Ocular Fungal Isolates and Antifungal Susceptibility

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Abstract: Fungal keratitis is a potentially sight threatening disorder and the leading cause of monocular blindness worldwide. This study analyzed the prevalence, microbiology and treatment of infectious fungal keratitis. A prospective review of cases presenting with keratitis at Cornea Outpatient Department of Research Institute of Ophthalmology, Egypt from September 2013 to March 2016 were included in this study. Full ophthalmic examination with slit lamp bio-microscopy was performed and corneal scrapings were sent for microbiological diagnosis. Results revealed that of the 87 patients who attended the outpatient Department Hospital 23 (26.5%) showed growth of fungi. The most common fungi was Aspergillus species (73.9%) shown as Asperagillus flaves (30.4%), Asperagillus fumigates (21.7%), Asperagillus niger (21.7%) and then followed by Candida species shown as Candida albicans (26%) in-vitro susceptibility tests were applied by Fluconazole (25µg), Itraconazole (50µg), Ketoconazole (10µg), Metronidazole (5µg), Voriconazole (1µg), Amphotercin and lemon essential oil as natural antifungal and compared with chemical antifungals, were found to be better efficacious drugs against fungal pathogens noted in in-vitro susceptibility testing.

Key words: Antifungals - Culture - PCR - Fungal Keratitis

INTRODUCTION

Fungi are opportunistic organisms which rarely infect healthy corneas of immunocompetent individuals. Systemic and local underlying diseases may render the corneal tissues susceptible to infections with these fungal organisms like Fusarium spp., Aspergillus spp. and Alternaria alternata [1]. Trauma, specially related to organic materials, topical use of steroids and antibiotics, contact lens wear, surgical procedures, contaminated eye solutions, inappropriate maintenance of contact lenses [2] are causes fungal infection systemic. Systemic infections as well as any disease associated with immunodeficiency are considered risk factors for fungal eye infection [3].

Fungal eye infection is known as fungal keratitis. It is an inflammation of the cornea that is caused by a fungal organism. There are more than 70 different types of fungus that can cause eye infection. It requires immediate treatment by an eye care professional. Fungal keratitis and endophthalmitis are the major ocular infections in developing countries. Fungi are identified as the principal etiological agent of corneal ulceration [4].

Etiological Agents and Epidemiology: The incidence of ocular fungal infections has increased substantially over the past decades because of the increased number of patients with acquired immunosuppression secondary to extended use of immunosuppressive agents, long term broad spectrum antibiotics and AIDS [5-9]. The pathogenesis of eye infections is linked to the epidemiology of disease. The term of endogenous endophthalmitis indicates to blood borne spread of microorganisms into the eye. Mainly, neutropenic immunosuppressive patients undergo blood borne infections and fungemia. Candida species are the most common cause of endogenous endophthalmitis which usually develop in immunocompromised patients having chronic underlying systemic disease, Aspergillus species are the second most common cause of endogenous fungal endophthalmitis. Aspergillus flavus, A. fumigatus, A. niger, A. terreus, A. glaucus, A. nidulans have been reported to cause endophthalmitis [10-12].

Exogenous fungal endophthalmitis occurs by inoculation of pathogens into the eye from, trauma or intraocular surgery and usually follows keratitis. Patients with exogenous endophthalmitis are rarely immunocompromised.
Therefore any of the saprophytic fungi found in natural habitats, may cause exogenous infection of the eye. The mycotic causes of exogenous endophthalmitis are mainly Candida species especially in postsurgical group [7, 12] whereas Fusarium species were found only in the posttraumatic and postkeratitis groups [13, 14].

Fungal keratitis or keratomycosis is the third clinical presentation of ocular fungal infections. Wearing of hard and soft extended-wear contact lenses is associated with bacterial infections usually caused by Pseudomonas aeruginosa [12]. Fungal keratitis usually occurs after trauma with fungus-contaminated plant material in agricultural workers. Majority of cases are due to soil saprophyte filamentous fungi belonging to nearly 56 genera which have been reported from the cases of corneal infections like filamentous fungi (Aspergillus spp, Fusarium and Candida spp).

**Treatment of Fungal Keratitis:** If direct microscopic examinations of corneal scrapes yield definite results that are consistent with the clinical picture, treatment should be initiated immediately [15]

The antifungal agents available today to combat fungal keratitis are not so well developed as those available against bacterial infection Most of the available agents only inhibit the growth of the fungus necessitating the host defense mechanisms to eradicate the infection [16]

The currently used antifungal agents belong to, (1) Polyenes, (2) Azoles, including newer azoles (3) Pyrimidines, (4) other derivatives.

**Polyenes:** The polyene bind to the ergosterol of fungal cell membrane, creating pores that disrupt the homeostatic mechanisms leading to cell death [16].

**Azoles:** These are the derivatives of imidazole ring with substitution mainly in position 2 Antifungal resistance is based on different mechanisms namely, (i) reduced drug intracellular accumulation, (ii) decreased target affinity/processivity for the drug, and (iii) counteraction of the drug effect. Particularly, the mechanism of resistance will be different depending on the mode of action of antifungal compounds.

**Patients:** A total of 87 clinically diagnosed patients of corneal ulcers of different age and sex who attended the Cornea Outpatient Department and also admitted in the Ophthalmology ward of Research Institute of Ophthalmology during 3 years study from 2013 till 2016 were included in the study.

**Collection of Samples:** It was ensured that clinical microbiology material was collected before giving antibiotic therapy.

All patients underwent thorough slit -lamp biomicroscopic examination by an ophthalmologist. After a detailed ocular examination, corneal scrapings were taken by an ophthalmologist with all aseptic precautions Five minutes after instillation of local anesthetic to the affected eye, corneal scrapings were taken. The material obtained was spread onto labeled slides for Gram stain and was also inoculated onto the surfaces of Sabouraud’s dextrose agar medium ( SAD) for fungal growth.

**Detection of Fungal Agents:** Three corneal scrapings were used for fungal detection. First corneal scraping was used for direct stain by gram stain, second scraping for fungus culture and third scraping for culture and PCR. Materials obtained by second scraping were spot inoculated on Sabouraud’s dextrose agar medium (SDA). Inoculated SDA media was incubated at 25°C and observed daily for the first 7 days and on alternate days for next 7 days for observing slow growing fungi. Only growth occurring on the “C” streaks was considered as significant and out growth away from the “C” streak was discarded as contaminants the plates which did not show any evidence of growth after 14 days were discarded. Fungal growth was grossly identified by its colony morphology, pigment production and microscopically by lacto-phenol cotton blue stain.

**All Fungal Isolates Were Confirmed by PCR Test:** All fungal isolates were tested for their antifungal susceptibility by disc diffusion method against Voriconazole (1µg), Fluconazole (25µg), Itraconazole (50µg), Ketoconazole (10µg), Metronidazole (5µg) and Amphotercin B (20µg), the results of susceptibility were recorded as highly sensitive and compared with the sensitivity of lemon essential oil.

**RESULTS**

Of all the 87 keratitis patients who attended the outpatient department reviewed from 2013 to 2016 a total of 23 (26. 5%), cases were culture positive fungi.
Table 1: Distribution of patients with corneal ulcer according to age groups

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Age Groups (Years)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10-20</td>
<td>8.6%</td>
</tr>
<tr>
<td>3</td>
<td>20-30</td>
<td>13.04%</td>
</tr>
<tr>
<td>4</td>
<td>30-40</td>
<td>17.39%</td>
</tr>
<tr>
<td>8</td>
<td>40-50</td>
<td>34.78%</td>
</tr>
<tr>
<td>4</td>
<td>50-60</td>
<td>17.39%</td>
</tr>
<tr>
<td>2</td>
<td>60-70</td>
<td>8.6%</td>
</tr>
</tbody>
</table>

The distribution of patients having corneal ulcer according to the various age groups is shown in Table (1) which reveals that the highest frequency belonging to the 40 to 50 years.

Table 2: Distribution of patients with corneal ulcer according to Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>53</td>
<td>60.92</td>
</tr>
<tr>
<td>Female</td>
<td>34</td>
<td>39.08</td>
</tr>
</tbody>
</table>

Table (2) describe the distribution of culture positive patients according to gender. It is seen that 60.92% of the cases were males while the females were 39.08% of cases so, in our study males had a tendency to get corneal ulcer more than females.

Fig. (1) Show the fungal isolates and their incidence *Aspergillus flavus* (30.4%), *Asperagillus fumigates* (21.7%), *Asperagillus niger* (21.7%) and *Candida albicans* (26%) the predominant fungus isolated in cases, (*Aspergillus flavus* (30.4%) being the commonest species. Candida was the second isolate (26%).

Identification of samples by detection of fungal DNA by polymerase chain reaction (PCR) to confirm the results of microscopic and culture examination at first general PCR was done followed by nested PCR to determine the species and PCR results were the same to microscopic and culture examination.

Fig. 1: % incidence of different isolated pathogens

Fig. 2: General PCR for *Asperagillus* spp and *candida albicans*
N: negative with water
P: positive control
Fig. 3: Nested PCR for *Asperagillus* spp (*Niger*, *fumigates* and *flaves*)

N: negative with water

P: positive control

Table 3: Comparison between the antifungal effect of chemical antifungals & natural antifungals

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Chemical antifungal</th>
<th>Natural antifungal</th>
<th>Mean of Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>4.00</td>
<td>1.00</td>
<td>2.50</td>
</tr>
<tr>
<td>A. fumigates</td>
<td>3.50</td>
<td>1.80</td>
<td>2.65</td>
</tr>
<tr>
<td>A. flaves</td>
<td>4.30</td>
<td>1.50</td>
<td>2.90</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>3.60</td>
<td>1.30</td>
<td>2.45</td>
</tr>
<tr>
<td>Mean of antifungal</td>
<td>3.85</td>
<td>1.40</td>
<td></td>
</tr>
</tbody>
</table>

F-value:
- Antifungal: 423.35
- Pathogen: 2.88
- Interaction between Antifungal x Pathogen: 5.94

P-value:
- Antifungal: 0.000
- Pathogen: 0.054
- Interaction between Antifungal x Pathogen: 0.003

LSD:
- Antifungal: 0.053
- Pathogen: 0.107
- Interaction between Antifungal x Pathogen: 0.213

Fig. 4: Comparison between the antifungal effect of chemical antifungals & natural antifungals
The susceptibility of the fungal isolates (Table 3) show the sensitivity to different antifungal to six antifungals, *Aspergillus niger* showed highly sensitive to voriconazole, *Asperagillus fumigates* showed highly sensitiv to ketoconazole, *Asperagillus flaves* showed highly sensitive to ketoconazole and Candida albicans showed sensitive to ketoconazole and this results were compared to sensitivity of lemon essential oil and the results were shown in Fig. (4) and Table (3).

**DISCUSSION**

Fungal keratitis represents is one of most infectious keratitis in tropical areas of the world, because of the divesting ocular damage that it can produce if it not diagnosed and treated promptly and effectively. Unfortunately, delayed diagnosis is common, primarily because of lake of suspicion and even if the diagnosis is made accurately, management remains a challenge due to the poor corneal penetration and limited commercial availability of fungal drugs [21].

Fungal keratitis affects both males and females A male preponderance has been noted. In our study males had a tendency to get corneal ulcer more than females which may be explained by males having more chances of accident or trauma than females due to more outdoor activities. This is similar to the other studies conducted in Peshawar [22] Thailand [23] India [24] and Oman [25]. The result of this study showed the highest frequency for corneal ulcer belonging to age group of 40-50 years.

Our study also revealed a high frequency in other age groups that ranged from 10 to 70 years and these findings are similar to studies from Thailand [23] and India [24] where corneal ulcer was common in middle age group and also in harmony with the study from Oman [25].

Increased awareness of the occurrence and frequency of fungal keratitis, better recognition of the clinical features of these infections, and improved laboratory diagnostic techniques of direct examination of stained smears and culture of the causative fungi have all led to an increased in the frequency of correct diagnosis [26].

In the present study 87 case of corneal ulcer were investigated. Positive fungal cultures on SDA media were obtained in 23 case (26.5%) of corneal ulcer cases.

Direct microscopic examination with Gram stain were show fungal elements and pus cells.

The most common fungi were *Aspergillus* species (74%) followed by Candida species (26%). In the study by Bahgat et al. [27] on 622 cases of diseased eyes, direct microscopic examination gave positive results for fungal elements in 20% of diseased eyes, while fungi were isolated from cultures in 37.5% of healthy eyes. The most common isolated were asperagillus species (46%), followed by candida albicans (9.8%), penicillum (9.8%) rhodtroularuba (7.3%), alternaria(6.6%) , and others (20.5%). Non fungal growth was obtained in (62.5%) of the diseased eyes.

The results are similar to our work this may be explained due to the same social-economic factors and the same environmental conditions (Climate and occupation).

**REFERENCES**