion, occasionally dark blood-tinged rhino rhea or epistaxis, sinus tenderness, retro-orbital headache, fever and malaise. Symptoms may progress to include facial or periorbital swelling and proptosis.

In solid organ transplant recipients, the sinus is the most frequently involved site. In normal hosts, a phagocytic response to colonization prevents infec-

tion. In immunocompromised hosts, on the other hand, the response is suboptimal and germination ensues.[3]

When the clinical picture includes the presence of sinusitis with black discoloration in the nose in addition to a predisposing factor, a diagnosis of RCZ should be highly suspected. Even so, a tissue biopsy is necessary to confirm the diagnosis.[4] Invasive hyphae can be seen as ribbon-like, 10 to 20 μ wide, haphazardly branched organisms with little or no septation. The fungus can be seen on H&E, PAS and GMS staining, and it is easily differentiated from *Aspergillus*, which has a thinner wall and characteristic regular septation.[5] In our patient, PAS and GMS staining readily demonstrated the fungus.

Blood vessel invasion (angioinvasion) with thrombosis is a peculiar feature of RCZ, and contributes to its necrotic, ischemic appearance. Histologically, angioinvasion was demonstrated in the present case (Fig. 3).

Ischemia favors the development of acidotic tissue, which is ideal for fungal growth. The infection spreads rapidly to adjacent sinuses and the orbit, and continues into the cranium via the ethmoid bone or orbital vessels. In our patient, the orbital manifestation occurred at almost the same time as did the nasal symptoms, yet the patient did not experience intracranial spread, hence, 'rhino-orbital' zygomycosis was the most appropriate terminology. Radiographic findings are helpful in assessing the different stages of the disease rather than making a definitive diagnosis because the radiographic features may be indistinguishable from those of simple rhinosinusitis. In fact, during the early stages of RCZ, imaging features may even be normal: it is only late in the progression of the disease that bony erosion appears.[6] In our patient, various degrees of mucosal thickening within the nose and sinuses were noticeable, and the appearance of orbital extension was similar to that seen in bacterial orbital cellulitis, yet the bony framework was intact.

Several risk factors might have favored this fulminant, invasive, fungal rhinosinusitis in our patient. Uremia implies alterations in the immune system, with granulocyte dysfunction and depressed cell mediated immunity.[7] Normal human serum inhibits the growth of *Rhizopus* spp.,[8] while the sera of uremic patients decrease the inhibitory effects of macrophages on spore germination.[9] Anti-rejection therapies with high doses of corticosteroids exert an additional important risk factor for opportunistic pathogens in transplant patients.[10]

The treatment of RCZ involves a combination of surgical and medical modalities, as survival increased to 70% for patients treated with a combination of surgery and antifungal therapy compared to 57% for those treated with surgery alone,[1] plus correction of the underlying medical problem if possible. The timing of surgery is very crucial; surgery should be instituted without delay once the condition is diagnosed. In the patient in the present study, endoscopic debridement was performed within 24 h of disease diagnosis. The standard medical therapy for RCZ is amphotericin B at a dose of 1.0 to 1.5 mg/kg/day for a period of several weeks to several months, depending on the clinical response and the degree of the drug's side effects, especially nephrotoxicity.[6] Less toxic forms of amphotericin B, such as liposomal amphotericin B, colloidal dispersion amphotericin B and amphotericin B lipid complex, can be used.[11]

In our patient, early debridement of the eschar followed by intravenous liposomal amphotericin B within 24 h appeared to be very effective in controlling RCZ. Amphotericin B remains the standard therapy for zygomycosis. New antifungal agents, such as voriconazole and caspofungin, are not effective against zygomycosis.[12] Furthermore, breakthrough zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole has been reported.[13] Posaconazole, a new triazole antifungal, has been used successfully in a number of cases.[14] The present case, and the other reported cases that have occurred in our hospital,[15] emphasize the fact that zygomycosis is an increasingly reported life-threatening disease. The underlying principles of therapy for this disease remain rapid diagnosis, urgent aggressive surgical debridement, rapid amphotericin B therapy and, if possible, reversal of underlying predisposition.

REFERENCES

1. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, Sein M, Sein T, Chiou CC, Chu JH, Kontoyiannis DP, Walsh TJ. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis 2005;41:634-53.

2. Wang JL, Hsiao CH, Chang SC, Hsueh PR, Chen YC. Diagnostic challenge of zygomycosis in compromised hosts. Med Mycol 2006;44:19-24.
3. Yohai RA, Bullock JD, Aziz AA, Markert RJ. Survival factors in rhino-orbital-cerebral mucormycosis. Surv Ophthalmol 1994;39:3-22.
4. Akoz T, Civelek B, Akan M. Rhinocerebral mucormycosis: report of two cases. Ann Plast Surg 1999;43:309-12.
5. Schell WA. Histopathology of fungal rhinosinusitis. Otolaryngol Clin North Am 2000;33:251-76.
6. Ferguson BJ. Mucormycosis of the nose and paranasal sinuses. Otolaryngol Clin North Am 2000;3:349-65.
7. Goldblum SE, Reed WP. Host defenses and immunologic alterations associated with chronic hemodialysis. Ann Intern Med 1980;93:597-613.
8. Chinn RY, Diamond RD. Generation of chemotactic factors by *Rhizopus oryzae* in the presence and absence of serum: relationship to hyphal damage mediated by human neutrophils and effects of hyperglycemia and ketoacidosis. Infect Immun 1982;38:1123-9.
9. Jorens PG, Boelaert JR, Halloy V, Zamora R, Schneider YJ, Herman AG. Human and rat macrophages mediate fungistatic activity against *Rhizopus* species differently: in vitro and ex vivo studies. Infect Immun 1995;63:4489-94.
10. Paya CV. Fungal infections in solid-organ transplantation. Clin Infect Dis 1993;16:677-88.
11. Strasser MD, Kennedy RJ, Adam RD. Rhinocerebral mucormycosis. Therapy with amphotericin B lipid complex. Arch Intern Med 1996;156:337-9.
12. Dannaoui E, Meletiadis J, Mouton JW, Meis JF, Verweij PE; Eurofung Network. In vitro susceptibilities of zygomycetes to conventional and new antifungals. J Antimicrob Chemother 2003;51:45-52.
13. Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. N Engl J Med 2004;350:951-2.
14. Brugiere O, Dauriat G, Mal H, Marrash-Chalha R, Fournier M, Groussard O, Besnard M, Leseche G, Dupont B. Pulmonary mucormycosis (zygomycosis) in a lung transplant recipient: recovery after posaconazole therapy. Transplantation 2005;80:1361-2.
15. Hilal AA, Taj-Aldeen SJ, Mirghani AH. Rhinoorbital mucormycosis secondary to *Rhizopus oryzae*: a case report and literature review. Ear Nose Throat J 2004;83:556, 558-60, 562.