A Clinical Microbiological Study of Corneal Ulcer Patients at Western Gujarat, India

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Abstract- Corneal ulcer is a major cause of blindness throughout the world. When the cornea is injured by foreign particles, there are chances of infection by the organism and development of ulcer. Bacterial infection in the cornea is invariably an alteration of the defense mechanism of the outer eye. It is essential to determine the local etiology within a given region when planning a corneal ulcer management strategy. Laboratory evaluation is necessary to establish the diagnosis and to guide the antibiotic therapy. One hundred corneal ulcer patients were studied by collecting their corneal scraping samples and processing at Clinical Microbiology department of Shree Meghaji Petharaj Shah Medical College, Jamnagar, Gujarat, India during a period of 17 months. All clinical microbiology laboratory procedures followed standard protocols described in the literature. 40 (40%) patients from the age group of 20-70 years had been confirmed as - any organism culture positive - within the corneal ulcer patient population. Fungi were isolated from 26 (26%) corneal ulcer patients. The bacterial etiology was confirmed in 14 (14%) corneal ulcer patients. The major risk factors for mycotic keratitis were vegetative injury (16, (62%)), followed by conjunctivitis (4, (15%)), and blunt trauma (3, (11%)). *Pseudomonas aeruginosa* was the most commonly isolated bacterium (6, (43%)), followed by *Proteus spp*. (4, (29%)). Corneal Infections due to bacteria and filamentous fungi are a frequent cause of corneal damage. Microbiological investigation is an essential tool in the diagnosis of these infections. The frequency of fungal keratitis has risen over the past 20 to 30 years. Prognosis of bacterial corneal infection has improved since the introduction of specific antibacterial therapy.

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Introduction

Corneal ulcer is the second most important cause of blindness throughout the world, after trachoma (1). An estimated 1,02,122 new blindness cases occur in Gujarat, India (2). Bacterial keratitis is considered a leading cause of monocular blindness in the developing world (3). A host of bacterial organisms can cause infectious keratitis. These organisms are commonly grouped by their staining pattern with Gram stain and their response to oxygen, that is, gram positive versus gram negative, and aerobic versus anaerobic. The incidence of infection by specific organisms varies by region, and practitioners should be aware of the local epidemiological patterns of corneal infection (4). Suppurative corneal ulcers may be caused by bacteria, fungi, and protozoa. However, within the tropics, filamentous fungi may account for as many as two thirds of ocular ulcers. This type of ulceration is commonly associated with ocular trauma. The microbial causes of suppurative keratitis vary considerably between continents and countries and also within countries. It is essential to determine the local etiology within a given region when planning a corneal ulcer management strategy (5). Comparable to other forms of microbial keratitis, initiation of fungal infection requires the presence of viable microorganisms and a mechanism of corneal inoculation. Fungi cannot penetrate the intact corneal epithelium and do not enter the cornea from episclerallimbal vessels. The principal routes of inoculation are introduction of fungi concurrent with a penetrating or perforating wound, either mechanical

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injury or surgery, and introduction through an epithelial defect. Post-traumatic infections are caused principally by filamentous fungi in the United States (6). It is necessary to use those antibiotics which are effective against the causative organisms (7). Laboratory evaluation is necessary to establish the diagnosis and guide the antibiotic therapy (8). Prognosis of bacterial corneal infection has improved since the introduction of specific antibacterial therapy.

This research study was undertaken to determine local bacterial and mycotic infectious agents in corneal ulcer patients, so that it becomes helpful in planning of corneal ulcer management strategy, before any permanent ocular damage takes place in the form of loss of vision and other abnormalities.

Materials and Methods

A clinical microbiological study was carried out to identify bacterial or fungal agents on 100 corneal ulcer patients at Ophthalmology out-patients department of Shree Meghaji Pethraj Shah Medical College and Guru Govindsingh Hospital, Jamnagar, Gujarat, India between September 2006 and February 2008. Guru Govindsingh hospital is a tertiary care teaching medical college hospital in western Gujarat, India.

One hundred corneal ulcer patients were studied by collecting their corneal scraping samples and processing at Clinical Microbiology department of Shree Meghaji Petharaj Shah Medical College, Jamnagar, Gujarat, during a period of 17 months. Demographic details like name, age, sex, clinical history, and associated findings were recorded onto standard clinical history form. The ophthalmologist inspected the corneal ulcer with the help of slit lamp microscope. Corneal ulcer was examined, presence of foreign body and hypopyon was looked for, and associated medical history was noted. It was ensured that clinical microbiology material was collected before giving antibiotic therapy. The ulcer was cleaned with sterile normal saline, and preservative free lignocaine (2%) was applied as a local anesthetic agent. With the help of slit lamp microscope corneal scraping was performed by the ophthalmologist using a sterile Bard-Parker surgical blade number 15. A plenty of clinical material was directly inoculated onto blood agar, chocolate agar, and Sabouraud's Dextrose Agar (SDA) making multiple "C" shape marks. Gram stain and 10% KOH smears were prepared right on the examination table. Clinical materials were not processed for the viral and anaerobic study. Inoculated blood agar and chocolate agar media were incubated at 37°C temperature aerobically. 24 hours later, the culture media were inspected for growth. If organism had not grown, plates were further incubated and finally declared as culture negative after 48 hours. Culture positive growth was followed for identification of the organism. Inoculated SDA was inspected daily for up to 10 days and declared as fungal culture negative after ten days. Fungal growth was grossly identified by its colony morphology on obverse, pigment production on reverse, and microscopically by lacto-phenol cotton blue stain. The clinical correlation of fungal growth was established as per the criteria in Box 1. All clinical microbiology laboratory procedures followed standard protocols described in the literature.

1. Direct smear examination is positive for fungal elements
2. Culture slope shows same fungal growth on many inoculation sites
3. Subculture from original slope yields same fungal growth
4. Same fungal growth is obtained from more than one slope

Box 1: At least one finding out of four should be established to diagnose mycotic keratitis.

Results

One hundred ophthalmic out-patients having clinical presentation of corneal ulcer were enrolled in the present study. 60 (60%) patients were male and 40 (40%) patients were female. 40 (40%) patients from the age group of 20-70 years had been confirmed as - any organism culture positive - within the corneal ulcer patient population.

Fungi were isolated from 26 (26%) corneal ulcer patients out of whom 18 (73%) patients were positive with 10% KOH direct smear examination. 14 (54%) fungal ulcers were diagnosed in male patients and 12 (46%) fungal ulcers were diagnosed in female patients. The maximum number (20, [77%]) of fungal corneal ulcer cases was documented in the age group of 31-60 years (Figure 1).

The bacterial etiology was confirmed in 14 (14%) corneal ulcer patients out of whom eight (57%) patients were positive with Gram stain-direct smear examination. Out of 14 bacterial ulcer cases, eight (57%) patients were male and six (43%) patients were female. The maximum number of bacterial corneal ulcer patients (11, [79%]) was observed in the age group of 31-60 years.

The major risk factors for mycotic keratitis were vegetative injury (16, [62%]), followed by conjunctivitis (4, [15%]), and blunt trauma (3, [11%]) (Figure 2).

Co-existing ocular diseases like conjunctivitis,

trachoma and dacrocystitis were major risk factors for bacteria keratitis (8, [57%]).

With 20 (70%) isolates *Aspergillus spp*. dominated the fungal keratitis isolate matrix, followed by *Fusarium spp*. with 3 (12%) isolates (Figure 3).

Among the bacterial keratitis patients *Pseudomonas aeruginosa* was the most commonly isolated organism (6, [43%]), followed by *Proteus spp*. (4, [29%]) (Figure 4).

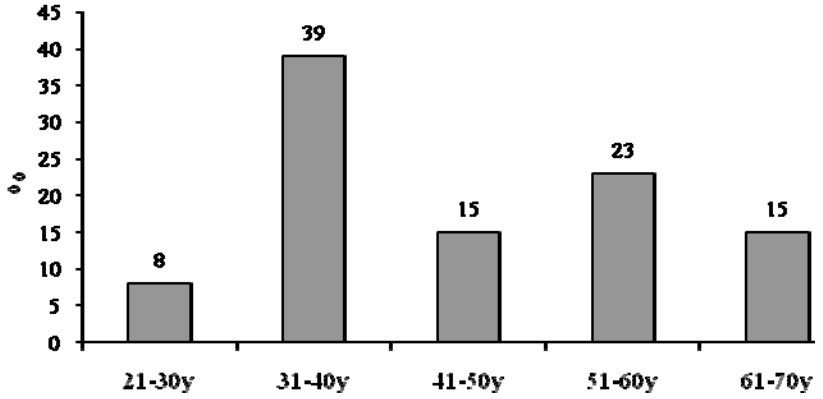

Figure 1. Age-wise pattern of fungus isolates.

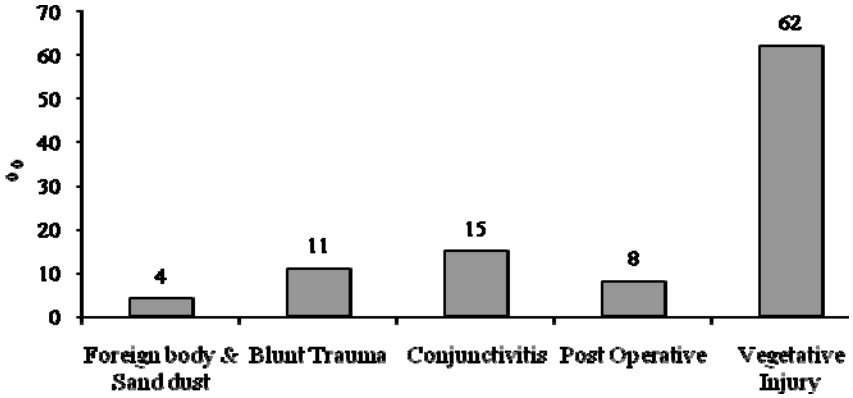

Figure 2. Predisposing factors for mycotic keratitis.

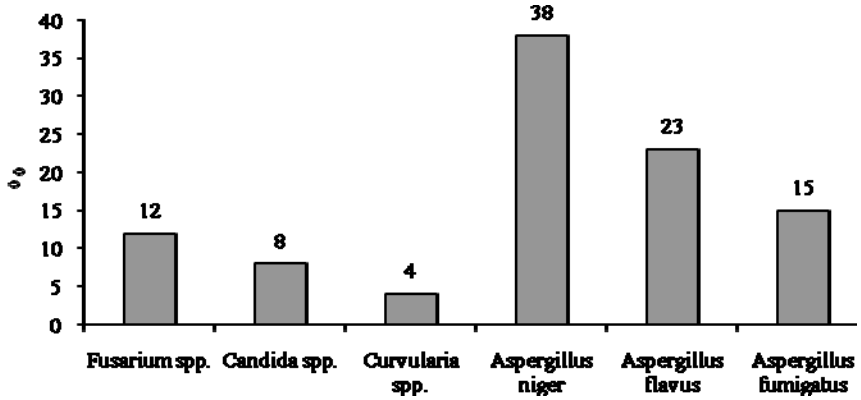

Figure 3. Fungal isolate matrix.

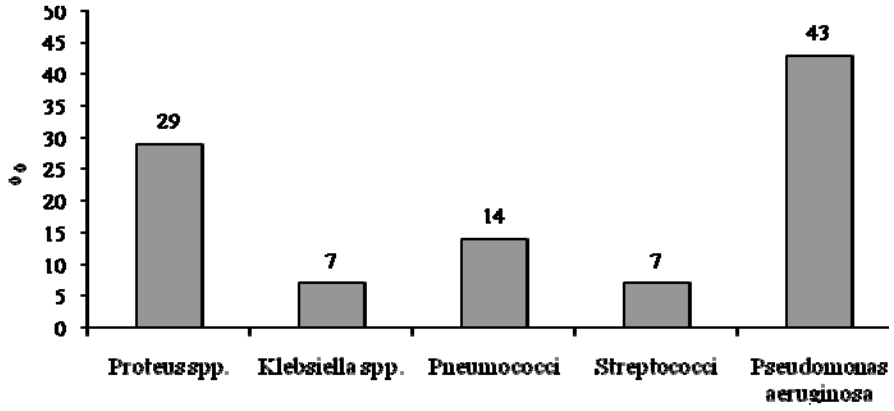

Figure 4. Bacterial isolate matrix.

Discussion

Fungal infections of the cornea are relatively infrequent in the developed world but constitute a larger proportion of keratitis cases in many parts of the developing world. Infections of the cornea due to filamentous fungi are a frequent cause of corneal damage in developing countries in the tropics and they are difficult to treat. Microscopy is an essential tool in the diagnosis of these infections. Knowledge of the local etiology within a region is of value in the management of suppurative keratitis in the event that microscopy cannot be performed (5). We isolated pathogenic fungi from 26 (26%) corneal patients with a maximum number (20, [77%]) of cases in the age group of 31-60 years. According to the world view observed by Leck AK *et al.*, filamentous fungi turned out to be the principal causative micro-organisms with 42% rate of isolation from 1090 corneal ulcer patients. In his study from Madurai, south India, Srinivasan M *et al.*, reported a similar trend of corneal ulceration in his study population of 434 ophthalmic patients (9). Similarly, Shoja MR *et al*., reported the mean age of corneal ulcer patient as 45.3±18.5 years with the highest frequency in the 40 to 50 age group (10).

The bacterial etiology was confirmed in 14 (14%) corneal ulcer patients of this study. Bacterial keratitis is considered a leading cause of monocular blindness in the developing world. In this environment, corneal infections often follow trauma, and they also contribute significantly to corneal blindness associated with trachoma and xerophthalmia. Infiltration that begins at the site of corneal injury can readily spread (often towards the center of the cornea), producing a deep stromal abscess, fibrin deposition, plaque formation, severe anterior chamber reaction, hypopyon, and iris synechiae (4).

We observed that vegetative injury (16, [62%]) was the major risk factor for corneal ulceration, followed by conjunctivitis (4, [15%]), and blunt trauma (3, [11%]). In the warmer states and in the tropics, corneal trauma, which might be trivial, frequently precedes infection. Concurrent contamination with plant material presents an increased risk for fungal keratitis. Srinivasan M *et al.*, reported a similar pattern of recent injury to the cornea in 284 (65.4%) of the 434 patients. Other complicating conditions predisposing to corneal ulceration were present in 65 (15.0%) of the 434 patients (9).

Mycotic keratitis, an ophthalmological problem, especially in outdoor workers in the tropics, is frequently caused by filamentous fungi such as species of Fusarium, Aspergillus and Curvularia, and by yeast like fungi such as Candida. The *Aspergillus spp*. (20, [70%]) was the commonest fungal pathogen, followed by *Fusarium spp*. (3, [12%]) in our study. *Fusarium* species was the commonest species isolated with the frequency of 32.3% during the previous study from the similar geographical area as to the present study region (11). Such phenomena may be the result of difference in climate and environment across time and space. Basak SK *et al.*, isolated *Aspergillus spp*. at 59.8% isolation rate and *Fusarium spp*. at 21.2% isolation rate (12).

We isolated *Pseudomonas aeruginosa* (6, [43%]) as the predominant bacterial pathogen, followed by *Proteus spp*. (4, [29%]) among bacterial keratitis patients. *Pseudomonas aeruginosa* is the most common gram-negative organism isolated from corneal ulcers. These aerobic bacilli are found in moist environments and frequently contaminate inadequately chlorinated swimming pools and hot tubs, ventilators, nebulizer and

vaporizer solutions, and ophthalmic solution bottles. The organism readily adheres to damaged epithelium, and stromal invasion is rapid. *Pseudomonas* keratitis tends to progress rapidly if inadequately treated (4). Basak SK *et al.*, isolated *Pseudomonas spp*. at 74% isolation rate (12). In Ghana, more than 50% of bacterial isolates were from *Pseudomonas* species (5). In conclusion, corneal Infections due to bacteria and filamentous fungi are a frequent cause of corneal damage. Microbiological investigation is an essential tool in the diagnosis of these infections. The frequency of fungal keratitis has risen over the past 20 to 30 years. Prognosis of bacterial corneal infection has improved since the introduction of specific antibacterial therapy.

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