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CORNEAL INFESTATION BY CILIATED PROTOZOA–FIRST CASE REPORT

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Abstract- ***Purpose: The purpose of the authors was to report the first case of ciliated protozoa diagnosed in corneal scrapings from keratitis from a hydrophilic contact lens wearer. Methods: The laboratorial research confirmed polymicrobial contamination with bacteria, fungi and protozoa. The results: A 27-year-old Brazilian woman was presented in the ophthalmology ambulatory with complaints of red eye, foreign body sensation, tearing and decreased visual acuity in the left eye during the use of hydrophilic contact lenses. A ciliated protozoan suggestive of Balantidium coli was found in the examination of corneal scrapings (Giemsa). Trophozoites of the ciliated protozoa were also observed in the direct examination and culture of the lens solution, while Acanthamoeba trophozoites were diagnosed only in the culture of the solution. The other agents were diagnosed by culture of material collected from the affected cornea and contact lens. Conclusion: it is necessary to consider ciliated protozoa as potential contaminants of solutions for hydrophilic contact lenses and possible infectious agents of the cornea since there are no reports in the literature of this fact.***

Keywords **-** Corneal Diseases; Keratitis; Contact Lenses; Parasitology; Protozoa**.**

I. INTRODUCTION

Contact lens use is the main risk factor for corneal infections [1]. Parasitic infections are not a frequent cause of keratitis, *Acanthamoeba* is a main etiological agent of this group [2]. The association between contact lens use and protozoan keratitis has been reported since 1984 [3]. The first cases of corneal infection in humans caused by protozoa were reported in 1973, one case by *Acanthamoeba* and the other by *Microsporidium* [4,5]. In Brazil, Nosé et al described the first cases of protozoan keratitis (*Acanthamoeba*) in 1988 [6]. Despite the diversity and heterogeneity of the kingdom protista, the *Acanthamoeba* represents the main cause of parasitic keratitis. Other protozoa such as *Microsporidium that* belong to the phylum Microspora have also been described as pathogenic agents in corneal infection.

The ciliated protozoa belong to the phylum *Ciliophora*, and, similar to other parasites, are ubiquitous and can potentially affect man. However, there is no reference in the literature of the presence of these parasites in scraped corneas with infectious keratitis.

II. CASE REPORT

A 27-year-old Brazilian woman was presented in the ophthalmology ambulatory with complaints of red eye, foreign body sensation, tearing and decreased visual acuity in the left eye during the use of hydrophilic contact lenses. The patient reported daily use of lenses for about 10 hours and monthly disposal. Maintenance and disinfection were carried out with multi-use solutions for soft lenses. There was no personal history of systemic or eye diseases. Her right and left eyes had respectively, 20/20 and 20/30 vision with best correction. Biomicroscopy of the left eye revealed a moderate hyperemia of the bulbar conjunctiva, a 02 mm diameter middle and lower white paracentral infiltrate with undefined edges, overlying epithelial ulceration and corneal edema around the lesion. Slit lamp examination revealed anterior chamber reaction with flare (+1 / +4) and cells (11 -20 cells / field) and intraocular pressure of 16 mmHg in both eyes by applanation tonometry. There were no biomicroscopic changes in the right eye.

We carried out the corneal scrapings with the material collected, stained with Gram and Giemsa (slides), and seeded in blood, Sabouraud and soy agars. The soy agar was enriched with bacterial substrate. The contact lens and its solution were placed separately in culture media. The solution was also analyzed by direct optical microscopy. The patient was treated with eye drops of vancomycin 25mg/ml and amikacin 20mg/ml once a day and atropine 1% twice a day.

The cytologic examination of tissue from the ulcer showed rare cells in keratinization and that were keratinized, complete and degenerated rare polymorphonuclears, mucus and fibrin. The bacterioscopy did not show any bacteria. The corneal scrapings culture grew *Nocardia sp*. confirmed by biochemical tests (Fig. 1 and 2). The contact lens culture grew *Pseudomonas sp*. and *Candida albicans*. The lens solution culture found trophozoites and cysts of *Acanthamoeba sp*. and trophozoites of a ciliated protozoan suggestive of *Balantidium coli* (Fig. 3 and see video,

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Supplemental Digital Content 1, that demonstrates the ciliated protozoan suggestive of *Balantidium coli* moving around among cysts of *Acanthamoeba* in soy agar culture which was seeded with the lens solution of the eye affected).

Fig. 1 Corneal scraping culture on blood agar with *Nocardia sp* growth.

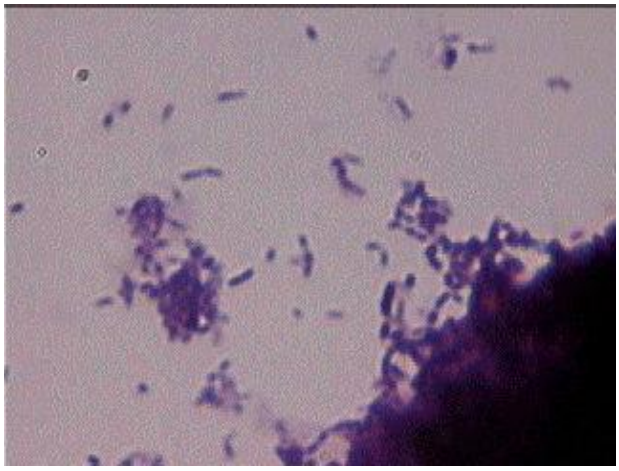

Fig. 2 Corneal scraping culture with *Nocardia sp*. identified through Kinyoum modified stain. (Optical microscopy 1000x).

The direct examination of the solution revealed trophozoites of the same ciliated protozoa (Balantidium coli?) and the direct examination of the slide stained with Giemsa revealed a trophozoite of a ciliated protozoan, that also seems to be Balantidium coli (Fig.4)(see table 1).

The antibiogram showed *Nocardia sp*. sensitive to amikacin, gentamicin, tobramycin, and imipenem. The patient had good improvement with the treatment with favorable remission of the symptoms and return of visual acuity to 20/20 (with correction) in the affected eye, a residual leucoma such as sequelae remaining in the area of previous injury.

Fig. 3 Trophozoites and cysts of *Acanthamoeba* (hollow arrow) and trophozoites of ciliated protozoan (full arrow - *Balantidium coli* ?) in soy agar culture media (Optical microscopy 1000x).

III. DISCUSSION

Pathogens can be carried to the eye for contact lenses, especially hydrophilic ones, due to their greater ability to retain deposits.

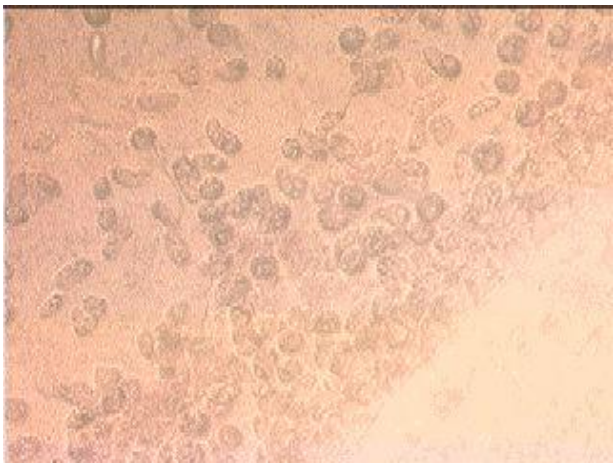

Fig. 4 Trophozoites of ciliated protozoan (*Balantidium coli*?) in corneal scraping - Giemsa stain (left) and direct examination of the contact lens solution (right) (Optical microscopy 1000x).

TABELA I LABORATORIAL DIAGNOSIS

	Cornea	**Contact lens**	**Solution**
Slide (Giemsa)	Trophozoites of *Balantidium coli* ?	---	---
Culture	*Nocardia sp*.	*Pseudomonas sp*. and *Candida albicans*	Trophozoites of *Acanthamoeba sp*. and *Balantidium coli*
Direct examination (solution)	---	---	Trophozoites of *Balantidium coli*

Therefore, the disinfection and maintenance of these lenses is essential. Several studies have shown that corneal infections among contact lens wearers are associated with contamination of their lenses and maintenance products by inadequate sanitation methods [7].

Bacteria, fungi and protozoa have been reported to cause keratitis in contact lens wearers. Among the bacteria, *Pseudomonas aeruginosa* is able to adhere to the surface of the lens especially in the presence of deposits, causing pyogenic keratitis with unfavorable prognosis [8]. In our report, this agent was only detected in the lens culture.

Nocardia has been described as a rare cause of keratitis, representing 1.7% of isolated cases in this type of infection [9]. Clinical diagnosis may be hampered by the lack of foresight by the ophthalmologist or similarity with keratitis caused by fungi or atypical mycobacteria [10]. Biomicroscopy shows disc-shaped gray infiltrate with a lacy aspect and "satellite" lesions. The conjunctiva may only present mild inflammation. Isolated cases of *Norcadia* keratitis have been reported in association with the use of contact lenses and patients who have undergone LASIK (*Laser in situ keratomileusis*). Traumas were the most important means of contamination in these cases [11]. *Nocardia* are aerobic and Gram-positive that grow as branching filaments and are stained with the Kinyoun modified method [12]. As they are weakly acid-resistant strains they may be mistaken with *Mycobacterium*. In culture, *Nocardia* grows commonly in various culture media such as blood agar, producing smooth and white colonies with a dry aspect as observed in the culture of corneal scrapings of the patient. Clinical studies indicate the sensitivity of *Nocardia* to drugs such as trimethoprim-sulfamethoxazole and amikacin [13]. This fact was confirmed in our case by antibiogram, which showed sensitivity of *Nocardia* to amikacin and a favorable clinical response to administration of this antibiotic.

Studies that assess the efficacy of disinfecting solutions for contact lenses show ineffectiveness of antifungal products against *Candida albicans*, which has been a frequent contaminant of contact lenses and a potential cause of keratitis in its users [7]. In our laboratory study, *Candida albicans* grew in Sabouraud agar from the culture of the contact lens of the affected eye, but was not found in other tests performed.

In the literature description, the main protozoa responsible for corneal infection is *Acanthamoeba*, especially in contact lenses wearers, and *Microsporidium* is more related to immunocompromised individuals, especially with acquired immunodeficiency syndrome (AIDS) [3,14]. *Acanthamoeba* is a cosmopolitan protozoa, in the form of either trophozoite or cyst, that does not require a host in its life cycle [15]. The trophozoite has protrusions on its surface like spines called acanthopodes that give it amoeboid movement and the name of the genre. The trophozoite measures approximately 30 to 40 μm and has a nucleus with single prominent nucleoli and contractile vacuoles in their cytoplasm that help in the laboratory diagnosis by optical microscopy. In the trophozoite form, *Acanthamoeba* moves around, feeds and reproduces by binary division. The ideal culture media for *Acanthamoeba* growth are soy agar or a non-nutritive culture media with bacterial substrate. In unfavorable conditions the trophozoite assumes the cystic resistant form, returning to the trophozoite condition when the environment becomes suitable again [16]. The cyst presents a spherical or polygonal oval form with double walls and sparse points of adhesion and measure from 15 to 25μm. As referenced in some studies, the presence of trophozoites of *Acanthamoeba* and other ciliated protozoa (*Balantidium coli*?) in the culture of contact lens solution demonstrates the tendency of this free-living amoeba for using other organisms as substrate nutrition, including protozoa [15,16]. Therefore, there is often the association of *Acanthamoeba* in polymicrobial infections, a fact in agreement with our laboratory findings. There are reports showing that *Acanthamoeba* can adhere to new and used contact lenses and contact lens cases. The use of all types of contact lens has been associated with *Acanthamoeba*. The risk is greater among hydrophilic contact lenses for daily or extended use as related in this case [17].

The ciliated protozoa had not been reported previously as a laboratory finding during the diagnosis of corneal ulceration. These ciliated parasites are, like the other protozoa species, widely distributed in nature [18]. They have been identified in soil, air, fresh and sea water, dust and as human parasites such as *Acanthamoeba*. Therefore, they are potential contaminants of contact lenses and their maintenance solutions, and can be carried to the cornea.

Balantidium coli is a ciliated protozoan that causes balantidiasis, an infection of the human intestine and an endemic in the Philippines. This protozoan is cited in the literature as the only ciliated protozoa that can cause infection in humans[19]. It is presented in the form of a cyst (a form of resistance) or a trophozoite (reproductive form), which multiplies in the large intestine and is eliminated with feces and can contaminate the environment and transmit the infection to other hosts. Its main reservoir is the pig, and the most common route of transmission is the fecal-oral ingestion of cysts present in contaminated water and vegetables. The diagnosis is confirmed by the observation of parasites in the examined material [20]. The trophozoite has a saccular shape, measuring about 60 to 100 μm in length and 50 to 80 μm in width with its surface covered by cilia, which gives it characteristic appearance and ability to move differently from *Acanthamoeba*. At its front end there a slot called cytostome through which debris and bacteria are ingested and stored in phagocytic vacuoles. Internally, it has two contractile vacuoles responsible for osmotic regulation, and a micro and macronucleus among other structures. The cyst is oval or spherical, measuring about 40 to 60 μm in diameter. Its wall is smooth and, internally, we note the macronucleus. As free-living amoebae, protozoa ciliates are associated with fungi, bacteria, other protozoa and even algae that are used as food substrate.

In our case, the ciliated protozoan found in the corneal scrapings was questioned as a trophozoite of *Balantidium coli*. The presence of the same organism in the culture and in the direct examination of the contact lens solution suggests that the parasite found in the cornea is the same. This finding has great importance, since it indicates the ability of accession of these parasites in the cornea and can cause a pathogenic process in this tissue. However, despite evidence of ciliated protozoa in the cornea, we cannot, in this case, attribute pathogenic properties to it, since *Nocardia sp.* was also detected as the etiologic agent in the sample of the same material. Therefore, this case consists of a keratitis caused by

Nocardia sp. An infestation of the cornea by ciliated protozoa in hydrophilic contact lens wearers with simultaneous contamination of their solution by the same parasite.

IV. CONCLUSIONS

In conclusion, it is necessary to consider ciliated protozoa as potential contaminants of solutions for hydrophilic contact lenses and possible infective agents of the cornea since there are no reports in the literature of this fact.

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REFERENCES

[1] Schein OD, Glynn RJ, Poggio EC, et al. The relative risk of ulcerative keratitis among users of daily-wear and extended soft contact lens. N Engl J Med 1989;321:773-778.

[2] Ma P, Visvesvara GS, Martinez AJ, et al. *Naegleria* and *Acanthamoeba* Infections: Review. Rev Infect Dis 1990;12:490-513.

[3] Moore MB, McCulley JP, Luckenbach, M, et al. *Acanthamoeba* keratitis associated with soft contact lenses. Am J Ophthalmol 1985;100:396-403.

[4] Jones DB, Visvesvara GS, Robinson NM. *Acanthamoeba polyphaga* keratitis and *Acenthamoeba uveitis* associated with fatal meningoencephalitis. Trans Ophthalmol Soc UK 1975;95:221-232.

[5] Ashton N, Wirasinha PA. Encephalitozoonosis (nosematosis) of the cornea. Br J Ophthalmol 1973;57:669-674.

[6] Nosé W, Sato EH, Freitas D, et al. Úlcera de córnea por *Acanthamoeba*: quatro primeiros casos no Brasil. Arq Bras Oftalmol 1988;51:223-226.

[7] Gopinathan U, Sharma S, Boghani S, et al. Sterility and the disinfection potential of Indian contact lens solutions. Indian J Ophthalmol 1994;42:65-70.

[8] Aswad MI, Jonh T, Barza M, et al. Bacterial adherence to extend wear soft contact lenses. Ophthalmology 1990;97:296-302.

[9] Garg P, Rao GN. Corneal ulcer: diagnosis and management. Community Eye Health; 1999;12:21-23.

[10] Huang AJW, Plugfelder SC. Nocardial and actinomycotic Keratitis. In: Pepose JS, Holland GN, Wilhelmus KR, eds. Ocular Infection and Immunity. St. Louis, Mosby; 1996:1043-1047.

[11] Douglas RM, Grove DI, Elliott J, et al. Corneal ulceration due to *Nocardia asteroides*. Aust NZJ Ophthalmol 1991;19:317-320.

[12] Berd D. Laboratory identification of clinically important aerobic actinomycetes. Appl Microbiol 1973;25:665-681.

[13] Boiron P, Provost F. In-vitro susceptibility testing of Nocardia spp. and its taxonomic implication. J Antimicrob Chemother 1988;22:623-629.

[14] Shadduck JA. Human microsporidiosis and AIDS. Rev Infect Dis 1989;11:203-207.

[15] Page FC. Taxonomic and ecological distribution of potentially pathogenic free-living amoebas. J Parasithol 1970; 56(suppl):257.

[16] 16. Illingworth CD, Cook SD. *Acanthamoeba* Keratitis. Surv Ophthalmol 1998;42(6):493-508.

[17] Alizabeh H, Niederkorn J, McCulley JP. *Acanthamoeba* Keratitis. In: Krachmer, Mannis, Hollan, Cornea. St Louis: Mosby, 1996:99.

[18] Finlay BJ. The global diversity of protozoa and other small species. Int J Parasitol 1998;28:29-48.

[19] Zaman V. *Balantidium coli*. In: Kreier JP, ed. Academic Press. Parasitic Protozoa, Vol.2. New York: 1978: 633.653.

[20] Clarck CG, Diamond LS. Methods for cultivation of luminal parasitic protists of clinical importance. Clin Microbiol Rev 2002;15:329-341.