Primary cutaneous zygomycosis from a tertiary care centre in north-west India

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**Background & objectives:** Zygomycosis is highly invasive fungal infection, with high mortality rate. In most of patients, diabetes mellitus is an underlying factor but in primary cutaneous zygomycosis, presentation may be different. Here we present the description of clinical presentation, fungi isolated and management of cases with cutaneous zygomycosis seen in a tertiary care hospital in north India during 2001-2007.

**Methods:** All patients diagnosed with primary cutaneous zygomycosis between November 2001 and September 2007 presenting with clinical diagnosis of necrotizing fasciitis were included. Detailed history of each patient was taken, clinical presentation, site of involvement, underlying illness and risk factor, if any were noted. The diagnosis was established by direct microscopic evidence of broad, aseptate or sparsely septate ribbon-like hyphae with right angle branching in KOH wet mount and histopathological examination of stained sections. Cultures were put up for fungal isolation and species identification. Outcome of the therapy was analysed.

**Results:** Of the nine patients reviewed, only one had diabetes mellitus. Commonest risk factor was injection abscess (33.3%). *Apophysomyces elegans* was isolated in four cases, *Saksenaea vasiformis* and *Absidia corymbifera* in one each. The fungal culture was sterile in three cases. Mortality rate was high with only four patients responded well to surgical and/or medical therapy.

**Interpretation & conclusion:** Primary cutaneous zygomycosis appears to be on rise in India that calls for high index of clinical suspicion and an early biopsy of the affected area for timely diagnosis. The standard treatment is a combination of amphotericin B therapy, surgical debridement, and reversal of the underlying disease or immunosuppression.

**Key words** *Apophysomyces elegans* - cutaneous - fungal infection - primary zygomycosis

Zygomycosis is a serious and often rapidly fatal infection usually seen in immunocompromised individuals. It is caused by fungi belonging to class *Zygomycetes*, order *Mucorales*. The genera reported to cause invasive infection are *Absidia*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Apophysomyces*, *Saksenaea*, *Cunninghamella*, *Cokeromyces* and *Syncephalastrum*. Commonly reported infections belong to the first three genera, *Rhizopus*, *Absidia* and *Rhizomucor*. These fungi are ubiquitous in environment and although humans have a strong natural resistance to infection, they can cause a rapidly progressive and
fatal disease in compromised hosts. The spectrum of diseases described includes rhinocerebral, pulmonary, gastrointestinal, cutaneous and disseminated infections. Patients who are at high risk of developing cutaneous zygomycosis are those with disruption of the normal protective cutaneous barrier. Local risk factors for cutaneous zygomycosis include trauma, burns, surgery, surgical splints, arterial lines, injection sites, biopsy sites, tattoos, and insect or spider bites. Systemic risk factors for cutaneous zygomycosis are hyperglycaemia, ketoacidosis, malignancy, leucopenia and immunosuppressive therapy. Infection in the immunocompetent host, however, is well described. The outcome of cutaneous zygomycosis depends, in part, on the prognosis of the underlying disease together with early diagnosis and treatment.

In our centre we diagnosed nine cases of primary cutaneous zygomycosis between November 2001 and September 2007. Here we describe clinical presentation, fungi isolated and management of these cases to raise clinical awareness.

Material & Methods

All patients diagnosed with primary cutaneous zygomycosis at Government Medical College & Hospital, Chandigarh between November 2001 and September 2007 were included in this study. They presented with clinical diagnosis of necrotizing fasciitis. Detailed history of each patient was taken. Clinical presentation, site of involvement, underlying illness and risk factor, if any were noted. Routine laboratory investigations were carried out. Aspirated pus, pus swabs or cutaneous tissue specimens were sent for microbiological examination. Cutaneous tissue specimens were sent for histopathological examination. The diagnosis was established by direct microscopic evidence of broad, aseptate or sparsely septate ribbon-like hyphae with right angle branching in KOH wet mount and histopathological examination of stained sections. To isolate fungi, the specimens were inoculated on to Sabouraud dextrose agar (SDA) and brain heart infusion agar (BHIA) and incubated at 25°C and 37°C. The growth obtained on culture was identified by colony characteristics, lactophenol cotton blue preparation and slide culture. For sporulation, agar floatation method was used i.e., one cm SDA agar blocks permeated with hyphae and accompanying aerial hyphae were cut and placed on surface of sterile water-yeast extract solution. Abundant sporulation was seen within 4 days when grown at 37°C. Additionally, the samples were inoculated on to the plates of blood agar, MacConkey agar and BHI broth and incubated at 37°C overnight to look for bacterial growth. The patients were managed surgically and/or medically along with correction of underlying illness, if any (Table). Outcome of the therapy was analysed.

Results & Discussion

The clinical and laboratory details of all nine patients diagnosed of primary cutaneous zygomycosis between November 2001 and September 2007 show that diabetes mellitus was present only in one patient (Table). None of the patients were neutropenic or on immunosuppressive therapy. A history of trauma as a risk factor was available in one patient, three had zygomycosis affecting intramuscular injection sites, one had involvement of appendicectomy wound and one had Plaster of Paris cast as the risk factor. The fungal culture was sterile in three cases, however, KOH wet mount and histopathological examination of stained sections confirmed zygomycosis. In the remaining six cases, fast growing, white and cottony growth was obtained from biopsy tissue, aspirated material or pus. Species identification was done by lactophenol cotton blue preparation, slide culture and spore induction. Apophysomyces elegans was isolated in four cases, Saksenaea vasiformis in one and Absidia corymbifera in one. Mortality rate was high. Only four patients responded well to surgical and/or medical therapy. Five patients expired either because of delay in diagnosis and treatment or septicaemia due to superadded bacterial infections or inability to afford costly antifungal treatment.

Cutaneous zygomycosis is characterized by pain, erythema and induration with varying degrees of central necrosis. More advanced lesions take on the appearance of necrotizing fasciitis which has a mortality approaching 80 per cent. Necrotizing fasciitis is mostly bacterial in origin. Progression to the advanced gangrenous or disseminated form may be rapid and occurs most commonly in the immunocompromised host.

The agents of zygomycosis are incapable of penetrating intact skin. A typical case results from traumatic implantation of soil. In diabetic and immunocompromised patients, cutaneous lesions may also arise at insulin injection or catheter insertion sites. Contaminated surgical dressings have also been implicated as a source of cutaneous zygomycosis.
<table>
<thead>
<tr>
<th>S. no.</th>
<th>Month &amp; yr</th>
<th>Age/yr</th>
<th>Sex</th>
<th>Area involved</th>
<th>Clinical presentation</th>
<th>Underlying illness</th>
<th>Risk factor</th>
<th>KOH mount &amp; HPE</th>
<th>Cultural growth</th>
<th>Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nov 2001</td>
<td>28/M</td>
<td></td>
<td>Anterior abdominal wall</td>
<td>Fever, swollen necrosed area 20×12×4 cm at site of appendicectomy wound 1 wk after operation, underlying muscles, tissues and fascia gangrenous</td>
<td>None</td>
<td>Appendicectomy wound</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>Absidia corymbifera, Escherichia coli</td>
<td>Incision &amp; drainage, local debridement AMB (i.v &amp; topically), KI orally (25 drops tds), Antibiotics</td>
<td>Recovery within 1 month</td>
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<tr>
<td>2.</td>
<td>Dec 2004</td>
<td>60/F</td>
<td></td>
<td>Right gluteal region</td>
<td>Fever, necrotic patch 6×6 cm with surrounding erythema &amp; swelling extending to ant. abdominal wall</td>
<td>None</td>
<td>i.m. injection at same site 7 days prior</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>Saksenaea vasiformis, Pseudomonas aeruginosa, E. coli</td>
<td>Local debridement, AMB (50 mg/day) Antibiotics</td>
<td>Expired after 2 months due to bacterial sepsis</td>
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<tr>
<td>3.</td>
<td>April 2005</td>
<td>32/F</td>
<td></td>
<td>Anterior abdominal wall (left side)</td>
<td>Fever, black discoloration 15×15 cm on ant. abdominal wall with surrounding oedema and induration</td>
<td>None</td>
<td>Massage on abdomen for abdominal pain 10 days prior</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>A. elegans</td>
<td>Local debridement, Antibiotics, No antifungal</td>
<td>Expired after 7 days</td>
</tr>
<tr>
<td>4.</td>
<td>August 2005</td>
<td>43/M</td>
<td></td>
<td>Anterior abdominal wall (right side)</td>
<td>15×15 cm debrided wound on right ant. abdominal wall, necrotic fat at margin present, laterally skin discolored</td>
<td>None</td>
<td>H/o of injection on right ant. abdominal wall which developed into small blister</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>A. elegans</td>
<td>Local debridement, AMB (50 mg/day) Antibiotics</td>
<td>Recovery within 1.5 months</td>
</tr>
<tr>
<td>5.</td>
<td>August 2005</td>
<td>30/F</td>
<td></td>
<td>Left gluteal region</td>
<td>Skin, superficial fascia, fat uptil gluteal muscle necrosed, covering area of 25×20 cm</td>
<td>None</td>
<td>i.m. injection over left gluteal region 10 days prior for fever</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>Sterile</td>
<td>Local debridement, AMB (50 mg/day) Antibiotics</td>
<td>Recovery within 1.5 months</td>
</tr>
<tr>
<td>6.</td>
<td>June 2007</td>
<td>29/M</td>
<td></td>
<td>Right arm, axilla and right part of chest wall</td>
<td>Necrosed area on right arm at the site of Plaster of Paris cast extending to axilla and right part of chest wall</td>
<td>None</td>
<td>Plaster of Paris cast applied at the site for fracture of right humerus</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>A. elegans</td>
<td>Local debridement, AMB (50 mg/day) Antibiotics</td>
<td>Expired within 15 days</td>
</tr>
<tr>
<td>7.</td>
<td>August 2007</td>
<td>35/F</td>
<td></td>
<td>Left thigh &amp; anterior abdominal wall</td>
<td>Massive wound on left thigh &amp; anterior abdominal wall with black necrotic areas</td>
<td>Diabetes mellitus</td>
<td>i.m. injection over left gluteal region 7 days prior</td>
<td>Broad, asceptate hyphae with right angle branching</td>
<td>A. elegans</td>
<td>Insulin, local debridement &amp; antibiotics No antifungal given as patient could not afford</td>
<td>Expired after 7 days</td>
</tr>
<tr>
<td>S. no.</td>
<td>Month &amp; yr</td>
<td>Age (yr)/Sex</td>
<td>Area involved</td>
<td>Clinical presentation</td>
<td>Underlying illness</td>
<td>KOH mount &amp; HPE</td>
<td>Cultural growth</td>
<td>Therapy</td>
<td>Outcome</td>
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<td>8.</td>
<td>Sep. 2007</td>
<td>85/M</td>
<td>Left leg</td>
<td>Necrosed and swollen left leg</td>
<td>None</td>
<td>Broad, aseptate hyphae with right angle branching</td>
<td>Sterile</td>
<td>Local debridement, antibiotics, Irrigation of wound with AMB &amp; topical application of AMB (Ambisome)</td>
<td>Wound started to recover within 15 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Sep. 2007</td>
<td>19/M</td>
<td>Right side of chest and abdomen</td>
<td>Fever, pain &amp; progressive black discoloration of right side of chest and abdomen</td>
<td>Unknown</td>
<td>Broad, aseptate hyphae with right angle branching</td>
<td>Sterile</td>
<td>Patient’s condition serious, put on ventilator and given adrenaline and effcorlin</td>
<td>Expired on day 1 of admission</td>
<td></td>
<td></td>
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</table>

Cutaneous zygomycosis has also been reported to occur due to contaminated tape used to secure an endotracheal tube in a ventilated patient\(^{18}\).

*A. elegans* is being reported with increasing frequency as a cause of infection typically in immunocompetent patients following trauma or invasive procedures. A fatal case of necrotizing fasciitis caused postoperatively by *A. elegans* in an immunocompetent patient was reported from south India in 1993\(^{19}\). In 1997 another case with striking similarity was reported from south India\(^{6}\). Chakrabarti *et al*\(^{20}\) reported three cases of cutaneous and subcutaneous infections due to *A. elegans*, two of whom were immunocompetent\(^{20}\). Jain *et al*\(^{21}\) reported 18 cases of zygomycotic necrotizing fasciitis, of whom, 15 were immunocompetent and of the eight cases cultured, five were positive for *A. elegans*. Of the present six cases showing growth, four were positive for *A. elegans*, and three were immunocompetent. *A. elegans* gives an excellent mycelial growth on routine culture media within 24 to 48 h but is notorious for not being able to produce asexual spores\(^{12}\). In general morphology, *A. elegans* closely resembles *Absidia* species in producing pyriform, apophyseal, multisporated sporangia on sporangiophores which arise on stolons but typically not opposite rhizoids.

![Fig. 1. Photomicrograph of the lactophenol cotton blue mount (40x magnification), showing typical pyriform, apophyseal, multisporated sporangia of *Absidia corymbifera* on sporangiophores which arise on stolons but typically not opposite rhizoids.](image1)

![Fig. 2. Photomicrograph of the lactophenol cotton blue mount (40x magnification), showing the characteristic funnel-shaped apophyses of *A. elegans*.](image2)
not opposite rhizoids (Fig. 1). It differs from Absidia species in that it has more pronounced apophyses, which are funnel or bell shaped (Fig. 2) and a hyphal segment reminiscent of the foot cells of Aspergillus species. The characteristic darkening and thickening of the sporangiophore wall below the apophyses that narrows the lumen of the sporangiophore differentiates the genus Apophysomyces from related genera.

A. Corymbifera grows readily upon subculture on routine mycology media, growing more rapidly at 37\(^\circ\)C than at 25\(^\circ\)C and it is capable of growth at temperatures up to 48 to 52\(^\circ\)C, which distinguishes it from other Absidia species\(^2\). It accounts for perhaps 2 to 3 per cent of culture confirmed cases of zygomycete infection. Subcutaneous infection due to A. corymbifera in a diabetic patient with multiple discharging sinuses has been reported from Chennai\(^2\). Post-traumatic cutaneous and subcutaneous infections due to A. corymbifera have been also reported\(^24\,25\).

Saksenaea vasiformis resembles A. elegans in gross colony morphology and in its inability to sporulate on routine culture media. However, the morphology of sporangia (flask shaped) on spore induction differentiates it easily from A. elegans. The first case of subcutaneous zygomycosis caused by S. vasiformis in India was reported in a rice mill worker in 1988\(^2\). More cases were subsequently been reported from Chandigarh in 1997\(^2\). A fatal case of necrotising fasciitis due to S. vasiformis has been reported from Visakhapatnam\(^28\).

Diagnosis of cutaneous zygomycosis is based clinically on the rapidly invasive course of the disease and demonstration of broad, aseptate or sparsely septate hyphae with right angle branching often with angioinvasion on histopathology and direct examination. Specimen should be taken from the leading edge rather than the centre of the lesion\(^29\). The tissue biopsy should be large enough to contain viable fungus. Zygomycetous fungi have primitive coenocytic hyphae that will often be damaged and become non-viable during the biopsy procedures or by the chopping up or tissue grinding process in the laboratory. That is why zygomycetous fungi that are clearly visible in direct microscopic or histopathological mounts are often difficult to grow in culture from clinical specimens\(^29\). This could be the reason why three of our cultures turned out to be sterile, despite best efforts to isolate the fungus while direct microscopic and histopathological mounts were positive for fungal hyphae suggestive of zygomycosis. So, in case of clinical suspicion, culture for zygomycetes should specifically be requested for, because homogenization of tissue in the laboratory can result in fungal destruction.

Treatment requires prompt and aggressive surgical debridement supported by administration of systemic antifungals and reversal of underlying risk factors\(^29\). Lipid formulations of amphotericin B are the treatment of choice. Posaconazole, a new triazole, can be a good alternative in patients with amphotericin B associated toxicity\(^30\). Hyperbaric oxygen has been a component of successful therapy in a few cases\(^31\). Cytokines such as gamma interferon and granulocyte-macrophage colony-stimulating factor increase the ability of phagocytes to kill agents of zygomycosis in vitro but their role as adjunctive therapy for zygomycosis is still understudied. Surgical debridement and antifungal therapy should be continued until follow up evaluation shows no residual fungal infection or necrosis\(^32\).

Zygomycosis must be considered in the differential diagnosis in any patient presenting with progressive, necrotic lesion whether immunocompromised or not. There is a need for awareness among the clinicians to have high index of suspicion to establish appropriate diagnosis at an early stage of the disease so that specific treatment can be instituted in time.

References


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