

Microbiological Pathogens Lessening the Ocular Perception of Ailing Fellow with ophthalmic infection, in and Around Rajkot District, Gujarat



Medical Science

KEYWORDS : Ophthalmic infections, corneal ulcer, Microbiological pathogens

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ABSTRACT

AIM : This study is contrived to find the aetiology of corneal ulcer clinically and by laboratory investigations. **MATERIAL AND METHODS:** 100 clinically suspected cases of ophthalmic infections at G.T. Sheth Eye

Hospital, P.D.U Government Medical College, Rajkot, Gujarat from January 2012 to April 2013 were studied. PDUMC is a referral center and postgraduate training institute that caters patients from all Saurashtra & Kutch regions. The clinical diagnosis was made with slit-lamp biomicroscope. **TYPE OF STUDY:** Cross sectional. **STATISTICAL ANALYSIS:** Student's t test. **RESULT:** 46/100 Gram stain Smear is positive. 21/100 KOH smears were positive. 100/21 cases were culture positive. Among them pure bacterial culture is 12(57.14%) and pure fungal culture is 9(42.8%). Bacterial isolate is *Pseudomonas aeruginosa* 5(5/21, 23.80%) and *Streptococcus Spp* in 3 patients(14.28%). *Fusarium spp* were 5(5/21, 23.80%) and *Aspergillus spp* were 3 cases(14.20%). **CONCLUSION:** Bacterial and fungal infections occurs almost equally. *Pseudomonas aeruginosa* cause majority of bacterial ulcers and *Fusarium spp* cause the fungal ulcers. **KEY MESSAGE:** This study is important in public health implications for the treatment and prevention of corneal ulceration in the developing world.

INTRODUCTION

Bacterial and fungal Infections are major cause of blindness in the developing country like India. In India there are 12 million people blind ⁽¹⁾. The incidence of corneal blindness is 15.4 %⁽²⁾.

Microbial infections and ulceration is a sight-threatening condition with significant morbidity ⁽¹⁾ Successful treatment depends upon accurate and rapid identification of the causative organism ⁽²⁾ to rescues vision. The rate of disease progression is dependent on the virulence of the infecting organism and on host factors

Highly virulent organisms such as *Pseudomonas* cause rapid tissue destruction, while other organisms like such as non-tuberculosis *mycobacteria* and *viridians* type *Streptococcus* are usually associated with a more indolent course. Some bacteria, which are considered to be normal flora (e.g. *Neisseria gonorrhoea*, *Neisseria Meningitidis* and *Corynebacterium diphtheria*), may become opportunistic pathogen in compromised eye. The role of microbiologists is a crucial, as microbial infections can differentiated from other non-infectious inflammatory conditions of the cornea resulting from immune-mediated reactions.

The present study is undertaken to evaluate the current concept of the aetiology, clinical characteristic, microbiological workup, trends in antibiotic susceptibility and management of ophthalmic infections.

MATERIAL AND METHODS

The Study Population:

This is a cross sectional prospective study, in which 100 patients of the ophthalmic infection presenting to the ophthalmology department of G.T. Sheth Eye Hospital, P.D.U Government Medical College, Rajkot, Gujarat from **January 2012 to April 2013** were included in the study. It is a referral center and postgraduate training institute that caters patients from all Saurashtra & Kutch regions. A total of 100 consecutive patients with ophthalmic infection were analyzed

The case definition and criteria for inclusion in the study:

Patients were seen after the initial clinical diagnosis of corneal ulceration, infiltration, suppuration, hypopyon, conjunctivitis contact lens.

The Study Samples :

Conjunctival swabs, Corneal scrape, biopsy, button, Aqueous paracentesis.

Clinical Techniques:⁽³⁾

Direct visualization in Slit Lamp Bio- microscopy of lesion.

Inoculations:

Scrapings were performed after instillation of 4% lignocaine from the leading edge and the base of each ulcer.

Material obtained was inoculated directly onto sheep's blood agar, chocolate agar, and potato dextrose agar (PDA) in a row of C shaped streaks and into brain heart infusion broth (BHI) without Gentamicin sulphate. Non-nutrient agar overlaid with *Escherichia coli* in an attempt to isolate *Acanthamoeba spp.* ⁽⁴⁾.

Smear on two separate glass slides: Gram stain and KOH wet mount. ⁽⁴⁾.

LABORATORY PROCEDURES:⁽³⁾

Transport Media: Either directly inoculated on blood agar, or glucose broth is used.

Culture:

Chocolate agar is incubated in a candle jar, which provides 5 % CO₂ and another blood agar is incubated in an anaerobic jar or cabinet if available at 35C (±1); SDA and PDA, are incubated at 27C (±1) in BOD incubator.

Blood agar, chocolate agar and BHI were evaluated at 24 hours and at 48 hours and then discarded at 7 days if no growth was seen.

Fungal cultures inoculated onto SDA were examined daily, and discarded at 3 week if no growth

Cultures on non-nutrient agar (NNA) overlaid with *E coli* were examined daily for the presence of *Acanthamoeba* spp. and likewise discarded at 3 week if no signs of growth.

Grams stain is for visualization and differentiation of bacteria into two groups : Gram positive appears purple and gram negative appears red, Lacto phenol cotton blue is for visualization of fungal structures: Fungal elements stain more intense blue against light blue background. KOH mount is for visualization of fungal hyphae fungal elements appear as hyphae structures

The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria.

Microbial cultures were considered significant when:

- Growth of the same organism was demonstrated on more than one solid phase medium on the C-streaks.
- If growth of one medium was consistent with direct microscopy findings
- If the same organism was grown from repeated scraping.
- Growth was consistent with clinical signs.
- Growth on liquid media appears as turbidity, subculture and gram stained for identification was done.

A negative culture may represent true sterile ulcers, although other factors to consider are.

- Prior antibiotic treatment
- Inadequate sampling methods
- Improper selection of media
- Improper interpretation of data.

Ethical Consideration:

The data collected for the purpose of current research is the part of the diagnostic techniques, so the ethical consideration is not needed. Patients privacies are secured and the results are kept confidential.

RESULT:

Out of 100 cases 21 cases showed positive culture. Among them pure bacterial culture is 12(57.14%) and pure fungal culture is 9(42.8%).

MICROBIOLOGICAL DIAGNOSIS

COMPARISON OF GRAM STAIN SMEAR:

46 Gram stain Smear shows the pathogenic organism from them: 21 showed fungal element, 16 were gram positive cocci, 7 were gram negative bacilli and 2 were yeast cells.

Microscopic examination of ophthalmic KOH MounT

21 KOH wet munt hyphae was present and 79 were fungal element negative.

Table1: MICROBIAL GROWTH PATTERN OBTAINED IN CULTURES

Growth pattern	Cases
All Culture Positive Cases	21
Pure bacterial growth (single species of Bacteria) in the culture	12
Pure fungal growth (single species of fungus) in the culture	9
All Culture negative cases	79

Total no of ophthalmic infection cases	100
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Table 2: ISOLATES FROM 21 CULTURE POSITIVE CASES

Bacteria	Pure isolate	% out Of 21 culture positive cases
(Gram positive isolated)		
Streptococcus pneumonia	1	4.70%
Streptococcus spp.	2	9.50%
Staphylococcus epidermidis	2	9.50%
Staphylococcus aureus	2	9.50%
(Gram negative organisms)		
Pseudomonas spp.	5	23.80%
Total	12	57.14%

Fungi	Pure isolates	% out Of 21 culture positive cases
Fusarium	5	23.80%
Aspergillus spp.	3	14.20%
Candida	1	4.70%
Total	9	42.85%

DISCUSSION:

TABLE3: COMPARISON OF MICROBIAL PATHOGENS ISOLATED IN THE CULTURES (In Percentage)

Result	Pinchare et al. ⁶	Dhakhwa et al. ⁷	Laspina et al. ⁸	Present study
Pure Bacterial	16.66	29.20%	51	57.14
Pure Fungal	20	33.30%	26	42.85
Mix	1.66	9.90%	23	0

TABLE4: Comparison of bacterial pathogen

Bacteria	Basak et al. ⁹	Bharati et al. ¹³	Srinivasan et al. ¹¹	Present study
Gram positive isolated	71.8	70.06	79	33.33
Staphylococcus aureus	42.6	2.52	12.5	9.5
Staphylococcus epidermidis	7	-	-	9.5
Streptococcus pneumonia	9.4	37.5	44	4.7
Streptococcus spp	15.7	17.4	10.2	9.52
Gram negative isolates	28.2	25.79	21	23.81
Pseudomonas spp.	21.1	18.03	14	23.81

TABLE 5: COMPARISON OF FUNGAL PATHOGEN

Fungi	Basak et al. ⁹	Bharati et al. ¹³	Chowdhary et al. ¹⁰	Present study 2013
Filamentous fungi	-	74.73	1	38.08
Aspergillus spp	59.8	26	-	14.28
Fusarium	21.1	42.82	12.5	23.80

ON COMPARISON OF CULTURE RESULTS:

In Pinchare et al.⁽⁶⁾ positive culture were 38.33%, in Bharati et al.⁽¹³⁾ positive rate was 70.59% and in Laspina et al.⁽⁸⁾ it is 79 %. While our study had 21% positive cultures.

ON COMPARISON OF KOH & GRAM STAIN SENSITIVITY:

Gopinathan et al.⁽¹²⁾ KOH sensitivity is 91% ,gram is 88.2%; In Bharati et al.⁽¹³⁾ KOH- 99.23%, Gram- 88.73%.While in our study its 100% for Gram and KOH.

CONCLUSION:

- Common clinical characteristics of fungal infections are long duration of onset of symptoms, dry with raised slough ulcer, satellite lesions and hypopyon present.
- Common clinical characteristics of bacterial infections were short duration of onset of symptoms, greyish white with curdy purulent slough presented with hypopyon.
- Among bacteria *Pseudomonas aeruginosa* and *Staphylococcus* spp were the commonest organisms isolated.
- Among fungi, *Fusarium* and *Aspergillus* were the commonest organisms isolated. *Fusarium* presented with greater severity and had poorer prognosis because of its predilection for the posterior chamber
- Fungal corneal ulcer should be diagnosed with a high index of suspicion especially in patients who have vegetable matter injury.
- Two cases had the history of wearing contact lenses out of them 50% were culture positive with *pseudomonas* infection.
- Early diagnosis and treatment of patients results in good visual prognosis and is the key in management

To conclude as **Helen Keller** has aptly said, **“The most pathetic person in this world is someone who has sight but no vision.”** So we as Microbiologist should pay more attention towards this neglected field of ocular microbiology and work as a team with our able Ophthalmologist colleagues, with a common vision of imparting evidence based treatment in all cases of corneal ulcers to save the sight of our patients thus playing our part in reducing the burden of blindness in the society.

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