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Epidemiological profile of infectious keratitis

Perfil epidemiológico da ceratite infecciosa

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Abstract

Objective: *To evaluate the epidemiologic aspects of cornea ulcers in a referred ophthalmology private practice center.* ***Methods****: Retrospective study over electronic files of patients treated for corneal ulcers during a period of 7 years by the same physician (RJMF) at Centro de Olhos São Francisco, between june 2007 and june 2014. These patients were evaluated for risk factors such as: trauma causes, co-morbidities and contact lenses use. They were also evaluated by the microorganism found at the smears, treatment and patient`s outcome. Patients that didn´t return with the smears results or that didn´t have a complete follow-up were excluded from the study.* ***Results****: A total of 242 patients were fully treated during this period. 55 patients were excluded and 187 patients were included. 28.88% (54/187) were positive for gram negative bacteria, 27.81% (52/187) for filamentous fungi, 16.04% (29/187) for gram positive bacteria and only 1.07% for acanthamoeba. Twenty-four patients had negative cultures and 21 patients had combined infections (bacteria + fungi or other combination). Corneal collagen Cross-linking was effective on melting arrest in 16 of the 16 patients that we have submitted to this procedure during 2008 and 2009. Among all the patients that had corneal infection and were previously using contact lenses, 81% were infected by pseudomonas or pseudomonas associated with other gram negative bacteria. Filamentous fungi had a strong association with physical trauma. While use of saline solution for contact lenses cleaning had a strong association with gram negative bacteria and biological trauma, a strong association with combined infection.* ***Conclusion:*** *Gram negative bacteria (Pseudomonas) followed by filamentous fungi (Fusarium sp) were the most frequent etiologic agent found in our study. Cross-link was effective on corneal melting arrest. Pseudomonas aeruginosa was the most frequent isolated agent on hydrophilic contact lenses wearers.*

Keywords: *Cornea ulcer/etiology; Microorganisms; Pseudomonas; Gram-negative bactéria; Contact lens*

Resumo

Objetivo: Avaliar os aspectos epidemiológicos de úlceras de córnea em um centro privado de referência oftalmológica. **Métodos:** Estudo retrospectivo a partir de arquivos eletrônicos de pacientes tratados por úlceras de córnea durante um período de 7 anos pelo mesmo médico (RJMF) no Centro de Olhos São Francisco, entre Junho de 2007 e Junho de 2014. Estes pacientes foram avaliados quanto a fatores de risco, trauma, co-morbidades, uso de lentes de contato, tipo de microrganismo encontrado, tratamento e evolução do doente. Os pacientes que não retornaram com os resultados das culturas ou que não possuíam um seguimento completo foram excluídos do estudo. **Resultados:** Um total de 242 pacientes foram tratados durante este período. 55 pacientes foram excluídos e 187 foram incluídos no estudo. 28,88% (54/187) foram positivos para as bactérias gram-negativas, 27,81% (52/187) para fungos filamentosos, 16,04% (29/187) para as bactérias gram-positivas e apenas 1,07% para *acanthamoeba*. Vinte e quatro pacientes tiveram culturas negativas e 21 pacientes tinham infecções combinadas (bactérias fungos + ou outra combinação). O Cross-link foi eficaz em interromper o melting em 16 dos 16 pacientes que se submeteram a este procedimento durante 2008 e 2009. Entre todos os pacientes que tiveram infecção de córnea e usavam previamente lentes de contato, 81% foram infectados por *pseudomonas* isolada ou *pseudomonas* associada ou não a outras bactérias gram-negativas. Os fungos filamentosos tiveram uma forte associação com trauma físico, uso de solução salina para a limpeza de lentes de contato teve uma forte associação com bactérias gram- negativas e trauma biológico, uma forte associação com a infecção combinada. **Conclusão**: Bactérias Gram negativas (em especial, *Pseudomonas*) seguido por fungos filamentosos (*Fusarium sp*) foram os agentes etiológicos mais freqüentemente encontrados em nosso estudo. O Cross-linking foi eficaz em interromper o *melting* de córnea. *Pseudomonas aeruginosa* foi o agente isolado mais frequente em usuários de lente de contato hidrofílica.

Descritores: Úlcera de córnea/ etiologia; Microorganismos; *Pseudomonas*; Bactérias gram-negativa; Lentes de contato

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Introduction

Infectious keratitis or corneal ulcers are one of the greatest causes of visual impairment in the world and important cause of blindness in underdeveloped countries.[1] Early treatment helps to reduce corneal scarring, visual impairment and to prevent severe complications such as endophthalmitis, and even the irreversible loss of the entire globe eyeball.[1]

The incidence of this disease in the world is difficult to obtain, some data indicates an incidence of infectious keratitis varies around the world. In the United States is 11 cases per 100.000 inhabitants in the United States.[2] In developing countries that number is far bigger, reaching 799 cases per 100.000 inhabitants per year in Nepal.[3]

Bacteria is the leading cause of infectious keratitis in the world. And among the bacterial ulcers, 90% are caused by Staphylococcus, Streptococcus sp and Enterobacteriaceae. After bacteria numbers, fungi take second place, particularly in cases of trauma with plants, in which cases, features filamentous fungi.[4,5]

The major risk factors associated with the onset of infeccious keratitis were: use of contact lens, ocular trauma, changes in the ocular surface (blepharitis, penetrating keratoplasty and dry eye) and systemic diseases (diabetes, rheumatoid arthritis, alcoholism and AIDS).[6] The history of contact lens use and ocular trauma are the most significant risk factors.[6]

Signs and symptoms of ulcers depend on the aggressiveness of the etiologic agent, but usually present as eye pain, ciliary injection, photophobia and blurred vision.[7]

This study aimed to characterize the epidemiological profile of patients with corneal ulcers treated in a unit of private ophthalmologic practice during the period of 8 years (between June 2007 and June 2015) in São Luís, since there are no previous reporting infectious keratitis epidemiology in our region.

Methods

A retrospective study based on the survey review of electronic medical records of all patients treated due to infectious corneal ulcers by a single examiner (RJMF) between June 2007 and June 2015 was held at São Francisco Eye Center in São Luis MA. São Francisco Eye Center (Centro de Olhos São Francisco) is a private company, but for two years, part of the sample was obtained also by the counter-reference coming from patients of the public health system.

It was found 242 records of patients treated for corneal ulcer in this period. Cornea cultures, cytology (giemsa) and bacterioscopy (gram) were performed in all patients, regardless of size and location of the lesion. Patients who did not return with the culture result or not followed the consults until the end of treatment were excluded from the study sample.

Patients were assessed for risk factors, such as types of trauma, coexisting diseases and the use of contact lenses. By statistical decision, the risk factors were classified into three groups, physical, which correspond to the traumas with woods and foreign bodies for example, chemical, represented by the use of contact lens cleaning solution, and biological, to traumas with insect and beetles. Regarding the type of trauma, physical trauma was categorized when was due to a foreign body or wood trauma. Chemical trauma was classified as the use of saline solution for cleaning contact lenses (not proper contact lenses cleaning solutions). Biological trauma refers to trauma due to insects or other bugs that triggered the infectious process. The greatest trauma recorded was the chemical trauma which was a trigger point to all etiologic agents, and was more related to infections due to gram-negative bacteria. In 2008 and 2009, some patients underwent further treatment with cross-link of the corneal collagen and the results were also analyzed.

All statistical analyzes were performed using the R software (R Development Core Team 2012), adopting the significance level of 5 % (Zar 2010).

Results

Two hundred and forty two patients were treated for corneal infections, 55 patients were excluded and 187 patients were included, negative culture was found in only 24 patients (12.83%) of the sample.

Cytology (bacterioscopy/fungal research) showed that 84 (44.91 %) patients only had bacteria in their samples, and of these 54 (28.88 %) presented with Gram Negative bacteria exclusively. The laboratory culture demonstrated growth of fungi in 56 patients (30%) and of *acanthamoeba* in 2 patients (1.6%). Twenty-one patients (11.22%) had combined infections (Gram positive plus Gram negative bacteria or fungus + bacteria). (Table 1)

Regarding fungi, Filamentous fungi were the most frequent in our region, with the highest frequency of *Fusarium sp*. whether as a single agent (61.90 %, n = 39) or associated to other infections (15.87%, n = 10) in the cultures (Table 2). For yeast-like fungi, we only isolated *Candida sp*. Among Gram negative bacteria, *Pseudomonas aeruginosa* or *P. aeruginosa* associated with other Gram negative were the most frequency etiologic agent with 44,62 % and 20 %, respectively (Table 3). Among the gram positive bacteria, the most frequent ones were *Staphlococos aureus* (40.82%) and *Staphlococos aureus* associated to other infections (22.45%) (Table 4). In combined infections, *S. aureus plus* associations (*Pseudomonas* or fungi) have the most frequent rate with 9 (36%) infected people, followed by *P. aeruginosa* combined with other infections with 5 (20 %) cases.

Corneal collagen cross-linking was performed only in patients with exclusive bacterial infections, who had severe pain and corneal melting. Antibiotics have not been discontinued in these patients. All patients who underwent cross-link showed pain relief within 24 hours of the procedure and significant improvement in melting (melting arrest) within 72 hours after cross-link application with a chi-square test showing significant association between data ($p < 0.05$).

In correspondence analysis, the following main associations were observed: Filamentous Fungi with physical trauma, while combined or mixed infections with biological trauma and chemical trauma had a strong association with Gram Negative and less extend association with yeast and Acanthamoeba. Gram Positive showed similar association with all kind of traumas (central position of the graph) (Figure 1).

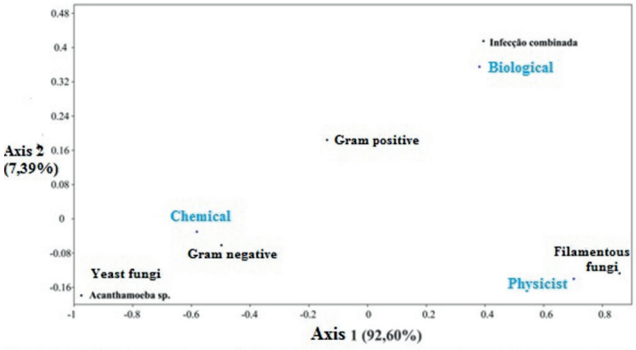

Figure 1: Biplot correspondence analysis for classes of types of trauma among the etiologic agent classes

Table 1

Causes of Infectious. keratitis

Cytology	Absolute Frequency	Relative Frequency			
Bacteria	84	44,91%	Gram positive	54	(28.80%)
			Gram Negative	30	(16.04%)
Fungi	56	30%	Filamentous	52	(27.80%)
			Yeast	4	(2.14%)
Acanthamoeba	2	1.06%			
Combined infections	21	11.22%			
Negative cultures	24	12.83%			

Table 2

Distrlbuition of fungi filamentous found in cultures of patients examined

Fungi.filamentous	Abs. Freq.	Rel.Freq.
Acremonium recifei	1	1.59%
AspergilJus niger	2	3.17%
AspergiJlus fumigatus	2	3.17%
Combined with Aspergillus	1	1.59%
Aspergillus sp.	3	4.76%
Bipolaris sp.	1	1.59%
Fusarium solani	2	3.17%
Association with Fusarium	10	15.87%
Fusarium sp.	39	61.90%
Penicillium spp.	1	1.59%
Scedosporim spp.	1	1.59%
Total	**63**	**100.00%**

Table 3

Disbiboition of gram-negative bacteria found in cultures of patients examined

Gram Negative	Abs. Freq.	Rel. Freq.
Burkholderia stabilis	2	3.08%
Burkholderia cepacia +other	1	1.54%
Corynebacterium jeikeium	1	1.54%
Corynebacterium +other	1	1.54%
Cryseobacterium gleum	1	1.54%
E. coli and A xylosoxidans	1	1.54%
E. meningoseptica	1	1.54%
Enterobacter+other	2	3.08%
Klebsiella pneumoniae	1	1.54%
Klebsiella + other	1	1.54%
Pseudomonas aeruginosa	29	44.62%
P. aeruginosa + Gram Negative	13	20.00%
P. aeruginosa + others	8	12.31%
Pseudomonas putida	1	1.54%
Sphingobacterium multivorum	1	1.54%
Stenotrophomonas maltophilia	1	1.54%
Total	**65**	**100.00%**

Table 4

Distribultion of gram positive bacterias

Gram positive	Abs Freq.	Rel. Freq
Staphylococcus aureus	20	40.82%
Staphylococcusaureus and Acanthamoeba	1	2.04%
Staphylococcusaureusand other	11	22.45%
Staphylococcus caprae and streptococcus sp.	1	2.04%
Staphylococcus coagulase negative	5	10.20%
Staphylococcus coagulase negative + other	5	10.20%
Staphylococcus epidermidis	1	2.04%
Staphylococcus epidermidis + other	2	4.08%
Streptococcus pyogenes (group A)	3	6.12%
Total	**49**	**100.00%**

Table 5

Contingency table of cases of occurrence of X-LInk and improvement response (Yes or No).

		X-Link'*		
		No	**Yes**	**Total**
Improvement	No	171	0	171
	Yes	0	16	16
Total		171	16	187

*Statistically significant association according to the Chi square test (p<0:.05)

Table 6

Contingency table of cases of co-morbidity in relation of improving the clinical profiles of patients

		Comorbidity				
		Diabetes	**Diabetes and others**	**Others**	**No**	**Total**
Improvement	No	4	4	12	151	171
	Yes	1	0	0	15	16
	Total	5	4	12	166	187

Table 7

Contingency table and multinomial proportion analysis for the number of cases of contact lens wearers in etiologic agent classes by bacterioscopy

		Contact Lens use		
		Inappropriate use	**No use**	**Total**
Bactenoscopy	Acanthamoeba sp.	2c	0	2
	Fungi Filamentous	0	51**a**	51
	Fungi Leveduriforme	1	0	1
	Gram negative	**A 42 a**	**B** 33 **b**	75
	Gram positive	**A** 9 **b**	**B** 22 **b**	31
	Combined infection	**A** 3 **c**	**B** 20 **b**	23
	Total	57	128	183

Different small letters among those who wear contact lens (separately,. per column) indicate statistically significant differences between the proportions ($p<0.05$). Different big letters in each bacterioscopy group (separately by lines) indicate a statistically significant difference between the proportions ($p<0,.05$)

When analyzing co-morbidities, diabetes was co-related with worsening prognosis in the treatment of ulcers, because most often these patients evolved to emergency corneal transplants (Table 5). There was no predisposition to a specific agent cause in the patients of our sample.

Regarding the use of Contact Lens, 128 patients did not wear contact lenses, while 57 patients used them incorrectly, which means, not adapted by ophthalmologists, bought online or in the hands of friends and/or being cleaned with saline solution. Of these 57 patients only one was using a rigid gas-permeable contact lens, cleaning it correctly.

Among these patients who misuse contact lenses, the highest proportion, according to the multinomial proportions test had infections caused by Gram negative bacteria. Gram Positive and combined infection showed similar and lower statistical proportions in contact lenses users. Among those who did not wear contact lenses, the highest proportion was for Gram negative and filamentous fungi.

Eighty-one percent (81%) of patients who used incorrectly their bad users of contact lenses presented in our study with Pseudomonas aeruginosa einfections and other gram negative bacteria. (Table 6)

Discussion

In our sample, the major incidence of infectious keratitis was caused by gram-negative bacteria and filamentous fungi, represented by Pseudomonas and Fusarium sp, which differs from Brazilian literature, where the majority of cases is by gram-positive bacteria, stanted by Staphylococcus epidermidis, Staphylococcus aureus and Streptococcus pneumoniae.[7] As regards to fungal ulcers, the result was similar to Brazilian literature, with prevalence of filamentous fungi, mainly Fusarium.[7]

An American study showed that the etiology depends on the geographic location of the study population. In the northern states, cooler, occurred more cases of bacterial keratitis, while in the southern states, in the hottest and rural areas, predominates infections of fungal origin.[8] This finding may fall in relation to our reality, since our state, Maranhão, is an extremely hot state and most of the population attended in this reference service, were from the rural areas of the state. Another American study showed that just as in Brazil, the major cause of fungal keratitis is the Fusarium (66 % of cases).[9]

A study in India described a statistic similar to ours, with 47.1% of bacterial involvement and 46.8 % of fungi infections.[10]

In our sample, Pseudomonas aeruginosa was present in 30% of patients and it is an important etiology in other studies, as shown in a paper published in 2015 where culture was positive for Pseudomonas in 25% of cases.[11] Pseudomonas aeruginosa is found, as in our study, strongly associated with cases of patients using contact lenses, and contact lens use is for itself the major risk factor commonly associated with the onset of infectious ulcer, as shown in several studies.[12-14]

Studies have shown that poor visual acuity at initial presentation, corresponds to worse patient´s visual prognosis.[11,15]

In our study, we apply the cross-link to stop melting in only 16 patients with bacterial keratitis, which markedly improved their evolution after it application. These data correlates with literature in which Tabibian demonstrated the use of cross-linking (Dresden Protocol) for melting corneas in melting secondary to infectious ulcers by fungi and mycobacteria, showing a melting demonstrating improvement in most cases, avoiding a tectonic transplant.[16]

A study in Nepal with 1644 patients with infectious keratitis over a period of 1 year showed that ocular trauma was the most common risk factor of associated with keratitis in 60.3 % of cases, appearing after 2 weeks after trauma of symptoms.[17] Another multicenter study in the United States over fungal keratitis characteristics reported that 25 % were associated with ocular trauma, and these, filamentous fungi were identified in 78 % of cultures made.[18] In our study, the main association observed was vegetable trauma with the filamentous fungi or mixed infections.

We also found a similar proportion of patients gender affected affection between male and female, with where 52.94 % of patients were male and 47.06 % female. Respectively, this finding which diverges from the literature, where most of the studies show a predominance of males, probably due to greater exposure to risk factors (trauma).[19]

Raica et al. studying 11 diabetic patients with corneal ulcer detected cytological changes consistent with the severity of cases and a poor visual prognosis.[20]

Most studies show ocular trauma as the main risk factor and associated with poor visual outcomes, there are few studies showing the association of diabetes mellitus with a worsening prognosis on corneal ulcers, which would be of great importance because it is increasing its frequency in the world.

Conclusion

In contrast to other studies in Brazil, we found gram negative bacteria as the most commonly isolated species in infectious keratitis cultures, followed by filamentous fungi, primarily Fusarium sp, which is presented as an important etiology. Physical trauma was strongly associated with fungal infections and incorrect cleaning of contact lenses with saline solution has been associated with infections by gram- negative bacteria. The incorrect use of contact lens is associated with infections caused by Pseudomonas aeruginosa and other gram-negative bacteria in 81% of our sample. Cross- link was effective in arresting melting and relieve pain of patients with bacterial keratitis.

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