

Reflections on mycotic keratitis based on findings from histopathologically examined specimens

Reflexões sobre a ceratite fúngica por meio dos achados de exames histopatológicos

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ABSTRACT

Purpose: The study of fungal invasion and pathogenicity in corneal tissue observed through the histopathological examination of specimens obtained through penetrating keratoplasty ('PKP') of samples obtained from an Eye Bank ('EB'), with the aim of applying findings in diagnosis and treatment of the condition. **Methods:** Retrospective non-comparative case studies on samples collected between January 2006 and June 2011 based on identification data comprised of scant historical information sent by surgeons and material obtained through PKP, consisting of 38 samples from 35 patients. Processing involved special stains for fungi in order to detect the presence thereof, with one to three colourations being performed in accordance with diagnostic difficulty in relation to each sample. **Results:** Patients were predominantly male (20 compared to 15 females), and the most represented age group was 60+ years of age (1/3 of the patients). Mycotic keratitis was detected in 6.4% (n= 597) of cases referred to the EB and in 1.65% (n= 2310) of transplants using corneal material provided by the EB over the last five years. According to historical information provided by surgeons, 39.5% (n= 38) of cases were due to perforation of the cornea. A statistical table was prepared using transplant data. 11 specimens (n= 38) were due to an anterior corneal graft. Yeasts were present in 63% (n= 38), and 50% (n= 38) of corneal tissue had mild or non-existing inflammation. 13% (n= 38) had whole Descemet layers, while 45% (n= 38) presented fungi on the corneal surface. **Conclusion:** Corneal grasping and confocal microscopy may be performed successfully after treatment has been initiated, although in corneal ulcers samples should ideally be collected with a spatula for laboratory testing *in vivo*. The high prevalence of yeasts in the samples we looked at may be due to morphologic changes in corneal tissue of fungal origin. Intraocular penetration of the fungi is facilitated by changes to the Descemet layer, and assisted by the fungi's own properties. Therefore systemic treatment is justified from the outset.

Keywords: Keratitis/diagnosis, Keratitis/pathology; Eye infections, fungal/diagnosis

RESUMO

Objetivo: Estudo de botões corneanos por meio do exame histopatológico para verificar as alterações ocorridas nos tecidos corneanos numa infecção fúngica e tirar desses achados orientações para o diagnóstico e o tratamento. **Métodos:** Trabalho retrospectivo, realizado num Banco de Olhos (BOO) entre janeiro de 2006 e junho de 2011, usando dados de prontuários a partir das informações enviadas pelos cirurgiões e sendo examinado material recebido de ceratoplastia penetrante com o exame de 38 peças de 35 pacientes, sendo processadas e feitas de uma a três colorações de acordo com as dificuldades diagnósticas. **Resultados:** Os pacientes eram na maioria homens, 57% (n=35), a faixa etária acima de 60 anos a mais numerosa com 1/3 dos pacientes. Os casos de ceratite fúngica correspondiam à média de 6,4% (n=597) do material recebido no BOO e 1,65% (n=2310) dos transplantes ocorrido com o material fornecido nos últimos 5 anos. Pelas informações dos cirurgiões 39,5% (n=38) dos casos deviam-se a perfuração corneana. Usando as datas dos transplantes foi feita uma Tábua de Observação. Em 11 (n=38) casos, a córnea procedia de transplante anterior. As formas leveduriformes nos tecidos corneanos eram de 63% (n=38). Em 50% (n=38) dos casos o infiltrado inflamatório era pequeno ou inexistente. A camada de Descemet estava íntegra em 13% (n=38), enquanto eram encontrados fungos na superfície corneana de 45% (n=38) dos casos. **Conclusão:** A coleta do material poderá ser feita com sucesso mesmo depois de instalado o tratamento, entretanto, nas úlceras de córnea deve ser feito preferentemente a coleta de material com espátula para exame laboratorial e a microscopia confocal *in vivo*. A predominância das leveduras poderá ser devido a alterações morfológicas do fungo sofridas no tecido corneano. A penetração intraocular é facilitada por alterações da Camada de Descemet e pela própria capacidade do fungo de penetrar nos tecidos justificando o tratamento sistêmico desde o início.

Descritores: Ceratite/diagnóstico; Ceratite/patologia; Infecções oculares fúngicas/diagnóstico

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The authors declare no conflicts of interest

Received for publication: 9/4/2012 - Accepted for publication: 29/5/2012

INTRODUCTION

The study of fungal keratitis used to be of little interest to research centres because the disease is more common in warm climates, in countries with little investment in research. The condition also used to affect rural populations, which have been decreasing in recent centuries due to urbanisation after the industrial revolution. However, the condition was described in a temperate country, where cases are rare. Fungal keratitis was first described in 1879 by Leber in a 54-year-old farmer who was working with a shredder (Dreschmaschine) when he suffered a mild corneal trauma by oat chaff (haferspelze)⁽¹⁾. In 1907, Zade presented the case of a 44-year-old female peasant and, upon consulting the literature, found 22 cases of fungal keratitis. In an Annex to the text he presented a drawing of the organism found in the laboratory, showing the appearance of *Aspergillus*⁽²⁾.

In the second half of the 20th century the number of cases increased, mostly due to the widespread use of antibiotics and topical corticosteroids and the increased number of transplants and immunosuppressed patients. This led to a relative resurgence of interest in the disease, as it started to affect urban populations.

Few published articles studied the histopathology of the cornea during fungal infections. The most comprehensive article to which we had access was Naumann et al.⁽³⁾, which presented the cases of patients in the Southern United States, African and South American countries and examined by the Armed Forces of Ocular Pathology Laboratory. Previously, Zimmerman had presented images of the corneal tissue of 7 patients using the same sample⁽⁴⁾.

There is a practical explanation for the lack of interest in the histopathology of fungal keratitis, apart from the ones presented above: the fact that histopathology does not reveal the aetiological diagnosis, which is more directly relevant for treatment according to the predominant thinking in medicine. However, as we had access to a satisfactory amount of cases, we decided to take advantage of our findings to reflect on the disease and to present suggestions aimed at minimising its harmful effects. We were moved by curiosity about what happens to the corneal tissue during this infection. The examined cases had no results of microbiological tests and little associated information, which allowed us to focus on the slides and try to learn lessons that could be of practical interest.

METHODS

The study was conducted in the Eye Bank of the Health Department of the State of Ceará, Brazil, which tracks emergencies by demanding that the corneas of recipients be submitted to the Eye Bank to undergo histopathological examination. The cases that were not urgent could be examined if the surgeon required some information about them.

The samples came only with the standard form with summary data about the case and no additional information. There was no detailed medical history, except for the clinical signs that could justify the examination, but without any chronological data describing the progression of the disease.

The samples were forwarded to the Laboratory of Pathology of the General Hospital of Fortaleza, where the Eye Bank is based. Macroscopic examination of the corneal buttons was done by an Eye Bank pathologist, who described the specimen and then sliced the material to examine all layers of the cornea.

The samples were embedded in paraffin and then cut into 5-µ thick slices. All the material was stained with haematoxylin-eosin (H&E). In cases where there remained doubts about the diagnosis of fungal keratitis, PAS (Periodic Acid of Schiff reaction), silver methenamine, Gomori's stain, and Masson's stain were used. In most cases there was no difficulty in establishing the diagnosis with routine staining (H&E), and the few doubts that remained were solved with the other stains. H&E was used alone in 30 cases (79%); H&E and silver methenamine were used in 3 cases (7.9%); H&E, silver methenamine and PAS were used in 4 (10.5%) cases; and H&E, silver methenamine and Masson's stain were used in 1 (2.6%) case.

The study began in January 2006 and ended in June 2011. 597 cases were assessed retrospectively, out of 2310 transplants done in the period. In total, 38 specimens had a histopathological diagnosis of fungal keratitis.

The medical records of patients were reviewed, extracting information provided by the surgeon about the transplant upon their request for a donor cornea.

With regard to the histopathological examination, the following data were considered: Staining, fungus morphology, general appearance of the cornea, location of the infection, appearance of Descemet's membrane, and description of the corneal inflammatory infiltrate. The data were plotted for presentation of the results.

RESULTS

The sample included 20 males and 15 females; there were three cases of relapse, which occurred in two men and one woman.

The youngest patient was 20 years old and the oldest was 81. Of these, 3 (8.5%) patients were aged 20-29 years; 8 (23%) were 30-39 years; 6 (17%) were 40-49 years; 5 (14%) were 50-59 years; and 11 (31.5%) were over 60 years — the age group with the largest number of cases.

General sample data included the annual distribution of corneal buttons obtained through transplantation in 35 patients, where 38 cases of fungal keratitis were diagnosed in the five and a half years during which the samples were submitted to the Eye Bank. Table 1 shows the number of cases of fungal keratitis as a proportion to the number of corneas submitted to the Eye Bank per year; the table also includes the number of transplants done in the period with corneas provided by the Eye Bank and the annual rate of fungal keratitis.

The information provided by the surgeons to justify the priority of their patients for transplantation is shown in Table 2.

In a large number of cases of fungal keratitis we were able to monitor the progress of the case chronologically by reviewing the medical record and the notes by the Eye Bank. In cases where a transplant was done after the initial operation where the fungal keratitis was diagnosed, it was possible to determine that the keratitis was cured. When the patient underwent a second operation and there was no evidence of relapse, the case was considered as cured; however, cases whose samples were not submitted were not counted as cured. As the number of operations seemed significant, we decided to examine it through an Observation Chart.

The table above shows that case 1 underwent two transplants without a histological diagnosis of fungal keratitis (yellow and orange) before it was diagnosed (red). In cases 2 and 10, fungal keratitis was found in two transplants, and no samples were submitted afterward (red). Cases 3, 5 and 7 had a diagnosis of keratitis, but the infection was not found in the

Table 1

Cases of fungal keratitis diagnosed by histopathological examination from January 2006 to June 2011 as a proportion to the number of samples examined per year, and the annual rate with regard to the total number of transplants

Year	Cases	Samples Examined	(%)	Transplants/Year	Rate
2006	3	74	4	253	11,9/1000
2007	2	77	2,60	382	5,2/1000
2008	5	76	6,60	444	11,3/1000
2009	17	201	8,50	443	38,4/1000
2010	6	121	4,95	465	12,9/1000
2011	5	48	10,40	323	15,5/1000
Total	38	597	6,40%	2310	16,5/1000

Table 3

Type of surgery before and after a corneal transplant in which it the fungal keratitis was diagnosed

Procedure	Number of procedures	Number of patients
Anterior transplant	11	7
Posterior transplant	5	3
Cataract+Transplant	6	3
Cataract	1	1
Corneal suture	1	1
Pterygium	1	1
None	22	22
Total	47	38

following transplant (green). In case 4, the time until diagnosis between the first and second transplant was long, and the infection was late (yellow followed by red), but the relapse was treated in less time. Still, in the chart it seems like the infection was treated for a long time, as the long period during which the patient received no treatment was included into the “transplant” column, so that we didn’t have to extend the chart, even though the recurrence was treated immediately. In cases 6, 8 and 11, the infection was noted in the second transplant but not in the first (yellow and red). In case 9 the infection was noted in the first transplant but not in the following two (two shades of green).

In the Observation Chart the data is not homogenous, therefore the dates of surgery were maintained without calculating the average interval between them. Due to the absence of chronological data about the onset of treatment, we could not determine the mean and standard deviation, as the data is largely about cases with markedly different progression.

As we had a relatively large number of operations before and after a transplant with a histological diagnosis, we assessed the reasons for the procedure, which are shown in Table 3.

In 24 (63%) cases only yeast forms were found, in 12 (32%) cases both yeast and hyphae were found, and in 2 (5%) cases only hyphae were found.

To determine the degree of vulnerability of the eyeball to a disseminated infection, we assessed Descemet’s membrane,

Table 2

Information included in the requests for donor corneas submitted to the Eye Bank among patients diagnosed with fungal keratitis

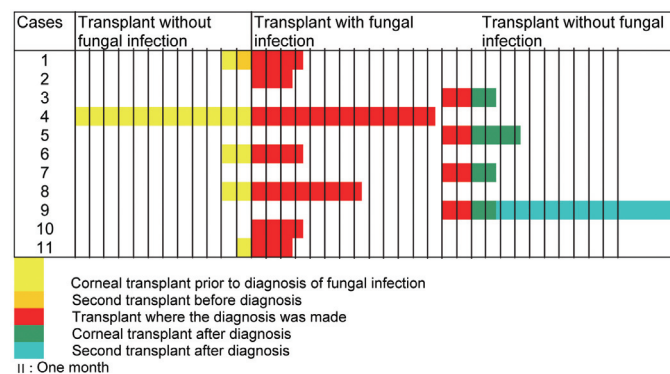
Information	N	(%)
Perforated ulcer	15	39,50
Keratitis with intense hyperaemia	15	39,50
Previous surgery	11	29
Keratitis without hyperaemia	10	26,30
Keratitis with moderate hyperaemia	8	21
Bacterial keratitis	7	18,40
Ulcer resistant to treatment	4	10,50
Fungal keratitis	2	5,30
Other	4	10,50

Table 4

State of Descemet’s membrane in corneal buttons removed from the receiver in a transplant where the fungal keratitis was diagnosed

State of Descemet’s Membrane	N	%
Normal	5	13
Ruptured	7	19
Ruptured with detachment	5	13
Detachment	6	16
Spores	13	34
Guttata	2	5
Total	38	100

Observation chart for the cohort of cases of fungal keratitis undergoing corneal transplant



which is considered one of the barriers against intraocular penetration of the parasite after rupture of the previous barrier – the epithelium and Bowman’s membrane. Table 4 shows that in only 13% of cases Descemet’s membrane was normal, and in 34% the fungus crossed the membrane, which is a cause of concern.

We also assessed the presence of an inflammatory infiltrate

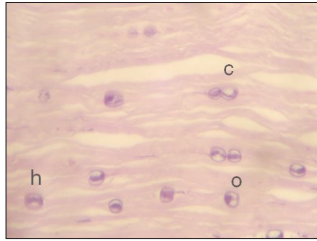


Figure 1A

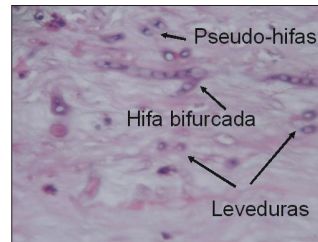


Figure 1B

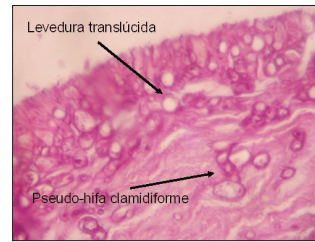


Figure 1C

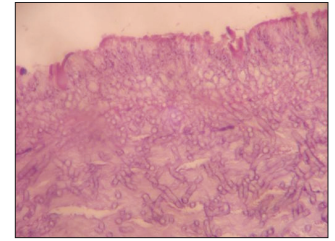


Figure 1D

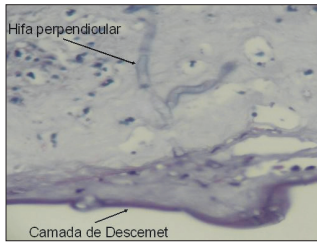


Figure 2A



Figure 2B

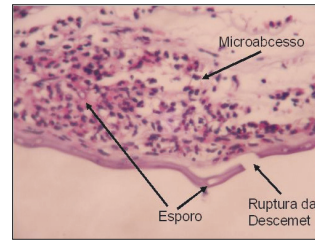


Figure 2C

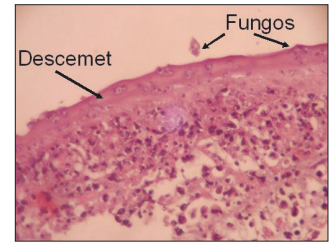


Figure 2D

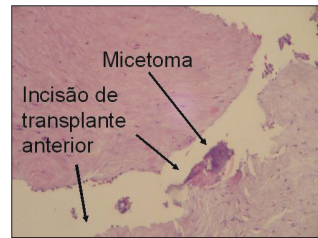


Figure 3A

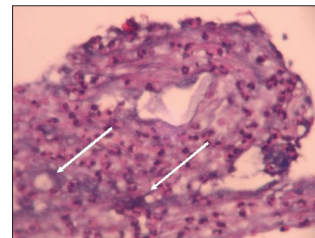


Figure 3B

and found that in half of the cases there was practically no hyperaemia, in 15 (39.5%) cases there was moderate hyperaemia, and in 4 cases there was intense hyperaemia (10.5%).

Many of the corneal buttons had fungi on the corneal surface, on the de-epithelised area or within ulcers. In total, 17 (45%) samples had superficial infections and 21 (55%) had deep infections.

As shown in Figures 1A, 1B and 1C, fungal organisms can have different shapes. Figures 1C and 1D show the parasite on the surface of the cornea both as yeast and mycelia, which consist of hyphae.

Figure 1 - A) Yeast of different appearance: (c) crescent-shaped nucleus, (o) triangular-shaped nucleus; (h) dumbbell-shaped nucleus. Note the absence of inflammatory reaction (HE x 200); B) Hyphae, pseudo-hyphae and septate hyphae, as well as crescent-shaped yeast. Note the absence of inflammatory reaction (HE x 200); C) Yeast on the de-epithelised surface and, deeper, translucent yeast with a thick capsule and a Chlamydia-shaped pseudo-hypha consisting of one larger and four smaller yeast cells (HE x 200); D) Mycelium on the ulcerated surface of the cornea, consisting of non-septate hyphae (HE x 120).

Deeper, beyond the hyphae that are parallel to the surface, perpendicular hyphae can be seen, as shown in Figure 2A. Table 5 shows that in 34% of cases the Descemet's membrane had been infected either with spores, hyphae, or yeast (Figure 2B). As shown in Table 7, several changes were found in Descemet's membrane: Figure 2C shows spores close to a rupture zone, and Figure 2D shows the various stages of penetration of the fungus

into Descemet's membrane.

Figure 2 - A) A hypha parallel to the corneal surface, which is common, and a perpendicular hypha in the deepest part of the stroma. Descemet's membrane has spores in its structure, with folds and increased thickness (HE x 200); B) Septate hypha within Descemet's membrane, underlying a microabscess in the innermost part of the corneal stroma (HE x 120); C) Microabscess in the innermost part of the corneal stroma, where a spore can be seen. Descemet's membrane is ruptured, and even though penetration could be easier through this breach, the fungus has penetrated the membrane's structure, showing its slow progression coupled with a great capacity of penetration. (HE x 120); D) Fungal spores, of which one has partially penetrated into the anterior chamber and the other is fully within the chamber (HE x 200).

A less common presentation is the formation of a mycetoma, which is present at the incision site of a previous transplant, indicating a recurrence (Figure 3A). Confirming that the parasite has entered the anterior chamber of the eyeball, hypopyon with the presence of parasites can be seen in Figure 3B.

Figure 3 - A) A mycetoma, which is a cluster of fungal cells, is situated within the keratoplasty incision done before the presentation of the fungal infection (HE x 80); B) Hypopyon, with arrows indicating yeast (HE x 200).

DISCUSSION

Studies on the aetiology of keratitis follow along two lines: they either focus on prevention or on aetiology in order to

implement treatment. By studying the flora of the conjunctiva, the preventive and epidemiological aspects of the disease are highlighted, which is important now that the frequency of opportunistic infections is increasing. Due to this, the focus is more directed to bacteriology⁽⁵⁻¹²⁾, and the presence of saprophytic fungi in the conjunctiva is stressed⁽¹³⁻¹⁶⁾. Some articles focus on fungal keratitis^(17,18).

Histopathological studies on fungal keratitis are rare. Still, the condition has already been studied in Brazil⁽¹⁹⁾, although with corneal buttons obtained immediately after transplantation, as is usually the case. This paper could confirm or express a more critical view on our sample, but unfortunately tissue changes were not explored. Therefore, it only served to confirm the previous diagnosis by examining the specimens collected from ulcers.

Having received a larger number of cases of fungal keratitis than expected, from samples submitted to the Eye Bank for control of emergencies and with almost no information presented by surgeons, we sought to do our research within a restricted universe, especially given the staggering number of cases in 2009, which raised the possibility of a problem of public health. From the findings, we tried to determine which preventive measures could be adopted and/or suggested to clinical ophthalmologists and anterior segment surgeons.

Nearly all articles report the results of laboratory tests identifying the species of fungus. Being deprived of this useful resource, we had to assess tissue responses to make a rough identification of the genus. Species identification was impossible because the pieces had been fixed with neutral formaldehyde, therefore there was no material suitable for tissue culture.

Our study has no value regarding the aetiological diagnosis, but it points to clinical procedures to be adopted in the treatment of keratitis and during the postoperative period of cataract surgery and corneal transplant. As we had scarce information, we had to do our research almost like the people in the cave examining the world outside in Plato's allegory (427-348 BC) in Book VII of the Republic. To support our research, we used histopathology to confirm the diagnosis and to determine what could be offered in terms of aetiology, epidemiology, treatment and prevention of relapse.

A fact that reveals an aspect of the change that has been detected in recent years, related to the risk factors for fungal keratitis, is the predominance of opportunistic infections, and no longer of trauma with plant materials. One reason for this is that the age group most often affected is over 60 years, even though this can also be related to cataract surgery, as seen in our results.

From the epidemiological point of view, it is important to determine whether our findings represent prevalence or incidence rates. As shown in Table 1, the samples received by the Eye Bank indicate an incidence, due to the demonstration of the fungal infection. However, they can also represent a point prevalence⁽²⁰⁾. The incidence rate was lower in 2009 than in 2011, while the point prevalence in 2009 was more than 2.5 times higher than in 2011, which would serve as an alert for health authorities. We can not speak in absolute terms because many of the cases were diagnosed clinically and treated until full recovery. We can use, in an inverted sense, the quote attributed to Diogenes (404-323 BC) by the Greek philosopher Diogenes Laertius. Upon visiting a temple, Diogenes was shown a large amount of ex-votos as a proof of benevolence of the gods towards the faithful who sought their help. The philosopher then asked: How many sought the gods and did not receive what they asked? In our sample, we saw the failures without having

access to cases with satisfactory results.

The cases we studied were those which did not respond to treatment, did not undergo microbiological studies and had a clinical picture compatible with bacterial keratitis, as was explicitly stated by 7 surgeons. Only two cases were treated for fungal keratitis, without adequate response to treatment (Table 2).

The small number of preoperative diagnoses of fungal keratitis should be considered with caution. On the one hand, in half the cases the disease progressed slowly, as shown in the results section. Surgeons reported intense hyperaemia, but little inflammatory infiltrate was found in many histopathological specimens. This shows a disagreement between the justification provided by surgeons and the histopathological findings, which leads to the following epistemological question: what needs to be investigated in a prospective study?

We cannot say there was an error, due to the lack of data. Even in the 10 cases (26.3%) where there was severe ulceration with little or no hyperaemia, we can not claim incompetence or ignorance by the surgeons as we do not have the medical record showing the disease's progression or symptoms. On the other hand, the impressive number of cases of enucleation for diagnostic confirmation cited by Naumann et al. showed not only the ineffectiveness of the existing therapeutic choices, but also a change in the management of the disease over time⁽³⁾. After that study there was a change of attitude among surgeons, who, counting on more accurate tests, started to perform enucleation only in suspected cases of intraocular tumour. Evisceration started to be used in cases of eye infection. In our context, eviscerated material is not routinely sent to histopathology; therefore, cases where recurrence was not demonstrated could not be considered as cured, as they may have had endophthalmitis and undergone evisceration.

Even universities may lack the laboratory tests for keratitis, despite favourable operating factors, as mentioned by Tanure et al.; and there are still cases where the fungus is only positively identified by histopathological examination of corneal buttons. Definitive identification through culture of corneal tissue may take several days or weeks⁽²¹⁾.

In Brazil there is an absolute predominance of infections by filamentous fungi, with some studies reaching figures above 70%^(11,13,14,18), although there is no consensus about which species are more frequent. Salera et al. found 60% of infections by *Fusarium* and 30% by *Aspergillus*, both filamentous fungi⁽¹⁷⁾; Santos et al. also found a predominance of filamentous fungi, but the most common were *Penicillium* and *Cladosporium*⁽¹⁶⁾; Carvalho et al. found that filamentous fungi were relatively more common, with 32% by *Fusarium*, 17% by *Aspergillus* and 10% by *Penicillium*⁽¹⁸⁾; Dalfré et al. found 44% by *Fusarium* and 23% by *Geotrichum*⁽¹⁴⁾.

With regard to other countries, in the United States *Aspergillus* was the most frequently isolated fungus; in Saudi Arabia, the most common were *Aspergillus* and *Trycophytum*, both filamentous⁽²²⁾; in Japan, *Fusarium* and *Aspergillus*⁽²³⁾; in India, *Fusarium* and *Aspergillus* were also the most frequent⁽²⁴⁾; and the same was found in China, where filamentous fungi were found in 90% of cases⁽²⁵⁾.

Table 3 shows the high number of cases related to previous surgery. In our sample, 20 operations out of 47 (42.5%) led to fungal infection of the cornea. Similar results were found in Saudi Arabia. Jastaneiah et al. found that 38.7% of cases had surgery prior to the diagnosis of fungal keratitis⁽²²⁾.

As can be seen in Figures 1C and 1D, the organisms remain on the surface of the cornea. Therefore, we decided to quantify

the possibility of diagnosing the presence of the fungus in cases where treatment had been instituted and found a significant number of cases (45%) where the parasite remained on the surface even though there was deep penetration in the cornea. Garg et al., which also did a study based on histopathology, found similar results⁽²⁴⁾. We support the mistaken statement by Naumann et al. that the organisms were found throughout the thickness of the cornea, but in almost all cases there were no fungi on the surface or in the corneal ulcer⁽³⁾, as those authors worked with samples from enucleation.

Our sample presented a different result from other studies, based on purely morphological findings of fungi with a predominance of yeast forms. Two issues have to be considered. (1) The growing number of cases of fungal keratitis due to the high prevalence of antibiotic use, especially broad-spectrum agents, and the indiscriminate use of steroids in diseases of the anterior segment and during the postoperative period, as well as aspects related to the urbanisation of populations; (2) The biological behaviour of the fungus within tissues as described by McGee et al.⁽²⁶⁾. According to these authors, morphological changes occurred due to an adaptation of saprophytic fungi to parasitism as they invade host tissues. Pathogens may present different types of dimorphism generally expressed by the transition from the filamentous form to yeast as they invade tissues.

This is supported by the fact that other studies on fungal keratitis usually perform laboratory tests on samples taken from the corneal surface, while we worked with material from inside the tissues. Figure 2, from Carvalho et al.⁽¹⁸⁾, is very different from the ones we presented because the material was collected with a spatula from the corneal surface for direct examination and culture.

Our findings are also supported by the work of Höfling-Lima et al., who found that yeasts were more frequent in patients who had previously had eye surgery or who were locally or systemically immunosuppressed. This is related to the use of topical or systemic drugs, debilitating diseases and the presence of systemic mycoses. Ocular surgical procedures prior to ocular infection were significantly more associated with infection by yeasts. Topical antibiotics were used both in both yeast and filamentous fungal infections⁽²⁷⁾. The large number of corneal buttons we examined came from patients with fungal infection who had undergone surgery, therefore these patients had used antibiotics and anti-inflammatory agents.

Based on these findings, we suggest the study of cases that do not respond to treatment for filamentous fungal keratitis to see if they would respond to the recommended treatment for yeast keratitis, based on the possible morphological changes of the fungus. In cases where the drug has so little specificity, it could be better to target the active parasite form, rather than its superficial one. This represents a different conceptual approach — to treat the morphological change, and not the aetiological manifestation. Even with the morphological changes of the fungus and the prevalence of filamentous forms, we still argue for the need to perform microbiological examination before treatment and to wait for the results in order to decide whether the therapeutic strategy has to be changed.

Clinical signs, which are more subjective, can be associated with the more objective histopathological findings, comparing the information provided by surgeons with the findings of histopathology. The information that about 40% of cases had an inflammatory process with intense hyperaemia contrasts with the histopathological examination, which only found intense infiltrate

in less than a third of cases. In half the cases there was of little or no inflammatory reaction. The number of cases cited as having moderate hyperaemia was almost half of those with moderate infiltrate in histological sections. These data may provide the level of reliability of the subjective information in relation to the reality inside tissues. It also shows the difficulty in finding the correct diagnosis when an aetiological diagnosis is not determined, especially in the case of fungal keratitis.

Figure 2C shows a ruptured Descemet's membrane and spores inside it. Table 4 gives an idea of the risk of intraocular penetration by the parasite, showing a high incidence of changes to of Descemet's membrane favouring the passage of the fungus. However, this would explain the presence of corneal oedema. Fungal keratitis can produce de-epithelisation or ulcers, which facilitates the passage of the fungus from the surface into the stroma. Penetration through the stroma can occur by thermotaxis or chemotaxis. Fungal proteases can also contribute to the destruction of the extracellular matrix, as reported by Hua et al.⁽²⁸⁾.

The importance given to Descemet's membrane as a barrier to intraocular penetration by the fungus can be seriously questioned, as suggested by the image shown here. It is not possible to assess the degree of protection it provides, as the fungus can be seen penetrating the membrane instead of taking advantage of the rupture point. According to the authors mentioned above, there are strains that, when inoculated experimentally, invade the cornea in 24 hours, whereas others advance rapidly over the first three days, then are slowed down until the fifth day; there are also strains that do not have penetrating hyphae. The authors also point out that conidial adhesion, germination of spores and hyphae with extensions facilitate the invasion. The presence of fungal spores in Descemet's membrane shows its great ability to invade the eye and justifies the use of systemic drugs from the beginning of treatment.

Although a literature review showed that endophthalmitis presents in patients with general health vulnerabilities⁽²⁹⁾, Godoy et al.⁽³⁰⁾ show a case of endophthalmitis following penetrating keratoplasty. Knowing the penetrating capacity of the organisms and the changes in Descemet's membrane facilitating intraocular invasion, and having no feedback on cases undergoing transplantation, we conclude that there may have been cases of fungal endophthalmitis in our sample without our knowledge. It is also important to note that *Fusarium* and *Aspergillus* can quickly reach the intraocular space, as reported by Uno⁽²³⁾.

Our results also show that 2/3 of infections were caused by yeasts. We do not have a mechanical explanation for this, although we can argue that as the fungus penetrates it changes from filamentous to yeast, as suggested by McGee et al.⁽²⁶⁾. However, Figure 2A shows hyphae perpendicular to the surface in the innermost part of the inner third of the corneal stroma. Hyphae are almost always parallel to the corneal surface; perpendicular hyphae were found in experimental studies in rabbits subjected to intense corticosteroid use⁽³¹⁾.

Figure 3A shows a mycetoma in the incision site for a previous transplant; this confirms the hypothesis by Garg et al. of contamination of the incision tunnel for cataract surgery with sutureless, self-adhesive material⁽³²⁾.

Xie et al. recommend penetrating keratoplasty for fungal ulcers of difficult treatment⁽²⁵⁾, which is reasonable, as we confirmed cure in four patients after the second transplant; however, in two others cases there was no confirmed cure,

although they have not been submitted to other transplants. Also, there was relapse in three cases, which are not evident in the Observation Chart because we used the same colour for cases of relapse. The success of those authors is due to the combination of topical and systemic antifungal agents for up to 2 months after transplantation, associated with cyclosporine and perhaps corticosteroids when the patient had significant inflammation before the transplant.

From what was discussed here we recommend that the aetiological diagnosis should be investigated in cases of keratitis, including tests for fungi. If this is not done, a sample can be collected from the ulcer, due to the significant probability of discovering the diagnosis even after the onset of treatment. As test results take a long time, and taking into account the urgency of the situation, *in vivo* confocal microscopy can be used to guide treatment earlier⁽²⁵⁻³³⁾.

CONCLUSION

In this paper we presented the reality of a sample, which in many aspects does not correspond to the common findings for this fungal keratitis. We presented our findings, starting from inferences that led to our deductions, therefore we conclude by simply suggesting experimental research that can clarify some of our findings.

The predominance of yeast forms, contradicting the findings of both national and international studies, can be attributed to the fact that other studies found the aetiological agent through microbiology, and the samples we received came from corneal buttons obtained through perforating keratoplasty. A field of research is thus opened up, aiming to determine whether a filamentous fungus tends to change into yeast once the hyphae penetrate tissues and whether they would respond to drugs targeting yeast.

A cause of concern is the ability of the infection to penetrate the intraocular tissues, which is facilitated by corneal oedema and changes in Descemet's membrane, requiring systemic medication from the onset of treatment.

Finally, we recommend that the aetiological diagnosis should be investigated of cases of keratitis, including through confocal microscopy.

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