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Clinical Aspects and Etiology of Mixed Microbial (Bacterial and Fungal) Keratitis

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Abstract:
Purpose: *To investigate the predisposing factors and clinical presentations of keratitis caused by mixed infectious agents (bacteria and fungi).*
Methods: *This was a prospective study of cases with mixed bacterial and fungal keratitis, done between May 2015 and February 2016 in outdoor unit of Department of Ophthalmology, Maharani Laxmi Bai Medical College, Jhansi. Thirty cases of mixed bacterial and fungal keratitis were identified. Samples (corneal swabs and*
scrapings) were collected aseptically from corneal ulcer patients. Isolation and identification of the microbial agents and antimicrobial susceptibility testing were done in the Microbiology and Pathology departments.
Results: *Nineteen cases (63.3%) were men, and the mean age was 54.2 ± 9.3 years. The affected people were mostly (25;83.3%) residing in the rural areas. 17 patients (56.7 %) were involved in agricultural activities . The people of 41- 60 years of age were particularly prone to this disease (20; 66.7%). The most common predisposing factor for mixed keratitis was a history of ocular trauma (21;70%) and 14 patients (46.7 %) had a history of trauma with vegetative matter. The incidence of the disease was highest in the monsoon season, between June to September (16; 53.3 %).The most common causative bacterial organisms was Staphylococcus aureus (13;43.3%) followed by Pseudomonas secies (5;16.7%) and among fungal organisms was Aspergillus fumigates (11;36.7%) followed by Fusarium species (6;20%).*
Conclusion: *The identification of the etiology and the predisposing factors of corneal ulcers in this region are important for the prevention and early treatment of the disease. Usually, patients with mixed bacterial and fungal keratitis have poor prognosis. Thus, when the infectious keratitis is running an atypical course or found unresponsive to the initial medical treatment, the possibility of a mixed infection by bacterial and fungal organisms should be considered.*
Keywords: *Fungal corneal ulcer, Bacterial corneal ulcer, etiological agent*

I. Introduction

Corneal ulcer is one of the important ophthalmic conditions causing significant morbidity especially in the developing countries[1]. It constitute the second most common cause of preventable blindness after cataract in tropical developing countries[2]. Corneal ulcerations can be caused by different microbial agents. Although any organism can invade the corneal stroma if the corneal protective mechanisms such as blinking, tear dynamics and epithelial integrity are compromised but microbial causes of suppurative corneal ulcers vary considerably in different geographical areas. Bacteria and fungi are frequently responsible for suppurative corneal ulcers especially in the developing countries[3]. Most of the organisms cultured from corneal infections are of the same species that are normally present on the lids and periocular skin, in the conjunctival sac or in adjacent nasal

passage. However, both gram-positive and gram-negative bacteria are responsible for causing suppurative corneal

ulcers with *Staphylococcus*, *Streptococcus* and *Pseudomonas* are the most frequent isolates[4]. While among the fungal causes of suppurative corneal ulcers, *Fusarium* and *Aspergillus* species are the predominant agents reported by many investigators[5]. The incidence of fungal corneal infection has increased remarkably in the recent years with

the wide spread use of broad spectrum antibiotics and corticosteroids. Injudicious topical application of cortisone

and its derivatives combined with antibiotics may not only favour the growth of fungi but may cause invasive infection[6]. Further, corneal ulcers are commonly associated with some predisposing factors. Among the important predisposing factors related to corneal ulcer are trauma (generally with plant materials), chronic ocular surface disease, contact lens usage, ocular surgery, corneal anaesthetics abuse, diabetes mellitus, vitamin deficiency and immunodeficiencies[7]. Patients with compromised cornea due to diseases such as Herpes

simplex keratitis or keratoconjunctivitis, bullous keratopathy are also at risk of developing corneal ulcers. Antibiotics are preferentially used in cases of bacterial corneal ulcers but in most of the instances, it is used empirically which may lead to resistant mutants with consequent treatment failure. Microbiological studies following the culture of corneal infiltrates are the gold standard for determining the etiology of infectious keratitis caused by bacteria or fungi; however, even if a culture of the corneal ulcer is obtained, subsequent growth and identification of microorganisms occurs in only 40% to 60% of cases[8]. Corneal ulcers are often treated empirically without the benefit of microbiological data and, even in cases where a specimen is collected, it is generally recommended that treatment be initiated as soon as possible before obtaining the results and continued even if no microorganism is identified[9]. The purpose of the present study was to find out the bacterial and fungal agents causing mixed corneal ulcers.

II. Materials And Methods

Patients:

A total of 77 clinically diagnosed patients of suppurative corneal ulcers of different age and sex who attended the Ophthalmology out patient department (OPD) of Maharani Laxmi Bai Medical College, Jhansi between May 2015 and February 2016 were studied and out of which 30 cases of mixed bacterial and fungal keratitis were included in this study. Corneal ulcer was defined as a loss of corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon[10].The typical or suspected viral ulcers, healing ulcers, Mooren's ulcers, interstitial keratitis, neurotrophic keratitis and any ulcer associated with autoimmune diseases were excluded from the study. The study was approved by the Institutional Review Board and written informed consent was taken from all the patients. A standardized proforma was filled up for each patient with documentation of sociodemographic features, duration of symptoms, predisposing factors, history of trauma, associated ocular and systemic conditions, prior therapy received and all other clinical findings including visual acuity.

Clinical examinations : Visual acuity at the time of presentation was recorded. All the patients were examined by slit lamp biomicroscope by an ophthalmologist. After staining the ulcer with sodium fluroscin the size of the ulcer, stromal infiltrate and depth was measured using the variable slit on the slit lamp and recorded in millimeter. The hypopyon if present was noted
and measured similarly in millimeters. The ulcer margin, thinning of the floor, satellite lesions, any retained foreign body and pigmentation over the ulcer surface was recorded. A diagram of each ulcer was drawn on the standardized form by performing frontal and cross sectional sketches. Associated ocular conditions like blepharitis, conjunctivitis, dacryocystitis, corneal anesthesia, dry eyes, lid abnormalities, lagophthalmos, past surgery in the cornea, use of contact lens and corticosteroids were noted.

Collection of Samples: One corneal swab and three corneal scrapings were collected from each patient by an Ophthalmologist with all aseptic precaution**s**. Corneal swab was taken by rubbing the ulcerated area of the cornea with sterile cotton swab soaked with sterile normal saline before instillation of local anaesthetic[11]. For taking corneal scrapings, two drops of preservative free local anaesthetic (0.4% oxybuprocaine) were given to the affected eye. Five minutes after instillation of local anaesthetic, three corneal scrapings were taken by sterile Bard Parker No. 15 scalpel blade under slit lamp. Great care was taken for not to touch the lashes or lids and to obtain material from the base and the peripheral margins of ulcer.

Bacterial culture test: The swab was inoculated onto Blood agar, MacConkey's agar, and Chocolate agar media and incubated aerobically at 370C for maximum up to 48 hours. To ensure 5-10% CO2, incubated Chocolate agar plates were put under candle extinction jar. All the bacterial isolates were identified by their colony morphology, gram staining, motility testing by hanging drop preparation, pigment production and relevant biochemical tests[12].

Detection of fungal agents: First corneal scraping was used for wet preparation in 10% KOH, second scraping for fungus culture and third scraping for lactophenol cotton blue staining. Materials obtained by second scraping were spot inoculated on plain Sabouraud's dextrose agar medium (SDA). The inoculation technique consisted of "C" streaks on the culture plate, with the idea to localize the site of implantation of the corneal scraping on the agar media. Inoculated SDA media was incubated at 250C 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[13]. The plates which did not show any evidence of growth after 14 days were discarded. For identification of fungal species that grown in SDA, microscopical examination in wet preparation and lactophenol cotton blue staining were used besides subculturing onto SDA media.

III. Results

Out of 30 patients included in this study (Table 1), nineteen cases (63.3%) were men, and the mean age was 54.2 ± 9.3 years. Most of the patients (25;83.3%) were residing in the rural areas. 17 patients (56.7 %) were involved in agricultural activities . The people of 41 - 60 years of age were particularly prone to this disease (20; 66.7%).

The most common predisposing factor for mixed keratitis (Table 1) was a history of ocular trauma (21;70%) and 14 patients (46.7 %) had a history of trauma with vegetative matter. The incidence of the disease was highest in the monsoon season, between June to September (16; 53.3 %).

The most common causative bacterial organisms were gram positive (22;73.3%) with Staphylococcus aureus (13;43.3%) followed by Pseudomonas secies (5;16.7%) as predominant ones and among fungal organisms was Aspergillus fumigates (11;36.7%) followed by Fusarium species (6;20%) (Table 2).

Table 3 shows the various laboratory results obtained from corneal scrapings of 30 patients of mixed keratitis with 21(70%) samples had positive fungal and bacterial growths in culture.

Most common findings on clinical examination were anterior chamber reaction and conjunctival injection seen in all the cases (Table 4). Other common findings (Fig.1,2) were stromal infiltration and hypopyon. On histopathological examination septate, slender, branching hyphae were seen in 11 cases where the fungus was typed as aspergillus along with gram positive cocci (Fig.3).

Figures and Tables

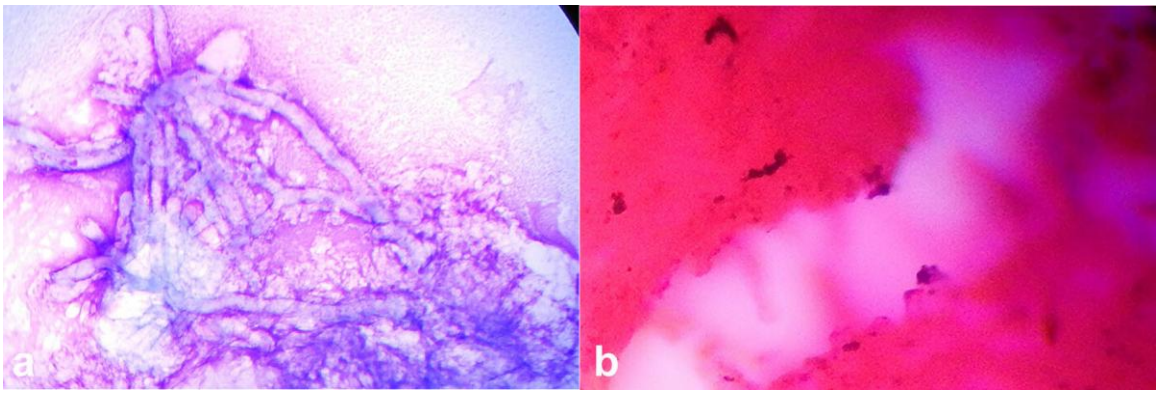
Figure 1: Clinical pictures of 3 patients with corneal ulcer (Left eye).

a. Corneal ulcer with dense stromal infiltrates and feathery margins.
b. Corneal ulcer with stromal infiltrates, feathery margins & thick slough
c. 2% fluorosceine dye stain positive corneal ulcer.

Figure 2: Clinical pictures of 2 patients with corneal ulcer (Left eye).

a. Corneal ulcer with dense stromal infiltrates, feathery margins satellite lesions and dense thick immobile hypopyon.
b. Corneal ulcer with stromal infiltrates, feathery margins & thick hypopyon.

Figure 3: Microscope pictures from stained corneal smear slide of same patient.



a. Branched filamentous septate hyphae.
b. Gram positive cocci.

Table 1: Demographic profile

Demographics	Particulars	Number (%)
Sex	Male	19 (63.3)
	Female	11 (36.7)
Age (years)	< 21 years	3 (1.0)
	21-40 years	7 (23.3)
	41-60 years	20 (66.7)
Residence	Rural	25 (83.3)
	Urban	5 (16.7)
Occupation	Agricultural workers	17 (56.7)
	Labourer	4 (13.3)
	Household	6 (20)
	Students/Children	2 (6.7)
	Service	1 (3.3)
Predisposing Factors	A)Corneal trauma & traumatic agents:	21 (70)
	i)Vegetative matter	14 (46.7)
	ii)Dirt/mud/sand/stone	3 (10)
	iii) Finger nail	2 (6.7)
	iv) Insects	1 (3.3)
	v) Animal tail	1 (3.3)
Seasonal variation	May-September	16 (53.3)
	October - February	14 (46.7)

Table 2: Microbial species isolated from 30 corneal ulcer patients

(A) Fungal species No. (%)	
Aspergillus Fumigates	11 (36.7)
Aspergillus Flavus	4 (13.3)
Aspergillus niger	2 (6.7)
Fusarium	6 (20)
Mucor	2 (6.7)
Rhizopus	2 (6.7)
Alternaria	1 (3.3)
Branching fungus (Unidentified)	2 (6.7)
(B) Bacterial species No. (%)	
Gram positive	
Staph. Aureus	13 (43.3)
Staph. Epidermidis	5 (16.7)
Strept. Pneumonia	4 (13.3)
Gram negative	
Pseudomonas spp	5 (16.7)
E. coli	2 (6.7)
H. influenza	1 (3.3)

Table 3: Correlation between 10 % KOH smear diagnosis, Gram-stained smear diagnosis, positive culture diagnosis from 30 corneal ulcers

Investigation	Results No. (%)	Fungal & bacterial growth in culture	
		Positive	**Negative**
Detection of fungal filaments in KOH smear	20 (66.7)	14 (46.7)	5 (16.7)
Detection of fungal filaments in Gram stained Smear	10 (33.3)	7 (23.3)	4 (13.3)

Table 4: Slit lamp examination findings in mixed keratitis patients

Signs	No of patients
Feathery infiltrate	6
Satellite lesions	3
Conjunctival injection	30
Immune rings	2
Endophthalmitis	2
Epithelial defect	12
Suppuration	4
Stromal infiltration	18
Anterior chamber reaction	30
Hypopyon	13
Vascularisation	6
Dry looking ulcer	3
Corneal thinning	12
Perforation	4

IV. Discussion

Males were affected more commonly than the females, which is usually the case[14]. However, a higher incidence amongst the females is reported in some studies . The disease was more common in the age group of 41-60 years which is in contrast to the observations by Chowdhary and Singh [14] where preponderance was seen between 31-40 years of age. The possible reason could be that our hospital caters to more patients who are from the rural background.

The most common predisposing factor found in our study was trauma to the cornea seen in 21 (70%) cases. The agents responsible for trauma were primarily thorns, tree branches and husk (14;46.7%). Other studies have also found trauma to be the commonest predisposing factor in the spectrum of fungal keratitis. The percentage of corneal trauma has been reported to be as high as 42% by Chowdhary and Singh[14]. Use of contact lenses by wearers practicing poor hygiene is another factor seen mostly in the developed world. Our study had no case with contact lens wear which is in congruence with some studies[15]. Clinical severity of corneal ulcer at presentation is a predictor of worst outcome[16]. A wide variety of fungi have been known to cause keratitis. The commonly implicated ones are aspergillus and fusarium. Various studies of mycotic keratitis implicate aspergillus species as the commonest incriminant[14].

V. Conclusion

The present study focuses on to the pattern of bacterial and fungal pathogens causing mixed corneal ulcers. It indicates that microbial etiology of corneal ulcer has a particular distribution with many predisposing factors that may contribute to it. Information about etiological agents that have been gathered in this study can help ophthalmologists for empirical antimicrobial therapy (both antibiotics and antifungals) and to take strategies for proper management of cases, specially where laboratory facilities are lacking. Although culture is the gold standard for definitive diagnosis of fungal and bacterial keratitis, direct microscopic examination of corneal scrapings or histomorphological evaluation of biopsies allow a rapid preliminary diagnosis. Thus, when the infectious keratitis is running an atypical course or found unresponsive to the initial medical treatment, the possibility of a mixed infection by bacterial and fungal organisms should be considered. Early administration of antifungal along with antibiotic treatment helps in preventing dreadful complications. To conclude, mixed corneal ulcers are a preventable cause of blindness. They need to be suspected clinically followed by laboratory confirmation. The accurate identification of causative agent helps in planning an appropriate treatment strategy.

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