PATTERN OF CAUSATIVE ORGANISMS IN INFECTIVE KERATITIS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: According to worldwide survey for blindness and visual impairment, the blindness and visual impairment, which is caused by corneal, scaring, is the second most common reason after cataract and this is accountable for around 20-30% of all optical harm in developing countries. After improvement in medical field technology and antimicrobial therapy, the occurrence of infective keratitis has been condensed in advanced countries. However, due to absence of medical training and therapy, keratitis is the foremost cause of monocular morbidity in most undeveloped countries like Pakistan.

Objective: To determine frequency and pattern of causative bacterial and fungal pathogens of infective keratitis in clinically diagnosed cases.

Study design, Place and Duration of Study: This is a descriptive study carried out in Lahore General Hospital, Lahore from June, 2016 – June 2017. According to standard operating procedures, the samples of corneal scrapings were processed at Microbiology laboratory of department of Pathology PGMI, Lahore.

Materials and Methods: The samples of corneal scrapings were collected from fifty patients of clinically identified cases of infectious keratitis by the ophthalmologists of Ophthalmology department of Lahore General Hospital, Lahore. These corneal scrapings, which were taken by the ophthalmologist, were immediately inoculated on Blood agar, Chocolate agar, MacConkay agar and Sabouraud's Dextrose agar, which had added antibiotics (chloramphenicol, gentamicin). The scrapes taken from cornea were immediately smeared on glass slides, they were air-dried and by heating method, they were fixed for Gram's staining and Kinyoun method of staining and with alcohol fixation for Giemsa stain. Identification of bacterial and fungal pathogens was done by Microbiological standard operating procedures in Microbiology lab of PGMI, Lahore.

Results: Out of 50 cases, 21 (42 %) patients were diagnosed as having fungal keratitis while 12 (24 %) cases were diagnosed as bacterial keratitis and 3(6 %) were having bacterial or/ and fungal pathogen. The commonest isolated pathogen was *Aspergillus spp*. 7 cases, which is followed by five cases of *Staphylococcus aureus* in keratitis.

Conclusion: The present study shows that the fungal keratitis is more common as compared to bacterial keratitis.

Key words: Infective keratitis, *Aspergillus spp.*

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INTRODUCTION

Infective keratitis is a dreadful, common ocular corneal infection. It is characterized by scaring of epithelium and inflammation of stroma, which is responsible for permanent defeat of vision [1]. Its causative microorganisms might be bacteria, viruses, fungi and parasites. Owing to breach in the corneal defense mechanisms, the above-mentioned microorganisms catch suitable and prime conditions for flourishing and lead to cause infections of the cornea [2].

Keratitis caused by these above-mentioned microbial agents is the leading fact of visual damage. After cataract, the corneal scaring is the second most common cause of blindness and leading to visual weakening [3].

Due to advancement in medical expertise and antibiotics treatment, the developed countries has reduced incidence of infective. However, due to lack of medicinal training and its management, keratitis is blamable to monocular disease in Asian, Middle Eastern countries and African [4]. Unfortunately, we are lacking of availability of local data related to pattern, frequency and etiological features of contributing pathogens of infective keratitis. Similarly, there are very few relevant studies are accessible, which are describing corneal opacity caused by infective keratitis as the second most communal cause of loss of sight after cataract [5].

There are countless etiological reasons, which are responsible in corneal breaching and then leading to keratitis. These factors include ecological factors, profession, age, contact lens, ocular surgeries, foreign body, trauma and chronic corneal diseases [6].

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The ulcer of cornea is the main clinical appearance of keratitis. It is characterized by epithelium defect of cornea along with stromal infiltration and suppuration. Bacterial keratitis caused by *Pneumococci, Staphylococci, and Streptococci* etc. are purulent ulcers [7]. While some bacterial pathogens, including *N. gonorrhea, Neisseria meningitides, Corynebacterium diphtheria*, and *Haemophilus influenza* are clever to breach an intact corneal epithelium in relationship with severe conjunctivitis. Keratitis can be poly-microbial, comprising both fungal and bacterial as combine infection [8]. Beside with bacteria, it might also cause via only fungus pathogens [9]. When pathogenic fungi gained access to its host cells, it causes damage to cornea. This is caused by molecules, having adhesive factors that interact with a variety of host proteins and glycoproteins [10].

The corneal scraping is the ultimate investigative procedure for infective keratitis [11]. Culture is considered the golden standard for, identification of causative pathogens of keratitis but microscopy of corneal scrapings smears delivers rapid information of contributing microbes. Simple microbiological modalities of direct microscopy for corneal scrapings are available for detection of microbes, which is helpful for eye health center for developing of appropriate and well-timed diagnosis and therapy, for averting visual loss and blindness [12].

The rationale of the study was to outline the etiological pattern of infective keratitis in corneal scrapings presented to Ophthalmology department of Lahore General Hospital. This would enable the ophthalmologists to know the prevailing trends of keratitis causing organisms and to start appropriate empirical treatment. This would go a long way to prevent permanent corneal damage and eventually blindness

MATERIALS AND METHODS

Clinically suspected cases of Infective keratitis were included in our study and scrapings of cornea were taken before commencement of therapy of Antifungal or Antimicrobial drugs. Patients who were on antifungal or antimicrobial drugs were not included in the present study.

The corneal scrapings were collected from the patients of keratitis in Eye operation theater, Lahore General Hospital, Lahore by the ophthalmologist. Present study included fifty patients for research. The name of patients, age, gender, collection date of sample, patients clinical history which include onset of disease, duration of illness, trauma history or foreign body injury to cornea, and any other related issues were inquired and documented in proforma. Topical anesthetic of Proparacaine was introduced in the affected eye of the patient. The necrotic material of corneal ulcer and its free mucus was detached from the ulcer. The borders and base of the ulcer were scraped by the ophthalmologist with the help of disposable scalpel blade no 15 [8].

The scrapings of corneal ulcer taken by the eye surgeon were instantly inoculated on the culture agar plates by sanitized wire loop in the eye OT:

Blood agar
Chocolate agar
MacConkay agar
Sabouraud's Dextrose agar with antibiotics (gentamicin, chloramphenicol)

After inoculating the sample of scrapings on the culture plates, then were smeared with the help of sterilized inoculating loop on slides. The smeared slides were dried and then fixed by hot flame for Kinyoun staining and Gram's Method of staining and with the help of alcohol fixation for Giemsa stains. These slides were labeled with serial numbers, patient's name, saved in slide box and were brought to the laboratory for further processing. The wet preparation of scrapings with 10% KOH and Lactophenol cotton blue stain was helpful for quick identification of any fungus in corneal scrapings.

With the help of Gram's staining identification of Gram positive and Gram-negative bacteria was done. Fungal detection was also done. If hyphae, yeast cells, fungal spores were present, they appeared as Gram positive and violet in color.

For identification of the inclusion bodies of Chlamydia trachomatis in infected epithelial cells of cornea Giemsa staining was done. Inflammatory cells were discriminated into polymorphs cells and mononuclear cells. This is appreciated on nuclear characteristics and variance staining. Kinyoun staining technique was done for identification of *Nocardia spp.* They appeared as weak acid-fast branching filaments. They may also marked as Gram-positive branching filaments [1][13][14].

The fungal pathogens were identified on positive culture of fungal growth by:

1. Growth rate of fungal pathogen as slow growing, moderate or fast growing.
2. The surface of growth, its aerial hyphae, their pigmentation and color was noted along with color on reverse surface of growth and

observing any pigment diffused in the culture medium.

3. Texture of the surface of the colonies of was observed.
4. Colonial topography was observed as heaping, raised or flat.

Microscopic analysis of fungal growths was done by tape preparation made by Lacto phenol cotton blue stain. The taped slide was observed under microscope first at lower power 10 X objective and then 40 X. Microscopic examination of the slides, revealed hypha, spores, arrangement of spores and presence or absence of conidia whether micro or macro conidia.

The fungi were identified only upto genus by:

- Their hyphae type i.e. hyphae were hyaline or having pigment, whether they are septate or aseptate.
- Presence of conidia; macro or micro and their hyphae arrangement.
- Their spores; presence or absence [1].

RESULTS

In the present study, fifty scrapings were taken from cornea of the patients who were having infective keratitis clinically. Figure-1 shows that out of 50 specimens, 21 fungi and 15 bacteria were identified and isolated with the help of staining and on cultures growth done in microbiology laboratory of department of Pathology, PGMI/AMC/LGH.

Figure no 2 of results shows the (% age) distribution of bacteria causing keratitis isolated from corneal scrapings of patients. Total 15 bacteria were isolated and identified as pathogens. Out of them five pathogens were identifies as Staphylococcus aureus (33.3%), followed *by 3* (20 %) *were identifies as Pseudomonas aeruginosa* and 3 (20 %) *Streptococcus pneumoniae, Klebsiella pneumoniae* were isolated from two cases (13.3 %) and one case of *Nocardia spp.* and one case of *Acinetobacter baumannii* was identified as pathogen.

* Two cases of infective keratitis were laboratory confirmed as mixed growth of *Staphylococcus aureus* with fungus on culture.

**One patient of keratitis identified in laboratory as having mixed growth of *Pseudomonas aeruginosa* with *Klebsiella Pneumoniae.*

Total fungal pathogen distribution, isolated from corneal scrapings in the present study is displayed in figure no 3. From total 21 fungi isolated in the present study, seven cases (33.3 %) were lab confirmed as *Aspergillus spp.*, followed by two (9.5 %) cases each of *Acremonium*, *Bipolaris*, *Rhizopus,* and *Candida species.* One cases each (4.8 %) of *Fusarium, Exophiala, Coccidioidies, Scedosporium, Curvularia* and *Cladophialophora species* was identified.

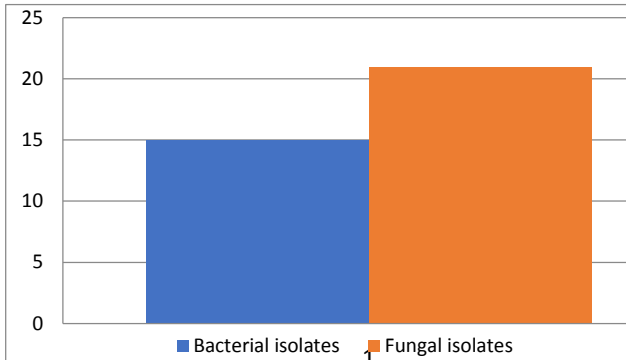

Figure-1: Frequency of fungal and bacterial pathogens identified microbiologically from corneal scrapings.

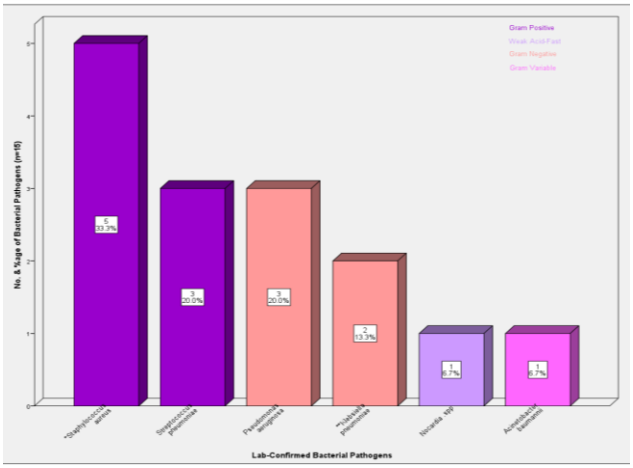

Figure-2: Frequency of different Bacteria isolated from the sample of corneal scrapings taken from the patients of clinical suspicion of Infective keratitis (N=50).

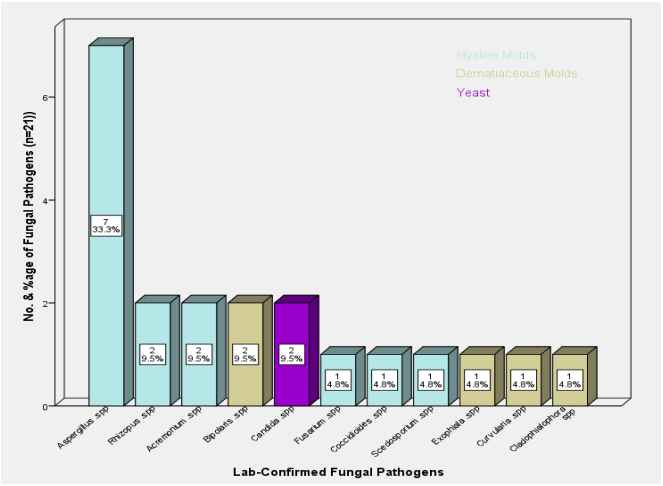

Figure-3: Frequency of different types of Fungi identified from scrapings of cornea from patients with clinical suspension of Infective keratitis (N=50).

DISCUSSION

Infective keratitis the commonest cause of unilateral blindness and is a foremost cause of ocular injury worldwide particularly in a low-socioeconomic settings and developing countries. The occurrence of infective keratitis in these backgrounds is assessed up to 800 per 100,000/year, which is 70 times as greater as equated to high-socioeconomic settings. The outcome of infections of cornea is seldom depends on the fundamental state of the cornea and the virulence factors of the infecting microorganisms. Its prospects is pitiable if correct and aggressive antibiotic therapy is not commenced instantly [15][16].

Similar to the findings of our study, Jeng et al (2010) isolated maximum yield of *Staphylococcus aureus* (27.6 %) in ocular infections from California [17]. Many researchers have documented *Staphylococcus* and *Pseudomonas species* as the most common causative microorganism of infective keratitis [18] [19]. *Pseudomonas aeruginosa* was found to be most frequent Gram-negative bacteria isolated from scrapings of cornea in our study. Many studies have described *Pseudomonas aeruginosa* as the contributing agent of corneal ulcers in the consumers of contact lenses, conducted by different researchers in Taiwan [19].

Streptococcus pneumoniae was the second commonest bacteria isolated from corneal ulcers in this study. Three cases of keratitis had *Streptococcus pneumoniae*, which were confirmed by Gram staining and on culture. Lin et al 2012 also reported *Streptococcus pneumoniae* (35.1%), similar to our results [20].

One case each of *Acinetobacter spp*. and *Klebsiella pneumoniae* was isolated. *Klebsiella pneumoniae* was also reported in different studies as a bacterial pathogen in infective keratitis [12]. *Acinetobacter baumannii* was also identified from one case of corneal scrapings, which was showing a low frequency of 6.7 %. Only one pathogen was identified as *Nocardia* from corneal scrapings in our study. Lalitha et al in their research, reported *Nocardia spp.* (11%), similar to our findings [21].

Aspergillus spp. was the most common fungus yielded in our research. This finding is also sustained by study on fungal keratitis from Nepal conducted by Amatya et al (2012) [22]. Out of 21 fungal pathogens, our results, showing *Fusarium spp.* as 4.8%, Bharati et al reported *Fusarium spp*. (45.85%) which was higher than the present study [12]. Two isolates of *Acremonium spp.* (9.5 %) were identified. *Rhizopus spp*. was isolated from 2 corneal scrapings and two cases of *Candida spp.* were confirmed in lab. Saha et al reported all these fungal pathogens in their researches [23].

Present study showed, only a case of *Curvularia spp*. (4.8%) which was confirmed from scrapings of cornea. Two cases of *Bipolaris spp.* were isolated. Our results were yielded one case each of *Exophiala spp.* and *Cladophialophora spp*. Researchers reported *Exopialia spp.* and *Cladophialophora spp*. in keratitis. *Scedosporium spp.* was confirmed in our research. Some studies have reported *Coccidiodes spp.*, which is similar to our results. Similarly, these isolated fungal pathogens were also reported by many researchers in their studies [24] [25] [26].

According to Shah et al fungal keratitis is more predominant in agricultural countries and tropical or subtropical regions [27]. Pakistan is an agricultural country and Punjab has climate favorable for fungal keratitis. Majority of our population belong to agricultural occupation and are more exposed to trauma related to vegetative injury that may leads to fungal keratitis.

CONCLUSION

The current study was primarily planned for determination of particular microorganism that is pathogenic and responsible for corneal ulceration in Lahore, as about etiology of keratitis no data form Punjab is available. More and more studies must be piloted to catch the occurrence and outline of etiology responsible for infective keratitis. This will aid to amend empirical management and generate data on keratitis. Additional researches should be designed on antimicrobial susceptibility and the pattern of microorganism causing corneal ulcers and hence we can launch standard susceptibility and resistance patterns of these pathogenic bacteria. This will be supportive in guaranteeing suitable management and limitation of spreading of resistant pathogenic strains.

AUTHORS CONTRIBUTION

Muna Malik: Entire research work, sample collection, analysis and writeup
Iffat Javed: Concept**,** Supervision, Literature review.
Sohaila Mushtaq: Supervision, Literature review.
M. Saeed Ahmed: Literature review, Data analysis.
Fahd Kamal Akhtar: Data analysis.

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