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Zygomycotic necrotizing fasciitis in immunocompetent patients: a series of 18 cases

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Necrotizing fasciitis is most often associated with bacterial infections. Zygomycosis is an uncommon infection causing necrotizing fasciitis. We report 18 such cases of zygomycotic necrotizing fasciitis, of these, 15 were immunocompetent. Of the eight cases cultured, five were positive for *Apophysomyces elegans.* A retrospective case review conducted at a tertiary referral center, from 1998 to 2004, 18 cases of fungal necrotizing fasciitis were diagnosed based on histomorphology of fungal organisms; and in few of the cases diagnosis was supported by mycologic culture reports. Of the total of 18 cases, culture report was available in eight cases, and out of which five of them grew *A. elegans*. Fifteen patients were immunocompetent. Clinical presentation, mycologic findings and histopathologic results were evaluated. A review of the literature pertaining to *A. elegans* infection was also done. Histopathologic examination showed broad, predominantly aseptate and occasional pauciseptate, thin-walled fungal hyphae with occasional angioinvasion. To the best of our knowledge, this is the first largest series of zygomycotic necrotizing fasciitis from India. Herein, we present data on 18 cases of necrotizing fasciitis assosiated with zygomycosis. Most of the cases in our series were immunocompetent. Nonsuppurative necrosis with presence of typical fungal profiles was important histologic feature. Zygomycosis must be considered in the differential diagnosis not only in immunocompromised patients but also in the absence of any underlying disorders.
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Necrotizing fasciitis is a rare and often fatal soft-tissue infection. It typically begins with trauma; although the inciting event may be as seemingly innocuous as a simple contusion, minor burn, or insect bite. The disease occurs more frequently in diabetics, alcoholics, immunosuppressed patients, i.v. drug users, and patients with peripheral vascular disease.[1] Although it can occur in any region of the body, the abdominal wall, perineum and extremities are the most common sites of infection.[2] Clinical presentation involves fever, cellulitis, edema, crepitus, bullae, necrosis and sepsis. Operative findings include fascial and subcutaneous tissue necrosis with or without myonerosis.[3] Necrotizing fasciitis may occur as a consequence of infection with *Streptococcus pyogenes* or as a result of a polymicrobial synergistic infection caused by aerobic, anaerobic, Gram-positive and Gram-negative organisms.[4,5] Fungal infection although reported, is not a common finding in necrotizing fasciitis; only a handful of case reports are available in literature.[6,7] In this study, we described a large series of fungal necrotizing fasciitis cases due to zygomycosis with their clinical, histopathologic and microbiologic findings. With the best of our knowledge this is the largest series of fungal necrotizing fasciitis from India.

Materials and methods

Cases of necrotizing fasciitis were analyzed over a period of 7 years (1998–2004) from the archives of Department of Histopathology, Post graduate institute of medical education and research, Chandigarh, India. A total of 58 cases of necrotizing

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Table 1 Clinical, microbiological and histopathologic data of 18 patients

SN	*Age/sex*	*Site/surgical procedure*	*Clinical details*	*Microbiology*				*Remarks*
					Smear report		*Culture*	
				Pus cells (neutrophills)	*Hyphae*	*Bact*		
1	50F	Lt Gluteal D	IM inj followed by abscess	+	Aseptate	–	No growth	
2	48M	Lt Gluteal D	Stab injury				NP	
3	72M	Rt interdigital cleft D	Case of adenoca caecum, Rt hemicolectomy done				NP	Immunosuppressed
4	20M	Lt Thigh D	RSA, motor vehicle	–	Septate	–	*A. flavum*	Coexistent *Aspergillus*
5	22M	Abdominal wall D	RSA, motor vehicle	++	—	+	No growth	
6	43F	Ant abdominal wall D	DM on insulin, asthmatic on prednisolone				NP	Immunosuppressed, coexistent *Candida*
7	45M	Flank Lt D	Splenic abscess and flank cellulitis	+	Aseptate	–	*A. elegans*	
8	55F	Rt loin D	Perinephric abscess	+	Aseptate	–	*A. elegans*	
9	60F	Rt thigh & leg amputation	# SOF compound Grade III	+	Aseptate	+	*A. elegans*	
10	30F	Chest wall D	RSA, motor vehicle	+	Aseptate	+	*A. elegans*	
11	65M	Lt gluteal D	IM inj followed by abscess				NP	
12	20M	Chest wall and abdomen D	Fall from height				NP	
13	60M	Rt leg D	Deep Burn	+	Aseptate	–	*A. elegans*	
14	20M	Chest wall D	Bullet injury				NP	
15	55M	Rt gluteal D	Gluteal abscess				NP	
16	35M	Rt gluteal D	Invasive gluteal mucormycosis with extensive necrosis of soft tissue				NP	
17	22M	Lt leg, disarticulation of gangrenous limb	RSA, motor vehicle				NP	
18	5M	Multiple subcut nodules	ALL-L1				NP	Immunosuppressed

Abbreviations: Adenoca, adenocarcinoma; *A. elegans, Apophysomyces elegans; A. flavum, Aspergillus flavum*; ALL, acute lymphoblastic leukemia; Bact, Bacteria; Crypto, *Cryptococcus*; D, debridement; DM, diabetes mellitus; F, female; #, fracture; IM inj, intramuscular injection; Lt, left; M, male; NP, not performed; Rt, right; RSA, road-side accident; SN, serial number; SOF, shaft of femur; Subcut, subcutaneous.

fasciitis were diagnosed. While searching for etiologic factors of necrotizing fasciitis, through SNOMED coding system, 18 cases of fungal necrotizing fasciitis were found from the database. Diagnosis was confirmed by combination of clinical and histomorphologic findings. Moreover in eight patients it was well supported by mycologic culture report.

Microbiologic Examination

Debrided tissues were inoculated onto blood agar, inhibitory mold agar and malt extract agar. The organism was subcultured to potato dextrose agar, and after 3 days of incubation at 30°C, a block of the medium was placed which was then incubated at 37°C.

Histopathologic Examination

Resected tissue from all cases was fixed in 10% neutral-buffered formalin, routinely processed and paraffin embedded. Sections (5 μm thick) were stained by hematoxylin and eosin (H&E) stain. Three histopathologists independently reviewed the H&E slides along with relevant histochemical stains and reconfirmed the original histopathologic diagnoses. Periodic acid Schiff (PAS) and Silver methenamine stain for fungus were performed in all cases. Neither immunohistochemistry nor molecular techniques were carried out for diagnosis of these cases.

Results

A total of 18 patients of fungal necrotizing fasciitis were analyzed at this tertiary care center, during a study period of 7 years, between January 1998 and December 2004.

Clinical Profile

The age ranged from 5 to 72 years (mean 40.3 years). There was male preponderance (13 cases) with male to female ratio of 2.6:1 (Table 1).

Of the total of 18 cases, three patients were immunosuppressed. Of these three, one patient was diabetic and the other two had history of malignancy and were receiving chemotherapy. In remaining 15 patients, no history of underlying immunosuppression was present. Four of the patients had history of motor vehicle road-side accident. Some kind of injury was present in 10 other cases. One patient had fracture neck of the femur. Lower extremities and gluteal region were involved in six and four cases, respectively. Eight cases presented with abdomen and chest wall involvement.

Microbiologic Analysis

Microbiologic data was available in eight patients (Table 1). Mycologic culture was not done in remaining patients as they were put on broad spectrum antibiotics, based on clinical diagnosis of polymicrobial necrotizing fasciitis. Macroscopically colonies were fluffy and cottony. Surface of the colony was initially white and turned brownish gray after some time. Sporangiophores with apophyses, and pyriform sporangia were identified. Smear examination revealed aseptate, broad (4–8 μm in diameter) and branching hyphae in six cases. Whereas one case showed septate hyphae on smear examination and grown as *Aspergillus flavum* on mycologic culture. Although on smear, bacteria were positive in three cases, bacterial cultures were sterile.

Histological Examination

Morphologically all cases exhibited predominantly aseptate and occasional sparsely septate, broad and branching hyphae, consistent with the morphology of zygomyces species (Figure 1). The angle of hyphal branching varied from 45° to 90°. At places the hyphae had a compressed distorted appearance owing to the thin hyphal walls (Figure 2). In addition to zygomycosis, one case showed septate acute angle branching hyphae of *Aspergillus* invaded only the superficial necrotic tissue; and one other case revealed yeast forms of *Candida.* Necrotizing inflammation involving skin, subcutaneous tissue and deeper tissue including fat, underlying fascial layers and muscle was seen in all of the cases. Four cases showed necrosis of skeletal muscle. Focal or diffuse nonsuppurative necrosis of subcutaneous tissue was noticed in all cases (Figure 3). Most areas of the tissue showed coagulative necrosis with focal suppurative inflammation.

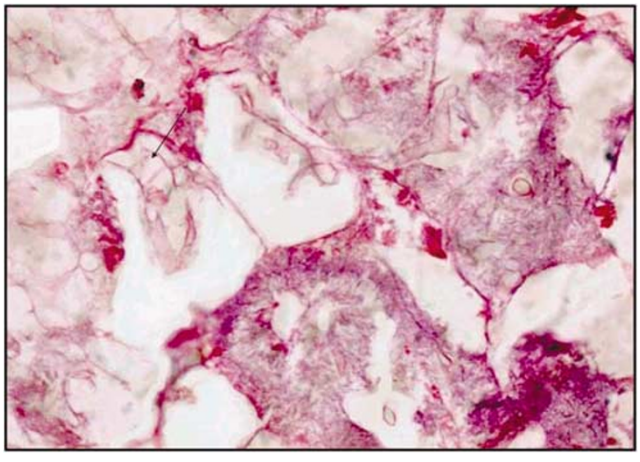

Figure 1 Acutely-to-obtusely branching, pauciseptate hyphae of zygomyces are present. The arrow designates a septum (H&E, × 200).

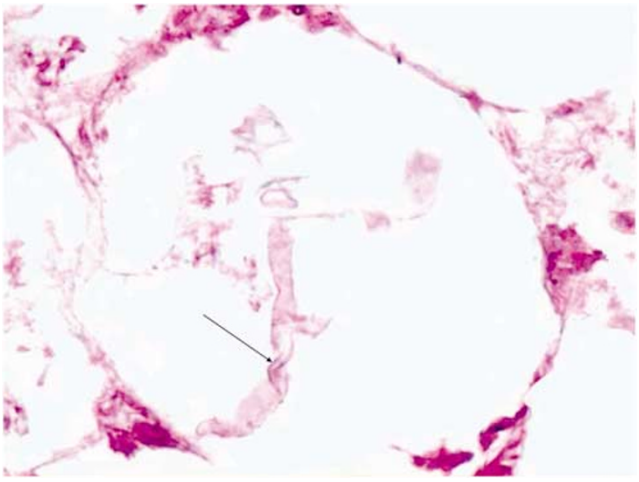

Figure 2 Broad aseptate thin walled fungal hyphae with collapse of their walls (arrow) (PAS, ×400).

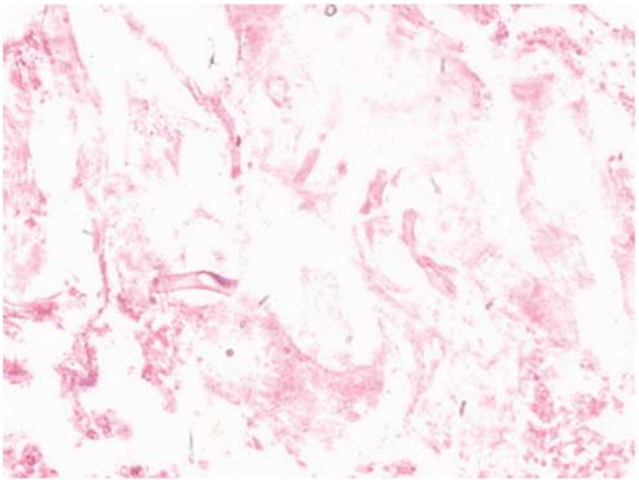

Figure 3 Photomicrograph shows subcutaneous non-suppurative necrosis with few fungal profiles (H&E, ×200).

The fungal profiles were more numerous in the area, where there was nonsuppurative necrosis, rather than in densely inflammed areas. Vascular invasion was noticed in three (16%) cases. Based on overall morphologic findings and in few cases with support of positive fungal culture, a diagnosis of zygomycotic necrotizing fasciitis was made.

Discussion

Necrotizing fasciitis is being attributed to a large variety of organisms, besides the originally described beta-hemolytic *Streptococcus*. Detailed microbiologic study of these infections revealed that these infections are polymicrobial; and fungal species are responsible for occasional cases.[4–7] However, in the present series, we have found zygomycosis in 31.03% cases of necrotizing fasciitis, over a period of 7 years, which is significantly high. There are only few case reports available on fungal necrotizing fasciitis, however, information on a large series of patients with clinical, histological and microbiologic details is scant in the literature.

Zygomycetes class of fungi includes three orders that is *Mucorales, Mortierellales* and *Entomophthorales.* The majority of human illness is caused by the *Mucorales.* While disease is most commonly linked to *Rhizopus* spp., other organisms are also associated with human infection, including *Mucor*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Cokeromyces*, and *Syncephalastrum* spp. *Mortierellales*, cause disease in animals, and *Entomophthorales* produce some relatively distinct syndromes and have dramatically different morphologies from *Mucorales*.[8] Human zygomycosis caused by the *Mucorales* generally occurs in immunocompromised hosts as opportunistic infections.[9] However, an increasing trend of mucormycosis in immunocompetent patients has been seen and reported recently by Sridhara *et al*[10] from this institute. In which authors concluded that mucormycosis must be considered in the differential diagnosis not only in immunocompromised patients but also in the absence of any underlying disease. In immunocompromised patients, risk factors for development of zygomycosis include diabetes mellitus, neutropenia, sustained immunosuppressive therapy, chronic prednisone use, severe malnutrition and primary breakdown in the integrity of the cutaneous barrier such as trauma and surgical wounds.[11,12] Histologically zygomycetes class of fungi exhibit broad, thin-walled, hyaline, often aseptate or pauciseptate hyphae with frequent angioinvasion. The hyphae of the zygomycetes appear thinner-walled compared to other fungal hyphae, which possibly accounts for their weaker staining with Gomori methenamine silver and PAS techniques relative to fungi with thicker cell walls.[13] In contrast to the zygomycetes, *Aspergillus* tends to demonstrate an acute branching pattern and has narrower, more uniform septa.[13] Vascular invasion that causes necrosis of the infected tissue, and perineural invasion are the most morbid features of these infections.[8] The vasoinvasion was seen in only 16% of our cases. In contrast to findings of Frater *et al*, we did not find perineural invasion in the present series.

Generally, diagnosing mucormycosis almost always requires histopathologic evidence of fungal invasion of the tissues. Culturing organisms from an infected site is rarely sufficient to establish the diagnosis because the causative agent is ubiquitous, may colonize normal persons, and is a relatively frequent laboratory contaminant. In addition, the organism may be killed during tissue grinding,[14] which is routinely used to process tissue specimens for culture. There are no reliable serologic,

PCR-based or skin tests for mucormycosis. Therefore, the diagnosis should be made by biopsy of infected tissues. The genus and species of the infecting organism may be determined by culture of the infected tissue. However, the organism is rarely isolated from cultures of blood, cerebrospinal fluid, sputum, urine, feces or swabs of infected areas.[15] As in our series, culture was available for only the minority of cases, hence the diagnosis of zygomycosis was based solely on histomorphology.

In the present series, most of the patients were immunocompetent (15/18), and had history of injury thus zygomycosis in these cases was attributed secondary to breakdown in the mucocutaneous barrier. Unfortunately microbiologic culture reports were available in only eight of these 15 patients. Of these eight, *A. elegans* was cultured in five (62.5%) cases. Although in remaining three immunosuppressed patients (cases—3, 6 and 18) microbiologic data was not available, but zygomycotic necrotizing fasciitis in these cases could be attributed either to other members of zygomycetes family such as *mucor* or to *Apophysomyces* itself. As morphologically *A. elegans* and *Mucor* are similar and can not be distinguished. Moreover *Apophysomyces* was described in immunosuppressed patients rarely.[8,16] Other members of *Mucorales* order besides *A. elegans* and *Mucor* are morphologically distinct, therefore might not be responsible pathogens in our cases.[8]

A. elegans is a rare cause of human zygomycosis, and the infection is usually acquired via traumatic implantations such as burns or invasive procedures, in previously healthy patients. By far the most common site of disease manifestation in *A. elegans* infections is the cutaneous and subcutaneous tissue, with local invasion resulting into necrotizing fasciitis.[17] *A. elegans* is an uncommon human pathogen that was first isolated in 1979 from soil samples collected in an orchard in northern India.[18] Kimura *et al*[19] reviewed 16 cases of *A. elegans.* While reviewing the literature further, we found 13 additional case reports; and two large series from this institute (Table 2). The study from this institute by Chakrabarti *et al*[29] highlighted the importance of increased awareness for early diagnosis of zygomycosis and aggressive management. The large number of cases in apparently healthy hosts and increased isolation of *A. elegans* in the same series were important characteristics of this disease in India.

Two patients in the present series revealed coexistence of *Candida* and *Aspergillus* (cases 4 and 6). One patient had evidence of a zygomycotic infection and *Candida* by light microscopy only, which was not confirmed by culture. A second patient's tissue grew *A. flavum* on culture but showed evidence of zygomyces by light microscopy only. Neither patient had both *Candida* and *Aspergillus*, in addition to a zygomycotic infection. Although immunosuppression is the major factor for *Aspergillus* infection but it may be local colonizer or might have occurred due to nosocomial infection in this case.[8]

Table 2 Literature review of *A. elegans* infection

SN	*Studies*	*Site of lesion*
1	Chakrabarti *et al*[20]	Craniofacial
2	Caceres *et al*[21]	Limb
3	Brown *et al*[22]	Rhinocerebral
4	Burrell *et al*[23]	Spine
5	Fairley *et al*[24]	Rhino-orbital-cerebral
6	Garcia-Covarrubias *et al*[25]	Rhino-orbital-cerebral
7	Blair *et al*[26]	Cutaneous
8	Lesueur *et al*[27]	Hand
9	Wang *et al*[28]	Cutaneous
10	Chakrabarti *et al*[29] (eight cases)	Cutaneous and subcutaneous, renal, rhino-orbital, disseminated
11	Carter *et al*[30]	Forearm
12	Kordy *et al*[31]	Deep soft tissue
13	Ruiz *et al*[32]	Lumbar region
14	Andresen *et al*[33]	Thigh
15	Sridhara *et al*[10] (three cases)	Rhino-orbital-cerebral
16	Present series (five cases)	Thigh, chest, gluteal region

The present series describes large number of patients of zygomycotic necrotizing fasciitis with their clinical, histopathologic and microbiologic features. The most important histological finding in all the cases was nonsuppurative necrosis of subcutaneous fat. In fact, presence of nonsuppurative necrosis of subcutaneous fat without much inflammatory reaction should raise the suspicion for zygomycosis. Patients with necrotizing fasciitis had an overall mortality rate of 47.7%. However, mortality in patients with superimposed zygomycosis rises to 80%.[34] So an early diagnosis and management is important in this group. Unfortunately no follow-up information was available for the patients of present series.

To the best of our knowledge, this is the first largest series of zygomycotic necrotizing fasciitis cases from India. This study is to alert the pathologists that one should be cautious in diagnosing necrotizing fasciitis cases because all the time these cases may not be due to bacterial infection and may not be in immunocompromised patients. We report a series of 18 cases of fungal necrotizing fasciitis, eight with confirmatory cultures and the remainder with characteristic histomorphology. All the cases showed ribbon-like hyphae typical of the zygomycetes. Occasional septations, thin walls and broad-angle branching with angioinvasion are the few features, important for differentiation from other group of fungi.

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