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Clinical Analysis of 137 Cases of Fungal Keratitis

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Abstract

Objective: To analyze the epidemiology, clinical diagnosis and treatment of fungal keratitis (FK) in hospitalized patients at the First Affiliated Hospital of Yangtze University in recent years. **Methods:** A retrospective investigation was conducted on the data of 137 cases of FK in our hospital from January 2019 to December 2022. The epidemiological characteristics, identification results of fungal strains, clinical treatment, and prognosis of the patients were analyzed. **Results:** Among the 137 FK patients, 89 were males and 48 were females, and the ratio of male to female was 1.85:1, The age of onset was the largest number of patients in 50 - 59 years old and 60 - 69 years old. The disease occurred most in autumn, winter and summer farming season (from September to December, January, May, June). 72 cases (52.6%) had a clear history of corneal injury, and 43 cases (31.4%) had a history of plant injury. Other risk factors include eye surface diseases, ophthalmic surgery, and wearing corneal contact lenses. The top three pathogens were *fusarium* (38.7%), *aspergillus* (23.3%), and *alternaria* (17.5%). 101 eyes showed improvement or cure after treatment with medication, 9 eyes underwent corneal stromal injection, 11 eyes were covered with conjunctival flap covering or amniotic membrane transplantation, 12 eyes were covered with corneal transplantation, and enucleation of ocular contents was performed in 4 eyes; Visual acuity was improved or maintained in 123 patients (about 89.8%). **Conclusions:** The incidence of FK in our hospital was mostly middle-aged and elderly men, mostly caused by corneal injury in the process of agricultural labor. The pathogens were mainly fusarium and aspergillus. The preferred treatment was medication, with severe cases requiring combined surgical treatment. Most patients can maintain or improve their vision after treatment.

Keywords

Fungal Keratitis, Retrospective Analysis, Epidemiology, Pathogens

1. Introduction

Fungal keratitis (FK) was a type of keratitis caused by pathogenic fungal, which was an important cause of blindness in corneal diseases in China, especially in areas with low medical care and underdeveloped economies, it often leads to visual loss and was the primary cause of eye removal [1] [2] [3]. The incidence of FK was increasing every year due to corneal injury, especially plant injury, abuse of glucocorticoids, broad-spectrum antibiotics and immunosuppressants, unregulated contact lens wear [4] [5]. According to a study [6], it was estimated that over 1 million eyes were affected by FK worldwide every year, with approximately 10% of patients suffering corneal perforation or requiring eyeball removal. The condition is most prevalent in tropical and subtropical locations and has been estimated to account for 20% - 60% of all culture-positive corneal infections in these climates. The epidemiology of FK varies with regional and climate differences, with *Candida albicans* predominantly found in developed countries and colder regions, while *fusarium* and *aspergillus* were the main pathogens in developing and warm regions. The Jing Zhou region was located in the Jiang Han Plain and grows a variety of crops such as rice, wheat, rape and cotton, FK was easily caused by plant injury during the harvesting season. This study retrospectively analyzed the case data of 137 FK patients in our hospital, providing a basis for clinical diagnosis and treatment of FK in this area.

2. Objects and Methods

2.1. Objects

A total of 452 patients (452 eyes) with FK in the First Affiliated Hospital of Yangtze University from January 2019 to 2022 were retrospectively collected, including 137 patients (137 eyes) with positive fungal culture. The pathogenetic diagnostic criteria were based on any of the following: 1) corneal ulcer curettage or biopsy of corneal tissue from the edge of the lesion, and the hyphae or spores was detected by microscopic of 100 g/L potassium hydroxide smears 2) corneal scrapings with positive fungal culture; 3) fungal hyphae or spores were observed by confocal microscopy; 4) If the pathogenetic results are negative, the clinical diagnosis of FK was mainly based on the patient's symptoms and signs consistent with the performance of FK. Symptoms such as ocular redness, eye grinding, photophobia, tears, blurred vision, and corneal ulcer with white or milky dense toothpaste like and lichen like appearance, with or without immune rings, satellite foci, pseudopodia, hyphema, corneal perforation, etc was found in eyes, and empirical antifungal drugs were effective. This study was approved by the hospital ethics committee.

2.2. Methods

2.2.1. Laboratory Examination Methods

1) Corneal scraping: after the patients were anaesthetized with proparacaine hydrochloride eye drops, the patients were operated under an operating micro-

scope, firstly, the necrotic tissue on the surface of the ulcer was scraped off. Then, then an appropriate amount of tissue was scraped from the basal layer and the edge of the ulcer with a razor blade, and then applied to slides, which were immediately sent to microbiology laboratory for 10% potassium hydroxide (KOH) wet film microscopic examination. 2) Fungal culture and identification: Scrape corneal tissue using the same method, immediately inoculated with Sabouraud dextrose agar, and send to the microbiology laboratory in 35˚C culture, daily observation of the agar whether there was fungal growth. If fungal growth was found, the colonies will be stuck with tape and identified under microscope after staining with lactic acid gossypol blue.

2.2.2. Drug Treatment

1) Corneal ulcer debridement: after surface anaesthesia, carefully scrape off the corneal ulcer Lesion with a blade under the microscope, avoiding damage to the normal corneal epithelium, and rinse the conjunctival sac with saline. Debridement should be performed once every 2 - 3 days until the wound surface improves significantly. 2) Drug treatment: When patients were highly suspected or diagnosed with FK, systemic and local antifungal therapy was given, including intravenous infusion of fluconazole injection or oral itraconazole capsules, eye drops of azoles (such as voriconazole eye drops, fluconazole eye drops), and polyene drugs (such as natamycin eye drops), assist with non-steroidal anti-inflammatory drugs (such as pranoprofen eye drops), antibacterial drugs (such as levofloxacin eye drops or tobramycin eye drops), if necessary, combine with dilaters (such as compound topicamide eye drops).

2.2.3. Surgical Treatment

1) Corneal stromal injection: On the basis of corneal ulcers debridement, voriconazole (0.5 mg/mL) or amphotericin B (0.1 mg/mL) was extracted with an insulin syringe. The needle was inserted into the transparent area of the cornea at a distance of 1.5 mm next to the lesion, with the tip pointing to the center of the lesion, then the medication was slowly pushed into the stroma of the cornea, the cornea could be seen to be white from oedema, and a total of 1 - 5 points could be injected, according to the scope of the lesion. The injection can be repeated according to the condition, and regular follow-up examinations can be conducted. 2) Other surgical methods: For patients who were not well treated with medication alone, combined surgical treatments were needed, including conjunctival flap masking or amniotic membrane transplantation and corneal transplantation. Additionally, enucleation of eyeball may be performed due to severe endophthalmitis, ulcer perforation.

2.2.4. Analysis Methods

Clinical data such as gender, age, month of high disease prevalence, onset triggers, visual acuity changes, fungal culture results, and treatment protocols of the patients were recorded and analyzed during their hospitalization.

3. Results

3.1. Gender and Age

Among 137 FK patients (137 eyes), 89 were males and 48 were females, the ratio of male to female was 1.85:1, the average age was 55.6 ± 11.7 years (21 - 81 years), 3 cases were 20 - 29 years old, 11 cases were 30 - 39 years old, 19 cases were 40 - 49 years old, 42 cases were 50 - 59 years old, 39 cases were 60 - 69 years old, and 12 cases were over 70 years old. The highest incidence of the disease was 50 - 69 years old (60.7%) (Figure 1).

3.2. Months of High Disease Incidence

The incidence of the disease in autumn and winter seasons (September to December, January) and the busy summer farming season (May to June) accounted for 83.2% of the year. The top four months were October (24 cases), June (20 cases), September (19 cases) and May (17 cases) (Figure 2).

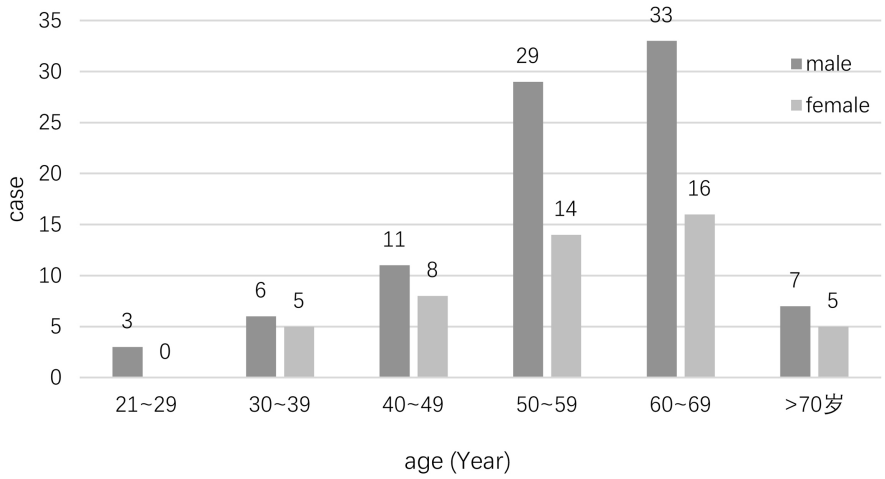

Figure 1. Age and sex distribution of patients with fungal keratitis.

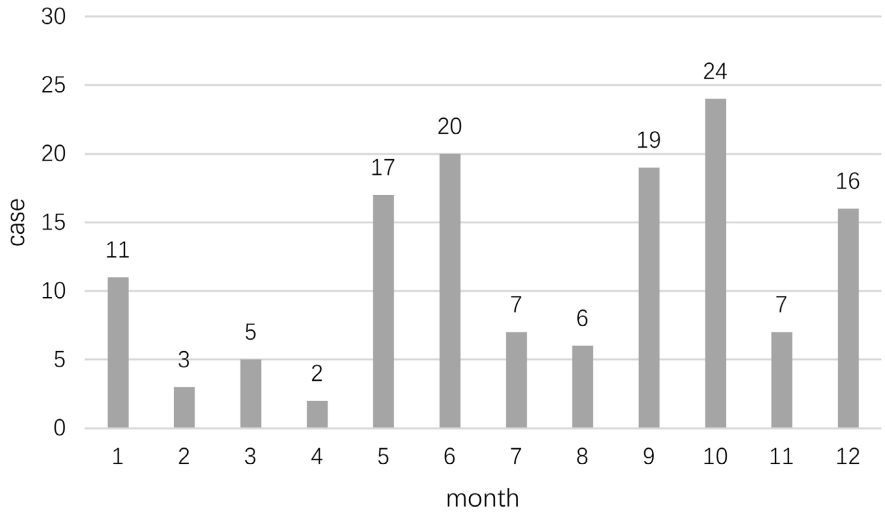

Figure 2. Distribution of visit time for fungal keratitis.

Table 1. Distribution of pathogens in patients with FK.

Pathogen	Cases	Percentage (%)
Fus spp	53	38.7
F. solani	29	21.2
F. oxysporum	17	12.4
others *Fus*	7	5.1
Asp spp	32	23.3
A. fumigatus	14	10.2
A. flavus	12	8.7
A. terreus	3	2.2
others *Asp*	3	2.2
Alternaria spp	24	17.5
Candida spp	11	8.1
others fungi	17	12.4

3.3. Risk Factors for Morbidity

In 137 eyes, 72 eyes (52.6%) had a definite history of trauma, of which 43 eyes (31.4%) had a history of plant trauma. 29 eyes (21.2%) had a history of associated ocular surface disease. 5 eyes (3.6%) were infected after ophthalmic surgery. 5 eyes (3.6%) were infected after contact lens wear. 3 eyes (2.2%) had a history of ocular substance abuse. Other unknown causes were present in 23 eyes (16.8%). A total of 27 eyes (19.7%) of all patients had a history of diabetes.

3.4. Distribution of Pathogens

343 FK patients were confirmed, but only 137 of them were positive for fungal culture, with a positive rate of 39.9%. The main pathogens were *fusarium*, *aspergillus*, and *alternaria*, as shown in Table 1.

3.5. Efficacy

All patients underwent corneal ulcer debridement, and visual acuity could be maintained or improved in 123 eyes (89.8%). 101 eyes were improved or cured by medication (natamycin eye drops, systemic antifungal therapy was given to those with severe conditions). After corneal injection of voriconazole or amphotericin B, the condition was stabilized in 9 eyes, conjunctival flap covering or amniotic membrane transplantation was performed in 11 eyes, and corneal transplantation was performed in 12 eyes. Finally, 4 eyes underwent enucleation, 3 eyes were infected with *aspergillus*, and 1 eye was infected with *fusarium*.

4. Discussion

FK was increasingly becoming the main cause of corneal blindness in developing countries due to its poor response to drugs [4]. Our region was located in the

Jiang Han Plain, with high precipitation, humid climate, rich vegetation, and dense distribution of water resource, which was a favourable environment for fungal growth. Corneal plant injury was more common due to the predominantly agricultural population, but the economic conditions were relatively low, resulting in relatively backward timeliness and accuracy of diagnosis and treatment [7].

Early diagnosis and treatment of FK was crucial for the prognosis of the disease. Traditional diagnostic methods, including microscopic examination of corneal scraping and culture of isolated fungal hyphae, had always been the gold standard for laboratory diagnosis of FK. As the culture of fungi was very time-consuming, it tends to delayed treatment and lead to false-negative results [8] [9]. Although corneal scraping examination can quickly obtain results, in cases of deep corneal infection, the superficial cornea sample may not contain fungal components. In addition, it was an invasive procedure with a poor patient experience, which was inappropriate for patients with near-perforation of the cornea. The recently developed cornea confocal microscopy was a supplementary diagnostic method for detecting fungi, which allows for non-invasive and direct visualization of potential fungal pathogens, enabling rapid and real-time diagnosis [10]. A study [11] has shown that the positive rate of FK detection was 88.8% using confocal microscopy, which was significantly higher than corneal scraping examination. The sensitivity and accuracy of confocal microscopy in the diagnosis of infectious keratitis had also been confirmed in many publications [12] [13]. However, the instrument was expensive and its application was limited in primary hospitals. Therefore, in order to improve the detection rate of FK, clinical doctors in our hospital directly inoculate corneal scrapings onto Sabouraud dextrose agar and applied to slides at the bedside, and send them directly to the laboratory within 2 hours, ensuring the timeliness of microscopy and culture.

This study shows that the age of FK in Jing Zhou was mainly concentrated between 50 and 69 years old, and the majority patients were male, with farmers as their main occupation, confirming once again that farmers were still the main occupation among FK patients. On the other hand, it indicated that young people were working in the cities, and those engaged in agriculture in the countryside were mainly middle-aged and old people. These people were prone to plant injury during agricultural work, and the symptoms were relatively mild at the early stage of infection. Due to their own cultural level and economic ability, they often failed to consult the doctor in time after infection, missing the critical period of early diagnosis and treatment, which may lead to severe fungal corneal infections, resulting in a poor prognosis or even enucleation of the eyeballs. The onset season was mainly concentrated in May to June and September to December, which may be related to the busy agricultural season during these two time periods. The risk factors for FK infection also include wearing corneal contact lenses, eye surface diseases, ophthalmic surgery, and misuse of eye medications.

These patients were prone to corneal damage and infection. Diabetes patients should pay attention to FK. Some studies have shown that diabetes can aggravate FK infection and affect prognosis [14].

The top three pathogens were *Fusarium*, *Aspergillus*, *Alternaria*, which were consistent with other literature reports [2] [15] [16], but also inconsistent with other studies showing that *Aspergillus* ranks the first [11] [17], indicating that there were geographical differences in the pathogens of FK in China. The clinical characteristics, treatment methods, and outcomes of keratitis caused by different fungal genera were different, which were related to the different growth modes of mycelium in the cornea and the immune status of the organism. The growth mode of *Fusarium* in the cornea was parallel growth, which can easily cause the area of ulcer to expand. The formation of mycelial tundra was thicker and easy to prevent the infiltration of drugs [18], and the mycelium that invaded the corneal tissue will appear mycelium wall thickening. This structure enhanced the pathogenicity and drug resistance of *Fusarium* genus, making treatment more challenging. *Aspergillus* grow vertically or diagonally in the infected cornea and secretes enzymes that degrade collagen fibers, making it more prone to corneal fusion, corneal perforation, fungal endophthalmitis, leading to a poor clinical prognosis and low drug cure rate. This was also the main reason for enucleation of the eyeball in FK patients. *Alternaria* has no membrane and low pathogenicity, the area and depth of corneal ulcers caused by *Alternaria* were smaller than *Fusarium* and *Aspergillus*, and were sensitive to various antifungal drugs. Its treatment was mainly drug therapy, with a high clinical cure rate [19].

The susceptibility of different fungal genus to commonly used antifungal drugs also varies, and the minimum inhibitory concentration (MIC) of *fusarium* to various drugs was higher than that of other genera [18]. Thet Tun Aung [20] found that voriconazole showed better effects than natamycin and amphotericin B in reducing wound size and viable fungal count in FK. Therefore, it was considered that natamycin and voriconazole could be the preferred drugs for empirical treatment [21]. Temporarily formulated 1% voriconazole eye drops can be stored for 30d at room temperature (25˚C ± 2˚C) or refrigerated (4˚C ± 2˚C) protected in the dark. It was worthy of clinical promotion and use. Now there were many new drug in research, such as novel water soluble sterile natamycin formulation, hybrid natural hydrogels, natamycin solid lipid nanoparticle, which also achieved good results. In addition, as a form of drug treatment, intracorneal stromal injection can achieve good therapeutic effects, with a success rate of 95% [22]. In this study nine eyes were injected into the cornea and all of them were all cured. When the effect of drug treatment was poor or even worsens, surgery should be combined as soon as possible, including conjunctival covering, amniotic membrane transplantation, corneal transplantation.

There were some limitations. The positive rate of fungal culture in FK was low, which needs to be further strengthened. Meanwhile, the sample size of this study is relatively small.

In summary, FK patients in our hospital were more common in the middle-aged and elderly population, and mostly of them were caused by plant injury in the process of farming, with *Fusarium* and *Aspergillus* as the main causes. Treatment was based on drug therapy, with natamycin or voriconazole as the first choice of drug therapy, combined with surgical treatment can also achieve good effects. It was necessary to strengthen the health education of patients and the diagnosis and treatment abilities of primary ophthalmologists on FK, early diagnosis and treatment should be made as far as possible, so as to avoid patients' vision loss or even eyeball enucleation.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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