CASE SERIES

RHINOFACIAL ENTOMOPHTHORAMYCOSIS; A CASE SERIES AND REVIEW OF THE LITERATURE

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Abstract. Rhinofacial entomophthoramycosis is an uncommon chronic mycotic disease caused by exposure to the organism *Conidiobolus coronatus*. The authors report a case series of 5 patients with rhinofacial entomophthoramycosis and review the literature. All patients had typical involvement of the rhinofacial area with formation of subcutaneous lesions causing a chronic granulomatous inflammatory response with tissue eosinophilia and Splendore-Hoeppli reaction. Diagnoses were made based on histopathologic examination in all cases and fungi were isolated and identified in one case. The clinicopathologic features and therapeutic management of rhinofacial entomophthoramycosis are described.

Key words: entomophthoramycosis, phycomycosis, zygomycosis, *Conidiobolus* spp, rhinofacial

INTRODUCTION

Rhinofacial entomophthoramycosis is an uncommon chronic mycotic disease in humans occuring in tropical and subtropical regions of the world. The term rhinofacial entomophthoramycosis encompasses various diseases caused by fungi of the order Entomophthorales and class Zygomycetes (Phycomycetes). The Entomophthorales has two genera, *Conidiobolus* and *Basidiobolus*, producing rhinofacial and subcutaneous entomophthoramycosis, respectively (Prabhu and Patel, 2004; Sugar, 2005; Chayakulkeeree *et al*, 2006). Entomophthoramycosis conidiobalae occurs predominantly in immunocompetent patients, and involves the head and face. The disease is characterized by slowly progressive swelling of the soft tissue of the rhinofacial area and is occasionally mistaken for malignancy or tuberculosis in clinical practice. Histopathologic features reveal broad mycelial filaments with infrequently septation surround by a Splendore-Hoeppli reaction.

The authors describe here five cases of rhinofacial entomophthoramycosis in immunocompetent patients, diagnosed over a 15-year period on the basis of the characteristic morphology of fungal elements in infected tissue and on histopathologic findings. In one case reported, tissue culture revealed *Conidiobolus coronatus*.

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Case 1

A 44-year-old Thai male teacher living in Saraburi Province, Thailand, first came to Ramathibodi Hospital in January 1996, complaining of a firm mass on the right side of the nose with gradually increasing nasal obstruction of one-year duration. There was no history of trauma or insect bites. Clinical examination revealed the patient was in good health. Physical examination showed a firm mass on the inferior turbinate expanding to the external opening of the right nose. The first biopsy revealed granulation tissue. Rhinoscopy disclosed an infiltrative mass of the lateral part of the nose compressing the right inferior turbinate, causing obstruction of the right maxillary and right anterior ethmoid openings. The mass was firm with contact bleeding. Relevant laboratory data included a hemoglobin of 13.1 g/dl, hematocrit of 41%, white blood cell count of 8,700 cells/mm^3, with a differential of 64% neutrophils, 30% lymphocytes, 5% eosinophils, and 1% monocytes. The antineutrophilic cytoplasmic antibody (ANCA) was negative. Anti-human immunodeficiency virus (HIV) was negative. Computer tomography (CT) of the paranasal sinuses showed an infiltrative soft tissue mass involving mainly the right nasal ala with extension to the overlying skin and subcutaneous tissue. The mass occupied the anterior aspect of the right nasal cavity and the right ethmoid sinus causing obstructive sinusitis of the right maxillary sinus. Repeat incisional biopsies were taken from the anterior end of the right inferior turbinate. The pathological diagnosis was rhinofacial phycomycosis. Histopathology revealed short broad hyphae ensheathing with amorphous eosinophilic Splendore-Hoeppli material. Tissue eosinophilia with granulation tissue was noted. A culture of the right inferior turbinate biopsy recovered *Conidiobolus coronatus*. The mass was completely removed and a right maxillectomy was performed. Medical therapy included a combination of a saturated solution of potassium iodide (SSKI) (40mg/kg/day) and trimethoprim/sulfamethoxazole. The treatment was continued for about 12 weeks. The patient was followed up in the Ear-Nose-Throat clinic for 14 years without any evidence of recurrence.

Case 2

A 20-year-old healthy Thai male came to Saraburi Hospital in September 1999 with a complaint of rapidly progressive left nasal swelling, starting 3 months prior to admission. There was no history of nasal discharge, epistaxis, trauma or evidence of insect bites. He had a biopsy of the left nasal mass and the pathological diagnosis was consistent with angiolymphoid hyperplasia with eosinophilia but could not completely rule out lymphoma or granuloma. He was transferred to Ramathibodi Hospital. Physical examination revealed marked swelling, deformity, and enlargement of the left side of the nose, obstructing the left nasal canal. The cervical lymph node were not enlarged. Relevant laboratory data included a hemoglobin of 14.9 g/dl, hematocrit of 45.6%, white blood cell count of 7,710 cells/mm^3, with a differential of 65% neutrophils, 25% lymphocytes, 6% monocytes, 3% eosinophils, and 1% basophils. ANCA and anti-HIV were negative. A CT of the paranasal sinuses revealed a homogenous enhancing, infiltrative soft tissue mass, involving the left alar nasi. A repeat biopsy was performed and histopathological findings revealed short, broad fragmented hyphal elements surrounded by a Splendore-Hoeppli reaction, compatible with entomophthoramycosis. Amphoteri-

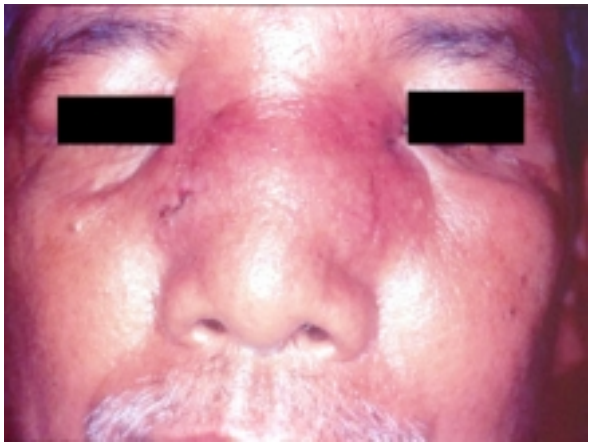

Fig 1–Case 3 when first seen, with swelling of the nasal and paranasal areas.

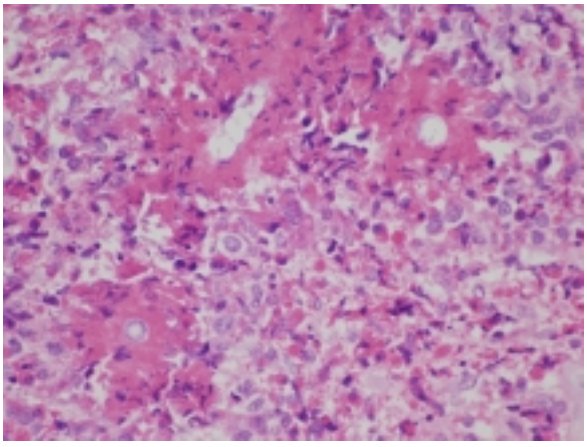

Fig 2–Section of the nasal mucosa showing hyphal elements surrounded by Splendore-Hoeppli material and inflamed stroma consisting of tissue eosinophilia (H & E, x 400).

cin B at a dosage of 1 mg/kg of body weight was given over a ten-week period with good response and there was no recurrence.

Case 3

A 55-year-old Thai male came to Chantaburi Hospital in January 2000 with bilateral nalsal masses for 3 months duration. The patient had no history of significant past illness, and no family history of diabetes mellitus, tuberculosis or malignancies. The first biopsy revealed a midline granuloma. His primary physician referred him to Ramathibodi Hospital. Physical examination showed swelling of the nasal and paranasal areas with redness and mild tenderness involving the frontoglabella (Fig 1). There were no skin or mucosal ulcerations, cervical lymphadenopathy or other clinical abnormalities. Relevant laboratory data included a hemoglobin of 13.1 g/dl, hematocrit of 38.7%, white blood cell count of 10,000 cells/mm^3, with a differential of 66% neutrophils, 30% lymphocytes, 2% eosinophils, and 2% monocytes. ANCA and anti-HIV were negative. Radiography of the paranasal

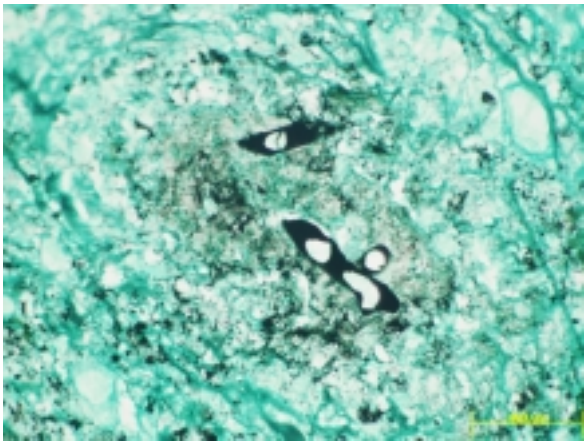

Fig 3–The GMS stained section of nasal mucosa showing short, broad, fragmented hyphal elements (x 400).

sinuses revealed mucoperiosteal thickening in the right maxillary sinus and mucosal thickening of the left maxillary, right frontal and right ethmoid sinuses. A repeat biopsy was performed and revealed irregular sparse nonseptate branching fungal hyphae, which was easily seen on a

Gomeri Methenamine Silver (GMS) stain at low magnification. The hyphal fragments were ensheathed by amorphous eosinophilic Splendore-Hoeppli material. The pathological diagnosis was rhinofacial phycomycosis. Bacteriologic and mycological cultures of the biopsy specimen were performed but there was no organism growth. The medical treatments included a combination of SSKI and trimethoprim/sulfamethoxazole with a gradual response to treatment by two weeks of medication. The treatment was continued for 12 weeks. At eight months follow-up there was complete recovery without recurrence.

Case 4

A 65-year-old Thai male came to Samut Prakhan Hospital in January 2002 with a complaint of a firm swelling of the right nose with gradually increasing nasal obstruction of three months duration. The patient had no history of significant illness in the past. There was no history of tuberculosis among family members. He was diagnosed with a right nasal mass. The first biopsy revealed chronic inflammation suggestive of tuberculosis. He was placed on antituberculous drugs for two months duration, but there was persistent enlargement of the right nasal mass. He was referred to Ramathibodi Hospital. Physical examination showed swelling, deformity, and enlargement of the right nasal and paranasal areas. A white blood cell count was 8,000 cells/mm^3, with 5% eosinophils. ANCA and anti-HIV were negative. CT of the paranasal sinuses showed a partially ill-defined, minimal enhancing lesion of the subcutaneous fat extending to the right lateral wall of the nasal cavity. Repeat biopsy revealed irregular short broad nonseptate branching fungal hyphae. Hyphal fragments were ensheathed by amorphous eosinophilic Splendore-Hoeppli material (Fig 2). A GMS-stain showed short, broad, fragmented hyphal elements (Fig 3). The diagnosis was rhinofacial entomophthoramycosis. Medical therapy included itraconazole for 3 months. He had a good response to treatment and there was no recurrence.

Case 5

A 30-year-old Thai male came to Nakhon Pathom Hospital in April 2008 with bilateral nasal swelling for 8 months. The patient had no history of significant illness in the past. There was no family history of diabetes mellitus, tuberculosis or malignancy. The first biopsy revealed squamous papilloma. His primary physician referred him to Ramathibodi Hospital. Physical examination showed swelling and redness of the left inferior nasal turbinate. There were no skin or mucosal ulcerations, cervical lymphadenopathy or any other clinical abnormalities. Relevant laboratory data included a hemoglobin of 13.1 g/dl, hematocrit of 38.7%, white blood cell count of 11,800 cells/mm^3, with a differential of 76% neutrophils, 14% lymphocytes, 6% monocytes, 3% eosinophils, and 1% basophils. ANCA and anti-HIV were negative. A CT of the paranasal sinuses showed an ill defined soft tissue mass at the left inferior nasal turbinate. A medial maxillectomy by lateral rhinotomy approach was performed and pathology revealed irregular sparse nonseptate branching fungal hyphae, which were easily identifiable on GMS stained sections at low magnification. Hyphal fragments were ensheathed by amorphous eosinophilic Splendore-Hoeppli material. The pathological diagnosis was rhinofacial entomophthoramycosis. Bacteriologic and mycological cultures of the biopsy specimen were performed but there was no organism growth. SSKI was given with

Table 1
Clinical and pathological findings of the five reported cases.

	Case no. 1	Case no. 2	Case no. 3	Case no. 4	Case no. 5
Age (years)	44	20	55	65	30
Sex	Male	Male	Male	Male	Male
Date of diagnosis	January 1996	September 1999	January 2000	January 2002	July 2008
Method of diagnosis	Histopathology and tissue culture grew *Conidiobolus coronatus*.	Histopathology	Histopathology	Histopathology	Histopathology
Histopathological findings	Short, broad hyphae ensheathing by amorphous eosinophilic Splendore-Hoeppli material.	Short, broad fragmented hyphal elements surrounded by Splendore-Hoeppli reaction.	Hyphal fragments ensheathed by amorphous eosinophilic Splendore-Hoeppli material.	Short, broad nonseptate, branching fungal hyphae surrounded by Splendore-Hoeppli reaction.	Hyphal fragments ensheathed by amorphous eosinophilic Splendore-Hoeppli material.
Clinical presentation	Swelling of the right nose with gradually increasing nasal obstruction of one-year duration.	Rapidly progressive left nasal swelling of three-months duration	Bilateral nasal swelling of three-months duration.	A firm mass of the right nose of three-months duration.	Bilateral nasal obstruction of eight-months duration.
White blood cell count (cells/ml)	8,700	7,710	10,000	8,000	11,800
Eosinophils (%)	5	3	2	5	3
Treatment	SSKI and T/S for 12 weeks	Amphotericin B for 10 weeks	SSKI and T/S for 12 weeks	Itraconazole for 3 months	SSKI for 12 weeks
Follow-up	Disease-free	Disease-free	Disease-free	Disease-free	Disease-free

SSKI, saturated solution of potassium iodide; T/S, trimethoprim/sulfamethoxazole

gradually response to treatment at two weeks. The treatment was continued for 12 weeks. At 18 months follow-up there was complete recovery without recurrence.

The clinical and pathologicaly characteristics of the five cases are summarized in Table 1. In all 5 cases, histopathologic examinations revealed severe infiltration with eosinophils and areas of Splendore-Hoeppli phenomenon containing some broad fragmented hyphae. The five patients showed a dramatic response to antifungal treatment. The patients were followed for 1.5 to 14 years. All 5 patients were HIV negative.

DISCUSSION

Rhinofacial entomophthoramycosis is a chronic infectious disease found predominantly in the tropical and subtropical regions of the world. The first report of a disseminated human infection caused by *Conidiobolus* spp was in 1965 (Bras *et al*, 1965). Rhinofacial entomophthoramycosis has occurred predominantly in males, with a male to female ratio of 10:1 (Martinson and Clark, 1967). The ages of patients range from 15 months to 79 years (Gilbert *et al*, 1970; Akpanonu *et al*, 1991). The occupational history is an agricultural lifestyle. The disease is usually seen in immunocompetent patients. However, there have been reports in HIV infected patients (Boonsangsuk *et al*, 2001) and renal transplant patients (Walker *et al*, 1992). Infection has been suggested to result from percutaneous inoculation of *Conidiobolus coronatus* via fungal spore inhalation or insect bites. It is a locally progressive infection of the nasal cavity, paranasal sinuses and soft tissues of the face that rarely extends to the intracranium, mediastinum or lungs (Gilbert *et al*, 1970; Hoogendijk *et al*, 2006). *Conidiobolus* spp rarely causes disseminated or localized deep-tissue invasive infection. Reports of gastrointestinal entomophthoramycosis (Chiewchanvit *et al*, 2002) and disseminated infection caused by *Conidiobolus* spp (Walsh *et al*, 1994) have been published.

It usually presents with painless, slowly progressive diffuse thickening of the skin and prominent distortion of face due to formation of chronic granulomatous inflammation involving the nasal and paranasal mucosa, extension to the skin of the nose, superior lip and frontoglabellar area. A common complaint is discomfort caused by swelling of the face and restriction of movement of the upper lip. Nasal obstruction in varying degrees of severity is also a common symptom. Nasal discharge is an early symptom that diminishes with increasing intranasal swelling. Rare clinical presentations include nasal polyps, epistaxis and pain (Martison, 1971; Moretz *et al*, 1987; Chiewchanvit *et al*, 2002).

The lesions seem to start in the inferior turbinate, which appears swollen, then spreads in several directions to the submucosa and through the ostia, sutures, and foramina into the paranasal sinuses, palate, cheeks, pharynx, dorsum of the nose, and upper lip. The smooth or lobulated tumor produced has distinct margins and is not movable. The lesion is occasionally mistaken clinically for malignancy, such as squamous cell carcinoma, lymphoma or angiocentric immunoproliferative lesions. Ulceration does not occur (Segura *et al*, 1981; Balraj *et al*, 1991), but there is scabbing of the surface when the intranasal tumor projects outside the nasal cavity (Martinson and Clark, 1967). The bones of the face are not involved. Kimura disease and angiolymphoid hyperplasia with eosinophilia (ALHE) are two major diseases in the differential diagnosis of entomophtho-

ramycosis. They typically show lymphoid infiltration with lymphoid follicles and tissue eosinophilia. In ALHE, thick-walled blood vessels are lined with hypertrophic low cuboidal to dome shaped "epithelioid" or "histiocytoid" endothelial cells, which can be misleading, causing a misdiagnosis of granuloma. Kimura disease and ALHE lack eosinophilic Splendore-Hoeppli material and have negative results on fungal stains. GMS stain and tissue culture should be performed in all cases with lymphoid infiltration with lymphoid follicles and tissue eosinophilia.

Histology reveals sparse hyphal elements which frequently occur as short, broad fragments and have conspicuous septation. Budding is not common, but irregular branching hyphae are seen frequently. The hyphae of *Conidiobolus* spp measure 2 to 6 μm in diameter with a mean of about 4 μm. They may be found singly or in clusters on stained specimens. Hyphal fragments are ensheathed by amorphous granulomatous or eosinophilic Splendore-Hoeppli material (Prabhu and Patel, 2004; Sugar, 2005; Chayakulkeeree *et al*, 2006), which is believed to be an antigen-antibody precipitate (Williams, 1969; Segura *et al*, 1981). The adjacent stroma consists of granulation tissue that is rich in eosinophils (Sugar, 2005). In the acute inflammatory phase, eosinophilic, lymphocytic and plasma cell infiltrations predominate, while the chronic phase reaction tends to be characterized by granulomatous infiltration with giant cell, mononuclear and histiocytic elements. The hyphae are not angioinvasive (Sugar, 2005), and there are no vascular thromboses, in contrast to what is observed in cases of mucomycosis. Rhinofacial entomophthoramycosis has a more indolent clinical course. The hyphae are readily visible on hematoxylin and eosin stains, because of their thin wall, but in contrast to other fungi, are not as well demonstrated by modified silver staining.

Only 15% of rhinofacial phycomycosis are positive on mycological culture for *Conidiobolus* spp (Akpanonu *et al*, 1991; Balraj *et al*, 1991). Immunoreagents for the identification of rhinofacial entomophthoramycosis in tissue sections are not presently available. This has subsequently made the histopathological diagnosis more important, because the clinical features of rhinofacial entomophthoramycosis appear similar to malignancy. Rhinofacial entomophthoramycosis can be diagnosed based on finding typical short, broad, fragmented hyphae with Splendore-Hoeppli material on histopathological specimens.

The most successful treatment, with the lowest incidence of recurrence, is oral antifungal therapy. Medications used to treat entomophthoramycosis include potassium iodide, trimethoprim/sulfamethoxazole, amphotericin B, terbinafine, ketoconazole, itraconazole, fluconazole, miconazole, voriconazole and hyperbaric oxygen (Prabhu and Patel, 2004; Sugar, 2005; Chayakulkeeree *et al*, 2006; Moore *et al*, 2007). The drug of choice is SSKI. Doses of 2 to 8 grams daily (40 mg/kg body weight) is continued from 4 weeks to one year or more (Krishnan *et al*, 1998). This is an efficient, inexpensive, easily administered therapeutic agent. In extreme cases drug therapy has been combined with surgical intervention. Patients generally responded well to appropriate treatment.

This report describes rhinofacial entomophthoramycosis in five men with histopathological diagnosis in all 5 cases and microbiologically identified on culture in one case. All cases responded well to management and there were no recurrences on follow-up.

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