Microsporidial Keratitis in Patients with Hot Springs Exposure

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This retrospective study included 10 eyes of 9 patients diagnosed with microsporidial keratitis. All of them were known to contract this disease after taking baths in hot springs. The disease was diagnosed based on detecting microsporidia in corneal scrapings using Gram stain and the modified Kinyoun’s acid-fast stain. The specimens from the last six patients were subjected to PCR and then sequencing. All of them revealed that the microorganism identified has a high similarity to Vittaforma corneae. Repeated debridement of the epithelial lesions successfully eradicated the microsporidial infection in all nine patients.

Microsporidia are tiny, obligate intracellular eukaryotic protozoan parasites that can produce infective spores. The organism is widespread in the environment (36), and it is considered a waterborne pathogen (9, 16, 31). Many species of microsporidia are pathogenic to animals (7, 28), and these are also confirmed to cause opportunistic infection in humans (36). These organisms cause infection not only of human digestive tracts but also of many other organs, including eyes (25, 36). In past reports, microsporidal keratitis usually was found to occur as epithelial keratitis in patients with AIDS (1, 11, 22–24, 37) and as stromal disease in immunocompetent individuals (34–35). Recently, it has been found to manifest as keratoconjunctivitis with involvement of the corneal epithelium mainly in healthy individuals (4, 13, 21, 30, 32), and it is considered an emerging ocular disease (21). To date, a number of studies were reported on the predisposing factors for microsporidal keratitis in immunocompetent individuals (2, 14, 15, 19, 21, 32). These include contact lens wearing, LASIK surgery, prior use of topical corticosteroids, and soil/mud or dirty water exposure.

Microsporidal keratitis typically manifests as disseminated, whitish, coarse punctate or target-shaped epithelial lesions (15, 21). It is usually not difficult to make the diagnosis based on the characteristic presentations. The infection can be confirmed by detecting microsporidia in the scraped corneal tissues with various staining techniques (6, 13, 15, 21). To date, a sensitive PCR technique has been developed for the diagnosis (14). DNA was extracted from the corneal scrapings using the Gentra Puregene kit manufactured by Qiagen (Valencia, CA). Pan-microsporidian primers targeting 16S rRNA were used. The PCR products were cloned with the TOPO TA cloning kit obtained from Invitrogen (Carlsbad, CA) and were sequenced by Mission Biotech (Taipei, Taiwan).

Nucleotide sequence accession number. The nucleotide sequences determined by PCR sequencing were deposited in the GenBank database under accession no. JN635526.

RESULTS

From May 2006 to April 2011, nine patients conformed to the diagnostic requirements of this study. All patients were immunocompetent. Only one patient showed bilateral involvement. There were six males and three females. They ranged in age from 23 to 71 years (mean, 54.6; median, 55). All patients disclosed having direct water contact with their eyes during bathing in hot springs spas. The length of time from spring water contact to onset of symptoms for each patient is shown in Table 1. Two patients (patients 1 and 4) bathed in hot springs frequently, from every day to three times per week. The other 7 patients bathed in hot springs between 3 days and 21 days (mean, 11.0 days; median, 14 days) before the onset of symptoms. The predominant chemical com-
position of the hot springs to which the patients were exposed was sulfate for six patients and carbonate for three patients. The nine patients bathed in at least four different spa resorts located in two different areas. They all denied having a history of ocular trauma, soil/mud exposure, or contact lens use.

The best corrected visual acuity at presentation ranged from 20/20 to 20/200 in 9 eyes of 8 patients and counting fingers in 1 patient with a history of advanced glaucoma. The initial symptoms of infection included red eye, pain, blurred vision, epiphora, and eyelid swelling.

Slit-lamp biomicroscopy disclosed mild-to-severe nonpulverulent conjunctivitis in all patients. The typical, multiple, whitish, round, target- or bizarre-shaped, slightly raised epithelial lesions, varying in size and having no significant stromal involvement, were noted in 9 eyes of 8 patients (Fig. 1A). Only one patient (patient 4) presented with atypical pattern of epithelial lesions with diffuse, whorl-like, fine or coarse punctate appearance (Fig. 1B). Three patients underwent superficial keratectomy with removal of almost the whole of the involved epithelium with the whitish lesions. The other 6 patients underwent debridement with removal of diseased epithelium only, by using cotton swabs.

Gram stains of the corneal scrapings revealed oval-shaped, Gram-positive microorganisms (Fig. 2A). Modified Kinyoun’s acid-fast stains showed bright red spores (Fig. 2B). No bacteria or fungi were observed in all the smears. The specimens of the last six cases were subjected to microsporidial PCR examinations which all showed positive results (Table 1; Fig. 3). Subsequent sequencing of the PCR products disclosed high similarity (98% to 100%) with that of Vittaforma corneae (www.ncbi.nlm.nih.gov/GenBank) in all of the 6 cases. The nucleotide sequences were assigned accession no. JN635526 in the GenBank database. The sequences of the sample rRNA gene aligned with the reference Vittaforma corneae sequence are provided in the supplemental material.

The patients were followed every day or every other day until the epithelial lesions were completely absent. New lesions ob-

### Table 1: Clinical characteristics of nine patients with microsporidial keratitis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Onset of disease</th>
<th>Hot springs chemical composition</th>
<th>Gram stain</th>
<th>Modified Kinyoun’s acid-fast stain</th>
<th>PCR</th>
<th>Treatment</th>
<th>Time to resolution (days)</th>
<th>Initial VA</th>
<th>Final VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71/M</td>
<td>&lt;3</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>D × 3, N, FML, GMO</td>
<td>4</td>
<td>20/63 → 20/20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23/M</td>
<td>7</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>D × 3, N, FML, Duratear</td>
<td>5</td>
<td>20/63 → 20/20</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>55/M</td>
<td>21</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>D × 5, N, FML, GMO</td>
<td>7</td>
<td>20/200 → 20/20</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>52/M</td>
<td>&lt;3</td>
<td>Carbonate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 1, N, EMO</td>
<td>5</td>
<td>20/200 → 20/40</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>55/F</td>
<td>14</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 1, CM, GMO</td>
<td>2</td>
<td>20/20 → 20/20</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>48/F</td>
<td>5</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 4, N, FML, GMO</td>
<td>6</td>
<td>20/25 → 20/20</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>57/M</td>
<td>14</td>
<td>Sulfate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 1, C, EMO</td>
<td>12</td>
<td>CF → 20/200 at 1 m</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>68/F</td>
<td>21</td>
<td>Carbonate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 4, N, FML, Duratear</td>
<td>6</td>
<td>Right: 20/20 → 20/20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>62/M</td>
<td>3</td>
<td>Carbonate</td>
<td>+</td>
<td>+</td>
<td>Vc</td>
<td>D × 3, N, FML, Duratear</td>
<td>7</td>
<td>20/100 → 20/20</td>
<td></td>
</tr>
</tbody>
</table>

a M, male; F, female.
b Days from last hot springs exposure to onset of symptoms.
c Bilateral eyes were involved.
d +, microsporidial spores were present; ND, PCR was not done; Vc, Vittaforma corneae was confirmed.
e D, debridement (× number of debridements); N, 0.3% norfloxacin eye solution; FML, 0.02% fluorometholone; GMO, 0.3% gentamicin eye ointment; EMO, 0.5% erythromycin eye ointment; CM, 0.25% chloramphenicol eye solution; C, 0.3% ciprofloxacin eye solution.
f Time to resolution is defined as the duration from starting treatment to observing complete disappearance of whitish epithelial lesions with no subsequent recurrence.
g VA, best-corrected visual acuity; CF, counting fingers.

**FIG 1** Slit-lamp photography. (A) Patient 1, showing typical patterns of whitish, small round, or larger target- or bizarre-shaped epithelial lesions. (B) Patient 4, showing atypical pattern of epithelial lesions with diffuse, whorl-like, fine or coarse punctate appearance.
served were removed with cotton swabs. The three patients who underwent total epithelium removal did not show reappearance of the lesions during and after complete healing of the epithelium. The other six patients underwent debridement from 3 to 5 times (median, 3.5 times). We also prescribed topical antibiotics (0.3% norfloxacin, 0.3% ciprofloxacin, 0.25% chloramphenicol) to prevent secondary infection, topical corticosteroids (fluorometholone 0.02%) to diminish subsequent nummular keratitis, and/or topical lubricant eye drops and ointments to enhance epithelium healing (Table 1). The clinical symptoms and signs improved rapidly. The epithelial lesions disappeared in around 1 week (median, 6 days; range, 2 to 12 days) and did not recur in all patients after then. Topical fluorometholone 0.02% was used in 6 patients for 5 to 30 days (median, 16 days). Patients with nummular anterior stromal infiltrates received a longer period of topical corticosteroids. At the follow-up (median, 1 month; range, 1 month to 47 months), no patient showed sequelae that would compromise the best-corrected visual acuity.

DISCUSSION

The emerging prevalence of microsporidial keratitis has been recently reported in Singapore (21) and India (5). A seasonal trend toward this disease onset during the rainy (monsoon) seasons in tropical areas was suggested (21, 26). Those cases in their series, however, were caused most likely by the contamination of the microsporidia in the soil/mud or dirty water. We have clinically diagnosed a total of 23 patients with microsporidial keratitis during the period of the last 10 years. Fourteen (60.9%) of these patients were hot springs spa attendees. Five of the 14 patients contracted microsporidial keratitis between May 2006 and June 2006. These patients’ eyes were contacted by the water in the spas due to their taking showers or submerging their faces in the hot springs. Exposure to the hot springs was never proposed as a predisposing factor to lead to the development of this disease. We are surprised to find this disease in people who have been exposed to the hot springs in health spas. As the result of economic improvement, attending hot springs spas has become a popular recreational and health care activity in Taiwan. Authorities of the Taiwan government check regularly the quality of the hot springs in spas open for the public. These checks include the pH value and the level of Escherichia coli colonies but not the presence of other microorganisms, e.g., microsporidia. The temperatures of the hot springs in the spas are usually kept at approximately 40°C (104°F). Results obtained from an in vitro study indicated that the microsporidial spores could be infectious even at temperatures higher than 50°C (122°F) (20). It is known that the polar tube of the microporidia is discharged through the thin anterior end of the spore, thereby penetrating into the new host cell to inoculate the infectious sporoplasm (10). Many conditions that promote spore discharge were investigated. These include shifting of pH, dehydration followed by rehydration, various cations and anions, mucin or polyanions, hydrogen peroxide, UV radiation, and calcium ionophore (36). Our patients attended two types of hot springs, one sulfate and the other carbonate in chemical nature. It is unknown whether the presence of sulfate and carbonate in concentrations higher than those in drinking water contribute to the growth of microsporidia. Other possible factors in the hot springs that may stimulate the growth of microsporidial spores remain to be determined.
The sterilization methods for hot springs are different from that for swimming pools. Chlorine-based disinfectants are commonly used for swimming pools. These disinfectants, however, are rarely used for hot springs due to the unpleasant odor. There are other methods that can be used to keep the hot springs clean. These include frequent recycling of the spring water through filters, reheating, photoirradiation, and treating with hydrogen peroxide. Microsporidia are much smaller than most other protozoan parasites. Because the spores of *Vittaforma cornea* were measured at 3.3 by 1.4 μm (25), recycling of the spring water through filters with pore sizes larger than 2 μm cannot remove the parasite. *Vittaforma cornea* was confirmed to exist in wastewater effluent that has undergone tertiary treatment by pressure filtration through mixed-medium filters (9). Therefore, recycled spring water contaminated by microsporidia and treated by passing through filters with too large a pore size can potentially be the source of infection. The effectiveness of other treatment methods on the germination and infectivity of the microsporidial spores requires further studies.

In relation to the large number of hot springs spa attendees, the incidence of microsporidial keratitis is low. Defects of the immune system have been considered one of the risk factors, as this disease was reported to occur in patients with AIDS (1, 11, 22–24, 37). However, most cases reported in recent years were healthy and serologically negative for human immunodeficiency virus (21). In the study by Loh et al., normal CD4/CD8 T-lymphocyte counts were found in all the cases tested (21). One of our patients (patient 2) also had a history of acanthamoeba keratitis associated with wearing of orthokeratology contact lenses 3 years before this microsporidial infection. His contact lens use was discontinued after the acanthamoebic infection. Infections by two parasites in this patient could have occurred coincidentally or could represent a defect of host innate immunity. Elements of innate immunity, including macrophages, immunoglobulin A, and neutrophils, are crucial in the defense against acanthamoebic keratitis (3, 12, 33). For microsporidial infection, a chain of immune responses triggered by host macrophages after microsporidial invasions is critical for host protection against parasite challenge (8, 18, 27). Epithelial barrier function is also an important host defense against parasite invasion. During collection of epithelium specimens, we noted that most patients had a very loose attachment of the entire epithelium, as we have frequently observed in cases of recurrent corneal erosion. This phenomenon could be explained as the result of a secondary change to infection. It could also be due to a preexisting defect of the host epithelium that facilitates parasite invasion (17, 29). None of our patients, however, had a history of previous recurrent corneal erosions. The chemicals in the vapor or water of the hot springs may have caused the detrimental effects to the corneal epithelium and further jeopardized its barrier function.

The clinical features of microsporidial keratitis are unique. As stated, these are characterized as disseminated, whitish, coarse punctate, or larger target- or bizarre-shaped epithelial lesions (21) (Fig. 1A). It is not difficult to make a correct diagnosis based on these clinical appearances. One of our patients (patient 4), presented with diffuse, whorl-like, fine to coarse punctate epithelial lesions, as discussed in Results. These features are generally seen in patients with toxic keratopathy or poor ocular surface condition. We observed features similar to these in some other patients who took baths frequently in hot springs. Corneal smears and PCR studies on microsporidia were done on two of them. One took baths in sulfate hot springs and the other in carbonate hot springs. The results were all negative. This atypical presentation of microsporidial keratitis has not been reported previously. It is possibly a result of combined irritations from the hot springs. Corneal smears and additional molecular diagnostic procedures, therefore, appear to be important for differential diagnosis.

The results of our studies indicate that *Vittaforma cornea* is the species that caused keratitis in those hot springs spa attendees. Whether there are factors present in the hot springs that make attendees more susceptible to microsporidial invasion requires further investigation.

REFERENCES


