

Acanthamoeba keratitis related to contact lens in a tertiary hospital in China

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Abstract

Background To report the clinical and microbiological features of *Acanthamoeba* keratitis (AK) related to contact lens in a tertiary hospital in China. **Methods** In this retrospective study, medical results of 61 cases of AK related to contact lens were reviewed from January 2000 to December 2017. The data included patients' demographics, lens type, history, risk factors, the disease stages, reports of corneal scrapings and culture, treatment. Moreover, the genotype identification of part of these isolates was carried out with a PCR assay and sequence analysis of the 18S ribosomal DNA gene. **Results** There were 64 eyes included in the study. And 32.8% of cases wore soft contact lenses, 67.2% of patients used overnight orthokeratology. In the cases (20eyes) of early stage, 65% (13 eyes) had positive results in Giemsa stained smears, trophozoites were seen in 7 eyes (35%) in 0.9% sodium chloride (NaCl) wet mounts. Six eyes (30%) were diagnosed by confocal microscopy together with clinical sign. In the orthokeratology patients, 87.8% (36/41) of cases rinsed the lenses and/or cases with tap water, while 55% of soft lens wearers wore their lens during showering. The genotype of 9 isolates was determined, all the strains belonged to genotype T4. In the orthokeratology related cases, patients required therapeutic penetrating keratoplasty after 2005 were less than before 2005 (Chi-square test, $\chi^2 = 4.209$, $P = 0.04$). **Conclusions** More than two thirds of cases were associated with orthokeratology. The examinations of Giemsa stained smears, 0.9% NaCl wet mounts and confocal microscopy should be done for the highly suspected cases of early stage to help the early diagnosis. The rate of therapeutic keratoplasty was decrease after 2005 compare with before 2005 in the cases of orthokeratology.

Background

Acanthamoeba keratitis (AK) is a severe and vision-threatening infection of the cornea. In the 1980s, AK was reported to be a dramatic rise in conjunction with the growing use of soft contact lens (SCL) [1]. Then, AK was thought to be most frequently in contact lens wearers in developed countries [2, 3]. In recent year, the incidence was increase [4, 5]. Moreover, the cases of AK related to contact lens were also reported in other countries [6, 7]. Although contact lens was not the most common risk factors in China [8], AK continued to be seen in contact lens wearers in recent years, especially the users of orthokeratology.

A good prognosis of AK depends on the diagnosis being early, but early diagnosis remains challenge. Several methods are fast and useful for the diagnosis in early stage, such as corneal scraping and confocal microscopy. In some cases of early stage, trophozoites were active and the number of cysts was small, diagnosis by *in vivo* confocal microscopy maybe difficult, directly examination of trophozoites by 0.9% sodium chloride (NaCl) may be a helpful method. In this retrospective study, the clinical and microbiological data of AK related to contact lens was analyzed in a tertiary hospital in Beijing, China.

Methods

A retrospective review was performed on the medical records of patients diagnosed with AK associated with contact lens during January 2000 to December 2017 at Beijing Tongren Hospital. This study was

approved by the Institutional Review Board of Beijing Tongren Hospital and adhered to the tenets of the Declaration of Helsinki.

Cases were included in this study if the corneal scraping and/or culture were positive for *Acanthamoeba*. The corneal scraping was stained with Giemsa for the detection of cysts. The material obtained by the next scrape was smeared into 0.9% NaCl on clean sterile glass slide for 0.9% NaCl wet mount in order to detect the trophozoites by direct microscopy (Olympus BX51, Tokyo, Japan), meanwhile, the cysts could also be detected. The images were captured using a color digital camera (DP-12, Olympus). The samples were applied onto the non-nutrient agar plates covered with *Escherichia coli* at 28°C for 5 to 15 days for the culture of amoebae. When the corneal scraping and culture were negative, the cases were identified if *Acanthamoeba* cysts were observed by *in vivo* confocal microscopy (Heidelberg Engineering GmbH, Dossenheim, Germany) together with a typical clinical sign. The following data were collected: demographic information, lens type, history, the risk factors, disease stages, reports of simultaneous cultures for bacteria and fungus. The DNA extraction and PCR assay were performed as described in our previous study^[9]. The PCR assay was performed with the genus-specific primers JDP1(5'-GGCCCAGATCGTTTACCGTGAA) and JDP2(5'-TCTCACAAGCTGCTAGGGGAGTCA). Then the direct sequencing of PCR products was performed with the conserved primer 892C(5'-GTCAGAGGTGAAATTCTTGG).

Treatment was also included. The disease stages (Table 1) and treatment referred to our previous study^[8]. The topical anti-amoebic therapy included chlorhexidine and/or polyhexamethylene biguanide (PHMB), the concentration of these drugs was according to the disease stages. Oral itraconazole was administered to some patients. If the infectious process spread despite an aggressive medical therapy, or in the cases of severe AK, therapeutic keratoplasty was performed. If the patients were coinfection with *Acanthamoeba* and other pathogens, appropriate treatment was used at the same time.

Statistical analysis was performed using the SPSS statistical software package (SPSS for Windows, version 17.0; SPSS, Inc, Chicago, IL). Independent-Sample t test and Chi-square test were conducted to compare the difference of clinical data. A *P* value less than 0.05 was considered statistically significant.

Results

There were 61 patients (64 eyes) included in the study, 24(39.3%) patients were male and 37(60.7%) patients were female. The age varied between 9 and 48 years old (mean 19.95±7.06 years old). Of the 61 patients, 20 patients (32.8%) wore SCL and 41(67.2%) patients used overnight orthokeratology. There were 3 bilateral cases in this study, all these patients used orthokeratology. The cases in each year were shown in Fig. 1, and the incidence peaked in 2001.

Table 2 showed the demographic characteristics, clinical feature, microbiological culture and treatment of all cases. There was no significant difference in gender between the SCL and orthokeratology (Chi-square test, $\chi^2 = 0.005$, *P* = 0.942). The orthokeratology wearers were younger than the patients who wore

SCL (Independent-Sample t test, $t = 4.338$, $P = 0.000$). The symptom duration was range from 5 days to 3 months. Fifty-four eyes (84.4%) had positive results in Giemsa stained smears. And trophozoites were detected in 19 eyes (29.7%) in 0.9% NaCl wet mounts. When the 0.9% NaCl wet mount was directly examined, the trophozoite had an oval to elongated outline, large central nucleolus and hyaline pseudopodia (Fig. 2), and moved slowly by its pseudopodia in the tissues. During the cases (20eyes) of early stage, 65% (13 eyes) had positive results in Giemsa stained smears, trophozoites were seen in 7 eyes (35%) in 0.9% NaCl wet mounts. Six eyes (30%) were diagnosed by confocal microscopy together with clinical sign. The culture-positive rate of *Acanthamoeba* was 76.6% (49/64). Six cases (9.84%) had polymicrobial infection, most (5/6) were soft contact lens wearers. The isolated organisms were *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. No fungal organisms were isolated. The genotype of 9 isolates from nine patients was identified, 4 patients were male and 5 were female. Six isolates were from soft contact lens wearers, 3 isolates were form the patients who used orthokeratology. All these *Acanthamoeba* strains belonged to the genotype T4. Among the 9 strains, two were identified as T4/6, and others were identified as T4/8, T4/9, T4/13, T4/24, T4/31, T4/34, T4/41 respectively.

The cases of different disease stages in each lens type were summarized in Table 3. In the orthokeratology related cases, patients required therapeutic penetrating keratoplasty after 2005(3/22) were less than before 2005(8/19) (Chi-square test, $\chi^2 = 4.209$, $P = 0.04$).

During the orthokeratology patients, 87.8% (36/41) of cases rinsed the lenses and/or cases with tap water, while in the SCL wearers, 11 patients (55%) wore their lens during showering (5 cases, 25.0%) or sleeping (6 cases, 30.0%).

Discussion

Acanthamoeba genus is free-living amoebae which can find in the air, soil and water. In China, the most common risk factor was ocular trauma, followed by contact lens wear^[8], however, the cases related to contact lens could be seen almost every year in this study. In addition, a notable finding of our previous study was that cases of AK were nearly one half of the cases of microbial keratitis related to contact lens wear^[10]. But so far, few studies reported the clinical and microbiological features of contact lens related AK in China.

In this study, the peak year for occurrence was 2001, which may be due to the outbreak of AK associated with orthokeratology in 2001^[11, 12]. Then the government intervened to regulate the orthokeratology market, the number of patients being fitted with orthokeratology was decrease. The cases of AK related to orthokeratology were decrease correspondingly^[13]. In this study, there were no patients related to orthokeratology in 2004 and 2005. But in recent years, the cases were reemerged, and increased slightly. This is also happened in other countries. It was reported that a new baseline incidence of AK in the United Stated was 10 times greater than before 2004^[14]. A study of AK among rigid gas permeable contact lens wearers showed that nearly a quarter of patients were orthokeratology wearers from 2005 to 2011^[15]. In

New Zealand, the incidence of AK from 2009 to 2016 had more than doubled when compared with the preceding 7-year period [4].

In previously studies, most cases related to contact lens involved soft lens [4, 6, 16, 17]. However, there were more than two thirds of AK cases related to orthokeratology in this study. Even in Hong Kong, 37.5% cases of contact lens-related AK were attributed to orthokeratology [18]. The reason may be that the myopic population in China has been increased in recent years, and there has also been an increase in the average degree of myopia, especially among young children [19]. Orthokeratology became one of the most popular choices for the parents. Consequently, many accompanying adverse effects associated with orthokeratology were observed, including AK. Moreover, most orthokeratology users are children and teenagers, the AK patients associated with orthokeratology were younger than the patients with soft lenses. Therefore, it is necessary that the patients and their parents should learn more regarding proper lens hygiene and care. Unlike other studies [4], the bilateral cases in this study were all associated with orthokeratology.

Early diagnosis is essential to secure a good prognosis. Some literatures suggested that it was important to remember and consider AK in all cases of contact lens keratitis [20, 21]. Clinical features of AK are similar to other keratitis sometimes, such as fungal and herpes simplex virus. Although confocal microscopy is a noninvasive technique [1], the images of cysts were atypical in some cases of early stage [22]. Microbiological testing of corneal scrapings by microscopy is easy, fast and efficient. In this study, the positive rate of Giemsa stained smears was as high as 84.4%. In the cases of early stage, the rate was lower, while the rate of 0.9% NaCl wet mounts for the detection of trophozoites was a little higher. Because drugs were not used in some cases of early stage, encystment did not occur. The trophozoites activity can be observed by 0.9% NaCl wet mounts directly. For the highly suspected cases of early stage, especially characterized by epitheliopathy, the examinations of Giemsa stained smears, 0.9% NaCl wet mounts and confocal microscopy should be done to help the early diagnosis of AK.

The culture of *Acanthamoeba* was required in any suspected infective keratitis. The culture-positive rate of *Acanthamoeba* had been reported as between 30% and 60% [20], and the culture requires long incubation time, it still thought to be a definitive diagnosis method [23]. In this study, the culture-positive rate (76.6%) was higher than previous reports, the reason may be that two thirds of cases were diagnosed with advanced and late stages, the corneal scrapings may include more trophozoites and cysts.

It is notable that AK can coinfect with other organisms. It was reported that the coinfections with bacteria were seen in 23% of cases [24]. In a study in Egypt, bacteria were isolated from all cases of AK [7]. In this study, 9.84% of cases had polymicrobial infection. This was close to the result of our previous study (15%) [25]. Most likely because this survey was conducted in a tertiary referral center, many cases had antibacterial treatment before antiamebic therapy. The result of genotyping analysis was consistent with our previous study [9] and other study in China [26]. Although the subtypes were different, the genotype

T4 is still the main AK-related genotype in China, furthermore, genotype T4 is the predominant genotype in other countries and areas^[27-30].

Therapeutic penetrating keratoplasty is required in cases of severe AK but not recommended as a method of removal of organisms from the cornea^[20], because it associated with some poor outcomes, such as poor graft survival, repeat transplantation and glaucoma. In this study, the rate of therapeutic keratoplasty was decrease after 2005 compare with before 2005 in the cases of orthokeratology. There are several possible reasons. First, since 2003, orthokeratology gradually entered in to a state of relative standard and healthy development in China^[31], clinicians were gradually highly suspicious of AK in the keratitis related to contact lens. Contact lens users were told to go to hospital in time if they had the symptoms. Second, as confocal microscopy has high sensitivity and specificity for AK diagnosis, some cases were diagnosed rapidly. Third, as the increasing number of cases, the clinicians were more experienced in the treatment.

The risk behaviors between orthokeratology and soft lens wearers were a little different in this study. About 87.8% of patients with orthokeratology rinse their lenses with tap water, while the most common risk behaviors in SCL wearers were showering or sleeping with their lenses. The risk behaviors are consistent with the survey in Egypt^[7]. But in the United States, storing lenses in tap water and topping off contact lens solution in the case were the most common risk factors for AK in rigid gas permeable contact lens wearers. Moreover, wearing lenses for orthokeratology overnight was also thought as a risk factor^[15]. Anyway, contact lens wearers should strictly adhere to good contact lens hygiene practices and avoid the exposure of tap water in order to minimize risk of AK, especially for the orthokeratology patients.

There are still some limitations. As a retrospective study, some information was incomplete during clinical data collections, such as the history. Because this survey was conduct in one hospital, there might be bias of the percentage of cases in each lens type. More work is needed to explain the difference of polymicrobial infection between orthokeratology and SCL.

Conclusions

In conclusion, AK is one of the most challenging infections to manage. Although contact lens wear was not the most common risk factor of AK in China, AK cases related to contact lens could be seen continuously in these years. More than two thirds of cases were associated with orthokeratology. The most common risk behaviors was rinsing lenses and/or cases with tap water in orthokeratology users while wearing lenses during showering or sleeping in SCL patients. For the highly suspected cases of early stage, especially characterized by epitheliopathy, the examinations of Giemsa stained smears, 0.9% NaCl wet mounts and confocal microscopy should be done to help the early diagnosis of AK. As the increasing vigilance of AK by clinicians, the early diagnosis and effective medical treatment, the rate of therapeutic keratoplasty was decrease in recent years in the cases of orthokeratology. Optometrists and ophthalmologists should teach contact lens wearers to strictly adhere to good contact lens hygiene

practices and avoid the exposure of tap water in order to minimize risk of AK, especially for the orthokeratology users.

Abbreviations

AK: *Acanthamoeba* keratitis; SCL: Soft contact lens; NaCl: Sodium chloride.

Declarations

Authors' contributions

WWL drafted the manuscript and performed the literature review. ZQW and YZ conducted *Acanthamoeba* lab laboratory tests and culture. JHQ participated in information gathering and editing. XGS conceived the idea, managed the patients' ophthalmic conditions. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Tongren Hospital, Beijing, China, and the approval number is TRCKY-033. Informed consent was obtained from all subjects after the aims and nature of the study were explained to the participants.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The authors declare no conflict of interest.

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Tables

Table 1 The disease stages [8]

Stage	Manifestation
Early stage	Pseudodendritic or punctate corneal epithelial lesions, dot infiltrations under the epithelium, recurrent epithelial erosion, radial corneal neuritis, corneal ulcer smaller than 4mm in diameter in the anterior stroma
Advanced stage	Deep stromal ulcer greater than 5mm and less than 8mm in diameter
Late stage	The deep stromal ulcer greater than 8mm in diameter and hypopyon, central corneal thinning and perforation

Table 2 Results of demographic characteristic, clinical feature, microbiological culture and the treatment in each lens type

Characteristic	Soft contact lens (n=20, ,eyes=20)	Orthokeratology (n=41,eyes=44)
Gender, n(%)		
Female	12 (60.0)	25 (61.0)
male	8 (40.0)	16 (39.0)
Age(years)	25.90±8.81	17.05±3.41
Positive result in culture for <i>Acanthamoeba</i> , eyes(%)	16 (80.0)	33 (75.0)
Positive result in culture for bacteria, eyes(%)	5 (25.0)	1 (2.3)
<i>Pseudomonas aeruginosa</i>	3(15)	0(0)
<i>Staphylococcus epidermidis</i>	2(10)	1(2.3)
Treatment, eyes (%)		
Medical therapy	16(80.0)	33(75.0)
Therapeutic penetrating keratoplasty	4(20.0)	11(25.0)

Table 3 The cases in each lens type with different disease stages

Lens type	Early stage, eyes(%)	Advanced stage, eyes(%)	Late stage, eyes (%)
Soft contact lens	6(30.0)	12(60.0)	2(10.0)
Orthokeratology	14(31.8)	20(45.5)	10(22.7)

Figures

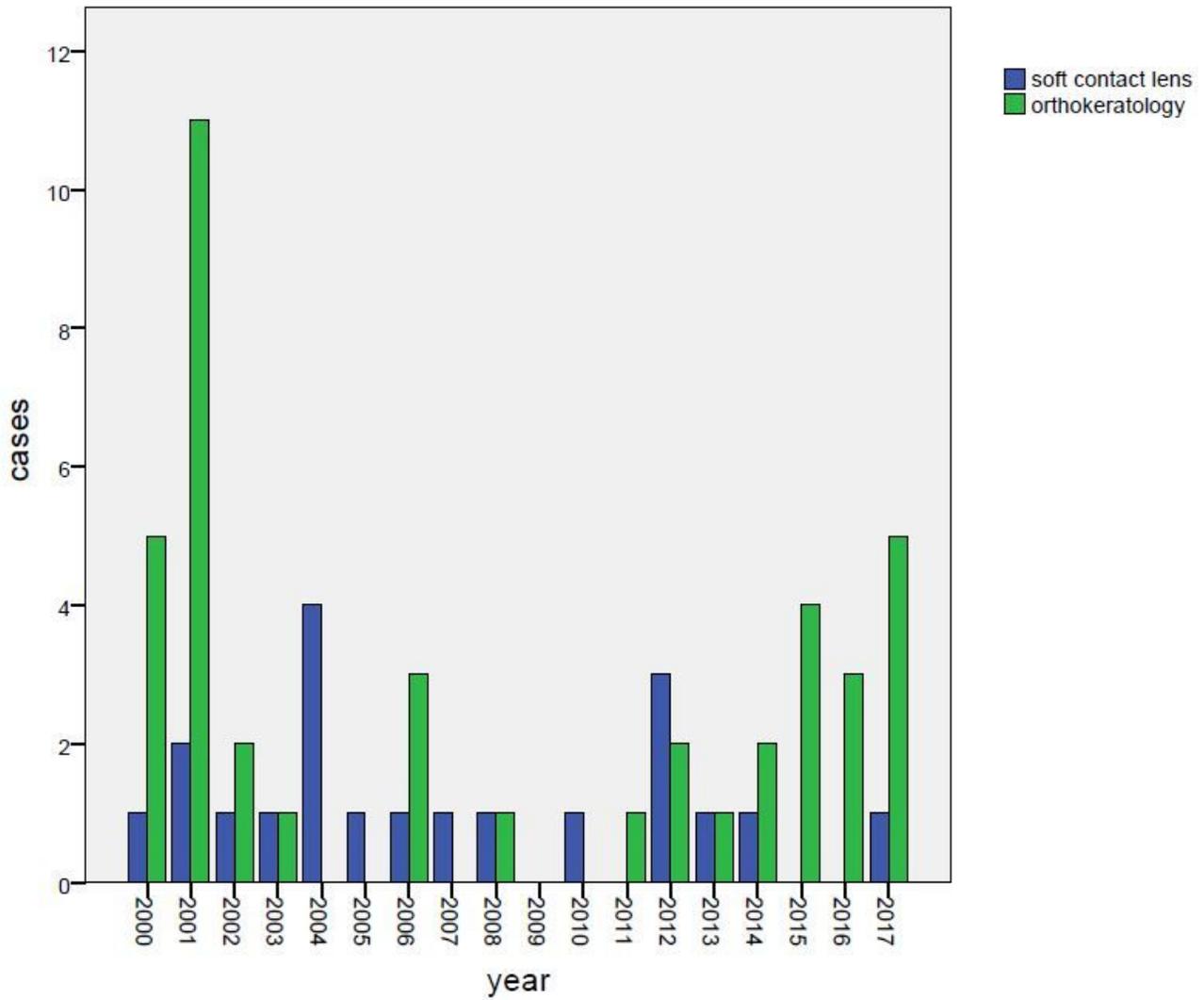


Figure 1

Cases of Acanthamoeba keratitis related to contact lens in each year



Figure 2

Acanthamoeba trophozoite in 0.9% sodium chloride wet mount (black arrow) a The trophozoite was oval with a large nucleus. b The same Acanthamoeba trophozoite became elongated in shape after several seconds.