Case Report

Introduction

Zygomycetes are filamentous fungi comprising two orders, Mucorales and Entomophthorales. Rarely, the genus Basidiobolus of the order Entomophthorales produces deeply invasive fungal infections (Wasim Yusuf et al., 2003). Basidiobolus ranarum is an environmental saprophyte found worldwide and isolated from decaying vegetation, foodstuff, fruits and soil, as well as from the gastrointestinal tracts of reptiles, amphibians, fish and insectivorous bats (Bigliazzi et al., 2004).

Basidiobolomycosis, caused by B. ranarum, is usually a subcutaneous infection, transmitted by traumatic inoculation, and gastrointestinal involvement is rare (van den Berk et al., 2006). To the best of our knowledge, fewer than 50 cases of gastrointestinal involvement with B. ranarum have been reported in the English literature to date (Vikram et al., 2012).

Here, we report our experience with 14 patients diagnosed with gastrointestinal basidiobolomycosis (GIB) over the last 10 years. Details of three of these patients have been published previously (Geramizadeh et al., 2007). However, we included these three cases here because they had the longest duration of follow-up.

Abbreviations: ESR, Erythrocyte sedimentation rate; GIB, gastrointestinal basidiobolomycosis.

Case reports

Over a period of 10 years (2002–2012), 14 cases of gastrointestinal zygomycosis with features characteristic of B. ranarum were diagnosed in university-affiliated hospitals at our centre at the Shiraz University of Medical Sciences, Iran, which is the largest referral centre in the south of the country. The cases comprised 12 males and two female patients. The mean age was 13.1 years (range 13 months to 52 years).

Table 1 shows the most important clinical findings of these patients. The most common presenting symptom was abdominal pain (100%), but other symptoms such as diarrhoea (21.4%), constipation (14.3%) and abdominal distension (14.3%) were also noted.

In all 14 patients, preliminary diagnosis before surgery was a neoplastic process, especially intestinal lymphoma. These diagnoses were based on pre-operative imaging studies, especially ultrasonography, which showed intestinal wall thickening or multiple intestinal masses.

One of the cases (no. 11) presented with simultaneous intestinal and liver masses (Fig. 1). A liver biopsy revealed granuloma with severe eosinophilic infiltration. An intestinal mass in this patient was also resected and the diagnosis of GIB was made after histopathological examination of the involved segment (Fig. 2a).
### Table 1. Important characteristics of the study patients with GIB

The laboratory findings shown in the table were obtained before surgery. WBC, White blood cells; ESR, erythrocyte sedimentation rate.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical presentation</th>
<th>Site of involvement</th>
<th>WBC  (ml⁻¹)</th>
<th>Eosinophils (%)</th>
<th>Haemoglobin (g dl⁻¹)</th>
<th>ESR  (mm h⁻¹)</th>
<th>Culture</th>
<th>Duration of follow-up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37 years</td>
<td>M</td>
<td>Abdominal pain</td>
<td>Colon</td>
<td>20 000</td>
<td>10</td>
<td>9.5</td>
<td>50</td>
<td>Negative</td>
<td>6 months</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>28 years</td>
<td>M</td>
<td>Abdominal pain</td>
<td>Colon</td>
<td>14 000</td>
<td>10</td>
<td>9</td>
<td>62</td>
<td>Negative</td>
<td>2 years</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>15 months</td>
<td>F</td>
<td>Abdominal pain and distension</td>
<td>Stomach, colon, mesentery</td>
<td>23 000</td>
<td>17</td>
<td>8.8</td>
<td>61</td>
<td>Negative</td>
<td>None</td>
<td>Died</td>
</tr>
<tr>
<td>4</td>
<td>5 years</td>
<td>M</td>
<td>Abdominal pain, weight loss</td>
<td>Colon</td>
<td>21 300</td>
<td>20</td>
<td>8.7</td>
<td>72</td>
<td>Negative</td>
<td>8 months</td>
<td>Alive</td>
</tr>
<tr>
<td>5</td>
<td>5 years</td>
<td>M</td>
<td>Abdominal pain</td>
<td>Colon</td>
<td>16 100</td>
<td>10</td>
<td>10</td>
<td>33</td>
<td>Negative</td>
<td>3 years</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>2 years</td>
<td>M</td>
<td>Abdominal pain and diarrhoea</td>
<td>Terminal ileum, colon</td>
<td>17 400</td>
<td>20</td>
<td>10</td>
<td>45</td>
<td>Negative</td>
<td>6 months</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>52 years</td>
<td>M</td>
<td>Abdominal pain, diarrhoea and vomiting</td>
<td>Colon</td>
<td>17 000</td>
<td>15</td>
<td>9</td>
<td>58</td>
<td>Negative</td>
<td>6 months</td>
<td>Alive</td>
</tr>
<tr>
<td>8</td>
<td>16 years</td>
<td>M</td>
<td>Abdominal pain</td>
<td>Colon</td>
<td>11 500</td>
<td>14</td>
<td>9</td>
<td>40</td>
<td>Negative</td>
<td>2 years</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>16 months</td>
<td>M</td>
<td>Abdominal pain and diarrhoea</td>
<td>Stomach, small intestine, colon, mesentery</td>
<td>18 200</td>
<td>8</td>
<td>9</td>
<td>110</td>
<td>Negative</td>
<td>1.5 months</td>
<td>Died</td>
</tr>
<tr>
<td>10</td>
<td>13 months</td>
<td>M</td>
<td>Abdominal pain and bloody stool</td>
<td>Colon</td>
<td>23 000</td>
<td>16</td>
<td>9</td>
<td>81</td>
<td>Negative</td>
<td>1 year</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>42 years</td>
<td>F</td>
<td>Abdominal pain</td>
<td>Small intestine, colon</td>
<td>17 000</td>
<td>15.9</td>
<td>9</td>
<td>48</td>
<td>Positive</td>
<td>1 month</td>
<td>Alive</td>
</tr>
<tr>
<td>12*</td>
<td>2.5 years</td>
<td>M</td>
<td>Abdominal pain and constipation</td>
<td>Colon</td>
<td>26 800</td>
<td>10</td>
<td>10.6</td>
<td>50</td>
<td>Not performed</td>
<td>10 years</td>
<td>Alive</td>
</tr>
<tr>
<td>13*</td>
<td>2 years</td>
<td>M</td>
<td>Abdominal pain and distension</td>
<td>Colon</td>
<td>29 400</td>
<td>16</td>
<td>9.1</td>
<td>55</td>
<td>Not performed</td>
<td>7 years</td>
<td>Alive</td>
</tr>
<tr>
<td>14*</td>
<td>18 years</td>
<td>M</td>
<td>Abdominal pain and constipation</td>
<td>Small intestine, colon</td>
<td>12 000</td>
<td>8</td>
<td>9</td>
<td>65</td>
<td>Not performed</td>
<td>7 years</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*These three cases have been described previously by Geramizadeh et al. (2007).*
All of the patients underwent surgical procedure to resect the involved segment.

In all of the cases, upper and lower gastrointestinal endoscopy was performed before surgery, but in none of the cases was this informative.

Surgical resection of the involved segment of gastrointestinal tract was performed in all of the cases. The specimens received in the pathology department showed diffuse thickening of the wall (Fig. 2b).

The diagnosis of fungal infection was based on the characteristic histopathological findings, i.e. granulomatous inflammation of the gastrointestinal wall with prominent eosinophilic infiltration, as well as degenerative fungal hyphae demonstrating the Splendore–Hoeppli phenomenon (Fig. 3). Grocott–Gomori methenamine silver staining also showed fungal hyphae (Fig. 4).

Culture was positive in just one case (no. 11); in all other cases, culture was either not performed or was negative. In case no. 11, which was our last case, the culture turned out to be positive and was identified as B. ranarum.

In all of our cases, long-term post-operative antifungal therapy was administered comprising itraconazole with or without amphotericin B. The duration of antifungal therapy was 0–2 years.

After surgery and long-term antifungal therapy, 10 patients were alive and completely free of symptoms after 6 months to 7 years of follow-up. One of our patients (case no. 13) had one episode of recurrence that needed surgery to resect the involved segment. Unfortunately, two of our patients died (case nos 3 and 9). Both of these patients were very young (15 and 16 months of age, respectively) and had disseminated disease with widespread involvement of the upper and lower gastrointestinal tract, as well as omentum and mesentery (Table 1).
None of our cases showed any history of pica. All of the cases were thoroughly investigated regarding immunological status for human immunodeficiency virus (HIV) and chronic granulomatous disorder (CGD). They were all HIV antibody negative, and a nitro blue tetrazolium test for CGD was unremarkable in all patients. In addition, levels of CD4-positive T cells were adequate in all of the cases. There was no history of recurrent infection or any other symptoms of immunodeficiency in our patients.

All of the patients were from Fars province with a temperate climate; otherwise, we did not find any common underlying participating risk factor.

Discussion

Entomophthoromycosis is an uncommon form of zygomycosis. One of the principal species responsible for this infection is B. ranarum (Nemenqani et al., 2009). B. ranarum occurs saprophytically in decaying plant material and in the intestinal contents of several species of reptiles and amphibians such as toads (Gugnani, 1999). The fungus causes infection in immunocompromised hosts such as diabetic patients, but is also an opportunistic pathogen in immunocompetent hosts (Hussein et al., 2007). The mode of transmission is not absolutely confirmed but seems to be via major trauma and insect bites. The organism can also be transmitted from soil or vegetation that is contaminated with animal faeces (El-Shabrawi & Kamal, 2011).

Basidiobolomycosis is an unusual chronic fungal infection mostly determined by dermatological manifestations. Most cases have been reported from tropical and subtropical regions of South America, Africa and Asia (El-Shabrawi & Kamal, 2011). Visceral involvement of B. ranarum in immunocompetent hosts is very rare.

The first report of GIB was in a 6-year-old boy from Nigeria (Edington, 1964). Following that report, over the past three decades, several documented cases have been reported worldwide (Vikram et al., 2012). GIB is defined and diagnosed initially on the basis of characteristic histopathological findings in tissue specimens, or isolation of the fungus from specimens (Lyon et al., 2001).

We evaluated the socioeconomic status of our 14 patients to determine a possible common route of transmission. However, they were from different family situations, i.e. from poor, intermediate and high economic levels, and were also from different geographical areas (urban and rural) of the Fars province in the south of Iran. Most of the patients were children below the age of 18 years; we had just four adults (aged 27, 37, 42 and 52 years) and the mean age of the patients was 13.1 years.

The most common presenting symptoms were various abdominal discomfors such as abdominal pain, distension, palpable mass and constipation or diarrhoea. Some of our patients also showed fever of ~38–38.5 °C. This is the same as earlier reports (Vikram et al., 2012).

The most common site of involvement was the colorectal area, and most of the patients were operated on with a preliminary diagnosis of malignancy (El-Shabrawi & Kamal, 2011). However, there are case reports of pre-operative diagnosis of inflammatory bowel disease (Saadah et al., 2012).

Common laboratory findings in our 14 cases, and also in most previous case reports, were leukocytosis, eosinophilia and a high ESR (Vikram et al., 2012).

In most of the previous reports, and also in our 14 cases, the diagnosis was made after surgical resection and pathological study of the resected specimen, with characteristic histopathological findings: (i) mixed suppurrative and granulomatous inflammation; (ii) prominent eosinophilic infiltration in the involved tissue; and (iii) the presence of degenerate thin-walled broad hyphae surrounded by eosinophilic amorphous material (Hussein et al., 2007). In our 14 cases, fungal elements were visible in the sections examined by haematoxylin and eosin staining, and were surrounded by eosinophilic sheath, histiocytes and eosinophils (Splendore–Hoepli body), which is relatively pathognomic for B. ranarum in the gastrointestinal tract (Hussein et al., 2007). Frequently, some giant cells were also present, which had engulfed the hyphal fragments.

In most of the previous case reports of GIB, the cultures were negative and the diagnosis was made based on histopathological findings, as described above (Bigliazzi et al., 2004; Geramizadeh et al., 2007; Nemenqani et al., 2009; van den Berk et al., 2006; Vikram et al., 2012). In all of our cases, the preliminary diagnosis was made by histopathological findings; however, in one case, culture confirmed the diagnosis, whilst in the others, culture was either not performed or was negative. As a positive culture of B. ranarum was obtained in only one case, the other cases should perhaps be properly described as presumed cases of GIB; however, the histological evidence of the characteristic hyphae in the gut tissue makes it highly likely...
that all cases were due to *B. ranarum*. It is noteworthy that the isolation medium used was Sabouraud’s dextrose agar (without cycloheximide).

The role of the pathologist is critical in the diagnosis of GIB, because most of the patients are operated on with a preliminary diagnosis of malignancy and the tissue specimen is the only method of diagnosis, with no fresh tissue available for culture. In a few studies, PCR or electron microscopy has been performed for confirmation of diagnosis (El-Shabrawi *et al.*, 2011; Hussein *et al.*, 2007).

The best treatment for GIB according to our experience and previous reports is surgical resection and antifungal therapy; however, the best antifungal drug is itraconazole, and most of the *B. ranarum* isolates are resistant to amphotericin B (Vikram *et al.*, 2012). Pre-operative antifungal therapy can also be helpful for a shorter course of the disease and better post-operative recovery, i.e. resolved abdominal pain and laboratory findings such as leukocytosis and eosinophilia. In all of the patients, it took at least 1 month for the signs and symptoms to be resolved.

According to previous experience, early diagnosis is critical and life-saving, and there are previous reports of post-mortem diagnosis of GIB by autopsy (Bigliazzi *et al.*, 2004).

In conclusion, GIB should be considered as a differential diagnosis of gastrointestinal masses in patients with leukocytosis, eosinophilia and high ESR, especially in children.

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**References**


