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Original Research Article

Infectious Keratitis A Prospective Study

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Abstract

Aim: The aim of the present study was to isolate causative pathogen in infectious keratitis.

Methods: This study was conducted in Department of Ophthalmology and Department of Microbiology, Sir Sunderlal Hospital, Institute of Medical Science, Banaras Hindu University, Varanasi. This study was conducted from May 2019 to September 2020.

Results: Amongst 30 patients, the age group most commonly affected, was found to be between 31 – 40 years i.e. 6 (20.00%) in our study, 41-50 years i.e. 8 (26.70%), 21-30 years i.e. 4 (13.30%), followed by >50-year patients i.e. 11 (36.70 %). The least commonly involved age group be in less than or equal to 20 i.e. 1 (3.30%). Gender wise analysis of corneal ulcer cases in our study showed 66.60% cases were Male and 33.30 % cases were Female. Our study showed a more common predisposition of corneal ulcer in right eye 20 cases (66.70%) as compared to left eye 10 cases (33.30%). In our study, 9 cases (30%) were known cases of diabetes mellitus and rest were nondiabetic. Out of 30 patients enrolled in our study, 8 (26.70%) had a history of instillation of corticosteroid drops at least once during course of disease and rest 22 cases (73.30%) had not used steroid drops. Amongst the included cases, 17 (56.70%) had already used antibiotic drops before presenting to our OPD, while 13 (43.30%) did not receive any form of treatment. Hypopyon at the time of presentation in OPD was present in 36.70% (11 cases) of cases and rest 63.30% (19 cases) were hypopyon free. In our study, 12 (85.70%) cases were gram stain positive while rest were gram stain negative 2 (14.30%).

Conclusion: Thus, the present study concludes that Aspergillus species are the most common cause for fungal keratitis in our region, with A. fumigatus and A. flavus being the prevalent species, followed by Fusarium. Bacterial isolates are responsible for considerably fewer cases of keratitis. Routine surveillance of fungal keratitis is necessary to know the existing and emerging pattern of pathogens. Trauma with vegetative matter was identified as the most common risk factor.

Keywords: isolate causative pathogen, infectious keratitis

Introduction

Infectious keratitis is a major cause of corneal blindness in both developed and developing countries.[1] The incidence has been estimated at 2.5–799 cases per 100,000 population/year.[1-4] Subject to geographical, temporal and seasonal variations, bacteria, and fungi are the most commonly implicated organisms in infectious keratitis.[4-8] The variations are mainly attributed to the difference in the climate of the studied region and the population-based risk factors, particularly contact lens wear, trauma, and agricultural activities.

Bacterial keratitis (BK) has been consistently shown to be the main causative organisms in the UK and other developed countries. Based on the recent literature, BK represents 90–93% and 72–86% of all culture-proven infectious keratitis cases in the UK and in North America, respectively. [4,9-13] Corneal ulcer is a sight threatening ocular condition that is the leading cause of monocular blindness. It is be caused by various pathogens i.e., bacteria, fungi, virus and parasites. Fungi and bacteria are the main causes of unilateral corneal scar. Suppurative Keratitis is the second most common cause of monocular blindness after un operated cataract in some developing countries in the tropics. [14-17] The reported incidence of corneal ulceration in India is 1130 per million population per year. The prevalence of blindness directly related from

complications of suppurative keratitis is estimated to be 5%; the associated ocular morbidity is the result of several factors and patients' management is directly affected by the lack of diagnostic facilities and initiation of appropriate antimicrobial therapy. Specific treatment requires quick and accurate identification of the causative microorganisms.

Minor injury to the eye is a critical predisposing factor. Indiscriminate use of steroids and antibiotics, use of contact lenses, immuno-compromised state and ocular surgery are other causes of increasing incidence of corneal infection.[18] Agricultural workers and laborers are the biggest occupational group as the highest incidence coinciding with the period of maximal activity. In India, a favourable tropical environment, coupled with a primarily agricultural population having a constant risk of plant exposure and low-income status are the predisposing factors for fungal infections. Aspergillus is one of the most common fungi in many parts of India, others being Fusarium, Penicillium, Candida, etc. Early diagnosis of fungal etiology is challenging due to mimicking symptoms with bacterial etiology in early stages. At the same time, culture for fungal pathogens takes a long time. So, it is necessary to comprehend the microbiological and clinical characteristics to start proper treatment. Knowledge of regional epidemiology and risk factors are essential to guide appropriate anti-fungal therapy.

The aim of the present study was to isolate causative pathogen in infectious keratitis.

Materials and Methods

This study was conducted in Department of Ophthalmology and Department of Microbiology, Sir Sunderlal Hospital, Institute of Medical Science, Banaras Hindu University, Varanasi. This study was conducted from May 2019 to September 2020.

Inclusion Criteria

Patients coming in Department of Ophthalmology OPD of Sir Sunderlal Hospital, BHU, Varanasi with complain of blurring of vision, watering, photophobia, redness which is presumed to be microbial keratitis were included in the study either associated with ocular injury, foreign body, contact lens users or installation of corticosteroid drugs.

Exclusion Criteria

- Viral ulcers
- Non healing ulcer
- Mooren ulcer
- Marginal keratitis
- Descemetocele
- Interstitial keratitis
- Neurotrophic keratitis
- Any ulcer associated with autoimmune disease.
- Ulcer with impending or actual perforation.

Selection of Patients

Patients with presumed infective microbial keratitis presenting to Ophthalmology OPD of Sir Sunderlal Hospital, B.H.U., Varanasi, India from May 2019 to September 2020 were enrolled in this prospective study. Sir Sunderlal Hospital, Varanasi is a tertiary centre where patients come from eastern Uttar Pradesh and Bihar. Total 30 cases of suspected fungal and bacterial corneal patients were included in this prospective study. Data related to socioeconomic features, patient's age, sex, residence, occupation, duration, use of any drugs treatment either allopathic or traditional eye medicine. A detailed history regarding predisposing factor like history of injury to the affected eye, nature of traumatizing agent, use of contact lenses, previous eyeor corneal disease, any surgery of the affected eye, with eye disorders like dryeye, use of corticosteroid and associated systemic diseases were asked. Apart from clinical history, ulcer characteristics were noted.

Sampling

Suspected infective keratitis were stained with fluorescent paper strips and examined under cobalt blue light. Ulcer gives green, fluorescent 2% paracaine (proparacaine) was instilled to desensitize the affected eye. Corneal scraping was done with a sterile Bard-Parker blade (15 No.) by using slit lamp biomicroscope. Material obtained from scraping of the edge and bases were directly inoculated on Sabouraud's Dextrose Agar and Blood Agar. Sample were also taken on slide for 10% KOH wet mount and Gram's stains.

Corneal scraps were directly inoculated in culture media in ―C‖ shaped or at one point (marked).Meticulous care was taken during corneal scraping and aseptic transfer of scrap to the culture Inoculated SDA and Blood agar with labelled slides with corneal scrap were transported to the mycology laboratory, where SDA and Blood agar was incubated at 25°C and 37°C respectively in BOD (Biochemical oxygen demand) incubator.

Processing of Slides

KOH Examination

2-3 drops of 10% KOH with 10% glycerol were placed over corneal scrap on a labelled slide and covered with a cover slip. Slide was kept for 5-10 minute to dissolve corneal material and tissue debris and examined initially at low power (10x) then at high power (40x). KOH solution serves to weak the corneal lamellar structure and epithelial cell outlines, rendering the background almost homogenous.

Fungal filaments appear retractile hyphae with characteristic features like septate or aseptate, branched or unbranched, hyaline or Phaeoid. Hyaline fungiare colourless whereas Phacoid fungi are brown black in colour due to presence of melanin in their cell wall.

Gram Stain

Second slide was processed for Gram's stain. Scrap material was heat fixed by passing it above the flame 3 times to retain the morphology of microorganism. Then smear was flooded by primary stain, crystal violet (1%) for 1 minute, followed by Mordant, Gram's iodine for 1 minute. Smear was decolorized with 95% alcohol and then counter staining was done with safranin (0.5%). Slide were allowed to dry. Later on, slide was examined under oil immersion lens at 100x. Filamentous fungi have a varying staining response. Most commonly fungal hyphae are gram positive but may be gram negative. However, this method is most commonly used to identify any concomitant bacterial infection. Yeast is gram- positive, round, or oval shaped structure. Yeast usually shows the budding.

Culture Methods

SDA and Blood agar media was used. SDA agar supports the growth of all fungal pathogens including yeast and fungi. Petri-dish containing media were commonly used for the culture of pathogens. Sample was inoculated on blood agar to see the presence of concomitant bacterial infection. Yeast and fungi also grow on Blood agar.

Incubation

Blood agar was incubated at 37°C and SDA were incubated at 25°C in BOD incubator. Petridishes with solid media were kept in such way that lid of the Petri dish was below and solid media above, to prevent the condensation of moisture onto the media. Most of the fungi grew within 1st week of incubation. Culture was declared negative if there is no growth after 28 days of continuous incubation.

Observation

Only growth on the inoculated ―C‖ region on solid media was considered significant. Plates were examined daily in 1st week then every 3rd day for next 3 weeks. Plated were observed in both obverse and reverse view. Subculture was done on SDA from primary growth.

Results

Table 1: Baseline characteristics

AGE	N=30	Percentage
< 20 years	1	3.33%
21-30 years	4	13.3%
31-40 years	6	20.0%
41-50 years	8	26.7%
>50 years	11	36.7%
SEX		
Male	20	66.6%
Female	10	33.3%
Domicile		
Urban	7	23.3%
Rural	23	76.7%
Occupation		
Unemployed	1	3.3%
Students	3	10.0%
Farmer	12	40.0%
Housewife/domestic	8	26.7%
Semiskilled Worker	5	16.7%
Professional	1	3.3%
Eye		
Right	20	66.7%
Left	10	33.3%

Amongst 30 patients, the age group most commonly affected, was found to be between 31 – 40 years i.e. 6 (20.00%) in our study, 41-50 years i.e. 8 (26.70%), 21-30 years i.e. 4 (13.30%), followed by >50-year patients i.e. 11 (36.70 %). The least commonly involved age group be in less than or equal to 20 i.e., 1 (3.30%). Gender wise analysis of corneal ulcer cases in our study showed 66.60% cases were Male and 33.30 % cases were Female, with male to female ratio being 2:1. Domicile wise analysis of corneal ulcer cases in our study showed 76.70% cases were Rural and 23.30% cases were Urban. Farmers constituted 40% (12) of the cases, followedby Housewife/domestic 8 (26.70%), Semiskilled worker 5 cases (16.70%), Student 3 case (10%) and Unemployed 1 (3.30%), Professional 1 (3.30%). Our study showed a more common predisposition of corneal ulcer in right eye 20 cases (66.70%) as compared to left eye 10 cases (33.30%).

Table 2: Causative Agents in Trauma, history of diabetes mellitus and History of Steroid installation

Causative Agents	N=25	Percentage
Vegetative matter	8	32%
Mud/soil dust	9	36%
Animal matter	5	20%
Metallic/ Foreign body	3	15%
Miscellaneous	0	0%
History of Diabetes mellitus		
Present	9	30%
Absent	21	70%
History of Steroid installation		
Used	8	26.7%
Not used	22	73.3%

About 25 cases had history of trauma to eye in any form. The most common agent being vegetative matter, in 8 cases (32.00%) followed by mud, dust and soil in 9 cases (36.00%), animal matter in 5 cases (20.00%) and metallic foreign body in 3 cases each (15.00%), and rest by miscellaneous object. In our study, 9 cases (30%) were known cases of diabetes mellitus and rest were nondiabetic. Out of 30 patients enrolled in our study, 8 (26.70%) had a history of instillation of corticosteroid drops at least once during course of disease and rest 22 cases (73.30%) had not used steroid drops.

Table 3: History of antibiotics, hypopyon, gram stain

History of antibiotics	N=30	Percentage
Used	17	56.7%
Not used	13	43.3%
Hypopyon		
Present	11	36.7%
Absent	19	63.3%
Gram Stain		
Positive	12	85.7%
Negative	2	14.3%
Blood Agar n=14		
Staph aureus	12	85.7%
Bacteroid	1	7.15%
Acinetobacter	1	7.15%
Miscellaneous	0	0%
KOH Stain n=18		
Positive	15	83.3%
Negative	3	16.7%

Amongst the included cases, 17 (56.70%) had already used antibiotic drops before presenting to our OPD, while 13 (43.30%) did not receive any form of treatment. Hypopyon at the time of presentation in OPD was present in 36.70% (11 cases) of cases and rest 63.30% (19 cases) were hypopyon free. In our study, 12 (85.70%) cases were gram stain positive while rest were gram stain negative 2 (14.30%). In our study 12 (85.70%) cases were staph aureus and followed by Bacteroid 1 (7.15%) and Acinetobacter 1 (7.15%) and rest of the miscellaneous. In our study 15 (83.30%) cases were KOH stain positive and rest were KOH negative.

Table 4: Age wise prevalence of Causative Agents

Age	Vegetative matter		Mud/soil/Dust		Animalmatter		Metallic Foreign body		*P*	*X2*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*		
<20 years	0	0	0	0	0	0	1	100		
21-30 years	0	0	0	0	2	50	2	50		
31-40 years	3	33.3%	5	55.6	1	11.1	0	0	38.456	0.001
41-50 years	5	55.6	3	33.	1	11.1	0	0		
>50 years	8	80	1	10	1	10	0	0		

In our study Total 25 participants had ocular injuries. In younger than 30 years causative agents are metallic foreign body and animal matter and older than 30 years causative agents are more Vegetative matter and dust/mud/ soil.

Table 5: Age wise prevalence of Bacterial infection

Age	Staphaureus		Bacteroid		Acinetobacter		Miscellaneous		*P*	*X2*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*		
<20 years	0	0	0	0	0	0	0	0	38.456	0.001
21-30 years	1	100	0	0	o	0	0	0		
31-40 years	2	66.6	0	0	1	33.3	0	0		
41-50 years	3	75	1	25	o	0	0	0		
>50 years	6	100	0	0	0	0	0	0		

In our study Total 14 participants had ocular Bacterial infection and in all age group maximum infection are caused by Staph aureus.

Table 6: Age wise prevalence of Fungal infection

Age	Aspergillus		Fusarium		Candida		Miscellaneous		*P*	*X2*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*		
<20 years	0	0	0	0	0	0	0	0	16.032	0.451
21-30 years	1	100	0	0	o	0	0	0		
31-40 years	3	100	0	0	0	0	0	0		
41-50 years	2	66.7	1	33.3	o	0	0	0		
>50 years	6	75	0	0	2	25	0	0		

In our study Total 15 participants had ocular Fungal infection and occurred in patients older than 20 years. In all age group maximum infection are caused by Aspergillus.

Discussion

Corneal opacification due to keratitis is a major cause of blindness and visual disability, in fact, second only to cataract in developing countries like Asia, Africa and the Middle East. A breach in common defense mechanism like lids, tear film and corneal epithelium leads to corneal invasion due to any microorganisms.[19] Fungal keratitis represents approximately 6% to 53% of all cases of culture-positive infectious keratitis.[20] Reports from different parts of the world suggest a paradigm shift, with an increasing incidence of fungal keratitis during the last four decades, possibly due to increased awareness and availability of fungal culture methods and identification. Mycotic keratitis is often associated with unfavourable outcomes due to the slower onset, long course, and the diversity of clinical presentations, presenting the greatest challenge to the ophthalmologists.

Amongst 30 patients, the age group most commonly affected, was found to be between 31 – 40 years i.e. 6 (20.00%) in our study, 41-50 years i.e. 8 (26.70%), 21-30 years i.e. 4 (13.30%), followed by >50-year patients i.e. 11 (36.70 %). The least commonly involved age group be in less than or equal to 20 i.e., 1 (3.30%). Gender wise analysis of corneal ulcer cases in our study showed 66.60% cases were Male and 33.30 % cases were Female. In our study, males outnumbered the females with a ratio of 2:1, perhaps due to greater involvement in outdoor work, with more chances of ocular injury. This has also been observed by several other authors. [18,21-23]

Domicile wise analysis of corneal ulcer cases in our study showed 76.70% cases were Rural and 23.30% cases were Urban. Farmers constituted 40% (12) of the cases, followed by Housewife/domestic 8 (26.70%), Semiskilled worker 5 cases (16.70%), Student 3 case (10%) and Unemployed 1 (3.30%), Professional 1 (3.30%). Our study showed a more common predisposition of corneal ulcer in right eye 20 cases (66.70%) as compared to left eye 10 cases (33.30%). History of trauma, especially with vegetative matter, maybe an essential risk factor for mycotic keratitis as shown by other authors. [24-26]

This is further emphasized by our study, as more than half of the patients with keratitis and more than two-thirds of the fungal keratitis patients had history of prior trauma with either vegetative matter or non-vegetative matter. Although, ocular trauma is the main culprit in developing countries, several studies suggest that contact lens users have more prone to develop infections in developed countries. [27,28]

Amongst the included cases, 17 (56.70%) had already used antibiotic drops before presenting to our OPD, while 13 (43.30%) did not receive any form of treatment. Hypopyon at the time of presentation in OPD was present in 36.70% (11 cases) of cases and rest 63.30% (19 cases) were hypopyon free. In our study, 12 (85.70%) cases were gram stain positive while rest were gram stain negative 2 (14.30%). In our study 12 (85.70%) cases were staph aureus and followed by Bacteroid 1 (7.15%) and Acinetobacter 1 (7.15%) and rest of the miscellaneous. In our study 15 (83.30%) cases were KOH stain positive and rest were KOH negative. In KOH wet mount preparation, hyaline, septate and branching hyphal filaments were commonly observed, while phaeoid, septate hyphae were relatively less frequent. Fruiting bodies were not seen in any direct KOH mount; except in one sample in which Aspergillus type conidiation was seen, though speciation was not possible. In our study cases showed presence of fungal hyphae in KOH mount but did not yield any fungal growth on culture.[30] This is similar to the observations of and Nath R et al and Tilak R et al where KOH mount had higher positivity.[29] However, Tahereh et al and Jose et al reported only 71.4% and 90.2% positive KOH smear compared to culture-positive fungal keratitis cases, respectively.[31,32]

In our study Total 14 participants had ocular Bacterial infection and in all age group maximum infection are caused by Staph aureus. In our study Total 15 participants had ocular Fungal infection and occurred in patients older than 20 years. In all age group maximum infection are caused by Aspergillus. Aspergillus spp. is the most common pathogen responsible for fungal keratitis followed by Fusarium. But some studies reported different types of results. Jampala S et al reported Candida spp as the most frequent isolate.[33] Nath R et al from Assam and Meena et al from Uttarakhand reported Fusarium as the most prevalent species followed by Aspergillus spp. and Curvularia spp.[34] Some studies found Aspergillus spp as common isolate in the north and western India and Fusarium spp. in South India.[35] Among the Aspergillus species, A. fumigatus was the most prevalent species in our study, as also seen by Sanjeev H et al from Mangalore; at the same time, Punia RS et al and Tilak R et al found A. flavus to be the commonest.[36] These findings suggest that although Aspergillus spp is the most common isolate, some variation may be possible.

Conclusion

Thus, the present study concludes that Aspergillus species are the most common cause for fungal keratitis in our region, with A. fumigatus and A. flavus being the prevalent species, followed by Fusarium. Bacterial isolates are responsible for considerably fewer cases of keratitis. Routine surveillance of fungal keratitis is necessary to know the existing and emerging pattern of pathogens. Trauma with vegetative matter was identified as the most common risk factor.

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