

Paediatric Fungal Keratitis—A Case Series of 45 Children Presenting to a Tertiary Referral Centre

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Abstract

Purpose To analyze the etiology, characteristics, and treatment outcomes of paediatric fungal keratitis in northern China.

Methods The medical records of children (< 18 years old) diagnosed with fungal keratitis at the Shandong Eye Institute from 1996 to 2017 were reviewed for demographic features, risk factors, seasonal variation, clinical characteristics, laboratory findings, and treatment strategies.

Results Forty-five children (46 eyes) were included. Fungal keratitis in children accounted for 16.4% of all of the children with infectious keratitis, which was lower than that in adults (59.4%, $p < 0.001$). Unexplained fungal keratitis accounted for 26.1%. Plant trauma (23.9%) and wind and sand into the eyes (21.7%) ranked second and third, respectively. Fusarium fungi infection was found in 69.9%, and 31.0% were infected by Aspergillus fungi. The corneal ulcer area was positively correlated with a peripheral blood neutrophil/lymphocyte ratio (NLR) at the first visit ($r = 0.310$, $p = 0.036$). Voriconazole had the highest drug sensitivity rate. A total of 67.4% (31 eyes) underwent therapeutic keratoplasty (PKP 21 eyes; LKP 10 eyes). The recurrence rate was 3.2% (due to an Aspergillus infection). The best corrected visual acuity (BCVA) after treatment in children ≤ 8 years (median 20/60) was lower than the BCVA in children > 8 years (median 20/50, $p = 0.035$).

Conclusions Fungal keratitis are less common in children than in adults. The main infection was due to Fusarium fungi. The preferred antifungal drug was voriconazole. LKP treatment is preferred for Fusarium infection keratitis.

Introduction

Fungal keratitis is a rare occurrence in children.¹ There was only one case (5.9%) of fungal infection in a five-year study of childhood infectious keratitis in Canada.² In a 23-year study of childhood infectious keratitis in the United States, only 12 cases of fungal infections occurred.³ These reports led to little knowledge of the pathogenesis of fungal keratitis in children. Is mycotic keratitis really lower in children than in adults? Are children different from adults regarding the characteristics of onset, treatment times, infection strains, antifungal drug susceptibility, and immune defense characteristics? If so, do they lead to the differences in prevalence between children and adults? The "cytokine storm" is associated with the severity of the disease,⁴ and severely ill patients often present with a decrease in lymphocytes.⁵ In children from 4–6 days to 4–6 years of age, the neutrophil-to-lymphocyte ratio (NLR) is inverted, leading to cellular immunity in childhood. The NLR as a classic inflammatory indicator has been used in recent years to predict the prognosis of hypertension and cardiovascular disease, tumor prognosis, keratoconus progression, and inflammation and oxidative stress in the corneas of patients with keratoconus indicators. We also determined whether it was related to the onset of fungal keratitis. We analyzed the data from children with mycotic keratitis who visited the Shandong Eye Institute from 1996 to 2017 to have a deeper understanding of the characteristics of fungal keratitis in children.

Methods

This study was approved by the Ethics Committee of Shandong Eye Institute and adhered to the tenets of the Declaration of Helsinki.

The medical records of children (< 18 years old) who were diagnosed with fungal keratitis at the Shandong Eye Institute from 1996 to 2017 were reviewed. The patient's demographic characteristics, pathogenesis, disease characteristics, laboratory tests, treatment methods, and outcomes were recorded. The methods of diagnosis, treatment, and laboratory examination of children with fungal keratitis were as described in previous studies.^{1,5} All subjects under the age of 18 had obtained the informed consent of their parents and/or legal guardians.

The diagnostic criteria for fungal keratitis were a 10% potassium hydroxide smear and/or confocal microscopy for hyphae, corneal scraper culture and/or corneal tissue culture with fungal growth, and corneal pathology with hyphae. A patient can be diagnosed based on one of these criteria.

As a drug susceptibility test, the M-38A protocol (microdilution method) developed by the American Society for Clinical and Laboratory Standards (CLSI) was used, and the MIC value was read. Image J software was used to analyze the corneal ulcer area percentage (corneal ulcer area percentage = corneal ulcer area/full corneal area x 100%).

All of the data were processed using SPSS 22.0 software. A Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. The best corrected visual acuity was expressed as the median (range), and other descriptive statistics were expressed as mean ± standard deviation (range). A Chi-squared test was used to compare the incidence at different times and the positive rate of different tests. A comparison of visual acuity between the groups was performed with a Mann-Whitney test. Spearman's correlation analysis was used to assess the correlation between the two sets of data. All of the tests were accurate on both sides; the difference was statistically significant at $p < 0.05$.

Results

Epidemiologic features

From 1996 to 2017, a total of 45 children (46 eyes) were treated for fungal keratitis, accounting for 16.4% (46/281) of the children with infectious keratitis. Among them, 24.4% (32/131) were assessed between 1996 and 2006, and 9.3% (14/150) were assessed between 2007 and 2017, which showed there was a decrease from 1996 to 2006 ($p = 0.001$, χ^2 -test). During the same period, there were 3,089 adult patients who presented with fungal keratitis (3,091 eyes), accounting for 59.4% (3091/5204) of the adult patients with infectious keratitis. The incidence of fungal keratitis was lower in children than in adults ($p < 0.001$, χ^2 -test).

Forty-five patients, including 30 males (66.7%) and 15 females (33.3%), were enrolled in the study. The mean age of onset was 12.2 ± 3.7 (4–17) years. A total of 95.6% (43/45) of the patients were from rural areas. No children with a history of diabetes were found.

From April to September, 69.6% (32/46) of the affected eyes of 47.8% (22/46) of the patients maintained symptoms from the time of symptom onset to presenting at our hospital within 1 week, and only 1 patient (2.2%) had contact lens-induced disease, which had a duration of more than 1 month.

Unexplained fungal keratitis accounted for 26.1% (12/46) of the cases, plant trauma (23.9%, 11/46), and dust, soil or a stone in the eye (21.7%, 10/46), respectively, occupied the second and third places, respectively (**Table 1**).

Forty-four eyes (95.7%) were treated at a local hospital. Among them, the correct diagnosis rate at the local hospitals was 50.0% (22/44); 27.3% (12/44) were misdiagnosed as bacterial keratitis; 2.3% (1/44) were misdiagnosed as viral keratitis, and undiagnosed accounted for 20.5% (9/44). Five eyes (11.4%, 5/44) had a clear history of hormone use.

Clinical features

At the initial visit to our hospital, we observed 26.1% (12/46) with anterior chamber empyema and 4.3% (2/46) had corneal perforation. We observed 6.5% (3/46) with endophthalmitis and all three eyes were treated with hormone therapy at a local hospital.

At the initial visit to our hospital, the area of corneal ulcers accounted for $21.5 \pm 22.7\%$ (1.6%–100%) of the total corneal area. The NLR averaged 2.4 ± 1.1 (0.7–7.0). The area of the ulcer was positively correlated with the NLR ($r = 0.310, p = 0.036$). There was a positive correlation between age and the ulcer area percentage ($r = 0.299, p = 0.043$) and NLR ($r = 0.394, p = 0.007$).

Microbiology results

The positive result rate for a fungal smear was 86.0% (37/43), the positive rate for a fungal culture was 81.4% (35/43), and the positive rate for confocal microscopy was 85.0% (17/20). There was no statistical difference ($p = 0.833$, χ^2 -test). Of the 29 eyes chosen for strain identification, 69.0% were shown to have a *Fusarium* infection (20 eyes) and 31.0% had an *Aspergillus* infection (9 eyes). Four patients had bacterial infections (one case each of *Staphylococcus epidermidis*, *Bacillus subtilis*, *Enterococcus*, and *Hafnia*; **Table 2**).

Antibiotic susceptibility

The geometric mean of common fungi for common antifungal MICs was voriconazole (0.909) and fluconazole (36.062). The geometric mean of the MIC of *Fusarium* against common antifungal drugs for voriconazole was 1.231, followed by 1.932 for amphotericin B. The geometric mean of the MIC of the

commonly used antifungal drug for Aspergillus (i.e., itraconazole) was the lowest (0.170), followed by voriconazole (0.463; **Table 3**).

From the results of the drug susceptibility tests, voriconazole was up to 65% sensitive to Fusarium, followed by natamycin and amphotericin B (50% sensitive). Fluconazole (85%), ketoconazole (75%), and terbinafine (75%) had a high rate of resistance. The highest sensitivity considering ketoconazole, itraconazole, and voriconazole to Aspergillus was 77.8%, and natamycin (100%) and amphotericin B (77.8%) were highly resistant to Aspergillus. All of the drug susceptibility results are summarized in **Table 4**. Voriconazole was the most sensitive to fungal treatment (69%).

Treatment and outcomes

Thirty-one eyes (67.4%) underwent therapeutic keratoplasty [21 eyes with penetrating keratoplasty (PKP), including 1 eye with crystal removal, vitrectomy combined with PKP, and 10 eyes with lamellar keratoplasty (LKP), 1 eye (2.2%) with enucleation and 14 eyes (30.4%) that improved after lesion removal and medication]. The follow-up time after treatment was 4.0 ± 3.9 (0.6–13.9) years. The recurrence rate of therapeutic keratoplasty was 3.2% (1/31), and the lesion reappeared three days after LKP treatment (10.0%, 1/10). The lesion improved after PKP treatment and the pathogen was Aspergillus.

The median best corrected visual acuity after PKP treatment was 20/50 (20/200–20/25), of which 42% were $\geq 20/40$ eyes; the median best corrected visual acuity after LKP treatment was 20/50 (20/100–20/40), whereas $\geq 20/40$ eyes accounted for 30.0%. The median best corrected visual acuity after drug treatment was 20/50 (20/400–20/20), eyes $\geq 20/40$ accounted for 21.4%. There was no significant difference between the best corrected visual acuity between PKP and LKP treatments ($p = 0.913$). There was no statistically significant difference between the best corrected visual acuity after drug treatment and corneal transplantation ($p = 0.951$). The best corrected visual acuity after treatment [median 20/60 (20/400–20/40)] for children ≤ 8 years old was lower than the best corrected visual acuity after treatment in children >8 years old [median; 20/50 (20/200–20/20); $p = 0.032$].

During the follow-up period of 3.8 ± 4.1 (1.2–13.9) years, three eyes (14.3%) developed immunostaining after PKP treatment, and the time of rejection occurred from 1 to 23 months after surgery, all of which were improved by drug treatment. With LKP, no immunosuppression occurred with the graft. At the last follow-up, all of the grafts remained transparent, with one ocular cataract occurring (3.2%, 1/31).

Discussion

Infectious corneal ulcers in northern China are predominantly due to fungal keratitis, and they mainly affect agricultural populations that are vulnerable to plant trauma.¹ Children's active nature and lack of life experience and self-protection make them more vulnerable to eye trauma. In this study, the children were mostly from rural areas and were exposed more to the natural environment. This study showed that the incidence of fungal keratitis in children was lower than that of adults, and the incidence gradually decreased with improvement of economic and public health levels. Especially in the past 11 years, the

incidence of fungal keratitis in children has dropped to 9.3%, which is close to the level reported in the United States.

As with other pediatric diseases, fungal keratitis, for which the history and cause cannot be determined, accounts for more than a quarter (26.1%), which increases the difficulty of a correct diagnosis of patients in local hospitals. Due to the presence of co-infection, it is easily misdiagnosed as a bacterial disease, such as sexual keratitis. The proportion of dust, soil, or stone causes (21.7%) was much higher than in our previous study that included all ages,¹ and the ratio reported in southern India was close to.⁷ This is related to children not knowing how to properly handle dust or sand, and false blinks increasing the chance of fungal infections. Fusarium infections were the main source of pathogenesis of childhood fungal keratitis, which was consistent with other reports,^{3,6} and the fungal spectrum was a single source, which was found less often than in adults.¹ Fungal corneal ulcers that occur in children do not appear to be more severe than fungal corneal ulcers in adults due to imperfect immune defenses in children. In addition to a more timely onset in children, a low childhood NLR may be a protective factor for fungal corneal ulcers in children. In some cases, a large number of immune cells are activated to secrete more cytokines, and more cytokines recruit a larger number of immune cells, forming an uncontrollable cascade reaction, which is called the cytokine storm. This causes the disease to become more severe, which causes serious damage to human tissues and organs.

In fungal corneal ulcers, neutrophils can both kill the fungal defenses and damage the cornea through reactive oxygen species (ROS) and the release of large amounts of protease.⁸⁻¹⁰ In this study, the higher NLR in the peripheral blood of children and the larger the area of corneal ulcers corresponding to it suggested that the level of neutrophils affects the outcome of corneal ulcers. At present, there are relatively few studies on lymphocytes and infectious eye diseases, which is the research direction that we need to pay more attention to in the future. Three children with endophthalmitis had severe eyeball penetrating injuries, injuries and crystals, or removal of crystals during surgery in the hospital as well as a clear history of hormone therapy, suggesting that damage to the crystal barrier and the application of hormones are fungal risk factors for intraocular infections¹¹.

Early diagnosis and treatment greatly reduced the proportion of children with fungal corneal ulcers treated with corneal transplantation, and the standardized use of antifungal drugs also played a beneficial role. Although voriconazole and itraconazole had the highest drug sensitivity to various fungi, *in vitro* drug sensitivity results are often inconsistent with the sensitivity of antifungal drugs *in vivo*.¹²

Children's corneal transplantation has higher risk than adult surgery, but in this study, the average age of the patients was around 12 years old, so the incidence of postoperative complications was not significantly different from that found in adults.^{7,13,14} Aspergillus is more erosive due to its oblique growth pattern, leading to a recurring need for a LKP, which is consistent with the findings of previous studies in our hospital.^{7,13} We therefore propose a viable LKP for corneal ulcers infected with Fusarium, reducing the chance of rejection and the risk of long-term corneal endothelial dysfunction.^{6,12}

Serna-Ojeda JC reported results from nine eyes after PKP treatment in children with HSK. The median best corrected visual acuity was 20/50, and none of the nine eyes had graft failure. The postoperative rejection rate (1 eye, 11.1%) was consistent with our treatment results.¹⁵ In our study, there were no children with abnormal intraocular pressure, which may be due to differences in total hormone use times and no history of recurrent episodes of fungal corneal ulcers. Monocular vision abnormalities in childhood can lead to amblyopia, which is the reason why we found the best corrected visual acuity of children aged \leq 8 years old was lower than that of children over 8 years old. This is the basic course of children's visual development.

In summary, the incidence of fungal keratitis in children was lower than in adults. A low NLR in childhood may be a protective factor for fungal corneal ulcers in children. The role of neutrophils in the pathogenesis of fungal keratitis in children should be taken seriously. Although early diagnosis and standardized treatment can help reduce the rate of therapeutic corneal transplantation in children, corneal transplantation can still provide children with long-term best corrected vision improvement. In particular, LKP treatment is preferred for keratitis due to the potential for *Fusarium* infection.

Declarations

Disclosures

Financial disclosures: No financial disclosures.

Author Contributions

Yanling Dong involved in design and conduct of study. Lixin Xie performed the keratoplasty and review the paper. Collection and management of the data were done by Huabo Chen, Xiaomei Wan, Mingming Jiang, Yichao Ding. Jing Zhang analyzed the data and wrote the paper.

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Tables

Table 1. Risk factors for fungal keratitis in children at the Shandong Eye Institute, 1996–2017

		No.of eyes	%
Trauma	Plants/agriculture	11	23.9%
	Stone	3	6.5%
	Glass	1	2.2%
	Firecracker	1	2.2%
	Dust, soil, or stone	10	21.7%
	Small winged insect	2	4.3%
Others	Post-Lasik	2	4.3%
	Contact lens	1	2.2%
	After respiratory tract infection	3	6.5%
Unknown		12	26.1%

Table 2. Fungal spectrum of children's fungal keratitis at the Shandong Eye Institute, 1996–2017

	No.of eyes	%
Fusarium species	20	
<i>F.moniliforme</i>	8	40.0%
<i>F.solani</i>	4	20.0%
Unknown	8	40.0%
Aspergillus species	9	
<i>A. fumigatus</i>	5	55.6%
Unknown	4	44.4%

Table 3. Children's fungal keratitis and commonly used antifungal drugs at the Shandong Eye Institute, 1996–2017 Minimum Inhibitory Concentrations, mg/ml

	Fusarium species		Aspergillus species		Total	
	n=20		n=9		n=29	
	MIC	GM	MIC	GM	MIC	GM
Amphotericin B	0.25-16	1.932	0.25-8	2.940	0.25-16	2.201
Ketoconazole	0.5-16	7.464	0.125-16	0.926	0.125-16	3.906
Itraconazole	0.25-32	2.071	0.125-0.5	0.170	0.125-32	0.953
Fluconazole	4-64	34.297	16-64	40.317	4-64	36.062
Voriconazole	0.25-16	1.231	0.125-8	0.463	0.125-16	0.909
Natamycin	1-16	2.378	8	8	1-16	3.466
Terbinafin	1-8	2.828	0.5-1	0.926	0.5-8	2

Table 4. Results of antibiotic susceptibility in children with fungal keratitis at the Shandong Eye Institute, 1996–2017

	Fusarium species n=20	Aspergillus species n=9	Total n=29
Amphotericin B			
S	10±50%	2±22.2%	12±41.4%
SDD	0±0%	0±0%	0±0%
I	3±15%	0±0%	3±10.3%
R	7±35%	7±77.8%	14±48.3%
Ketoconazole			
S	5±25%	7±77.8%	12±41.4%
SDD	0±0%	0±0%	0±0%
I	0±0%	0±0%	0±0%
R	15±75%	2±22.2%	17±58.6%
Itraconazole			
S	3±15%	7±77.8%	10±34.5%
SDD	5±25%	2±22.2%	7±24.1%
I	0±0%	0±0%	0±0%
R	12±60%	0±0%	12±41.4%
Fluconazole			
S	3±15.0%	0±0%	3±10.3%
SDD	0±0%	3±33.3%	3±10.3%
I	0±0%	0±0%	0±0%
R	17±85%	6±66.7%	23±79.3%
Voriconazole			
S	13±65%	7±77.8%	20±69%
SDD	0±0%	0±0%	0±0%
I	2±10%	0±0%	2±6.9%
R	5±25%	2±22.2%	7±24.1%
Natamycin			
S	10±50%	0±0%	10±34.5%

SDD	5±25%¶	0±0%¶	5±17.2%¶
I	5±25%¶	0±0%¶	5±17.2%¶
R	0±0%¶	9±100%¶	9±31%¶
Terbinafin			
S	0±0%¶	1±11.1%¶	1±3.4%¶
SDD	5±25%¶	8±88.9%¶	13±44.8%¶
I	0±0%¶	0±0%¶	0±0%¶
R	15±75%¶	0±0%¶	15±51.7%¶

S=susceptible; SDD=susceptible-dose dependent; I=intermediary; R=resistant