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Original Article

Etiological agents of suppurative corneal ulcer: Study of 56 cases

Laila Akter[1], M. A. Salam[1], Bulbul Hasan[1], Nurjahan Begum[2], Iftikhar Ahmed[2]

[1] Department of Microbiology, Rajshahi Medical College, Rajshahi-6000,[2] Department of Microbiology, Enam Medical College, Savar, Dhaka.

Abstract

This study was carried out to isolate and identify the etiological agents causing suppurative corneal ulcers and to perform the antimicrobial susceptibility testing of the bacterial isolates. Samples (corneal swabs and scrapings) were collected aseptically from 56 suppurative corneal ulcer patients attending Rajshahi Medical College Hospital (RMCH). Isolation and identification of the microbial agents and antimicrobial susceptibility testing were done in the Microbiology laboratory of Rajshahi Medical College. Culture yielded growth in a total of 47(83.93%) cases out of 56 patients with pure fungal growth, pure bacterial growth and mixed microbial growth (both bacteria and fungi) in 24(42.86%), 14(25.0%), and 09(16.07%) cases respectively. Among the fungal isolates, *Aspergillus fumigatus* was the leading agent detected in 10(30.30%) cases followed by *Fusarium spp, Mucor spp, Aspergillus flavus, Aspergillus niger, Rhizopus spp,* Unidentified branching fungus and *Alternaria spp.* found in 08(24.24%), 04(12.12%), 03(9.09%), 02(6.06%), 02(6.06%), 03(9.09%) and 01(3.03%) cases respectively. *Staphylococcus aureus* was the leading bacterial pathogen found in 10(43.47%) cases followed by *Pseudomonas spp., H. influenzae, Staph. epidermidis, Strept. pneumoniae* and *E. coli* detected in 05(21.73%), 03(13.04%), 02(8.69%), 02(8.69%) and 01(4.35%) cases respectively. Lomefloxacin, Tobramycin and Gentamicin were found to be better efficacious drugs against most of the bacterial pathogens noted in in-vitro susceptibility testing. This limited study has revealed and reinforced that suppurative corneal ulcers are caused by both bacterial and fungal agents with fungal preponderance in this geographical area and Lomefloxacin, Tobramycin and Gentamicin are better choice of antibiotics to treat bacterial suppurative corneal ulcers patients.

Key words: Suppurative corneal ulcer, Etiological agent, Antibiogram

Introduction

Corneal ulcer is one of the important ophthalmic conditions causing significant morbidity especially in the developing countries[1]. Scarring of the cornea developed as a result of suppurative corneal ulcer is the second commonest cause of preventable blindness after unoperated cataract among people in Asia, Africa and in the Middle East[2]. In Bangladesh, 33.55% of all cases of unilateral blindness were reported due to complication of corneal ulcer[3]. 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[4]. Bacteria and fungi are frequently responsible for suppurative corneal ulcers especially in the developing countries[5].

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[6]. While among the fungal causes of suppurative corneal ulcers, *Fusarium* and *Aspergillus* species are the predominant agents reported by many investigators[1,7].

✉ ***Correspondence:***
Dr. Md. Abdus Salam, Assistant Professor
Dept. of Microbiology, Rajshahi Medical College, Rajshahi-6000, Bangladesh
Tel. 0721 810983; 751234, 01916089071 (Cell); E-mail: salamrmc@yahoo.com

In a study conducted in Bangladesh, *Pseudomonas spp*. (24%), *Strept. pneumoniae* (17%), *Aspergillus spp*. (13%), *Fusarium spp*. (7%) and *Curvalaria spp*. (6%) were found as pathogens causing suppurative corneal ulcers[8]. Although the etiology of corneal ulcer varies widely in different geographical areas but 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[9]. 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[10]. 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. Like all other areas of bacterial infections, there is no alternative of antimicrobial susceptibility testing for corneal ulcer patients too, for proper selection of antibiotics to treat this condition successfully.

The purpose of the present study was to find out the bacterial and fungal agents causing suppurative corneal ulcers among patients of the Northern part of Bangladesh and to carry out antibacterial susceptibility testing for the bacterial isolates.

Methods

Patients: A total of 56 clinically diagnosed patients of suppurative corneal ulcers of different age and sex who attended the Ophthalmology out patient department (OPD) and also admitted in the Ophthalmology ward of Rajshahi Medical College Hospital (RMCH) during July, 2006 to June, 2007 were included in this study.

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 and sensitivity test: The swab was inoculated onto Blood agar, MacConkey's agar, and Chocolate agar media and incubated aerobically at 37^0C for maximum up to 48 hours. To ensure 5-10% CO_2, 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 [12,13] . All bacterial isolates were tested for their antimicrobial susceptibility by disc diffusion method against Chloramphenicol (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Lomefloxacin (10µg), Tobramycin (10µg) and Erythromycin (10µg). The results of susceptibility were recorded as *Sensitive* or *Resistant*.

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 25^0C 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[14]. 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.

Results

Culture of corneal swabs and scrapings taken from 56 corneal ulcer patients yielded pure fungal growth, pure bacterial growth and mixed microbial growth (both bacteria and fungi) in 24(42.86%), 14(25%) and 9(16.07%) cases respectively**. W**hile, no growth was observed in 9(16.07%) cases (Table-1). Omit **.** & small w

Microbial growth patterns in culture of corneal ulcer patients

Culture result	No. of case	Percentage
Pure fungal growth	24	42.86
Pure bacterial growth	14	25.00
Mixed microbial growth	9	16.07
No growth	9	16.07
Total	56	100.00

Fungal species isolated in both pure and mixed growths are shown in Table-2. Out of total 33 fungal isolates, *Apergillus fumigatus, Aspergillus flavus, Aspergillus niger, Fusarium,*

Mucor, Rhizopus, Unindentified Branching fungus and *Alternaria* were detected in 10(30.30%), 3(9.09%) 2(6.06%), 08(24.24%), 04(12.12%), 02(6.06%), 03(9.09%) and 01(3.03%) cases respectively.

Fungal species isolated from suppurative corneal ulcer patients

Fungal Species	Pure fungal growth	Mixed growth	Total
Aspergillus fumigatus	8	2	10(30.30)
Aspergillus flavus	2	1	03(09.09)
Aspergillus niger	2	0	02(06.06)
Fusarium	5	3	08(24.24)
Mucor	4	0	04(12.12)
Rhizopus	2	0	02(06.06)
Alternaria	0	1	01(03.03)
Branching fungus (Unidentified)	1	2	03(09.09)
Total	24	9	33(100)

Figures within parenthesis indicate percentage

Culture revealed 23 bacterial isolates (including both pure and mixed cultures) with *Staphylococcus aureus* as the leading bacterial pathogen representing 10(43.47%) followed by *Pseudomonas spp.* 05(21.73%), *H. influenzae* 03 (13.04%), *Staph. epidermidis* 02 (8.69%), *Strept. pneumoniae* 02 (8.69%) and *E. coli* 01 (4.35%) (Table -3).

Bacteria	Pure bacterial growth	Mixed with fungus	Total
Gram positive			
Staph. aureus	6	4	10 (43.47)
Staph. epidermidis	1	1	02 (08.69)
Strept. pneumoniae	*2*	*0*	02 (08.69)
Gram negative	3	2	
Pseudomonas spp	3	2	05 (21.73)
E. coli	1	0	01(04.35)
H. influenzae	1	2	03 (13.04)
Total	14	9	23(100)

Figures within parenthesis indicate percentage

In-vitro antimicrobial susceptibility patterns of the bacterial isolates from corneal ulcer patients revealed Tobramycin, Gentamicin and Lomefloxacin as better effective drugs against both leading gram-positive (*Staph. aureus*) and gram-negative (*Pseudomonas species)* bacteria. While, efficacy of Chloramphenicol, the frequently used ophthalmic antibiotic was found to be poor against most of the isolates except *Staph. epidermidis* and *Strept. pneumoniae* (Table-4).

Antimicrobial agents	Susceptibility pattern	*Staph. aureus* n=10 (43.47)	*Staph. epidermidis* n=2 (8.69)	*Strept. pneumoniae* n=2 (8.69)	*Pseudomonas spp.* n=5 (21.73)	*H. influenzae* n=3 (13.04)	*E. coli* n=1 (4.35)
Cloramphenicol	S	4 (40)	2(100)	2(100)	1(20)	1(33.33)	0
	R	6(60)	0	0	4(80)	2(66.66)	1(100)
Gentamicin	S	7(70)	1(50)	0	4(80)	2(66.66)	1(100)
	R	3(30)	1(50)	2(100)	1(20)	1(33.33)	0
Ciprofloxacin	S	5(50)	0	1(50)	1(20)	1(33.33)	1(100)
	R	5(50)	2(100)	1(50)	4(80)	2(66.66)	0
Lomefloxacin	S	6(60)	1(50)	2(100)	3(60)	1(100)	1(100)
	R	4(40)	1(50)	0	2(40)	0	0
Tobramycin	S	8(80)	1(50)	1(50)	4(80)	1(100)	2(66.66)
	R	2(20)	1(50)	1(50)	1(20)	0	1(33.33)
Erythromycin	S	2(20)	0	2(100)	0	0	0
	R	8(80)	2(100)	0	5(100)	1(100)	3(100)

Discussion

The present study focuses on to the pattern of bacterial and fungal pathogens causing suppurative corneal ulcers and the antibiogram of the bacterial isolates among patients attended in a teaching hospital in the Northern part of Bangladesh. A total of 56 samples obtained from corneal ulcer patients were analyzed of which 47 (83.93%) yielded growth of bacteria and fungi. Lone fungal and bacterial growths were detected in 24 (42.86%) and 14 (25%) cases respectively, while 09 (16.07%) cases revealed mixed fungal and bacterial growths. Taking the mixed growths into account, the total fungal and bacterial culture positive cases were 33 (58.93%) and 23 (41.07%) respectively. As far as the bacterial and fungal causative agents for suppurative corneal ulcers are concerned, similar figures were also noted by authors from India[15]. Further, fungi were identified as principal etiological agents of corneal ulcer, isolated from 58.93% cases in this study is consistent with the findings of researchers from different parts of the world [4, 15]. The frequency of distribution of fungal species detected in the present **study** is equally comparable with that of others[16, 17].

When compared to the number fungal isolates, less bacterial pathogens **were** detected in the present study. This result can be correlated with the fact that, 40 (71.42%) patients enrolled in this study have had introduction of antibiotics before samples were collected and as a consequence fewer bacteria were isolated. Regarding distribution of the bacteria causing suppurative corneal ulcers, similar pattern was reported by other investigators[8, 18].

Antimicrobial susceptibility pattern of the bacterial isolates carried out in this study revealed that Lomefloxacin, Tobramycin and Gentamicin were better effective drugs against most of the gram-positive and gram-negative bacteria. Chloramphenicol, the frequently used ophthalmic

antibiotic was found to be less effective against most of bacterial isolates except *Streptococcus pneumoniae* and *Staph. epidermidis*. This poor performance of Chloramphenicol may be due to its inappropriate and over ensuthiastic use seen in common practice leading to drug resistance. Similar pattern of antibacterial susceptibility among corneal ulcer patients was reported by other authors[19].

The present study was an attempt to explore the base line information about the major microbial etiological agents causing suppurative corneal ulcers among patients attending Rajshahi Medical College Hospital. The facts and figures that have been revealed are quite consistent with similar studies done at home and the neighboring countries. It indicates that microbial etiology of corneal ulcer has a particular geographical distribution with many predisposing factors that may contribute to it. Information about etiological agents and antibiogram that have been gathered in this study can help ophthalmologists for empirical antimicrobial therapy and to take strategies for proper management of cases, specially where laboratory facilities are lacking.

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