

Research Article**Zygomycosis in Immunocompetent Children in Iran: Case Series and Review**Abdolkarim Ghadimi Moghadam¹, Abdolvahab Alborzi², Owrangle Eilami³, Parisa Bediee²,
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Abstract: The class Zygomycetes is divided into two orders, Mucorales and Entomophthorales. Members of these two orders produce dramatically different infections. The fungi in Entomophthorales which encompass 2 genera: *Conidiobolus* and *Basidiobolus* are uncommon pathogens causing chronic cutaneous and subcutaneous infections in immunocompetent patients and rarely disseminate to internal organs and typically are restricted to the tropical and subtropical regions. In the present report, four cases of zygomycosis in immunocompetent children have been presented, which were diagnosed over two-year period 2010 -2012 in southern of Iran on the basis of the histopathologic findings. According to a PubMed search of the English language literature, three other cases of zygomycosis in immunocompetent patients in Iran reported. Surgical resection of the infected tissue and prolonged treatment with AmB followed by a combination of itraconazole and TMP/SMX had an excellent result in these patients. In an immunocompetent patient who presented with eosinophilia, anemia and a mass in different sites such as liver, intestine and other soft tissues in tropical and subtropical area, obtaining specimens for fungal culture and histopathologic examination with special fungal stains seems crucial.**Keywords:** Zygomycosis, Entomophthorales, Immunocompetent, Children, Iran.

INTRODUCTION

Zygomycosis includes infectious syndromes caused by Zygomycetes, a class of fungi that produce predominantly aseptate or pauciseptate, wide and irregularly branching ribbon-like hyphae [1]. The class Zygomycetes is divided into two orders, Mucorales and Entomophthorales. Members of these two orders produce dramatically different infections [2]. The fungi in Entomophthorales are uncommon pathogens causing chronic cutaneous and subcutaneous infections in immunocompetent patients and rarely disseminate to internal organs and typically are restricted to the tropical and subtropical regions [1, 3]. The members of Mucorales cause acute angioinvasive infections in immunocompromised patients and are rare in immunocompetent hosts which are often related to trauma [2, 4].

In the present report, four cases of zygomycosis in immunocompetent children have been presented, which were diagnosed over two-year period 2010 -2012 in southern of Iran on the basis of characteristic morphology of fungal elements in the infected tissues and on the histopathologic findings. Other cases of zygomycosis in immunocompetent patients in Iran reported previously according to a

PubMed search of the English language literature were reviewed.

METHODOLOGY

A PubMed search of the English language literature was conducted using the search terms "Iran" in combination with each of the following terms: zygomycosis, mucormycosis, phycomycosis, *Rhizopus*, *Mucor*, *Rhizomucor*, *Cunninghamella*, *Absidia*, *Apophysomyces*, *Syncephalastrum*, *Saksenaee*, *Cokeromyces*, *Entomophthora*, *Conidiobolus*, and *Basidiobolus*. To be eligible for inclusion, only those cases between 0 and 18 years which had no risk factors such as immunodeficiency state, diabetic and metabolic acidosis, iron overload, deferoxamine use, burns, wounds, severe malnutrition, and intravenous drug abuse included. The zygomycete infection had to be confirmed either histologically or by culture.

Case Series Data Collection

All patients were diagnosed and followed in Pediatric Infectious Ward of Namazi Hospital, a tertiary hospital affiliated to Shiraz University of Medical Sciences. The patients referred from Southern Iran from Fars, Bushehr, Hormozgan, Khozestan

provinces. The inclusion criteria used were the same as for the literature review.

RESULTS

Case Series

In November 2010, a 5-year-old male child from southern of Iran who was a known case of cerebral palsy, presented with fever, anorexia and abdominal discomfort for two months. Physical examination was unremarkable. Blood testing showed anemia (Hb = 8 gr/dl), leukocytosis (23000/ml) with 8% eosinophils and high erythrocyte sedimentation rate (ESR) (76 mm/hr) and C reactive protein (CRP) 192 mg/dl, total serum protein 9.5 mg/dl, serum albumin level 2.9 mg/dl, normal serum liver enzyme levels and blood glucose level. Abdominal computed tomography (CT) scan and ultrasonography showed a large liver mass (90 x 20 mm). Broad-spectrum antibiotics were started with suspicion of pyogenic abscess and with no good response. By the end of the second week of antibiotic therapy, the patient's condition improved slightly with no change in liver mass. Exploratory laparotomy and liver wedge biopsy were performed.

In July 2010, a 1.5 year -old male infant from south of Iran was presented with acute gastroenteritis and history of anorexia, abdominal distention and intermittent episodes of diarrhea and constipation for 4 months. Physical examination was unremarkable except for the presence of suprapubic abdominal mass which was rubbery and mobile (10 x 50 cm). White blood count (WBC) was 16,500/ml with 7% eosinophils and ESR 38 mm/h. The patient had normocytic anemia (Hb= 8.5 gr/dl). Blood glucose level was normal. Microscopic stool exam revealed few red blood cells and moderate puss cells and stool culture was negative. Abdominal CT scan and ultrasonography showed a mass in sigmoid colon (110 x 70 mm) . A surgical intervention was decided and sigmoid colon resection and end-to-end anastomosis were done.

In February 2011, a 5-year-old male child from southern of Iran presented with anorexia, nausea, vomiting, abdominal pain and diarrhea for 2 months. Physical examination was unremarkable. He had anemia (Hb = 10 gr/dl) and normal leukocyte count (6500/ml) with 10% eosinophils. ESR and CRP were 33 mm/hr and 6 mg/dl, respectively. Blood glucose level was normal. Microscopic stool exam revealed moderate puss cells and stool culture was negative. Abdominal CT scan and ultrasonography showed some masses in colon (...29 x 28... mm) . A subtotal colectomy was performed.

In November 2012, a 2.5-year-old male child from rural area of southern of Iran, presented with anorexia, low grade fever, periorbital swelling, pain with eye movements and decreased vision of the left eye which developed gradually in two months. Physical examination of the affected eye revealed proptosis,

erythema and swelling of the involved eyelids, chemosis of the conjunctiva, limited eye movements and decreased vision. Physical examination of right eye was normal. The laboratory finding were as follows: hemoglobin 8.5 gr/dl, WBC 14000/ml with 1.7% eosinophils and ESR 90 mm/hr and CRP 192 mg/dl. Blood glucose level was normal. Orbital and paranasal sinus CT scan showed a mass (34... x 28... mm) in retro-orbital area which extended to left maxillary sinus . Left ethmoid sinus was also involved. The magnetic resonance imaging scan of the brain was unremarkable. *Acinetobacter* species were isolated directly from the left maxillary sinuses. Broad-spectrum antibiotics were initiated. Surgical debridement of the left orbit and adjacent infected maxillary sinus were done and biopsy was taken. The procedure was repeated for several times.

Clinical and paraclinical characteristics of the four cases are summarized in Table 1. In the those cases, histopathological examinations revealed intact and ulcerated mucosa, beneath which there were numerous granulomas with necrotizing centers, as well as severe infiltration of eosinophils and areas of Splendore-Hoeppli phenomenon containing some broad sparsely septate hyphae which were in favor of zygomycosis.

All patients received amphotericin B (AmB) deoxycholate (1.5 mg/kg/day) for 3-7 weeks (Table 1). Patient no. 1 showed dramatic response to the antifungal treatment, i.e., decrease in white blood cell count, in ESR and CRP and in liver mass size, 3 weeks after the onset of the treatment. The patient was discharged with a combination of itraconazole (5 mg/kg/day) and trimethoprim-sulfamethoxazole (TMP/SMX) (12 mg/kg/day) for 4 months. The liver mass size decreased to 15 x 10 mm after 3 months. After a 1-year follow-up, he had no complications, minimal residual liver mass in abdominal ultrasonography and normal ESR and CRP levels.

Patient no. 2 was discharged after 3 weeks of AmB with a combination of itraconazole (5 mg/kg/day) and TMP/SMX (10 mg/kg/day) regimen for 4 months.

Patient no.3, a three-week course of AmB was followed by a four-month course of a combination of itraconazole (5 mg/kg/day) and TMP/SMX (10 mg/kg/day). The patient was symptom free after ten-month follow up.

For patient no.4, AmB was administered at the highest tolerated dosage, 1 to 1.5 mg/kg/d for 7 weeks. Nasal washing with AmB was performed, too. Afterwards, a combination of itraconazole (5 mg/kg/day) and TMP/SMX (10 mg/kg/day) was given up to 6 months. In 13-month follow up, the patient was symptom-free except for occasional epiphoria.

Immunologic work-ups such as serum immunoglobulins, nitroblue-tetrazolium test, CH50, and

flow cytometry of leukocytes in all four patients were inconclusive. The HIV tests were negative.

Table 1: Clinical and paraclinical presentation of the four cases

	Case No. 1	Case No.2	Case No. 3	Case No.4
Age (year old)	5	1.5	5	2.5
Sex	Male	Male	Male	Male
Date of diagnosis	2010	2010	2011	2012
Geographic area	Boushehr province	Fars Province	Fars Province	Hormozgan province
Method of diagnosis	Histopathology	Histopathology	Histopathology	Histopathology
Histopathologic findings	Necrotizing granulomas, Ribbon-like aseptat hyphae	Necrotizing granulomas, Ribbon-like aseptat hyphae	Acute and chronic inflammation granulomas, Ribbon-like aseptat hyphae	Necrotizing granulomas, Ribbon-like aseptat hyphae
Clinical presentation	Fever, anorexia and abdominal discomfort	Aanorexia, abdominal distention and intermittent episodes of diarrhea and constipation	Anorexia, nausea, vomiting, abdominal pain and diarrhea	Anorexia, low grade fever, unilateral periorbital swelling, pain with eye movements and decreased vision
Duration of symptoms (month)	2	4	2	2
White blood cell Count (ml)	23000	16500	6500	14000
Chest XRay	Normal	Normal	Normal	Normal
Immunological work up	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Radiologic findings	liver mass	Mass in sigmoid colon	Mass in colon	Retroorbital mass with maxillary invasion
Surgical procedure	Wedge liver biopsy	Resection of sigmoid colon	Subtotal colectomy	Surgical debridement of the orbit and adjacent maxillary sinus
Treatment (duration by weeks)	Amphotericin B (4) followed by Itraconazole and TMP/SMX (16)	Amphotericin B (3) followed by Itraconazole and TMP/SMX (16)	Amphotericin B (3) followed by Itraconazole and TMP/SMX (16)	Amphotericin B (7) followed by Itraconazole and TMP/SMX (24)
Follow-up (month)	12, disease-free	5 , disease-free	10, disease-free	13, symptom-free

Literature Search

Thirty six cases from 29 articles were identified using the PubMed search strategy outlined above. Of these, 3 cases from 1 articles met study inclusion criteria which were published by authors from this center in 2007.

A total of 7 patient were analyzed, four our patients and three which reported previously. All cases were male, with a mean age of 5.2 years (range 1.5 to 18 y;). All patients survived, only one case reported to have recurrence during follow up. All patients had

visceral involvement except a patient who had cutaneous involvement in rhinofacial region. The time to diagnosis ranged from 2 to 4 months in our cases. All Patients underwent surgery. All patients received itraconazole in treatment regimens. One patient in previous report developed recurrence with itraconazole only. None of our patients developed recurrence during follow up after treating with AmB followed by combination of itraconazole and TMP/SMX.

DISCUSSION

Zygomycosis includes infectious syndromes caused by Zygomycetes; a class of fungi is divided into two orders, Mucorales and Entomophthorales. The diagnosis of zygomycosis relies on a constellation of a high index of suspicions, assessment of presenting signs and symptoms, imaging studies, cultures and direct examinations of clinical specimens, and histopathology [5]. Zygomycetes are characterized in tissue by the formation of wide, ribbon-like, hyaline, aseptate or sparsely septated hyphae with wide-angle (approximately 90°) branching. The lack of septation and the tendency of hyphae to branch at right angles usually serve to distinguish them from *Aspergillus* species, which are septate and smaller and branch at acute angles [6]. During one year, we diagnosed four cases of zygomycosis on the basis of the typical morphological characteristics of fungal elements and histopathologic findings [1, 3] and in a PubMed search of the English language literature, three other case were described from southern of Iran.

The Mucorales and the Entomophthorales have distinct morphological, epidemiological and pathogenic characteristics [7]. The fungi in the order Entomophthorales (*Basidiobolus* and *Conidiobolus* spp.) are uncommon pathogens causing chronic cutaneous and subcutaneous infections in immunocompetent patients and rarely disseminate to internal organs and typically are restricted to tropical and subtropical regions [1, 3]. The members of the order of Mucurales cause acute angioinvasive infections in immunocompromised patients and are rare in immunocompetent hosts and is often related to trauma [2, 4]. All the patients in the present report and three cases previously described were living in subtropical region, with no risk factor for developing invasive zygomycosis according to clinical evidence and laboratory work ups, although the patient with liver zygomycosis was a case of cerebral palsy with moderate malnutrition. Risk factors associated with invasive zygomycosis include immunodeficiency state, diabetic and metabolic acidosis, iron overload, deferoxamine use, burns, wounds, malnutrition, extremes of age, i.e. prematurity or advanced age, and intravenous drug abuse [8].

Gastrointestinal zygomycosis is uncommon and it is the most common predisposing factors are prematurity, malnutrition, ingestion of contaminated food or of non-nutritional substances, severe underlying illness and immunosuppression [8-11]. Gastrointestinal zygomycosis in an immunocompetent host is a rare clinical event which is most commonly caused by *Basidiobolus ranarum* [12]. No other fungal species have been reported to invade the gastrointestinal tract of the immunocompetent patients [3, 13-15].

Subcutaneous nasofacial zygomycoses are caused by the fungi of the order Entomophthorales,

which encompass 2 genera: *Conidiobolus* and *Basidiobolus* [16]. Entomophthoramycosis is a rare granulomatous disease caused by the fungus *Conidiobolus*, and usually manifests as chronic, indolent, and localized infection of the rhinofacial region [17]. The condition usually presents in immunocompetent individuals. Quite often, those affected are farm workers or agriculturists or had a history of trauma [16, 18, 19]. Diagnosis is made by demonstrating distinctive non-septate hyphae with surrounding eosinophilic sleeve (Splendore-Hoepli phenomenon) in tissue sections. Vascular invasion and thrombosis as well as tissue infarction which are usually seen in acute rhino-orbito-cerebral mucormycosis are notably absent [17].

Entomophthorales are uncommon pathogens causing chronic cutaneous and subcutaneous infections in immunocompetent patients and rarely disseminate to the internal organs [20]. Liver involvement with gastrointestinal entomophthomycosis and one isolated hepatic involvement have been reported in Saudi immunocompetent pediatric patients [21]. Our case with isolated hepatic zygomycosis share many features with the Saudi case. Both were young children and immunologically grossly normal and hence, with no obvious risk for fungal infections. Prolonged history of abdominal pain and fever, hepatomegaly, leukocytosis with eosinophilia, high ESR, large liver mass, liver biopsy consistent histopathologically with entomophthomycosis were also similar features.

The early diagnosis and immediate initiation of treatment with an antifungal agent in combination with surgical intervention has proved critical for the favorable outcome of zygomycosis [21, 22]. The best choice of antifungal agents is not clear yet [23]. Conventionally, the agent of choice was AmB used at higher than normal doses of up to 1.5 mg/kg/day. However, the availability of the less toxic lipid formulations, backed by clinical data to support their use in zygomycosis [22]. There are limited and variable experiences with regard to the treatment of the infections caused by the fungi of the order Entomophthorales, in part because of the small number of cases reported in the literature [16]. Though potassium iodide (KI) has been the traditional drug used in the treatment of infections by the orders Entomophthorales, several other drugs, viz, amphotericin B, cotrimoxazole, ketoconazole, itraconazole and fuconazole have been successfully tried [24]. Posaconazole, a recently approved, orally available broad-spectrum triazole seems to have potent activity against Zygomycetes [25, 26]. A further consideration can be a combination antifungal therapy, and there are some case reports that describe successful outcomes with the combinations of various antifungal drugs [22, 23].

Mortality or recurrence had been observed with single therapy in some case series; therefore, we administered a course of AmB for 3-4 weeks, followed by a 4-6 month course of combination of itraconazole and TMP/SMX. No case of recurrence or treatment failure was detected with this regimen (Table 1). One patient in previous report developed recurrence with itraconazole only [3].

CONCLUSION

In an immunocompetent patient who presented with eosinophilia and a mass in different sites such as liver, intestine and other soft tissues in tropical and subtropical area, obtaining specimens for fungal culture and histopathologic examination with special fungal stains seems crucial. Surgical resection of the infected tissue and prolonged treatment with AmB followed by a combination of itraconazole and TMP/SMX had an excellent result in these patients.

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