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Epidemiologic aspects and clinical outcome of fungal keratitis in southeastern Brazil

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PURPOSE. *Fungal keratitis (FK) is a sight-threatening disease, more prevalent in developing regions. The present retrospective study was conducted in order to evaluate the epidemiologic and clinical aspects and the progression of FK in patients treated at two ophthalmologic reference centers in Southeast Brazil.*
METHODS. *The charts of patients with infectious keratitis treated between 2000 and 2004 were reviewed. For the 66 cases of FK confirmed by microbiological analysis, data related to patient, disease, and therapeutic approaches were obtained.*
RESULTS. *Mean patient age was 40.7±16 years. Fifty-three were men and 13 were women. Ocular trauma occurred in 40% of cases (27). Previous medications taken by the patients were quinolone in 72.5% and antimycotics in 30%. Visual acuity (VA) at presentation was >0.3 in 16% and <0.1 in 74.5%. Penetrant keratoplasty was performed in 38% and evisceration in 15%. The causing agents were Fusarium sp in 67%, Aspergillus sp in 10.5%, and Candida sp in 10%. Medication alone resolved 39% of cases within a mean period of 24.5±12 days. Final VA was >0.3 in 28%, and <0.1 in 63%.*
CONCLUSIONS. *Fungal keratitis presented as a disease with severe complications, predominantly among young males, and was mostly caused by filamentous fungi. The present information permits the establishment of preventive strategies. Reducing the time between onset and treatment and using more accessible specific medication would reverse the negative prognosis. (Eur J Ophthalmol 2009; 19: 355-61)*

KEY WORDS. *Fusarium* sp*, Penetrant keratoplasty, Microbial keratitis, Blindness prevention*

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INTRODUCTION

In general, microbial keratitis is characterized by a corneal epithelial defect with underlying stromal inflammation caused by replicating microorganisms. In some tropical areas, fungal keratitis (FK) may account for more than 50% of all cases of culture-proven microbial keratitis and of ophthalmic mycoses (1).
Most of the studies done exclusively on mycotic keratitis (2, 3) have listed trauma as the most common associated factor, occurring in 44 to 55% of patients. Less frequently reported associated factors include prolonged use of topical corticosteroids or antibacterial agents, systemic diseases such as diabetes mellitus, and preexisting ocular diseases. In developed countries, contact lens use is referred as an important associated factor (4, 5). Moreover, treatment continues to be a source of debate (6-8).
Southeastern Brazil is the area with the largest population (about 78 million people) and concentration of people (almost half the total Brazilian population) in this vast country located in South America. Although this area contains the major industrial facilities, a significant part of its econ-

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omy is based on extensive agriculture, predominantly sugar cane, coffee, and fruit monoculture or animal husbandry, with an intense involvement of manual workers.
The objective of the present study was to identify associated factors and to compare the clinical and microbiological presentation and visual outcome of patients treated in two ophthalmic reference center settings in São Paulo state, Southeastern Brazil.

METHODS

The present retrospective study was conducted on corneal ulcers treated and investigated from January 2000 to December 2004 at two tertiary reference centers located in São Paulo state, Southeastern Brazil. The inclusion criterion was the presence of FK as determined by positive microbiology result. Patients with fungal ocular infection affecting areas other than the cornea were excluded. The study was approved by the Research Ethics Committee of the hospitals and the research was conducted in accordance with guidelines for confidentiality of medical records. A total of 66 inpatient records with a relevant diagnosis of FK were identified and reviewed, 44 at the first center and 22 at the second one. Sometimes there were missing data and the results were expressed in a percentage of totals observed.
Epidemiologic and clinical data were used for the present study. The patients included in the study may self-present but are also referred by general practitioners and ophthalmologists. When the patients were sent by other professionals, data were obtained from their reports and directly from the patient. The cases were grouped according to documented associated factors and the groups were compared in terms of clinical presentation, epidemiology, microbiological data, hospital resource use, and vision loss. Data collected included age, sex, information on the history of the infectious process, as well as the use of corticosteroids, topical antibiotics, previous eye surgeries or any immunosuppression condition, or use of contact lenses.
Initial and final visual acuity (VA) was recorded and divided into three categories: blindness, ranging from no light perception to Snellen 0.1; subnormal vision, ranging from 0.1 to 0.3; and normal vision, when better than 0.3.
In all cases, collection of corneal scrapings for analysis was carried out directly from the base and margin of the ulcers aseptically using a metal blade under direct vision through a slit lamp after instillation of anesthetic eyedrops (0.5% proxymetacaine hydrochloride, Anestalcon®, Alcon Labs, Brazil). Direct microscopy was performed on 10% KOH wet mounts and Gram stains. The material obtained by next scrapes was directly inoculated onto the surface of solid media such as chocolate agar, Sabouraud dextrose agar, and also in liquid media such as thioglycollate medium. All laboratory methods were performed under standard protocols, which have been discussed in detail in previous studies (9, 10). The microbiology results were considered positive when smear results were consistent with culture, or growth of the organism was demonstrated in two or more media with negative smear results, or after repeated appearance in smears with negative culture results. The fungal etiology was defined if microbiology results were considered positive, or if fungal hyphae were observed in corneal tissue, but failed to grow in culture or isolation in smear.
Patients were treated with amphotericin B 0.15% and natamycin 5% used topically. Surgical therapy was applied when no response to clinical therapy was observed. PKP was performed in presence of corneal melting or ocular perforation. Conjunctival flap was applied when there was no margin to PKP. Evisceration was performed in presence of severe endophthalmitis or after multiple failures in PKP.
Statistical analysis was done using the Modules Exact test of SPSS (SPSS Inc., Chicago, IL) for descriptive statistics.

RESULTS

Epidemiologic findings

During the study period, 478 cases of clinically diagnosed infectious keratitis were conducted in both centers. Epidemiologic data were obtained for 66 cases microbiology positive for FK, which represented 36.6% (66/180) of clinical diagnosed cases of FK. The total FK clinical diagnosed represented 37.6% (180/478) of all infectious keratitis and the microbiology positive FK cases represented 13.81% (66/478) of all infectious keratitis from which microbial samples were collected. The yearly distribution was 9 cases in 1999, 11 in 2000, 15 in 2001, 12 in 2002, 9 in 2003, and 10 in 2004.
The male: female ratio was 4.1:1. The most common age group affected was between 31 and 50 years, mean pa-

tient age was 40.7 ± 16 years. The youngest patient was 6 years old and the oldest was 77 years old. No case of bilateral fungal infection was observed. Epidemiologic features are presented in Table I.

Table II illustrates the seasonal variation in the occurrence of FK. Incidence tended to be higher during the winter and spring (June to November in the Southern Hemisphere) than during summer and fall (December to May). Time since beginning of symptoms until seeking any kind of treatment is shown in Table III. The duration of symptoms prior to arrival at our centers was documented in 61 patients, represented in Table IV.

TABLE I - EPIDEMIOLOGIC ASPECTS OF PATIENTS WITH FUNGAL KERATITIS TREATED IN SOUTHEAST BRAZIL BETWEEN 2000 AND 2004

	No.	Percent
Sex	66	100.00
Male	53	80.30
Female	13	19.70
Recent injury	63	100.00
Plant	12	19.01
Other	14	22.20
Absent	37	58.70
Medication	66	100.00
Antibacterial	45	68.2
Antifungal	12	18.2
Antiviral agents	10	15.2
Steroids	8	12.1
Combined treatment	6	9.0
More than one medication	26	39.4
No medication	16	24.24
Prior ocular surgery	66	100.00
Present	3	4.50
Absent	63	95.50
Ocular predispose factor	66	100.00
Present	8	12.10
Absent	58	87.90
Systemic predispose factor	66	100.00
Present	5	7.60
Absent	59	92.40

Microbiological data

Filamentous or mycelia fungi were shown in 60 (91%) cases and yeasts in 6 (9%) cases. Among the 66 specimens, the most frequent agent isolated was *Fusarium* species, followed by seven other genera, as detailed in Table V.

Clinical findings and therapeutic approaches

At presentation, 34 patients (54.8 %) were defined as blind, 18 (29.0%) were found to have subnormal vision, and 10 (16.1 %) to have normal vision. After treatment, the blind category was reduced to 29 (51.8%), the subnormal vision category to 11 (19.6 %), and normal vision category increased to 16 (28.8%) individuals. Thus, visual improvement to at least one upper category occurred in 30 patients (63.0%) and reduction occurred in 24 (44.4%). Therapeutic penetrating keratoplasty (PKP) was performed in 26 eyes (39.4%), a conjunctival flap was applied in 7 (10.6%), and evisceration was performed in 10 (15.2%). Among the 26 eyes receiving PKP, fungal infection recurred in 6 (23.07%), 3 had a second PKP, 2 received a conjunctival flap, and 4 had evisceration. PKP was performed between the 1st and 40th day of treatment, at 14.96 days on average. Among the above six cases of recurrence after PKP, the second surgery was performed between the 8th and 52nd day of treatment, at 25.8 days on average. We followed 23 of 26 eyes that received PKP. During follow-up (1 year ± 3 months), 11 eyes (47.8%) remained clear after PKP, without immune rejection. At last follow-up, the best-corrected VA after PKP was between 20/200 and 20/40 in 5 patients and better than 20/30 in 2 patients.

TABLE II - SEASONAL VARIATION IN THE OCCURRENCE OF FUNGAL KERATITIS IN SOUTHEAST BRAZIL BETWEEN 2000 AND 2004

Season	Absolute frequency	Relative frequency (%)	SE	95% CI
Fall	12	18.2	0.0478	(8.6; 27.7)
Winter	21	31.8	0.0578	(20.28; 43.4)
Spring	23	34.9	0.06	(23.1; 46.7)
Summer	10	15.1	0.05	(6.3; 24.0)
Total	66	100.0	—	—

TABLE III - TIME SINCE BEGINNING OF SYMPTOMS UNTIL SEEKING ANY KIND OF TREATMENT FOR FUNGAL KERATITIS IN SOUTHEAST BRAZIL (2000–2004)

Duration (days)	No. of patients	Percentage
0–7	29	70.73
8–15	8	19.51
16–30	2	4.88
31–90	2	4.88

TABLE IV - DURATION SINCE BEGINNING OF SYMPTOMS UNTIL PRESENTATION AT TWO REFERENCE CENTERS IN SOUTHEASTERN BRAZIL (2000–2004)

Duration (days)	No. of patients	Percentage
0–7	18	29.5
8–15	13	21.3
16–30	24	39.3
31–90	5	8.2
>90	1	1.6

TABLE V - FUNGAL ISOLATION IN CULTURE FROM PATIENTS WITH FUNGAL KERATITS AT TWO REFERENCE CENTERS IN SOUTHEASTERN BRAZIL (2000–2004)

Fungi	No.	Percent
Fusarium sp	44	66.7
Aspergillus sp	7	10.6
Candida sp	6	9.1
Penicillium sp	3	4.6
Cladosporium sp	3	4.6
Cylindrocarpon sp	2	3.0
Acremonium sp	1	1.5

DISCUSSION

The present study evaluated epidemiologic and clinical aspects and the progression of cases of FK treated at two reference ophthalmologic centers in Southeastern Brazil. Associated factors such as delayed diagnosis, poor responses to antifungal agents, and low availability of these drugs make FK an important cause of corneal-related visual loss in many South American developing countries, as pointed out in previous studies and confirmed here (Tab. VI).

As shown in other South American studies, *Fusarium* sp and *Aspergillus* sp were the pathogens most frequently isolated in FK. In fact, they are the most frequently reported fungal pathogens isolated from cases of FK in the tropics over the last decades (11). *Fusarium* sp have also been found to be the principal fungal pathogen in Florida, Nigeria, Tanzania, Hong Kong, and Singapore. *Aspergillus* species predominate in Northern India, Nepal, and Bangladesh (12-16). This phenomenon may be explained by differences in climate and in the natural environment (17). In addition, *Candida* species have been reported to be most common in developed countries of temperate climate (18, 19).

In the present study, FK was reported more frequently during the winter and spring than during the summer and fall, Brazilian harvest season. In other studies, a significant increase in the number of reported cases of suppurative keratitis was observed during the harvest period and windy seasons (17, 20). Other authors have made similar observations, noting an increase in cases of FK during the dry, windy seasons compared with the wet, humid months of the year.

Male patients, in the middle decades of life, predominated in our series. This is usually attributed to the fact that men in this age are more actively involved in outdoor activities, which increase their vulnerability to this disease (21). Undoubtedly, the most common predisposing factor for corneal ulceration in Southeast Brazil, and probably other areas, is vegetal corneal injury, as also observed in other studies (22-24).

It is of interest that one third of the present patients did not receive any kind of treatment within 1 week of the onset of symptoms and a half spend more than 2 weeks to reach specialized treatment, which may reflect socioeconomic barriers to access to eye health care and/or the fact that ocular injury will often resolve spontaneously, with its real frequency not being recorded in this population. Before their initial visit to our hospitals, referred from primary and secondary health care centers, more than two thirds of the present patients had used topical medication. As is also the case for developed countries with better health systems, this reflects the high level of unnecessary prescription (25). Long-term use of antibiotics and corticosteroids is believed to compromise the cornea and to worsen the disease condition in FK, although a

TABLE VI - RECENT DEMOGRAPHICS OF FUNGAL KERATITIS IN SOUTH AMERICA

Place, study, population, duration (reference)	Demographic details	Fungus/total infectious keratitis	Associated factors	Principal isolates in culture (% of total)
Asunción-Paraguay: 146 children under 18 years with infectious keratitis, 1988 to 2002, 14 years (22)	Details not provided	Cultures positive in 113 (77%), 70% bacterial and 30% fungal	Rural area, 45 (30.8%); trauma, 64 (43.8%)	*Acremonium sp* (25%), *Fusarium sp* (14%), *Aspergillus fumigatus* (14%)
Curitiba-Paraná, Brazil: 49 patients, from 1975 to 2003, 14 years (23)	Male/female ratio, 5:1; average, 49 years; farm workers (46%)	16.4% (49/298)	Rural area (71.4%), trauma (57%), immunosuppression (18.4%)	*Fusarium sp* (32%), *Aspergillus sp* (16.5%), *Penicillium sp* (10%)
São Paulo, Brazil: 275 patients with fungal keratitis, from 1983 to 1997, 28 years (24)	Male/female ratio: 2:1	Details not provided	Filamentous fungi: trauma in 79 (36.6%), vegetal trauma in 46/79 (58.2%), contact lenses in 6.5% (14/216); yeasts: trauma in 4 (8.2%), contact lenses in 8.2% (4/49)	Total positive fungal cultures, 275; *Fusarium* (n=137; 58.8%), *Aspergillus* (n=28; 12.0%), *Penicillium* (n=12; 5.2%), yeasts: *Candida* (n=59; 93.7%)
Paraguay, 45 patients with microbial keratitis, 1 year (32)	Details not provided	Details not provided	Details not provided	Total fungal culture positives, 26; *Fusarium sp*, 11 (42%)

larger series would be necessary to confirm this observation (26).

There was some limitation to the method of estimating vision loss employed here, since there was no knowledge of pre-event VA in the affected eye. There was a significant portion of patients with severe and moderate vision loss. For most cases reviewed, documented vision loss was largely attributable to corneal scarring. It was encouraging to observe that, before referral, efforts were made on the part of these patients to treat this disease, which otherwise would progress to complete blindness and culminate with evisceration.

The literature does not contain a detailed analysis of the use and justification for performing PKP in patients with FK. Some studies have suggested that early surgery is preferable and others have suggested that an early diagnosis and antifungal therapy are critical for controlling FK (3, 20, 27, 28). Compared to the whole group, PKP patients had the worst outcome, although it should be pointed out that patients undergoing PKP had a more complicated disease course.

Although not supported by statistical analysis due to the limited sample size, the present report suggests that patients who presented with poor VA, independent of the time of onset of the disease, frequently presented large ulcers that are often refractory to antifungal therapy and therefore were more likely to undergo complications and PKP.

The present study confirms the associated factors for a poor outcome of FK at reference centers despite standard treatment (2). We also attempted to highlight the importance of suspicion as the key to an early diagnosis of FK. An early use of antifungal therapy based on clinical impressions, as recently suggested (29), would help to improve the results, but is limited by the few and restricted accessible formulas for antifungal therapy. Moreover, as previously suggested and confirmed here, at reference centers the clinical findings are modified by previous clinical interventions (30).

We also observed a trend for earlier PKP in the most

complicated cases, although larger comparative studies are necessary to confirm the benefits of this procedure (31). Adequate protection during outdoor activities and the availability of topical antifungal medication are practical means that may reduce or prevent the incidence of this disease.

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