

Organism Isolation in Corneal Ulcer: Utility of Different Techniques

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ABSTRACT

Introduction: Various techniques are available for isolation of microorganism in cases of microbial keratitis including conventional methods of scraping, re-scraping inoculation in different media and corneal biopsy.

Methods: This was a cross sectional descriptive study conducted from February 2002 to July 2003. A total of 161 eyes of corneal ulcers were evaluated with scraping inoculation. Re-scraping and corneal biopsy were performed in those cases which did not grow any organism in the first scraping or which did not respond to initial treatment.

Results: Microorganisms were isolated in 116 patients (72.04%) after first scraping. *S pneumoniae* was seen in 50(42%), *Fusarium* in 21(18%) and *Aspergillus* in 16(14%) cases. Re-scraping was done in 22 culture negative and non improving cases of which 14 (63.63%) showed isolation of organisms. After re-scraping, *Fusarium* and *Aspergillus* were isolated in four patients each (18% each) followed by *S. pneumoniae* in 3 (14%), *S. aureus* in 2 (9%), and *Penicillium* in 1(5%) case. Corneal biopsy was done in 10 cases with isolation of microbial agents in 7 (70%) with *Aspergillus* and *S. aureus* in three each, and *S. epidermidis* in 1(10%).

Conclusions: Apart from conventional scraping, other isolation techniques should be considered in the treatment of non-responding and culture negative cases of corneal ulcers. Compared to bacteria, fungi are difficult to isolate in the first scraping and requires more frequent repeat scraping and corneal biopsy.

Keywords: Corneal biopsy, corneal scraping, corneal ulcer, microbial keratitis, organismal isolation

INTRODUCTION

Corneal ulcer is one of the important causes of blindness worldwide.¹ The WHO bulletin, 2001 stated that annually about two million people go blind due to various causes of ocular trauma and corneal ulcers.² In Nepal, corneal trauma and ulcers were found to be the leading causes of unilateral blindness after cataract,

responsible for 7.9% of total blindness.³ Despite proper facilities to detect microbial agents and improved treatment modalities, corneal ulcer still remains a major cause of visual impairment in our country.⁴

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The clinical presentation of microbial keratitis, at times follows the classical description but it's not always diagnostic and must be confirmed with isolation of infective organism.⁵ There are reports on high isolation of causative microorganism by corneal biopsy, where mere scraping did not isolate any organisms.⁶ So, we aimed to investigate the role of corneal biopsy in isolation of microorganism in selected cases, in addition to other modalities like scraping, and re-scraping.

METHODS

This cross sectional study was conducted at out-patient department of BP Koirala Lions Center of Ophthalmic Study, Tribhuvan University Teaching Hospital (TUTH) and Lumbini Eye Institute, Bhairahawa from February 2002 to July 2003. Consecutive cases of corneal ulcer were enrolled in the study after informed consent. However, those who had already undergone corneal biopsy before presenting to us and those with viral corneal ulcers were excluded from the study.

Detail history of ocular and systemic illnesses and any risk factors were noted according to a specially designed Proforma. Visual acuity was taken with the help of Snellen's chart, E chart. Slit lamp examination of lids, conjunctiva, cornea, iris and lens were done using Haag Streit 900 slit lamp. Syringing was done in all cases to rule out lacrimal drainage passage blockade. Random blood glucose was also checked in every case.

After proper anesthesia with topical 4% lidocaine, corneal scraping was done in supine position. With the universal eye speculum in place, scraping of the ulcer was done with help of heat sterilized platinum Kimura spatula. Culture plates were inoculated in standard "C" streak pattern. The growing infiltrative edge and base of the ulcer were scraped. Staining was carried out using Gram's stain and Geimsa stain. Culture was performed in blood agar, Chocolate agar, Sabouraud dextrose agar and Brain Heart infusion (BHI) broth following standard protocols. The culture was considered positive only if the growth of the same organism was demonstrated on two or more solid media.

Those corneal ulcers, which were not culture positive and were not responding to treatment, were subjected for re-scraping and culture sensitivity. The time lapse between scraping and re-scraping varied from 10 days to 4 weeks depending on the status of corneal ulcer. Early re-scraping was done in those cases that deteriorated clinically even after the treatment. Re-scraping was done deeper with the needle or spatula. Anaerobic culture using Baltimore biological laboratory gas pack system, non-nutrient media inoculated with E-coli for Acanthamoeba was used in addition to the

culture media used with initial scraping. In suspicious case it was cultured in Lowenstein-Jensen media for mycobacterium as well.

Corneal biopsy was also done in those cases, which failed to give positive culture result of scraped material and did not respond to treatment. The time lapse between re-scraping and biopsy varied from 2 weeks to 4 weeks. Biopsy was done in two ways, one by excising the part of infected cornea and other by examining the corneal button in those cases where penetrating keratoplasty (PK) was done. That biopsy material was put in BHI broth and cultured; if there was a growth of microorganism, it was sub cultured in other media. Corneal ulcer treatment was not stopped before the re-scraping and corneal biopsy.

RESULTS

A total of 161 eyes of 160 patients were included in this study. 88 (55%) of them were male. The average age of the patients was 40. Of the total cases, 63 (39%) had traumatic ulcer (Table 1). 78 (48%) of the cases were using some medication at the time of presentation. More than half of them were using antibiotics alone. Table 2 shows the type of treatment they were undergoing at the time of enrollment.

Table 1. Age and sex distribution of cases with corneal ulcers

Age (yrs)	Male		Female		Total(n)	
	Total	Traumatic	Total	Traumatic	Total (%)	Traumatic (%)
0-10	6	2	3	1	9 (6)	3
11-20	6	2	10	7	16 (10)	9
21-30	19	11	11	3	30 (18)	14
31-40	10	2	15	2	25 (16)	4
41-50	15	6	11	7	26 (16)	13
51-60	15	6	8	3	23 (14)	9
61-70	12	6	8	3	20 (13)	9
71-80	4	1	2	1	6 (4)	2
81-90	1	0	4	0	5 (3)	0
Total	88	36	72	27	160 (100)	63

Table 2. Treatment at the time of the presentation

Medicine	Number (%)
Antibiotics	40 (51)
Steroid	13 (17)
Antibiotics + Antifungal	12 (15)
Antibiotics + Steroid	3 (4)
Antibiotics + Antiviral	3 (4)
Antibiotic + Antifungal + Steroid	1 (1)
Antibiotics + Traditional	2 (3)
Traditional	1 (1)
Unknown	3 (4)
Total	78 (100)

A little more than half of the cases (57%) were having conjunctival abnormalities in addition to the corneal ulcer. Conjunctivitis was frequently associated with corneal ulcer. Table 3 shows ocular surface abnormalities in study population. Conjunctival evidences of trachoma, xerophthalmia that may lead to corneal ulcer were not seen. Likewise, 6% (n=10) of the cases had preexisting chronic dacryocystitis. Out of them, seven *Strep. pneumoniae*, one alpha hemolytic *Streptococcus*, one *Fusarium* and one with mixed growth of *Strep. pneumoniae* and *Staph. Epidermidis* were isolated.

Table 3. Different ocular surface abnormalities noted in the cases

Ocular surface abnormality	Number (%)
Conjunctival abnormality	91 (57)
Lid abnormality	21 (13)
Lacrimal drainage system abnormality	10 (6)
Corneal abnormality	10 (6)
Without any ocular surface abnormality	29 (18)
Total	161 (100)

First corneal scraping gave positive culture in 116 (72%) of the eyes (Table 4).

Table 4. Organism isolated by various techniques

Technique	Total	Culture positive (%)
Scraping	161	116 (72)
Rescraping	22	14 (63.6)
Biopsy	10	7 (70%)

Among these 116 positive cultures, 63(54%) were gram positive bacteria, 2 (2%) were gram negative bacteria, 48 (41%) were fungus and 3(3%) had mixed growth. *S. pneumoniae* was the most commonly isolated organism. Cultures with mixed growth contained *Aspergillus* and *Penicillium* in one, *S. pneumoniae* and *S. epidermidis* in the next and *S. pneumoniae* and *Culvularia* in the last one. (Table 5)

Table 5. Organism isolated in scraping

Organism	Number (%)
<i>S pneumoniae</i>	50 (42)
<i>Fusarium</i>	21 (18)
<i>Aspergillus</i>	16 (14)
<i>S aureus</i>	11 (9)
<i>Penicillium</i>	5 (4)
Yeast	2 (2)
<i>Pseudomonas aeruginosa</i>	2 (2)
<i>Curvularia</i>	1 (1)
<i>Nocardia</i>	1 (1)
Alpha-haemolytic streptococcus	1 (1)
Unidentified pigmented fungus	3 (3)
Mixed growth	3 (3)
Total	116 (100)

Amongst remaining 45 patients, only 22 underwent re-scraping as others were improving with treatment. Of those 22 patients who needed re-scraping, micro-organism was isolated in 14 patients. Table 6 shows the list of organisms isolated after re-scraping.

Table 6. Organisms isolated in re-scraping

Isolation in re-scraping	Number (%)
<i>Fusarium</i>	4 (18)
<i>Aspergillus</i>	4 (18)
<i>Strep.pneumoniae</i>	3 (14)
<i>Staph.aureus</i>	2 (9)
<i>Penicillium</i>	1 (5)
No growth	8 (36)
Total	22 (100)

Gram stain showed 35 (21.7%) positive cases of bacterial infection, however; only 21 of them had positive cultures. Similarly, Geimsa stain showed 23 (14.3%) positive cases of fungal infection, among them, 21 had positive cultures.

Altogether, 10 corneal biopsies were taken. Five of them were done by excising the part of the infected cornea and five by examination of the corneal button after the penetrating keratoplasty. Histo-pathological

evaluation did not show micro organisms in any of the cases. However, seven cases had positive cultures. Of them, three were *Aspergillus*, three *S. aureus*, and one *S. epidermidis*.

The cases, which were already on treatment before presenting to us had similar pattern of microbial growth as other cases, who had not received treatment elsewhere. In those 78 cases, microbial isolation could be done in 71% (*Strep pneumoniae* in 26%, *Fusarium* in 17%, *aspergillus* in 13%, *S. aureus* in 7%, *Penicillium* in 4%, yeast in 2%, *pseudomonas* and *Alpha-hemolytic streptococcus* in 1% each were isolated).

DISCUSSION

Suppurative keratitis is a major cause of visual impairment and blindness in the developing world.¹ For the prevention of visual impairment and blindness from corneal ulcer, prompt detection and appropriate therapy directed towards causative organism should be considered. This study attempted to isolate organism by utilizing different techniques in cases of suppurative keratitis that may help in treating the condition.

Majority of corneal ulcer patients were in economically productive age group i.e. 21-60 years. This may be due to the fact that at extremes of the ages people are less involved in agricultural tasks and other physical works, which could be responsible for ocular trauma and infection that may follow the trauma. In our study there were 63 cases of traumatic corneal ulcer. A study done in Nepal earlier showed similar finding.⁷ In developed countries one of the most important risk factors for corneal ulcer is contact lens wear (50% in the study in Paris⁶, 36% in the study in Switzerland⁹). However, in developing countries, trauma is the most important risk factor.^{7,10} Most of them sustained trauma while working in the farms. Traumatic ulcers were seen more in males than females. This correlates with the other study done in Nepal¹¹, where most of the trauma was seen in the age group of 11-30 years. This may be due to their outdoor and sports activity in this age group. In this study there were three cases with traumatic ulcer under 10 years of age, out of which one had trauma with pencil. None of the children had agricultural trauma.

Growth pattern of the microorganisms did not reveal any significant relationship with trauma. Even in cases of traumatic ulcers, the most common isolate was *Strep. pneumoniae* followed by *Fusarium* and *Aspergillus*, similar as in the cases of non traumatic ulcer. At the time of presentation, many of the patients were already using either antibiotics, anti-fungals, anti-virals and steroids, alone or in combinations. This injudicious use of steroid may be one of the causes of non healing corneal ulcers. Out of 17 patients using steroids five

had fungal corneal ulcer. The cases that were already on treatment at the time of presentation had similar pattern of microbial growth as virgin cases. *Strep pneumoniae* was the most common isolate followed by *Fusarium* and *aspergillus*.

Gram stain yielded 35 positive cases, and Geimsa stain showed 23 positive cases out 130 culture positive cases. Thus, the stains have poor sensitivity in identifying the infective causes. First corneal scraping gave positive culture in 116 (72%) of the eyes. Among these 116 positive cultures, 65(56%) were bacteria, 48 (41%) were fungus and 3(3%) had mixed growth. Srinivasan et al also reported that out of 434 patients with central corneal ulcer, 68.4% were culture positive. Among them, 47.8% were purely of bacterial origin, 46.8% purely fungal, 5.1% had organisms of mixed growth and 1% had growth of *Acanthamoeba*.¹⁰ Among the culture positive cases, the most common isolate was *Streptococcus pneumoniae* (50, 42%), which correlates with other studies done in Nepal.⁷ In India, a study done at Aravinda Eye Hospital, showed *Strep. pneumoniae* in 41.85% of 626 cases.¹² Srinivasan et al also showed a high number of *S. pneumoniae* isolates in their study.¹⁰ However, a study at Beijing Institute of Ophthalmology showed low bacterial isolates of 18% and fungus in 34.8% of the cultures, the most common bacterial isolate being *Staph epidermidis* accounting for 27.5%, followed by *Pseudomonas* 21.7% and *Staph aureus* 6.2%.¹³ Another comparative study in Ghana and India showed that in Ghana, *Pseudomonas* was frequently isolated whereas in India *Streptococcus* species was the commonest.¹⁴ *Staphylococcus* and *Pseudomonas* were the most frequently isolated organisms in a study carried out at Department of Ophthalmology, University of Patras, Greece.¹⁵ One study conducted at Quinze Vingts National Center of Ophthalmology, Paris⁸ showed the use of contact lens as the most common predisposing factor and coagulase negative *Staphylococcus* as the commonest isolate followed by *Propionibacterium* and *Pseudomonas*. This shows that *Strep pneumoniae* is more common in developing country than in developed countries.

Fusarium was the most common isolate among fungus, which accounted for 21(18%) and *Aspergillus* was seen in 16 eyes (14%). Previous studies in Nepal⁷ had shown *Aspergillus* to be the commonest cause of fungal infection. This may be due to the fact that 37 cases in this study were collected from Lumbini Eye Institute, Bhairahawa where *Fusarium* was the commonest fungus. Literature has shown climatic preference among the fungus and *Fusarium* is more common in hot and humid climate,¹⁶ just the climate in Lumbini, where the study was carried out. Likewise, a study done at Beijing institute of Ophthalmology also showed

that *Fusarium* was the commonest isolate in cases of corneal ulcer followed by *Aspergillus*.¹³ Another multicentric study done in Ghana and south India also showed that filamentous fungus was the commonest isolate, *Fusarium* species being more than *Aspergillus*.¹⁴

In this study there were 22 cases in which re-scraping was done, out of them 14 came culture positive. In re-scraping, nine patients (41%) grew fungus. It shows that fungal growths can be obtained after repeated scraping in the cases where first scraping did not yield organisms. Thus, it can be concluded that deep scraping is needed to obtain fungus and repeated scraping may help in obtaining material from deeper tissue. Re-scraping is needed not only to debride the ulcer for good penetration of the drugs but also to obtain the deeper corneal specimen for culture. It may be possible that due to prolonged antibiotic use superficial corneal layer becomes sterile while deeper tissue may still be infected with microorganism, which needs to be removed for isolation of the causative agent. That is the reason for recommendation of deeper scraping during rescraping.^{17,18}

Of the 10 biopsies performed, seven showed positive cultures including three *Aspergillus*. High isolation rate of fungus in corneal biopsy again showed that fungi are difficult to isolate from the corneal ulcer and corneal biopsy may help in isolation. There was a study published in American Journal of Ophthalmology where corneal biopsies were taken in cases where there was poor response to the conventional treatment. Out of 33 cases they isolated the organisms in 27 cases by biopsy. Out of remaining six patients, isolation was done in five cases by corneal button examination.⁶ Though this study did not prove the superiority of any of the technique over the other, it definitely suggested

the usefulness of re-scraping and corneal biopsy in those cases which do not respond to treatment or which did not grow any organism in the first scraping. Although the sample size of 161 for study of corneal ulcer is reasonably good number but cases undergone re-scraping and biopsy were comparatively less to prove the superiority of one technique over the other. Although various literatures have suggested stopping of topical medications upto 72 hrs before the re-scraping and biopsy, the treatment was stopped only for 24 hrs in our study. Antibiotics were not stopped before corneal biopsy. It may have had some implications in subsequent growth of micro organisms. The clinical diagnosis should have been compared with the etiological diagnosis of corneal ulcer by lab confirmation, so that this study could have suggested the guidelines for clinical suspicion of various micro organisms causing the corneal ulcer.

CONCLUSIONS

Apart from the methods of conventional scraping, other isolation techniques should be seriously considered in the treatment of non-responding and culture negative cases of corneal ulcers. Re-scraping and corneal biopsy, either done by excising the part of infective cornea or by corneal button examination, seem to be good alternatives in identification of microbial agent. Also compared to bacteria, fungi are difficult to isolate in the first scraping and more frequently they may require repeat scraping and other techniques like corneal biopsy. This study also showed that culture of the biopsy material is more important than histopathological examination of the same for the isolation of microorganisms in cases of microbial keratitis.

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