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Epidemiology of fungal keratitis treated with penetrating keratoplasty by means of histopathologic findings

Epidemiologia de ceratites fúngicas tratadas com ceratoplastia penetrante através de achados histopatológicos

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ABSTRACT

Objective: *To study, by means of histopathological examination, the epidemiology of fungal keratitis treated with penetrating keratoplasty therapy, emphasizing the presence of previous ocular surgery.* ***Methods:*** *Initially, we made an observational and cross-sectional study of corneal buttons from penetrating keratoplasty in the 2006-2015 period sent for histopathological examination at the Hospital Geral de Fortaleza Eye Bank. Tissues were stained with hematoxylin-eosin, PAS or Grocott, and examined with an optical microscope. We selected the cases with histopathological diagnosis of fungal keratitis. After the selection, we carried out a review of records seeking for age and sex of patients, date(s) of the graft(s) made to treat fungal keratitis, preoperative clinical diagnosis, presence/type of earlier and/or subsequent surgeries. We included 62 corneal buttons from 55 patients.* ***Results:*** *Most patients were male. Only 7 (11.29%) cases had recurrence of the surgically treated infection. 10 (16.13%) cases had eye surgery prior to fungal keratitis treated by transplant. No cases had fungal keratitis as preoperative clinical diagnosis. The main form of fungus in histopathological examination was isolated yeast form, followed by the yeast form associated with the filamentous form. The predominant aspect of Descemet's membrane was free of fungus.* ***Conclusion****: We demonstrated the healing potential of fungal keratitis when treated with penetrating therapeutic keratoplasty and the possible association of previous eye surgery factor for the development of these infections. Characteristics of histopathological examination have been approached differently from other studies that mostly specify only the microbiological examination. The difficulty in preoperative clinical diagnosis was highlighted, which may have contributed to the evolution of the cases studied for surgical treatment.*

Keywords*: keratitis/epidemiology; Keeratitis/pathology; Eye infections, fungal; Keratoplasty, penetrating*

RESUMO

Objetivo: Estudar, através do exame histopatológico, a epidemiologia de ceratites fúngicas tratadas com ceratoplastia penetrante terapêutica, enfatizando a presença de cirurgia ocular prévia. **Métodos:** Inicialmente, o estudo foi observacional e transversal de botões corneanos provenientes de ceratoplastia penetrante no período de 2006-2015 enviados para exame histopatológico ao banco de olhos do Hospital Geral de Fortaleza. Os tecidos foram corados com Hematoxilina-eosina, PAS ou Grocott, e examinados com microscópio óptico. Foram selecionados casos com diagnóstico histopatológico de ceratite fúngica. Após a selecão, realizamos revisão de prontuários buscando idade e sexo do paciente, data(s) do(s) transplante(s) por ceratite fúngica, diagnóstico clínico pré-cirúrgico, presença/tipo de cirurgias anteriores e/ou posteriores. Incluímos 62 botões corneanos de 55 pacientes. **Resultados**: A maioria dos pacientes era do sexo masculino. Apenas 7 (11,29%) casos tiveram recidiva da infecção tratada cirurgicamente. 10 (16,13%) casos possuíam cirurgia ocular prévia a ceratite fúngica tratada por transplante. Nenhum caso teve ceratite fúngica como diagnóstico clínico pré-cirúrgico. A principal forma de fungo no exame histopatológico foi forma leveduriforme isolada, seguida pela leveduriforme associada à filamentosa. O aspecto predominante da membrana de Descemet foi livre de fungos. **Conclusão:** Demonstramos o potencial curativo das ceratites fúngicas quando tratadas com ceratoplastia penetrante terapêutica e uma possível associação do fator cirurgia ocular prévia ao desenvolvimento dessas infecções. Características do exame histopatológico foram abordadas diferente de outros estudos que, em sua maioria, citam apenas o exame microbiológico. A dificuldade no diagnóstico clínico pré-cirúrgico foi ressaltada, o que pode ter contribuído com a evolução dos casos estudados para tratamento cirúrgico.

Descritores: Ceratite/epidemiologia; Ceratite/patologia; Infecções oculares fúngicas; Ceratoplastia penetrante

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Introduction

Diseases affecting the cornea are a leading cause of blindness in the world, only behind cataracts in global importance[1]. Among the cornea diseases, microbial keratitis is the main one leading to blindness[2]. Ocular trauma and ulceration are important causes of corneal blindness that are often sub-related, but which may be responsible for 1.5 to 2.0 million new cases of monocular blindness each year. In addition, the prevalence of corneal diseases varies from country to country and even from one population to another[1]. Literature studies report a high proportion of bacterial ulcers in developed countries (North America, Australia and Western Europe), and the highest proportions of corneal infections are related to fungi in eastern India[3].

Fungal keratitis is an important cause of microbial keratitis[2]. An increase in its incidence has been reported in recent decades[4-6], but the disease is still a challenge in terms of diagnosis and treatment[5,7]. Regarding microbial ceratites, fungal ceratites are reported in 16.4% of cases in Parana (Brazil)[8], 10.3% and 14.0% of cases in Saudi Arabia[5,9], 8% of cases in San Francisco (California, United States)[10] and 38.06%, 39.8% and 36.79% of cases in India[2,11,12].

Two basic forms of fungal keratitis are recognized: one due to filamentous fungi (especially Fusarium and Aspergillus) which commonly occurs in tropical and subtropical zones, and another due to yeast fungi or related (in particular Candida)[13]. Corneal cultures have been used for laboratory confirmation of fungal keratitis[4,8,14]. The fungal form found in the culture also has helped guide choosing the antifungal medication to be used in the treatment[15].

Early diagnosis and treatment of cases of fungal keratitis are key for obtaining a better visual prognosis[8]. The management of mycotic keratitis involves drug therapy or surgery. Drug therapy consists of specific measures (such as the use of cycloplegic eyedrops to relieve the anterior uveitis) and the use of specific antifungal agents. Surgery attempts to remove antigenic and/or infectious elements, necrotic tissue and other debris that can difficult healing[13]. Penetrating keratoplasty has been described as an effective treatment for fungal keratitis[16], which was held in 12.20% to 70% of cases in some [2,4,6,9,16-19]. Some indications mentioned for penetrating keratoplasty in fungal keratitis were drilling[4,13,16-18,20], imminent drilling[13,16,17] and absence of response to clinical treatment[4,13,16,17,20].

The key element for the diagnosis of mycotic keratitis is the clinical suspicion[18]. The clinical presumption of the agent for microbial keratitis is possible based on clinical and epidemiological data, and the clinical suspicion based on these data may be beneficial to guide the antimicrobial treatment and early therapy[21]. Reports on etiological and epidemiological data of patients with fungal keratitis provide significant insights to the understanding of this potentially devastating corneal disease[11], and a proper understanding of the agent and host factors involved in these infectious processes may improve the outcome of this condition[13].

However, most epidemiological studies on mycotic keratitis are based on observations of corneal material used to prepare the slides and/or culture[4,8,9,11,12,18]. Few studies talk about histopathological characteristics of fungal keratitis [22] or mention a histopathological examination carried out during the study of the epidemiology of fungal keratitis[2,5,16,20]. Among the latter, none uses exclusive information for the histopathological exam, and some do not describe in details the findings of this exam[2,5]. It is important, however, to submit surgical samples of microbial keratitis cases to histopathological examination, especially if the microbiological diagnosis is unknown[23]. The histopathological examination of corneal buttons can show the presence of fungal elements in 75% of patients[6]. Histopathological studies offer some advantages over the culture in the diagnosis of mycotic keratitis, since contamination is avoided, penetration of the tissue can be assessed, and the results of surgical procedures can be anticipated [24].

Therefore, an epidemiological study of cases of fungal keratitis treated with penetrating therapeutic keratoplasty was carried out, and the tissues removed in the surgery were sent for histopathological examination at Banco de Olhos do Hospital Geral de Fortaleza. The study is based on the findings of the histopathological examination, and we try to emphasize the presence of prior eye surgery as a possible predisposing factor for this type of corneal infection.

Methods

With the approval of the Research Ethics Committee of Hospital Geral de Fortaleza, first we made an observational, cross-sectional, retrospective and descriptive study of corneal buttons for detecting cases of fungal keratitis from penetrating keratoplasty in the period between 2006 and 2015, and sent them for histopathological examination at Banco de Olhos do Hospital Geral de Fortaleza. As a routine and control method of emergency transplants, all corneas of recipients subject to emergency keratoplasty should be directed to Banco de Olhos for histopathological examination.

The tissues, once fixed in 10% neutral formalin in the surgical center, were forwarded to the pathological anatomy department and clipped. The inclusion was made in paraffin, with subsequent cuts of 2μm, and the routine staining was made with Hematoxylin-eosin. After preparation, the tissues were examined with an optical microscope by the authors. In case of doubt about the confirmation of diagnosis of fungal keratitis, additional staining was made: PAS (*Periodic Acid of Schiff reaction*) and silver methenamine (Grocott's method).

Only those cases (corneal buttons) with a diagnosis of fungal keratitis by histopathology were selected, i.e., those in which fungal elements were found in the corneal tissues examined with optical microscope by the authors. The slides were examined for information about the fungal form found (yeast isolated, filamentous isolated, or yeast associated to filamentous) and status of the Descemet's membrane (free of fungi, with fungal elements in its interior or not displayed).

After selecting the cases, the medical records were revised in search of information on age and gender of the patient at the time of the transplant by fungal keratitis, date and preoperative clinical diagnosis for said surgery, presence and type of previous and/or subsequent surgeries to this transplant. We considered the preoperative clinical diagnosis informed on the medical report corresponding to the probable etiology of the transplant. In the records of Banco the Olhos, we also searched for the total number of transplants performed per year in the State of Ceará.

Were then included 62 corneal buttons with histopathologic diagnosis of fungal keratitis in 55 patients, in which 36 patients were male and 19 female. The ages ranged from 19 to 89 years old, with an average of 48.76 ± 17.78 (average ± SD). The patients

selected were divided into groups according to the presence of previous surgeries or after the episode of fungal keratitis had been treated with penetrating keratoplasty or the recurrence of the fungal infection treated with therapeutic corneal transplant (Figure 1). We call previous or subsequent surgery any ocular surgery different from the therapeutic corneal transplant due to fungal keratitis and that were stated in the records of Banco de Olhos.

Results

The patients were divided into groups: single surgery (penetrating therapeutic keratoplasty due to fungal keratitis), previous surgery, recurrence of fungal keratitis treated with penetrating therapeutic keratoplasty and subsequent surgery (Figure 1 - in green). The group of recurrent fungal keratitis treated with penetrating therapeutic keratoplasty was also subdivided into subgroups: no other surgeries, previous surgery and subsequent surgery (Figure 1 - in pink)

The corneal buttons (cases of fungal keratitis represented by blue circles in Figure 1) were divided into the same groups and subgroups as the patients (Figure 1 - in green and pink). Since we had a corneal button from each surgery, the subgroups of corneal buttons from the group of recurrent fungal keratitis needed to be subdivided into 1st infection and 2nd infection (Figure 1 - in orange).

The distribution of patients into groups and subgroups and their respective age and gender can be found in table 1. A greater number of patients was found in the group of single surgery (n=32), and a smaller number in the group of recurrent fungal keratitis (n=7). All groups, including the total sample but excluding the group of previous surgery, showed a predominance of male patients. The highest average age was also observed in the group of previous surgery. A more detailed distribution of patients by age group can be found in Graph 1. The predominance of patients with fungal keratitis in the sample studied was observed in the age group from 21 to 60 years old (n = 39). A small difference is observed between the number of patients aged d”50 years (n = 30) and e”51 years (n = 25).

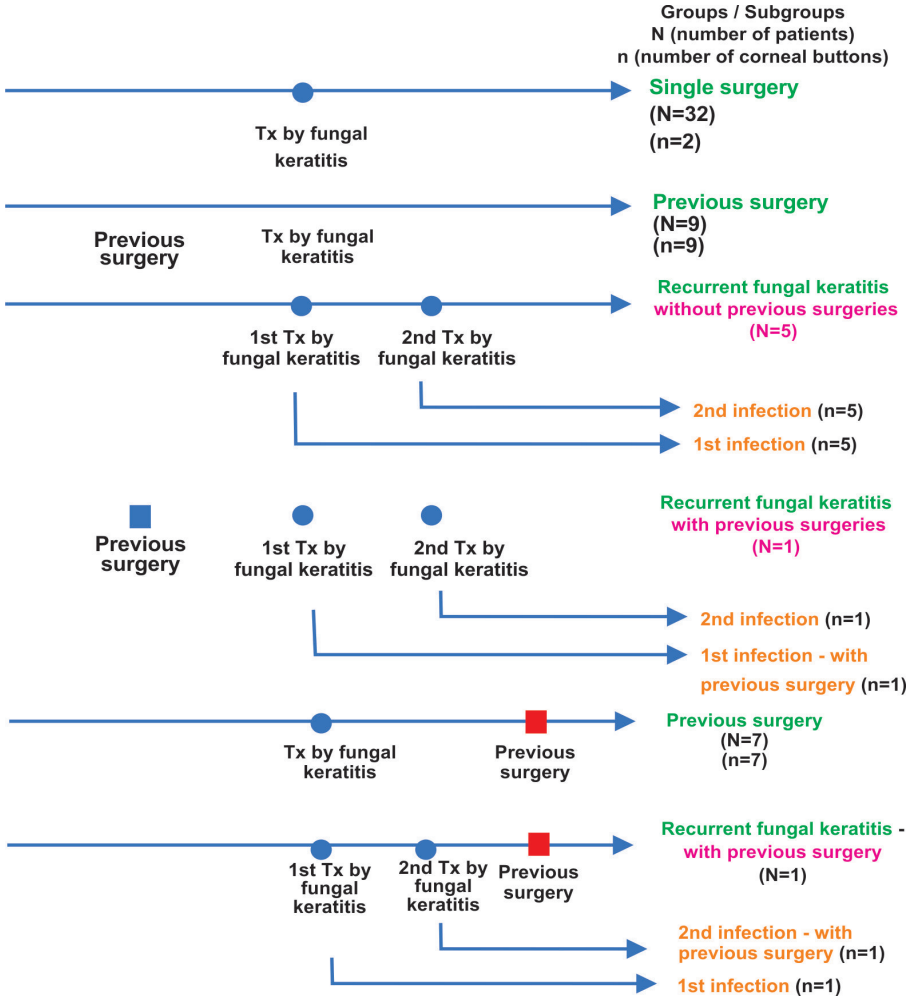


Figure 1: Division scheme of patients and corneal buttons (cases of fungal keratitis treated with penetrating therapeutic keratoplasty) into groups and subgroups. Groups and subgroups in which the patients and corneal buttons were divided (green and pink). Subgroups of corneal buttons from the group of recurrent fungal keratitis (in orange). Corneal buttons corresponding to the tissues removed from each transplant for fungal keratitis (blue circles). Number of patients in each group / subgroup (N). Number of corneal buttons (cases of fungal keratitis) of each group / subgroup (n).

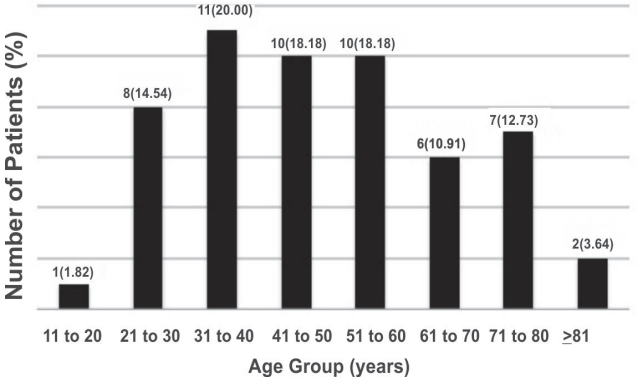
Table 1

Distribution of patients with fungal keratitis from the sample studied by the authors into groups / subgroups according to the presence of surgeries, age and gender

Groups	Subgroups	Nº of Pacients(%)	Age (average ± SD)	Gender (M/F)
Single surgery	-	32 (56.18)	43.75 ± 17.04	22/10
Previous surgery Recurrent	-	9 (16.36)	70.78 ± 8.23	3/6
fungal keratitis	No others surgeries	5 (9.09)	50.20 ± 15.24	4/1
	With previous surgeries	1 (1.82)	49.00 ± 0.00	1/0
	With subsequent surgeries	1 (1.82)	49.00 ± 0.00	1/0
Subsequent surgery		7 (12.73)	43.14 ± 14.00	5/2
Total		**55 (100)**	**48.76 ± 17.78**	**36/19**

Graph 1

Composition of the sample of patients studied by the authors with fungal keratitis treated by penetrating therapeutic keratoplasty between 2006 and 2015 according to the age group.



In table 2, we have the distribution of cases of fungal keratitis (corneal buttons) studied in groups and subgroups. In the group of recurrent fungal keratitis there are 14 cases, since 7 patients had recurrent fungal keratitis treated with penetrating therapeutic keratoplasty once. The second keratoplasty for treatment of recurrent fungal keratitis occurred between 15 days and 2 months after the first.

The distribution of cases of fungal keratitis (corneal buttons) with previous surgery regarding the type of surgery previous to fungal keratitis treated with therapeutic keratoplasty can be found in Table 3. Here, there is an almost equal number of cases had facectomy and corneal transplant prior to the infectious episode (n = 5) or just previous corneal transplant (n = 4). We emphasize that the reference to transplant + facectomy is not necessarily referring to the combined surgery, the surgery could have been performed at different times. Facectomy without previous transplant was found in only 1 case.

Table 2

Distribution of cases of fungal keratitis (corneal buttons) from the sample studied by the authors into groups and subgroups

Groups	Subgroups		Number of cases
Single surgery			32
Previous surgery			9
Recurrent fungal keratitis	No others surgery	- 1st Infection	6
		2nd Infection	6
	With previous surgery	- 1st Infection	1
	With subsequent surgery	- 2nd Infection	1

Table 3

Distribution of cases of fungal keratitis (corneal buttons) with previous surgery regarding the type of surgery prior to fungal keratitis treated with penetrating therapeutic keratoplasty

Type of surgery	Number of cases
Transplant(s)	4
Facectomy	1
Facectomy + Transplant	5

As for the cases that had surgery after the fungal keratitis treated with penetrating therapeutic keratoplasty, we observed that the predominant type of subsequent surgery in the records of Banco de Olhos was the corneal transplant by late or primary failure found in 7 cases (Table 4). Most of the cases (n=4) presented only one transplant after the fungal infection. In 1 case there was records of three transplants after the fungal keratitis. Only in 1 case there was also records of development to evisceration.

Table 4

Distribution of cases of fungal keratitis (corneal buttons) with subsequent surgery regarding the type and number of subsequent surgeries

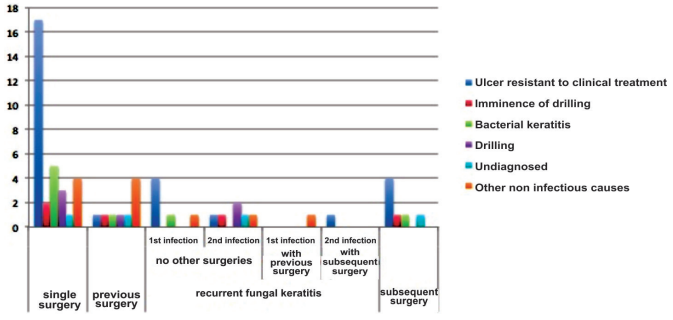
Type of surgery	Number of surgery	Number of cases
Transplant by failure	1	4
Primary or late	2	2
	3	1
Evisceration	-	1

The distribution of cases of fungal keratitis with regard to preoperative clinical diagnosis is found in graph 2. In most cases (n=47), infectious (bacterial keratitis) or possibly infectious (treatment-resistant ulcer, imminence of drilling and drilling) etiologies were thought in pre-operatory period. In any case, the fungal etiology was mentioned. Bacterial keratitis was found as a preoperative clinical diagnosis in various groups. Among the cases with previous surgery (with or without recurrent fungal keratitis), we found non-infectious causes in 5 (50%) of these cases. Other non-infectious causes considered were: interstitial keratitis, non-infectious ulcerative keratitis, corneal dystrophy, bullous keratopathy, rejection, corneal thinning and corneal degeneration. Finally, and still with regard to the preoperative clinical diagnosis, in 4 cases it was not present in the medical records of Banco de Olhos.

The form of more fungus mostly found in the histopathological exam was the isolated yeast form, followed by the yeast form associated to filamentous, found in 33 (53.23%) and 28 (45.16%) cases, respectively (Graph 3). The isolated filamentous form was found only in 1 case that had previous surgery, which accounted for 10% of cases among those who had previous surgery (with or without recurrent fungal keratitis) and 1.61% of total cases in the sample. Not always the form of fungus found in the corneal tissue of the 1st infection repeated in the 2nd infection, as shown in cases 4, 5 and 6 of table 5.

Graph 2

Distribution of cases of fungal keratitis in each group/ subgroup as for preoperative clinical diagnosis



Graph 3

Distribution of cases of fungal keratitis in each group/ subgroup regarding the form of fungus found in the histopathological exam

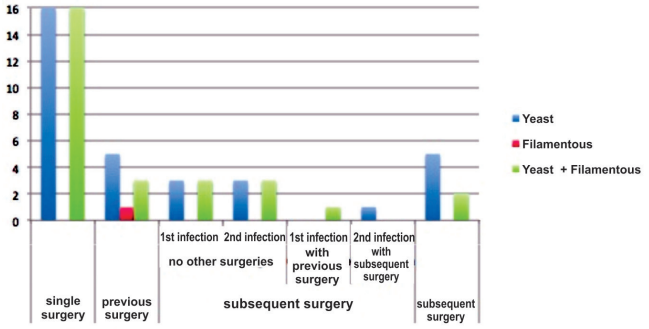


Table 5

Distribution of cases of fungal keratitis in the group of recurrent infection and its subgroups regarding the form of the fungus and the aspect of the Descement's membrane found in histopathology

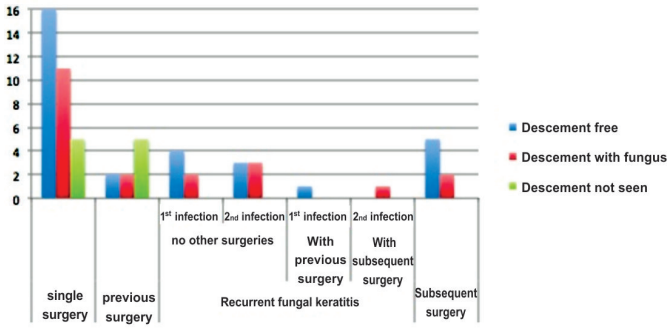
Number of cases	Form of the fungus		Aspect of the Descement's membrane	
	1st Infection	2nd Infection	1st Infection	2nd Infection
Case 1	yeast	yeast	free	with fungus
Case 2	yeast + filamentous	yeast + filamentous	free	free
Case 3	yeast + filamentous	yeast + filamentous	free	with fungus
Case 4	yeast + filamentous	yeast	with fungus	with fungus
Case 5	yeast + filamentous	yeast	free	free
Case 6	yeast	yeast + filamentous	with fungus	with fungus
Case 7	yeast	yeast	free	free

Note: The case of 1st infection in case 2 (in red) had previous surgery, and case of 2nd infection in case 4 (in green) had subsequent surgery.

The Descemet's membrane free of fungi was the predominant aspect of this membrane in the histopathological exam in most cases of the sample studied, having been found in 31 (50%) cases (Graph 4). The Descemet's membrane free of fungi was predominant in the group of single surgery, recurrent fungal keratitis – 1st infection (with or without previous surgery) and subsequent surgery (no recurrent fungal keratitis). Still in the Graph 3, 21 (33.87%) cases of the sample showed fungal forms inside the Descemet's, and in 10 (16.13%) cases of the sample this membrane was not present in the cohort. Not displayed Descemet was found in 5 (50%) cases among the total who had previous surgery. Descemet with fungal forms inside predominated in the total cases of Group of recurrent fungal keratitis - subgroup 2nd infection (without other surgeries or with subsequent surgery) (Graph 4 and Table 5). Fungus inside the Descemet's membrane in the tissue of the first surgery was repeated in the second surgery as demonstrated in cases 4 and 6 of table 5.

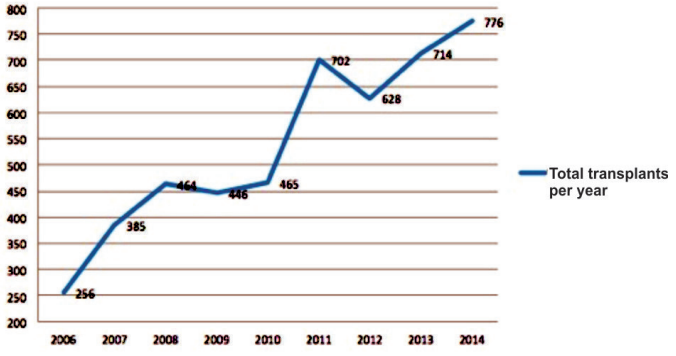
Graph 4

Aspect of the Descemet membrane in the histopathological exam for the presence of fungus in each group/subgroup.



Graph 5

Development of the total amount of corneal transplants per year in the State of Ceará in the period between 2006 and 2014.

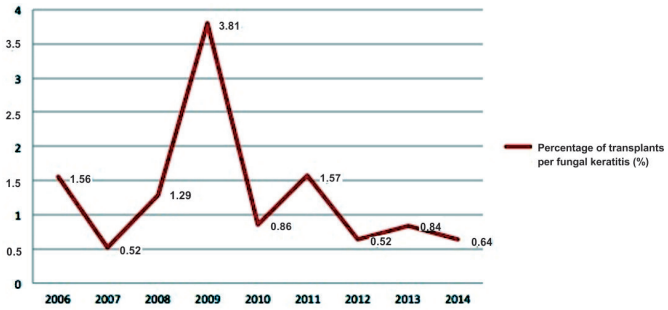


The development of the total amount of corneal transplants and the percentage amount of corneal transplants due to fungal keratitis is found in charts 5 and 6, respectively. Between 2006 and 2014, we observed an increase in the total amount of corneal transplants carried out in the State, except for the years 2009 and 2012, when we observed some reduction (Graph 5). Transplants due to fungal keratitis, in turn, kept in the same period a relatively constant percentage in relation to the total transplants, except for the peaks observed in 2009 and 2011. The peak in 2011 was accompanied by a significant increase of the total corneal transplants, which was not observed in 2009.

Finally, we observe that, in the period between 2006 and 2014, the cases of fungal keratitis treated with penetrating therapeutic keratoplasty predominated in the second half, when we found 34 (57.63%) cases (Graph 7).

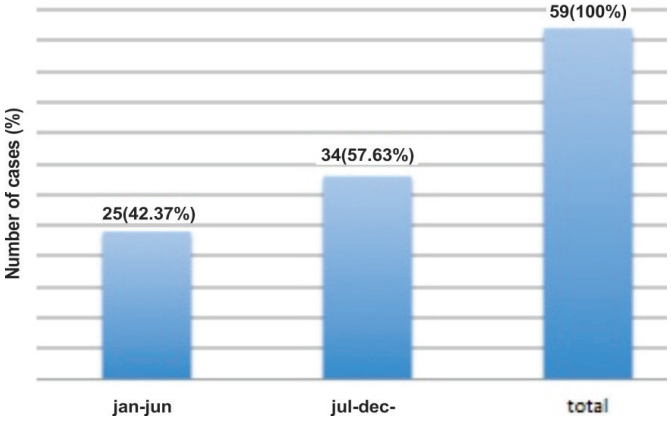
Graph 6

Development of the total percentage (%) of corneal transplants due to fungal keratitis in the State of Ceará in the period between the years 2006 and 2014.



Graph 7

Distribution of total cases of fungal keratitis studied by the authors between 2006 and 2014 in relation to the months of the year.



Note: The year 2015 is not part of graphs 5, 6 and 7 because since it is not finished and we could not have the annual percentage analysis.

Discussion

This epidemiological study of fungal keratitis showed predominance of male patients aged between 21 and 60 years, which is compatible with the professionally active population. The average age was 48.76 ± 17.78 (average ± SD) years. Other studies show the prevalence of fungal keratitis in similar age group[8,16,20] an in males[2,4,5,8,9,11,12,16,18,20,25]. Tanure et al. show in their study, however, most cases of fungal keratitis in females[17].

A study of microbial keratitis in the elderly also showed a predominance of cases in females[26].

The patients and their corneal buttons (cases of fungal keratitis) were divided into groups according to the presence of previous surgeries or after the episode of fungal keratitis had been treated with penetrating therapeutic keratoplasty or the recurrence of the fungal infection treated with therapeutic corneal transplant. The recurrence of 7 (11.29%) cases of infection surgically treated among the 62 cases studied (Table 2) shows the possible high curative potential of fungal keratitis when treated with penetrating therapeutic keratoplasty. Among the cases with subsequent surgery (n=8), just 1 developed to evisceration; the others (n=7) developed to late or primary failure, and consequently to cure of the infection (Table 4). We accept that a recurrence of the infection medically treated may have happened, and the eye database did not make access to this information possible. We understand, however, that recurrent fungal keratitis after therapeutic corneal transplant responding to treatment could mean a lower severity of the infection. As previously mentioned, penetrating therapeutic keratoplasty has been described as an effective treatment for fungal keratitis. Xie et al. show recurrent fungal infection after penetrating therapeutic keratoplasty therapy only in 8 (7.4%) eyes studied, and 3 of these infections were controlled by repetition of penetrating therapeutic keratoplasty, 4 eyes were enucleated because of endophthalmitis, and a recurrent infection was controlled in a patient by intense antifungal therapy[16]. Salera et al. report, however, recurrent fungal infection in the transplanted cornea in 7 (50%) of the cases studied, with prescription for a second therapeutic transplant in 3 cases, and 1 case a third transplant followed total conjunctival covering was necessary[4]. Regarding the evisceration of fungal keratitis, Carvalho et al. report a prescription of eviscerated or a consolidated poor prognosis subject to cornea transplantation in 71.5% of the cases studied[8]. In the study Khairallah et al., only 3 (11%) eyes, however, required evisceration / enucleation due to uncontrolled keratomycosis leading to fungal endophthalmitis[9].

We understand that the 10 (16.13%) cases with surgery prior to the episode of fungal keratitis surgically treated (Table 2) may also indicate that this is a possible factor associated to corneal fungal infections. Interestingly, as shown in Table 1, most patients with surgery prior to the infection episode studied had a higher average age (70.78 years). Previous ocular trauma is a recognized factor associated to fungal keratitis[2,4,6,8,9,11,16-18,20,25], including children[27]. Eye surgery prior to fungal keratitis has also been reported[2,5,11,26]. Jastaneiah et al. found previous eye surgery as the main factor associated to fungal keratitis, before ocular trauma, as well as the main factor for the development of endophthalmitis[5]. In a study of microbial keratitis in the elderly, there was an increase in the importance of the factor associated to previous eye surgery and an decrease of the factor ocular trauma, which is consistent with our finding of a higher average age in patients who had previous surgery[26]. In a retrospective analysis of microbial keratitis in southeastern Brazil, however, there was a more frequent corneal trauma in cases of fungal keratitis than in cases of bacterial keratitis; and the coexistence of systemic diseases, eye diseases and previous ocular surgery were more frequent in bacterial keratitis[21]. This last quote may also be what happens in our service, and was not demonstrated in this study because we focus only on cases of fungal keratitis. As an example of surgery prior to fungal keratitis, these infections have been reported after phacoemulsification[28] and refractive surgery[29]. Most of the previous surgeries found in our study were corneal transplants in 9 cases and cataract surgery in 6 (Table 3). According to our findings, in a study of microbial keratitis the main surgeries prior to the infectious process were penetrating therapeutic keratoplasty, followed by cataract surgery in the form of extracapsular facectomy[26]. Jastaneiah et al., in turn, found extracapsular cataract extraction as a main intraocular surgery prior to fungal keratitis, followed by penetrating therapeutic keratoplasty (5). We emphasize that our study did not record if the facectomy had been held by extracapsular extraction or phacoemulsification, as the standard form of Banco de Olhos does not require this detail.

Although in most cases infectious or potentially infectious etiologies (n = 47) had been thought as the preoperative clinical diagnosis (Graph 2), in no case the fungal etiology was mentioned, which demonstrates the difficulty of a purely clinical diagnosis for this kind of infection, even in reference centers for corneal transplant in our State, which may have contributed to the development of cases for corneal transplantation. As mentioned above, the corneal culture was used for laboratory confirmation of fungal keratitis[4,8,14]. The lack of reference to fungal keratitis as a preoperative clinical diagnosis in any of the cases studied leads us to assume that the vast majority of reference centers for the treatment of corneal diseases in our State does not offer this test. The difficulty in performing the clinical diagnosis of fungal keratitis is mentioned in other studies[17], but generally referring to the primary or secondary centers for ocular health[11]. The use of treatments prior to the referral to the corneal services is reported in the form of topical anti-bacterials, corticosteroids, antiviral and anti-protozoal and systemic use of antivirals, antibiotics and anti-protozoal isolated or combined[4,11,17]. Gopinathan et al. show a tendency of general practitioners and ophthalmologists at primary or secondary centers for ocular health to prescribe a drug "cocktail"; and when they chose antifungal drugs alone the dosage is often too low[11]. A study of ocular mycoses in Saudi Arabia including cases of fungal keratitis with or without endophthalmitis found that only 19.6% of the reference letters specify keratitis or endophthalmitis related to fungal etiology. The others were diagnosed as non-specific keratitis or microbial keratitis (5). In our study, however, the difficulty in having the clinical diagnosis of fungal keratitis was found in corneal services, there is, tertiary centers for ocular health. Still in Figure 2, the fact that in half the cases with previous surgery have shown noninfectious etiologies also leads us to believe that the factor of previous eye surgery possibly associated to corneal infections and especially fungal keratitis is still not well recognized in the medical practice, even by ophthalmologists of corneal transplant centers, hence our intention to emphasize this possibility in our study.

The most frequent fungus form in histopathology was the yeast in 61 (98.39%) cases, being common the association of the yeast form to filamentous found in 28 (45.16%) cases. The isolated filamentous form was found in only 1 case (Graph 3). Studies on fungal keratitis in various parts of the world show the

predominance of filamentous form[2,4-6,8,11,12,16,18,20]. A study in Philadelphia, in turn, found corneal infections caused by yeasts and filamentous fungi, each in half of the cases studied[17]. Specifically regarding gender, Fusarium is the most frequent in studies in Brazil[4,8] and China[16]; Aspergillus predominated in Saudi Arabia[5]; and Candida in Australia (Melbourne)[19]. In some countries, the most found gender varied according to the study and the region studied[2,6,11,12,17]. In the United States, Fusarium predominated in South Florida[6] and Candida was the most commonly isolated fungus in Philadelphia[17]. In India, Fusarium predominated in the south[11,12], and Aspergillus in West Bengal[2]. We emphasize, however, that most studies on fungal keratitis is based on observations made from the material collected from the corneal material used for making slides and / or culture[4,8,9,11,12,18]; and our findings are from histopathology. A few studies on mycotic keratitis using and specify information of histopathology, contrary to what was observed in our study, showed the prevalence of fungi in the filamentous form[16,20]. Still regarding the form of the fungus, as shown in Table 5 in cases 4, 5 and 6, not always the form found in the first infection was repeated in the second one. This last observation leads us to suggest that the second infection could not be a mere recurrence of the initial keratitis, but a possible new infectious episode having saprophyte fungi of the ocular surface as the etiology. The filamentous form found in the second infection of case 6 did not appear before in the initial fungal keratitis, but it reinforces our hypothesis.

The aspect of the Descemet's membrane in histopathology is observed in Figure 4. Although in most cases of the sample studied the fungi-free aspect prevailed; the fungal forms inside the Descemet in 21 (33.87%) cases lead us to suggest that the fungal infection often deeply penetrates into the cornea / eye. A deep penetration of the fungus in the cornea / eye, in turn, seems to go against the use of new transplant techniques such as lamellar keratoplasty for the treatment of fungal keratitis. Although lamellar keratoplasty will be described as an effective method for the treatment of fungal keratitis unresponsive to drug treatment[30-32] even in deep cases[31], cases with deep infection seem more likely to recur after lamellar keratoplasty[33]. Shi et al. show that partial lamellar keratoplasty is effective for fungal keratitis in which the infected injury lies in the middle or upper layers, especially when the corneal ulcer is quite widespread and non central[32]. A high rate of inadequate treatment with lamellar keratoplasty was also found in cases with *Aspergillus* species, those in which glucocorticoid or immunosuppressive were used and those with hypopyon or endothelial plate prior to lamellar keratoplasty (34). Still analyzing the same graph, we try to justify the absence of Descemet in 50% of cases with previous surgery due to potential surgical trauma that could have led to the detachment, or the way the cohort was done during the preparation of tissues. Cases of fungal forms were prevalent inside the Descemet's membrane between the total cases in the group of recurrent fungal keratitis - subgroup of 2nd infection, i.e., cases which probably evolved to cure. It shows that even when the fungus has penetrated the Descemet's membrane, the corneal transplant can be curative. The predominance of free Descemet in tissue from the first infection (Table 5), that is, cases that developed to recurrent infection, also seem to reinforce the possible absence of a relationship between the presence of the fungus within the Descemet and the recurrent fungal keratitis after treatment with penetrating therapeutic keratoplasty. The last two findings are contrary to previous reports showing that penetrating therapeutic keratoplasty seems to be more effective if performed before the fungus penetrates the Descemet's barrier[35].

In the literature, fungal keratitis has been mentioned as endemic in specific geographic regions[36], for example, in warmer weather such as in India[37]. In our study we also found, as shown in the graphs 5 and 6, that fungal keratitis seems to have an endemic behavior in our State, even when treated with therapeutic penetrating corneal transplant. However, the peak in the percentage of transplants due to fungal keratitis not followed by an increase in the total amount of corneal transplants occurred in 2009 leads us to think of a possible outbreak of these corneal infections that year. A keratitis outbreak by Fusarium associated to use of ReNu contact lens solution with MoistureLoc was described in the United States in 2006[38]. Regarding the possible outbreak of fungal keratitis in our State previously mentioned, we could not find any factors associated to this date.

Regarding the possible seasonality of the cases studied, we found a predominance in the second half of the year (Graph 7), which corresponds to the drier months in the state of Ceará. A study about fungal keratitis in Paraná, however, showed uniform incidence of infection in different months of the year, with a similar number of cases in the four seasons (8). Ibrahim et al. studied the epidemiology of fungal keratitis by monitoring the sales of anti-fungal eyedrops in Brazil, and found an increase in sales in the third quarter, which somehow is in line with the findings of our study. The third quarter of the year is the harvest season for many crops such as corn, soybeans, sugarcane, coffee, rice and orange[39], which leads us to think of a possible relation between the increase in the number of cases of fungal keratitis and the harvest season. Our findings are also in line with the hypothesis that in the tropics, during the dry season when agricultural activities are more frequent, there are more cases of corneal fungal infections[39,40].

Finally, we must mention that the number of cases in the present study could have been underestimated at times, but we emphasize those with previous surgery, with subsequent surgery, or those developing to evisceration. The preoperative clinical diagnosis may also not fully correspond to reality. Possible underestimation and difference in the preoperative clinical diagnosis emphasized here are due to the fact that our findings were based on the records of Banco de Olhos, where sometimes the information provided on the forms filled out by cornea surgeons and forwarded said institution is incomplete or not fully reliable. The completion of a comprehensive study of fungal keratitis in our State, especially considering histopathology information that are still poorly addressed in other studies, needs to be made on the records of Banco de Olhos, as we still do not have other kind of database on these infections in our locality. Due to this possible underestimation or due to being a descriptive study, we raise hypotheses or suggestions in many instances. Since we had several findings, from our point of view, a considerable number of cases emphasize the credibility of our suggestions. We also hope that this study is likely to stimulate corneal surgeons to more careful filling the forms which are routinely sent to Banco de Olhos, thus contributing to the development of future researches.

Conclusion

We conducted an epidemiological study of fungal keratitis treated with penetrating therapeutic keratoplasty with the use of histopathology findings. Thus, we demonstrated the healing potential of fungal keratitis when treated with penetrating therapeutic keratoplasty, and the possible association between the factor ocular surgery prior to the development of said infections. The characteristics of histopathology were discussed and detailed, unlike other literature studies which mostly mention only the aspects of microbiological examination. The difficulty in preoperative clinical diagnosis was highlighted, which may have contributed to the development of the cases studied for surgical treatment.

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