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Spectrum of causative agents of suppurative keratitis in sub-Himalayan region of North India – a prospective study

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Abstract:
Purpose: *The causative agents of suppurative keratitis vary from region to region. We undertook this study to formulate empirical management and comprehensive strategy for lab diagnosis in remote areas.*
Material & methods: *Corneal scrapings were collected under strict aseptic conditions by the ophthalmologist. Whenever possible multiple scrapings were collected and processed in a sequential manner. Selection of media and tests were modified according to individual need. Report of microscopy was provided immediately. Culture reports were provided on day 1, 2 and 7 and after 4 weeks. Results: Most of the cases were from rural background with trauma with vegetative matter. Growth of microorganisms was obtained in 57.5% (23/40) of corneal ulcer scrapings. Pure bacterial growth was seen in 19 of these 23 cases and rest four showed fungal growth. Gram positive organisms predominated (16 of 19) and Streptococcus pneumoniae (6) was the commonest isolate. Fusarium spp. was the common fungal isolate. Microscopy was found to be more sensitive for the diagnosis of fungal keratitis and culture for bacterial keratitis. Conclusion: At tertiary level sequential use of C-streaks on chocolate agar (candle jar incubation), 3 smears (one for KOH mount, one for gram staining & one smear kept unstained for future use) and inoculation in one ml BHI broth are sufficient to reach diagnosis of most of causative agents in our region. At rural hospitals use of 10% KOH wet mount will help in ruling out fungal and Acanthamoeba keratitis.*
Keywords- *corneal ulcer, etiology, Himalayan region, suppurative keratitis*

I. Introduction

Corneal blindness accounts for 20-30% of all blindness in the developing countries of the world. Infective corneal disease is the leading cause of this problem in South Asia.[1] The incidence of suppurative keratitis varies from 11 per 100,000 persons/year in the United States to 799 per 100,000 persons/year in Nepal. The epidemiological pattern and causative agents for suppurative corneal ulcer varies significantly from country to country, and even from region to region within the same country. It is important to determine the "regional" aetiology for making comprehensive strategy for the diagnosis and treatment of corneal ulcers.[2]
The purpose of this study was to evaluate all cases of suppurative keratitis seen at a tertiary level hospital over a period of one year. The aims and objective of this study were to study the spectrum of causative agents i.e. bacteria, fungi and parasites causing corneal ulcers, to compare sensitivity of culture and microscopy, to characterize predisposing factors and demographic character.

II. Materials & Methods

All clinically diagnosed cases of suppurative corneal ulcer were included in this study. A standardized form was filled for each patient documenting socio-demographic information as well as clinical findings including duration of symptoms, past treatment, time of presentation, predisposing ocular conditions and associated systemic risk factors amongst other clinical details.[2]All patients were examined under slit lamp biomicroscope by an ophthalmologist and clinical details were noted.

Corneal scrapings were collected under strict aseptic conditions by the ophthalmologist using a sterile Bard-Parkar blade (No. 15). If the lids were sticky or loose debris was found over the ulcer it was cleaned with sterile cotton swabs before collection of corneal material. The edge of the spatula or blade were not allowed to touch the lid margins or conjunctiva.[1] The procedure was performed following instillation of 2% lignocaine hydrochloride. Material obtained from scrapings of the leading edge and base of each ulcer was inoculated in the media and smeared onto slides.[2]Whenever possible multiple scrapings were collected and processed in a sequential manner. The scrapings were first inoculated in a row of C-streaks onto chocolate agar without cutting the agar and then smears were made on at least three slides for staining and lastly sample collected with a moistened swab was placed in BHI broth.[1,3] Earlier blood agar was being used but it did not support growth of *Streptococcus pneumoniae*always therefore chocolate agar was used. These two media also support growth of all the causative fungi of keratitis which are also rapid growers therefore Sabourauds dextrose agar was not used

initially. Direct inoculation of culture plates or tubes was done at patient's side in the operating room. Selection of media and tests were modified according to individual need.

Glass slides were cleaned and then sterilized in hot air oven after wrapping in aluminium foil. The slides were marked and labeled on its reverse with a wax pencil. Coverslips were flamed before using. Gram stain and 10% Potassium hydroxide (KOH) mount containing 0.1% glycerol were made for preliminary reporting.[1] One smear was kept unstained so that later it can be used for a specific stain i.e. Giemsa stain, Z.N. stain, etc. The presumptive report was provided to the clinician within minutes.

All media were incubated aerobically at 37°C. Chocolate agar was incubated in a candle jar. The plates were examined at 24 hours under magnification and daily thereafter for 7 days and then discarded if there was no growth. The specific identification of bacterial pathogens was based on microscopy, colony characters and biochemical properties using standard laboratory protocols. The antimicrobial sensitivity testing was done by Kirby Bauer's disk diffusion method.[3] Prior to final report a written preliminary report was provided at 24 hours and after that whenever a negative culture turned positive.[1] After one week if no growth of fungi was obtained on chocolate agar then subculture from BHI broth were made on SDA and incubated at 25˚C for three weeks.

Cotton swabswere used to culture material from the conjunctival sacs of both eyes and plated directly onto blood agar.[1]

Cultures were considered positive if they met one of the following criteria:-[1,3]

1. Growth of the same organism on more than one culture medium.
2. Semi-confluent growth on two or more C-streaks on one solid medium. Growth off the streak was taken as contaminant.
3. Heavy growth within the liquid medium confirmed by a positive stained corneal smear.
4. The smear results consistent with culture.
5. The same organism was grown from repeated samples.

Culture reports were provided on day 1, 2 and 7 and after 4 weeks.

III. Results

A total of 40 patients were seen with a corneal infiltrate that was compatible with a diagnosis of suppurative keratitis during the study period of 12 months (September 2006 to August 2007). There were 25 males and 15 females with age ranging from seven to ninety three years. Nearly half the cases (47.5%) were more than 50 years in age. Almost 90% cases were from rural area. The majority (60%) were agricultural workers. There was a significant increase in patients during the months of harvesting. Interestingly, 20% cases were from neighboring districts, they all had received trauma with vegetative matter and their cultures were positive for gram positive cocci.

Out of the total cases 50% cases reported within seven days, 40% between one to two weeks and 10% within one to two months after the onset of illness. Primary care was sought by 28 patients before presenting to our institute. Out of these 14 were treated by eye specialists, eight by general physicians and six by village healers. The patients who were on some form of topical medication (n=28), 50% were taking antibiotic drops, 14% were taking antifungal eyedrops and 25% were on topical steroids. Out of these 28 patients, 17(60%) showed growth of microorganisms despite antimicrobial use. Whereas in 12 patients with history of no drug use, growth was seen in eight cases (66%). All the six patients on steroids showed microbial growth (*Pseudomonas aeruginosa* -1, *CoNS* -2, *Streptococcus pneumoniae* -1, *Fusarium spp*. -1 and *Candida albicans* -1).

Almost 85% patients had one or more predisposing factors contributing to ulcerative keratitis. Trauma with vegetative matter (75%) was the most common cause of corneal injury (Table 1). None of the patients wore contact lenses. There was no case of diabetes mellitus. The risk of suppurative keratitis associated with these predisposing conditions are presumptive.

A total of 40 eyes were examined, left eye was more commonly involved. Visual acuity on presentation ranged from 6/12 to no perception of light. Satellite lesions were present in two cases. The infiltrate surface was $<5mm^2$ in 23 cases, 5-15 mm^2 in 15 cases and >15 mm^2 in two cases. Hypopyon was present in 25 cases. Corneal neovascularization was observed in six cases. One patient had corneal perforation. Descemetocoel was present in seven cases. In both the cases of *Pseudomonas aeruginosa* there was full chamber hypopyon and whole cornea was involved.

A significant growth of microorganisms was yielded in 57.5% (23/40) of corneal ulcer scrapings. The 19 (82.6%) of these 23 isolates exhibited pure bacterial growth while pure fungal growth was seen only in four samples. None of the patients showed mixed infection. Of the 19 bacterial isolates 16 (84%) were gram positive organisms and three were gram negative. *Streptococcus pneumoniae* was the most commonly isolated bacterium, accounting for 31.5% of all bacterial cultures. Other isolates are shown in Table 2. Antimicrobial sensitivity testing was done by Kirby Bauer's disc diffusion method. Gram positive cocci were most sensitive to cotrimoxazole (75%), gentamicin (75%), clindamicin (100%) and cefoperazone (100%). Ciprofloxacin showed

activity against only half of these isolates. They were uniformly resistant to ampicillin. For *Streptococcus pneumoniae* and diphtheroids sensitivity testing was not performed. One isolate of *Pseudomonas aeruginosa* was resistant to all drugs tested including second line of drugs, whereas other isolate showed sensitivity to ciprofloxacin and ceftazidime. The single isolate of *E.coli* was sensitive to ciprofloxacin and gentamicin. *Pseudomonas aeruginosa* was the only organism grown from both conjunctival sac and corneal ulcers. Other pathogens were isolated only from the ulcer. With healthy eyes eight controls were included in the study, their conjunctival swabs grew diphtheroids.

There were total seven cases of fungal keratitis on the basis of microscopy and culture, whereas fungal growth was obtained only in four. Results are shown in Table 2. Correlation of culture and microscopy shows microscopy is more sensitive for fungal keratitis and culture is more sensitive for bacterial keratitis (Table 3). The sensitivity of KOH wet mount was higher (7/7) than of gram staining (2/7) in the detection of fungal structures (Fig. 1-4).

IV. Discussion

Globally, it is estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new cases of corneal blindness annually. Ninety percent of them occur in developing countries and it has been recognized as a silent epidemic. A recent national survey by the Government of India (1991-2001) estimated that corneal lesions are responsible for 9% of all blindness in our country. The real concern is to explore the possibility of effective management of infective keratitis, as done in developed countries.[4]

In our prospective study 40 cases were seen during a period of one year which corresponds with 80 patients studied during two years in Srinagar by Bashir et al.[3] Corneal ulcers were seen in all age groups with a preponderance for > 50 years, which is similar to study done by Chanderet al[5] in Northern India but is different from other studies.[2,3,6,7] This can be attributed to predisposing ocular surface diseases and eyelid diseases in older age group.

The prevalence was seen to be higher in males than in females. Similar observations were made by others in their studies.[2,3,5,6,7] In our study, 90% cases were from rural area with agriculture background and therefore more cases were seen during harvesting. It is similar to studies done by Bashir et al[3] and Basaket al.[2]

Trauma as the most common predisposing factor for corneal ulceration in our study is also reported in other studies of Indian subcontinent[2,3,7,8,9] whereas contact lens wear is the main risk factor in developed countries.[4,6] There was no case of contact lens wear in our study.

Microbial growth was seen in 57.5% of corneal scrapings, this rate of recovery is also reported by Bashir[3] (57.5%) while a higher rate is reported by Basak[2] (67.7%), Bourcier[6] (68.2%), Bharathi[7] (70%), Upadhyay[8] (80%) and Khanal[10] (67.8%).

Pure bacterial growth was seen in 47.5% of corneal ulcers & pure fungal growth in 10%. No case of mixed growth was obtained. This prevalence is similar to studies done by Bashir[3] (40% & 12.5%, respectively) & Upadhyay[8] (63.2% & 6.7%) but different from Basak[2] (15.3% & 42.5%) and Khanal[10] (23% & 32%). Reason could be climatic conditions, which are similar at Srinagar[3]& Kathmandu[8] in comparison to Dharan[10]& West Bengal.[2]

Isolation of *Streptococcus pneumoniae* as most common bacterial pathogen in patients of corneal ulceration is also reported from Srinagar, Nepal, South India & South Africa.[3] Other frequently isolated bacteria were *CoNS, S. aureus*&*Ps. aeruginosa* which also correspond with these studies. Manikandanet al[11] suggest *CoNS* are leading cause of suppurative keratitis in elderly but our 50% cases of *CoNS* were among young adult males who responded to treatment.

Though drug sensitivity testing is important for bacterial isolates but according to Sharma et al[12] ocular antibiotic levels achievable by topical administration may be considerably higher and therefore conventional criteria of resistance may not directly apply. Indeed there have been many reports of pathogens with in-vitro resistance to an antibiotic which have been successfully treated in-vivo by that antibiotic.[12]To minimize complications and permanent squealae timely antimicrobial treatment must be started. Apart from its diagnostic value, corneal scraping allows improved antibiotic penetration and therapeutic debridement of necrotic tissue. The base and leading edges of the ulcer are scraped from the periphery to the center. Several studies mention that as a result of pretreatment, culture positive rate may not be significantly decreased, but a delay in pathogen recovery may occur.[13]But in our study, pretreatment did not make any difference in rate of recovery and neither was there any delay. Use of steroid was definitely associated with recovery of pathogens in all the cases. In a study Upadhyayet al[14] suggest that post traumatic corneal ulceration can be prevented by topical application of 1% chloramphenicol ophthalmic ointment in a timely fashion to the eyes of individuals who have suffered a corneal abrasion in a rural setting. Maximum benefit is obtained if prophylaxis is started within 18 hours.

On the basis of microscopy and culture there were 7 cases of fungal keratitis. *Fusarium spp.* was commonest isolate similar to prevalence reported by Bashir[3] from J&K and Bharathi[7] from South India and unlike cases reported by Basak,[2] Chander,[5]Upadhyay[8] and Verenkar.[9]*Candida albicans* was isolated in one

case, it was post-op steroid induced. Though Thomas et al[15] suggest serrated margins, raised slough, dry texture, satellite lesions and coloration other than yellow occur more frequently in cases of filamentous fungal keratitis and thereby a scoring system can be used. We did not find any such correlation in our study which is supported by Garg et al[16]that there are no exclusive clinical signs in microbial keratitis to diagnose the etiological agent. Fungal filaments are usually absent on the surface therefore scrapings should be taken from corneal stroma.[17]Good visual outcomes were achieved by aggressive dual topical antifungal therapy.[18]Ndoyeet al[19] suggest use of topical povidone iodine alone or with azoles may be an alternative treatment for fungal keratitis in intertropical areas.

Though at tertiary levels in-vivo confocal microscopy is a useful adjunct to slit lamp bio-microscopy for supplementing diagnosis in most cases and establishing early diagnosis in many cases of non-responding fungal and amoebic keratitis. At primary level two simple smear examinations i.e. 10% KOH and gram staining can identify majority cases of bacterial, fungal, nocardial and acanthamoeba keratitis. They can be correlated clinically and empirical therapy can be given.[13]India has pioneered in development of a DNA macrochip for the diagnosis of eye infections in a research funded by CSIR.[20]

V. Conclusion

We recommend use of following steps in lab diagnosis of suppurative keratitis at tertiary level in our region:-

1. C-streaks on chocolate agar (candle jar incubation),
2. Three smears (10% KOH wet mount, gram staining & one kept unstained) and
3. Inoculation in one ml BHI broth.

At primary level (rural/remote areas) a single wet mount with 10% KOH is sufficient for experienced eyes to make diagnosis of fungal and Acanthamoeba keratitis and thereby narrowing down the diagnosis. And if possible in addition they can send a sample in one ml BHI broth (it will serve both as transport and culture media) to a Microbiology laboratory.

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Conflict of interest: None.

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Table 1 Predisposing factors

I. Trauma with vegetative matter:
a. Grass-12
b. Woodstick -06
c. Removal of foreign body with grass – 03
d. Flying stones – 03

II. Ocular problems:
a) Chronic meibonitis – 01
b) Therapeutic CL – 01
c) Allergic eye – 01
d) Post-op:
i. suture material – 02
ii. steroid induced – 01
iii. others - 02

III. Hansen's disease – 02

Total – 34

Table 2 Causative microorganisms obtained in cultures

I. Bacterial: Total - 19

A. Gram positive:
1. *Streptococcus pneumoniae* – 6
2. *Staphylococcus aureus* – 4
3. *CoNS* – 4
4. *Diphtheroids*– 2

B. Gram negative: (GNB)
1. *Pseudomonas aeruginosa* – 2
2. *Escherichia coli* – 1

II. Fungal: Total - 4

A. Filamentous:
1. *Fusariumsolani* – 1
2. *Fusariumchlamydosporum* – 1
3. *Curvularia spp*. – 1

B. Yeast:
Candida albicans – 1

Table 3Correlation of culture & microscopy

I. Microscopy Positive + Culture Positive = 17 (4 fungal)
II. Microscopy Positive + Culture Negative = 13 (3 fungal)
III. Microscopy Negative + Culture Positive = 06 (all bacterial, mostly GNB)
IV. Microscopy Negative + Culture Negative = 04 (inadequate sample)

Legends of Figures

Figure 1: 10% KOH mount (×400) of corneal scraping showing hyaline septate hyphae with chlamydospores.
Figure 2: 10% KOH mount (×400) of corneal scraping showing hyaline septate hyphae with dichotomous branching
Figure 3: 10% KOH mount (×400) of corneal scraping showing hyaline septate hypha ofdoubtful significance
Figure 4: Repeat sample of figure 3 showing better arrangement and presence of septate hyaline Hyphae

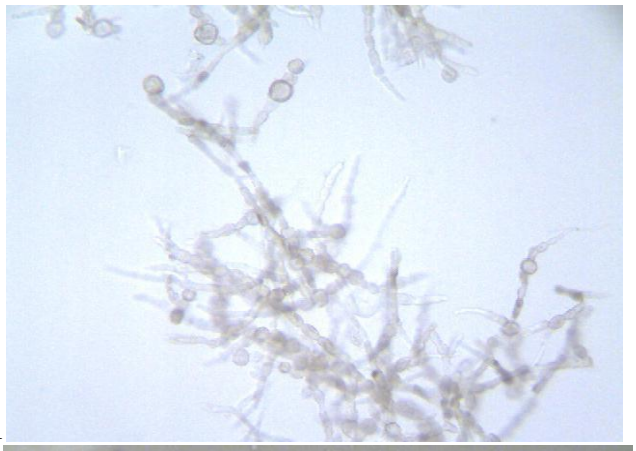

Figure 1

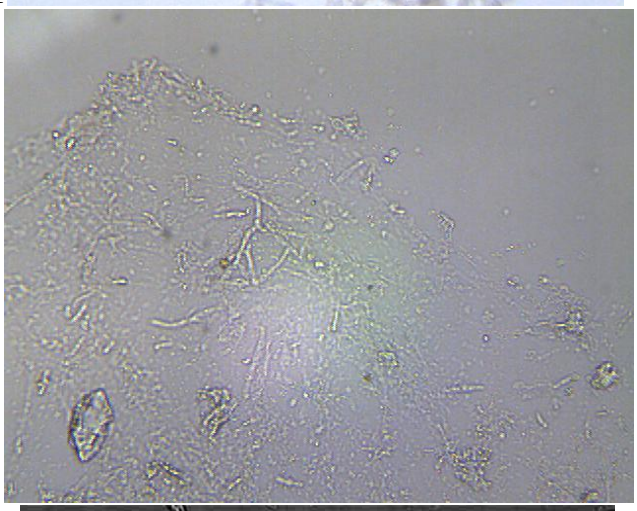

Figure 2

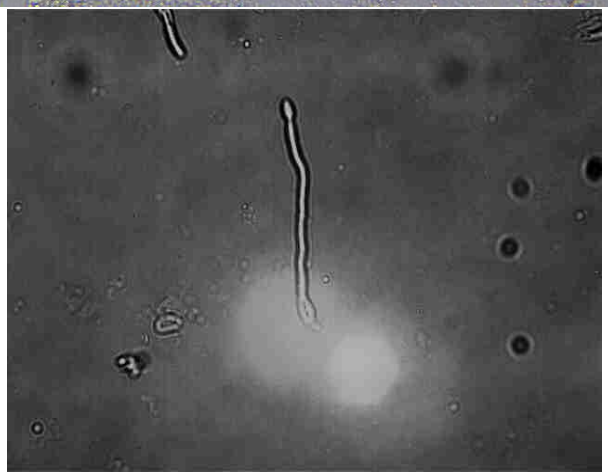

Figure 3

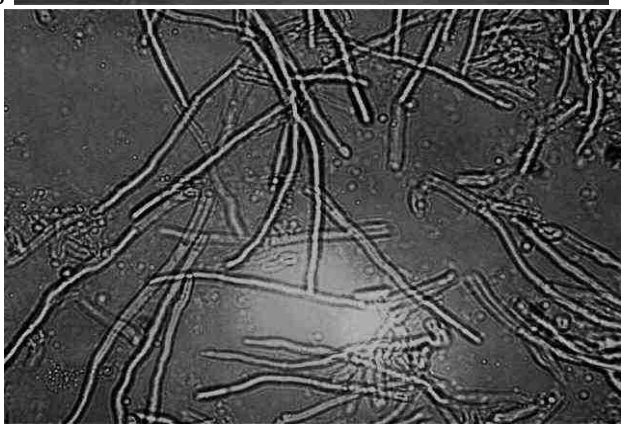

Figure 4