Original articles

Epidemiology and laboratory diagnosis of fungal corneal ulcer in the Sundarban Region of West Bengal, eastern India

Bandyopadhyay S[1], Das D[1], Mondal KK[1], Ghanta AK[2], Purkait SK[2], Bhaskar R[1]
[1]Departments of Ophthalmology and Microbiology, RG Kar Medical College and Hospital, Khudiram Bose Sarani, [2]N.R.S. Medical College & Hospital, Kolkata -700 004, India

Abstract

Introduction: Corneal ulcers are the second most-common cause of preventable blindness after cataract in tropical developing countries. Fungal corneal ulcers constitute 30 to 62 % of the total microbial culture-positive corneal ulcers.

Objective: To study the epidemiological characteristics, risk factors and laboratory diagnosis of fungal corneal ulcer in the Sundarban Region, West Bengal, eastern India.

Materials and methods: A retrospective review of 399 culture-positive, fungal corneal ulcers out of a total 928 corneal ulcer patients attending a tertiary care hospital in Kolkata, eastern India, over a period of four years from February 2007 to January 2011.

Results: Males (246; 61.65 %) were more commonly affected than females (P < .0001). The affected people were mostly (342; 85.71 %) residing in the rural areas (P < .0001). 196 patients (49.12 %) were involved in agricultural activities (P < .0001). The younger people of, 21 - 50 years of age, were particularly prone to this disease (269; 67.41 %). Corneal trauma (354; 88.72 %) was the commonest risk factor (P < .0001) and 261 patients (61.41 %) had a history of trauma with vegetative matter (P< .0001). The use of topical corticosteroids was implicated in 65 (16.29 %) cases. The incidence of the disease was highest in the monsoon season, between June to September (192; 48.12 %). The aspergillus spp was the most common fungal growth (151; 37.84 %), followed by an Fusarium spp (81; 20.3 %).

Conclusion: The fungal corneal ulcers are an important cause of ocular morbidity in people residing in the Sundarban Region. 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.

Key-words: Fungal corneal ulcer, epidemiology, culture

Introduction

Corneal ulcerations constitute the second most common cause of preventable blindness after cataract in tropical developing countries (Upadhyay et al, 1991, Whitcher et al, 2001, Gilbert et al, 1995). Fungal corneal infection represents 30 % to 62 % of culture positive infectious keratitis in different Indian studies (Srinibasan et al, 1997, Gopinathan et al 2002, Bharathi et al, 2003, Basak 2005, et al) and at least 70 genera of fungi have been isolated from corneal specimen (Agarwal et al, 2001).The filamentous fungi, Aspergillus and Fusarium contribute up to 70 % of the cultures (Agarwal et al, 1994). The determination of regional etiology is important

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Address for correspondence: Dr Sabyasachi Bandyopadhyay, MS (OPH) 11/11, Ghosal para Road, Dakshin para, Barasat. Kolkata-700124
Mobile:+91-9434189563.
E-mail: sabyasachi.bandyopadhyay@yahoo.com

as the causative fungi differ from region to region and within the same region over time. Various studies have been undertaken to highlight this issue in South India (Srinibasan et al, 1997; Leck et al, 2007; Bharathi et al, 2002) but only two study reports were found in West Bengal, eastern India (Basak et al, 2005; Das, 1972). However, some epidemiological studies of fungal keratitis in neighboring states of Assam (Dutta et al, 1981) and countries like Nepal (Upadhyay et al, 1991) and Bangladesh are available (Dunlop et al, 1994). Our tertiary eye care center caters patients from six districts of gangetic West Bengal, eastern India including Sundarban region of north and south 24 pargana districts. The Sundarban region is the largest single block of tidal mangrove forest in the world and extends from the north and south 24 pargana districts of West Bengal to the coastal areas of Bangladesh. This region is a part of the large gangetic delta of West Bengal and Bangladesh. As there were only two epidemiological study reports (Basak et al, 2005; Das, 1972) of fungal corneal ulcer in this region were available, we intended to study the epidemiological characteristics and laboratory diagnosis of the disease from Sundarban area which will help in the treatment and prevention of the fungal corneal ulcer in this region.

Materials and methods

Patients

Our tertiary eye care center mainly caters patients from north and south 24parganas and neighboring four other districts of Gangetic West Bengal. Among them, 923 patients with corneal ulcer coming from Sundarban region of north and south 24parganas over a period from February 2007 to January 2011 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 (Srinibasan et al, 1997).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 (Srinibasan et al, 1997; Bharathi et al, 2003).

Corneal scrapings were taken after instillation of 4 % preservative free lignocaine by an ophthalmologist using no.15 Bard-Parker blade with strict asepsis under slit-lamp biomicroscope (Srinibasan et al 1997; Sharma & Athmanathan, 2002). The material collected from the leading edge and base of the ulcer was inoculated into solid/liquid culture media and two smears made onto two slides. One slide was stained with Gram stain and the other with 10 % KOH preparation for direct microscopic examination (Srinibasan et al, 1997; Sharma & Athmanathan, 2002).

Laboratory procedure

Aerobical incubations were done for all inoculated media (Srinibasan et al, 1997). The inoculated

Sabouraud's dextrose agar media were incubated at 27^0 C and after daily examination they were discarded after 3 weeks, if there was no growth. The blood agar, chocolate agar, thioglycolate broth and brain-heart infusion broth after inoculation were incubated at 37^0 C, examined daily and if no growth was seen in 7 days were discarded. When KOH wet mount preparations were positive for amoebic cysts, scrapings were inoculated in non-nutrient agar media overlaid with Escherichia coli. If, there was no growth in 3 weeks they were discarded. All laboratory methods were performed following standard protocols (Srinibasan et al, 1997; Sharma & Athmanathan, 2002). Microbial cultures were considered positive if there was growth of same organism in more than one solid phase media, and/or confluent growth at the site of inoculation on one solid medium and/or growth in one medium consistent with direct microscopic observations as in KOH preparation or Gram stain (Srinibasan et al, 1997; Sharma & Athmanathan, 2002).

Results

A total of 928 patients with corneal ulcer attending a tertiary care hospital in Kolkata from Sundarban region over a period of four years from February 2007 to January 2011 were studied. Of the total, 399 (43 %) patients had fungal growth among which 363 (39.12 %) were pure fungal and 36 (3.88 %) were mixed fungal and bacterial growth (Table 1). Pure bacterial isolates were found in 272 (29.31 %) patients. Six (0.65 %) cultures were positive for Acanthamoeba and 251 (27.04 %) cases were culture negative.

Detection of fungal elements in KOH smear had 98.5 % sensitivity and 98.3 % specificity. Gram stained smear was 86.47 % sensitive and 99.62 % specific in diagnosing fungal ulcers. Clinical diagnosis by slit lamp bio-microscopy had 92.98 % sensitivity and 91.3 % specificity (Table 2).

On culture, there was single fungal species in all the 399 fungus positive isolates. The non-pigmented filamentous hyaline fungi (mostly Aspergillus and Fusarium) were isolated in 292 cases. 95 cases showed pigmented filamentous growth of dematicious fungi (mainly Curvularia, Bipolaris and Botryodiplodia). Candida was diagnosed in 12 cases. In our study, Aspergillus spp. were most common fungal growth (151 cases; 37.84 %) followed by Fusarium spp (81 cases; 20.3 %) (Table 3).

Males (246; 61.65 %) were more affected than females. 269 patients (67.41 %) were among 21-50 years of age group. Majority of the patients were residing at the rural area (342; 85.71 %) with agricultural work (196; 49.12 %) as their commonest occupation (Table 4). Corneal trauma was identified as the commonest predisposing cause (354; 88.72 %) and use of topical corticosteroids were found in 65 cases (16.29 %). The vegetative matter inflicted corneal trauma constituted 65.41 % (261 cases) of the total fungal ulcers. Paddy/hay, jute plants, other tree branches and broom sticks were common vegetative matters causing corneal injury. Children less than 11 years of age were most vulnerable to broom stick injuries (7 out of total 8 cases).

84 patients (21 %) reported within first one week, 82 patients (20.55 %) in second week, 105 patients (26.32 %) in third week, 73 patients (18.3 %) in fourth week and 55 patients (13.78 %) came after fourth week of their illness.

Table 1
Causative micro-organisms in cultures from 928 corneal ulcers in Sundarban, eastern India

Type of micro-organism	Number with (%)
Pure fungal culture	363(39.12 %)
Pure bacterial culture	272(29.31 %)
Acanthamoeba	6(0.65 %)
Mixed fungal and bacterial	36(3.88 %)
Patient with negative cultures	251(27.04 %)
Total fungal growth	**399(43 %)**
Patient with positive cultures	677(72.96 %)
Total number of corneal ulcer from Sundarban region	**928(100 %)**

Table 2

Correlation between 10 % KOH smear diagnosis, Gram-stained smear diagnosis, clinical diagnosis and positive fungal culture diagnosis from 928 corneal ulcers in Sundarban, eastern India

Sl. No.	Name of the Investigation	Results	Number	Presence of fungal growth in culture		Sensitivity (%)	Specificity (%)
				Positive	*Negative*		
1	Detection of fungal filaments in KOH smear	Positive	402	393 (357* +36**)	9	98.5 %	98.3 %
		Negative	526	6*	520		
	Total		928	399	529		
2	Detection of fungal filaments in Gram stained smear	Positive	347	345 309* +36**	2	86.47 %	99.62 %
		Negative	581	54*	527		
	Total		928	399	529		
3	Clinical diagnosis of fungal keratitis on Slit Lamp examination	Positive	417	371 335* +36**	46	92.98 %	91.30 %
		Negative	511	28*	483		
	Total		928	399	529		

* Pure fungal growth
**Mixed with bacteria

Table 3

Identification of fungal pathogens isolated from 399 fungal culture positive cases of corneal ulcers from Sundarban region treated at a tertiary eye care center in eastern India

Fungal isolates	Pure isolates	Mixed with bacteria	Total (%)
A)Hyaline fungi	263	29	292 (73.18 %)
i)Aspergillus spp.	133	18	151(37.84 %)
Aspergillus flavus	62	10	72 (18.05 %)
Aspergillus fumigatus	53	5	58 (14.54 %)
Aspergillus niger	18	3	21 (5.26 %)
ii)Fusarium spp	74	7	81 (20.3 %)
iii)Penicillium spp	11	1	12 (3 %)
iv)Unidentified hyaline fungi	45	3	48 (12.03 %)
B) Candida spp	11	1	12 (3 %)
C) Dematicious fungi			95 (23.81 %)
i) Curvularia spp	21	3	24 (6 %)
ii) Bipolaris spp	17	-	17(4.26 %)
iii) Botryodiplodia spp	26	2	28 (7 %)
iv) Unidentified dematicious fungi	24	2	26 (6.51 %)
Total no of isolates	363	36	399 (100 %)

Table 4
Demographic characteristics of 399 cases of culture positive fungal keratitis from Sundarban region treated at a tertiary eye care center in eastern India

Demographics	Particulars	No (%)
Sex	Male	246(61.65 %)
	Female	153(38.35 %)
Age (years)	< 11 years	13(3.26 %)
	11-20 years	58(14.54 %)
	21-30 years	72(18.04 %)
	31-40 years	105(26.31 %)
	41-50 years	92(23.06 %)
	>50 years	59(14.79 %)
Residence	Rural	342(85.71 %)
	Urban	57(14.29 %)
Occupation	Agricultural workers	196(49.12 %)
	Labourer	89(22.31 %)
	Household	38(9.52 %)
	Business/Professionals	32(8.02 %)
	Students/Children	19(4.76 %)
	Service	18(4.51 %)
	Unemployed/Unknown	7(1.76 %)
Predisposing factors	A)Corneal trauma & traumatic agents:	354(88.72 %)
	i)Vegetative matter-	261(65.41 %)
	Paddy/hay	135(33.83 %)
	Jute plant	59(14.79 %)
	Tree branch	42(10.53 %)
	Broom sticks	8(2.01 %)
	Other vegetative matter	17(4.26 %)
	ii)Dirt/mud/sand/stone	51(12.78 %)
	iii) Finger nail	7(1.76 %)
	iv) Insects	12(3.01 %)
	v) Cow tail	9(2.26 %)
	vi) Miscellaneous items	14(3.51 %)
	B)Coexisting ocular diseases:	76(19.05 %)
	i)Chronic dacryocystitis	22(5.51 %)
	ii)Lid abnormalities(trichiasis/entropion)	13(3.26 %)
	iii)Lagophthalmos	6(1.5 %)
	iv)Dry eye	12(3.01 %)
	v)Bullous Keratopathy	6(1.5 %)
	vi)Spheroidal degeneration	3(0.75 %)
	vii)Post operative	9(2.26 %)
	viii)Post viral corneal anesthesia	5(1.25 %)
	C)Use of topical steroids	65(16.29 %)
	D)Systemic diseases	51(12.75 %)
	i)Diabetes Mellitus	47(11.78 %)
	ii)Tuberculosis	4(1 %)
Time of first presentation	1-3 days	23(5.76 %)
	4-7 days	61(15.29 %)
	8-14 days	82(20.55 %)
	15-21 days	105(26.32 %)
	22-28 days	73(18.30 %)
	After 28 days	55(13.78 %)
Seasonal variation	Feb-May (summer)	78(19.55 %)
	June-Sep (monsoon)	192(48.12 %)
	Oct-Jan (winter)	129(32.33 %)

The disease was most prevalent during the monsoon (192; 48.12 %) followed by winter (129; 32.33 %) and was least common in summer (78; 19.55 %). On slit-lamp bio-microscopy among 399 fungal positive cases the clinical pictures observed were: thick, and dry with raised corneal surface in 311 cases (77.94 %), stromal infiltration with feathery margins in 287 cases (71.93 %), dendritic patterns in 21 cases (5.26 %), immune ring in 7 cases (1.75 %), satellite lesions in 42 patients (10.53 %), deep stromal infiltration in 153 cases (38.35 %), hypopyon in 228 cases (57.14 %), corneal abscess in 8 cases (2 %) and corneal perforation in 13 patients (3.26 %).

Discussion

The pathogenic fungi infecting cornea can be yeasts or moulds. The yeasts are oval or round unicellular fungi having blastoconidium. Candida species represents the yeasts in corneal pathology. The moulds have filamentous structure (hyphae) which entangles to make mycelium. Filamentous fungi may be septate or non-septate, pigmented or non-pigmented (hyaline) according to the presence or absence of pigmentation on the reverse of the culture plate. In

culture they are in the asexual phase of the life cycle.

In our study, 677 (72.96 %) of total 928 corneal ulcers were culture positive. This figure corroborates with previous studies in West Bengal (Basak et al 2005, 67.7 %), south India (Srinibasan et al 1997, 68.4 % and Bharathi et al 2003, 70.6 %) and Ghana (Leck et al 2002,57.3 %). Fungal cultures were positive in 399 (43 %) cases, which is comparable to several studies from South India {Srinibasan, 1997 (51.9 %); Bharathi, 2003 (34.4 %) and Leck, 2002(44.1 %)}. But, the previous study in Gangetic West Bengal by Basak et al (2005) reported slightly higher results (59.3 %). Similar studies in other tropical countries like Bangladesh (Williams et al, 1991; Dunlop et al, 1994) and Nepal (Upadhyay et al 1991) reported the incidence from 17 % to 40 %.

The most common fungal isolate in our study was Aspergillus spp (151; 37.84 %) followed by Fusarium spp. (81; 20.3 %) which together constituted 58.14 % (232) of cases. Aspergillus was the predominant isolate in West Bengal (Basak et al 2005, 59.8 %), Mumbai, parts of South India, Nepal and Bangladesh (Upadhyay et al, 1991; Williams et al, 1987; Despande & Koppikar, 1999; Venugopal et al, 1989.). But, Fusarium spp was found to be more common in other studies at South India {Bharathi et al, 2003 (42.82 %); Leck et al, 2002 (39.9 %); Srinibasan et al, 1997(47.1 %)}.The Fusarium spp preponderance was also found in Paraguay, Florida, Hong Kong and Singapore (Mino de Kasper et al, 1991; Liesegang & Forstor, 1980; Houang et al, 2001; Wong et al, 1997). These differences in predominance of fungal isolates could be attributed to different climatic conditions.

Similar to previous studies, males, particularly residing in rural areas and involved in agricultural works were most commonly affected. Most patients belonged to 21-50 years of age group (67.41 %). Corneal injury by vegetative matters (65.41 %) was the most common offending factor. Paddy/hay, jute, tree branches and broom sticks were the main vegetative matters causing corneal injuries. Children below 11 years of age group were particularly prone to broom stick injury with poor prognosis. Indiscriminate use of corticosteroid as over the counter drug also predisposed to fungal keratitis (65; 16.29 %). Prior to definitive fungal culture report, KOH wet mount preparation was a very sensitive method of fungal ulcer diagnosis (sensitivity of 98.5 % as opposed to Gram stained smear of 86.47 %). This result corroborates with study by Bharathi et al (2003). The slit-lamp bio-microscopy was very helpful to diagnose the fungal ulcer clinically. This is particularly important where laboratory facilities are scanty. The typical fungal ulcer was characterized by dry, thick lesion raised from the surface (311; 77.94 %), stromal infiltration with feathery margins (287; 71.93 %), satellite lesions (42;10.53 %), immune ring (7; 1.75 %) and Hypopyon (228; 57.14 %). Some patients showed dendritic patterns (21; 51.26 %) causing diagnostic dilemma with herpetic and acanthamoeba keratitis.

The incidence of fungal keratitis is quite common during the monsoon time corresponding to paddy harvesting in Sundarban region. Next to monsoon the incidence is more common in winter than summer. Winter season also corroborates with high agricultural activity. In this regard our study tallies with Hyderabad studies (Bharathi et al, 2003). A windy climate, dry or humid, associated with high agriculture based population favors fungal ulceration in tropics. Only 21 % of the culture positive patients came within 1st week of their illness. Thee majority (26.32 %) came in the 3rd week and 13.78 % of the patients attended after 4th week, in contrast to Madurai studies (Srinibasan et al 1997) where 60 % of the patients attended within 1st week of their illness. Transportation in the Sundarban region was not very good and the region was divided by numerous rivers. That could account for their late arrivals at the tertiary care eye hospital at Kolkata.

Conclusion

Fungal corneal ulcers are important cause of ocular morbidity of people residing in Sundarban region of West Bengal as in other parts of the tropics. Aspergillus spp is the most common fungal pathogen. The farmers are at particular risk. The healthcare awareness program among the villagers and improvement of transportation facilities can help prevent and treat the disease thereby significantly reducing the ocular morbidity due to corneal ulcer.

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