

Clinical and Microbiological Profile of Keratitis in Menelik II Memorial Hospital, Addis Ababa, Ethiopia

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Abstract

Background: Microbial keratitis is an infection of cornea that is associated with a risk of permanent visual impairment. It can be caused by bacteria, virus, fungi, and protozoa.

Objective: To determine risk factors, clinical and microbial pattern of keratitis.

Methods: A cross sectional study was conducted at Menelik II memorial hospital. A convenient sampling was used from January to September 2016. Corneal scrapping was taken by ophthalmologist on duty after installation of preservative free anesthesia. Scraping was smeared on to two slides for preparation of gram stain and 10% KOH wet mount and also inoculated in to 0.5 ml BHI broth. Broth samples were inoculated on to bacterial and fungal solid media and incubated at appropriate and duration. Data were analyzed by SPSS version 20 software.

Result: Of 60 patients, fungi and bacteria were recovered from 29 (48.3%) and 18 (30%) respectively. Potential risk factors were identified in 80% of cases. Corneal trauma was the main risk factor constituting 55% of all. The commonest fungal pathogen isolated were *Aspergillus* species (41.4%) followed by *Fusarium* species (24.1%). Coagulase negative *Staphylococci* and *Staphylococcus aureus* were common isolates accounting for 44.4% and 22.2% respectively.

Conclusion: High prevalence of infectious keratitis recorded in the present study, warrants the need for nationwide study on infectious keratitis and precise identification of the causative agents for efficient treatment.

Keywords: Microbial Keratitis; Fungal Keratitis; Bacterial Keratitis; Ethiopia

Introduction

Microbial keratitis is an infection of the cornea that is associated with a risk of permanent visual impairment. It can be caused by bacteria, virus, fungus and protozoa [1]. The severity of corneal infection depends on the underlying condition of the cornea and pathogenicity of the infecting organism [2].

The spectrum of microbial agents associated with corneal ulcer is wide and varies from one geographical location to another [3]. Gram positive bacterial species are more frequently recovered in temperate zones, and gram-negative species in tropical climates. Humid atmosphere and outdoor occupations make the population more vulnerable to fungal infections [2]. Several risk factors such as contact lens wear, trauma, ocular surface disease, ocular surgery, and systemic disease have been reported to predispose patients to corneal infections [4,5]. In developing countries although, trauma to the eye accounted for 48 to 65% of all corneal ulcers [6].

High rainfall, longer rainy season, and high humidity throughout the year have been identified as favorable environmental conditions for bacterial fungal and growth in countries located in tropical and sub-tropical regions such as Ethiopia. Therefore, conducting a search for the actual magnitude of microbial keratitis and its etiological agents appears to be timely and appropriate.

Methods

A total of 60 clinical samples were collected conveniently from patients visiting Menelik II Memorial Hospital ophthalmology clinic from January to September 2016. Patients underwent full bio microscopic examination to record the location and type of the ulcer. Duplicate corneal scraping was collected under aseptic conditions with a sterile 21 gauge needle, following the instillation of a local preservative free anesthetic (Tetracaine Hydrochloride 0.5%, Alcon laboratories Inc.) by ophthalmologists on duty under the magnification of a slit lamp from patients with presumptive diagnosis of infectious keratitis. Clinical diagnosis, socio-demographic data and risk factors, history of antibiotic treatment were obtained from laboratory request form.

Samples from corneal ulcers were directly smeared onto two separate labeled slides to prepare a 10% potassium hydroxide (KOH) wet mount and a gram stain. Another scraping was inoculated in to 0.5 ml brain heart infusion (BHI) broth and transported to the Department Medical Laboratory Science, Addis Ababa University within 2 - 3 hours. All clinical samples were then inoculated onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (Oxoid, Basingstoke, UK) and incubated at 25°C for 4 weeks aerobically, checking for fungal growth every other days. Brain infusion broth was also inoculated onto Blood Agar base (Oxoid, Basingstoke, UK) to which 10% sheep blood is incorporated, MacConkey agar, (Oxoid, Basingstoke, UK) and chocolate agar. All plates except MacConkey agar were incubated at 35 - 37°C for 18 to 24 hours in 5% carbon dioxide incubator. MacConkey agar was incubated at 35 - 37°C for 18 to 24 aerobically. Plates with no growth after 24 hrs were re-incubated for another 24 hrs.

Fungi were preliminary identified by examining their cultural characteristics such as topography, texture, rate of growth, pigmentation on the front and reverse side. Then microscopic identification was performed by placing pieces of a colony from SDA to clean slide and staining with lacto-phenol cotton blue. Preliminary identification of bacterial isolates was carried out by examining their colony morphology, gram stain and hemolytic reactions on blood agar plates. Bacterial identification down to genus and/or species level was carried out by array of routine biochemical tests. Data were analyzed by SPSS version 20 software.

Ethical clearance

The study was approved by the Department of Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Science, Collage of Health Sciences, and Addis Ababa University. Informed written consent was obtained from the patients. The participants were informed their right to withdraw at any time during study period. Participants who were positive for bacterial pathogen were linked to the hospital clinicians and received proper treatment.

Result

Socio-demographic characteristic, occupation and risk factors associated with fungal infection are shown in table 1. Among 60 study patients 38 (63.3%) were male and 22 (36.7%) were female. The median age was 46 years with a range of 16 to 75 years. Male patients with the age group of over 45 years were more affected (36; 60%) than other age groups. Thirty three (55%) patients were farmers, 14 (23.3%) were laborers, 9 (15%) were housewives, the remaining 4 (6.7%) patients were students, office workers and unemployed. Thirty seven patients (61.7%) were rural and 23 (38.3%) urban residents. The infection rate was more in rural patients. Risk factors for microbial keratitis were documented in 48 (80%) patients, among these patients 3 harbor more than one risk factor. Corneal trauma was the commonest risk factor, being caused by vegetative material 20 (33.3%), stone, sand and soil 8 (13.3%), thermal burn 3(5%) and miscellaneous material 2 (3.3%), respectively. Overall, corneal trauma accounted for 55% of the predisposing factor. Systemic illness was the second commonest risk factor (7; 11.7%), followed by previous corneal surgery (5; 8.3%) and previous use of drug like steroid (3; 5%) by patients, respectively.

Demographic	Variable	Number	Fungal keratitis		P value	COR	95% CI
			Yes	No			
Sex (n = 60)	Male	38	22	16	0.022	3.667	1.175 - 11.442
	Female	22	7	15			
Residence (n = 60)	Rural	37	18	19	0.951	1.033	0.365 - 2.929
	Urban	23	11	12			
Age(n = 60)	15 - 24	5	4	1	0.168	0.200	0.020 - 1.971
	25 - 34	9	3	6	0.548	1.600	0.345 - 7.418
	35 - 44	10	6	4	0.388	0.533	0.128 - 2.219
	> 45	36	16	20	0.506	1.250	
Occupation (n = 60)	Farmer	33	17	16	0.999	-	-
	Laborers	14	9	5	0.999	-	-
	House wife	9	2	7	0.999	-	-
	Student	2	1	1	0.999	-	-
	Office worker	1	-	1	-	-	-
	Unemployed	-	-	1	-	-	-
Risk factor (n = 48)	Trauma	33	19	14	0.229	2.375	0.580 - 9.720
	Systemic illness	7	3	4	0.783	0.438	0.189 - 9.101
	Ocular surgery	5	1	4	0.519	1.313	0.035 - 5.395
	Steroid usage	3	2	1	0.635	1.750	0.173 - 17.686

Table 1: Association of fungal keratitis with demographic characteristics and risk factors (n = 29).

Fungi and bacteria were recovered from 29 (48.3%) and 18 (30%) patients, respectively, with an overall prevalence of 78.3%. Of 29 patients with fungal keratitis, 7 (24.1%) were female and 22 (75.9%) were male patients. The association of fungal keratitis with gender was statistically significant [(COR = 3.667, 95% CI, 1.175 - 11.442) (P = 0.022)]. Eighteen (62.1%) and 11 (37.9%) patients with fungal keratitis were inhabitants of rural and urban areas respectively. There was no statistically association between residence and fungal keratitis. Fungal keratitis was the highest; 55.2% in patients over 45 years old followed by age groups of 35 - 44 (20.6%). The association of fungal keratitis with age was not statistically significant. Similarly, fungal keratitis was higher in farmers accounting 58.6%, followed by daily laborers 31%. Fungal keratitis was not statistically associated with occupation. Trauma was the predominant risk factor accounting for 55% fungal keratitis, but the association of trauma with fungal keratitis was not statistically significant. Bacterial keratitis was not significantly associated with neither gender (p = 0.257) nor residences (p = 0.603%). However, it was significantly associated in patients with age groups of 25 - 34 (p = 0.026). With regards to occupations, farmers were more affected (61.1%) than patients engaged in other activities.

As far as clinical characteristics infectious keratitis is considered, hypopyon was the predominant clinical manifestation with fungal keratitis accounting for 79.3% and this was followed by upper eye lid edema (55.2%), anterior chamber reaction (48.3%), and endothelia plaque (24.1%). None of the clinical manifestations was significantly associated with fungal keratitis. Hypopyon was also, the predominant clinical manifestation with bacterial keratitis accounting for 77.8% and this was followed by upper eye lid edema (44.4%), anterior chamber reaction (38.9%), and endothelia plaque (5.6%). None of the clinical manifestations was significantly associated with bacterial keratitis (Table 2).

Demographic	Variable	Number	Bacterial keratitis		P value	COR	95% CI
			Yes	No			
Sex (n = 60)	Male	38	14	24	0.257	1.983	0.600 - 6.554
	Female	22	4	18			
Residence (n = 60)	Rural	37	12	25	0.603	1.360	0.427 - 4.328
	Urban	23	6	17			
Age (n = 60)	15 - 24	5	1	4	0.808	0.750	0.740 - 7.613
	25 - 34	9	6	3	0.026	6.000	1.238 - 29.069
	35 - 44	10	2	8	0.744	0.134	0.134 - 4.203
	> 45	36	9	27	0.567	1.612	1.345 - 5.400
Occupation (n = 60)	Farmer	33	11	22	0.998	-	-
	Laborers	14	4	10	0.998	-	-
	House wife	9	1	8	0.998	-	-
	Student	2	1	1	0.998	-	-
	Office worker	1	1	-	0.998	-	-
	Unemployed	1	-	1	0.998	-	-
Risk factor (n = 48)	Trauma	33	10	23	0.999	-	-
	Systemic illness	7	3	4	0.999	-	-
	Ocular surgery	5	3	2	0.999	-	-
	Steroid usage	3	2	1	0.99	-	-

Table 2: Association of bacterial keratitis with demographic characteristics and risk factors (n = 18).

Of a total of 60 clinical samples 47 (78.3%) were culture positive, but organisms were detected microscopically in only 27 (45%). Among organisms detected in direct microscopy only three organism failed to grow in culture. Among clinical sample fungi and/or bacteria were neither detected nor showed visible growth in 13 (21.7%) samples despite being obtained from lesions compatible to keratitis (Table 3).

Clinical manifestation	Number	Fungal keratitis		P value	COR	95%CI
		Yes	No			
Hypopyon	44	23	21	0.314	0.548	0.170 - 1.779
Anterior chamber reaction	31	14	17	0.611	1.301	0.471 - 3.591
Upper eyelid edema	28	16	12	0.307	0.587	0.211 - 1.631
Endothelial plaque	9	7	2	0.466	0.177	0.121 - 1.798
		Bacterial keratitis				
		Yes	No			
Hypopyon	44	14	30	0.61	0.714	0.195 - 2.614
Anterior chamber reaction	31	7	24	0.199	2.095	0.489 - 5.052
Upper eyelid edema	28	8	20	0.693	1.250	0.412 - 3.790
Endothelial plaque	9	1	8	0.231	3.750	0.429 - 32.760

Table 3: Association of clinical manifestation with fungal keratitis (n = 29) and bacterial keratitis (n = 18).

A total of 47 fungal and bacterial isolates were recovered, of which 29 isolates were fungi and the remaining 18 isolates were bacteria (Table 4). Of the total fungal isolates *Aspergillus* and *Fusarium* spp. accounted for 41.4% and 24.1% the total fungal isolates, respectively. Of 18 bacterial isolates Gram- positive bacteria accounted for 83.3% (coagulase negative staphylococci 44.4%, *Staphylococcus aureus* 22.2% and *Streptococcus* species 16.7%) while gram negative bacteria consisted of 16.7% (*Klebsiella* species 11.1% and *Pseudomonas aeruginosa* 5.6%).

Isolates	Number (%)
Fungal species (n = 29)	
<i>Aspergillus specie</i>	12 (41.4)
<i>Fusarium species</i>	7 (24.1)
<i>Penicillin species</i>	3 (10.3)
<i>Aurobasidium species</i>	2 (6.9)
<i>Acromenium species</i>	2 (6.9)
<i>Scedosporium apiospermum</i>	1 (3.5)
<i>Cladosporium species</i>	1 (3.5)
<i>Rhizopus species</i>	1 (3.5)
Total	29 (100)
Bacterial species (18)	
Coagulase negative staphylococci	8 (44.4)
<i>Staphylococcus aureus</i>	4 (22.2)
<i>Streptococcus species</i>	3 (16.7)
<i>Klebsiella species</i>	2 (11.1)
<i>P. aeruginosa</i>	1 (5.6)
Total	18 (100)

Table 5: Spectrum of fungal and bacterial isolates from patients with microbial keratitis.

Discussion

Infectious keratitis (microbial keratitis) as the most frequent cause of keratitis is a sight threatening process characterized by defects of corneal epithelium with inflammation of underlying corneal stroma. Bacteria, viruses, fungi and parasitic organisms are all possible causes of this medical emergency condition. In the present study, the overall prevalence of microbial keratitis was found out to be 78.3% of which 30% was caused by bacteria. Our finding was not in line with previous local studies that reported a prevalence rate of bacterial keratitis in the range of 59.4 - 83% [7- 10]. Regional difference, methodology, geographical location and/or seasonal variations may be possible explanations for such variations among studies. The highest frequency of bacterial keratitis (50%) in our study, was in the age groups of over 45 years. The association of bacterial keratitis was statistically significant with age groups of 25 - 34 (p = 0.026). This could be explained by the fact that patients in the age group of 25 - 34 years in this study are the main force of manual works, especially in agricultural and outdoor activities. Our result did not corroborate the finding of Birtukan., *et al.* [10] who reported that bacterial keratitis is statistically significant with age groups over 55 years.

Our study revealed that 66.7% of bacterial isolates were recovered from rural population. Furthermore, patients engaged in farming accounted 61.1% isolates followed by daily laborer 22.2% together accounting 83.3% of the total bacterial isolates. This is obvious because rural parts of Ethiopia are inhabited by farmers and daily laborer who are more frequently exposed to materials that are etiological

agents of trauma. We found that corneal trauma was the commonest predisposing factor for bacterial keratitis. Out of 18 patients with bacterial keratitis, trauma was a predisposing risk factor in 10 (55.6%) patients. This is consistent with similar studies [9,11]. Contrary to our find, other related studies. Bourcier, *et al.* [12] and Siriku, *et al.* [13] reported that contact lens wear as the major predisposing factor for bacterial keratitis. Hypopyon was the predominant clinical manifestation associated with bacterial keratitis and our result was in line with the results noted by Bourcier, *et al.* [12] and Siriku, *et al.* [13].

Out of 18 bacterial isolates, Gram positive bacteria accounted for 83.3%. This is in good agreement with the findings of studies conducted in France [11], Nigeria [14] and Ethiopia [15]. Among these, coagulase negative staphylococci (CoNS) accounted for 44.4% and our result supports the findings of other studies [12,16]. As opposed to our finding, *Staphylococcus aureus* as a predominant isolate has been reported by many other studies [2,3,14]. *S. pneumoniae* as the commonest Gram positive bacteria in external ocular infections was documented from studies conducted in India [17], Nigeria [18] and Ethiopia [19]. The prevalence of Gram negative bacteria in the present study was 16.7% and this was less than the prevalence rates reported from studies conducted in Ethiopia; 48% [9], India; 39.7% [20].

Fungal keratitis is a very serious, potentially sight-threatening corneal infection which most commonly develops in patients after trauma or those with a compromised corneal surface. In current study, the prevalence of fungal keratitis was found to be 48.3%. Our finding was consistent with the results of previous internal studies who reported 30.4% [13], 37.5% [21] and 36.8% [22]. In contrast to our study, a higher fungal keratitis (83%) was reported by Mirshahi, *et al.* [23]. Discrepancies in the prevalence among different studies may be explained on the basis of differences in geographical location life style of the inhabitants and/or seasonal variations. Age has been reported as one of risk factor for microbial keratitis. Although the association of age and fugal keratitis was not statistically significant a prevalence 20.8 fungal keratitis observed in the age groups of 36 - 44 in the present study was comparable with the findings reported in many developing countries such as South India [17], North China [24] and Southeast Brazil [25]. This could be explained by the fact that patients in the age group of 35 - 44 in this study were the main force of manual works especially in outdoor activities. Residents of rural areas were significantly affected by fungal keratitis than urban residents even if there is no statistically significant association between residence and fungal keratitis. Similarly, fungal keratitis was higher in farmers accounting 58.6%, followed by daily laborers 48.2%, similar to a study in South India [17]. This might be due to the fact that rural parts of Ethiopia are inhabited by farmers and daily laborer. Similar to bacterial keratitis, hypopyon was the predominant clinical manifestation with fungal keratitis and our finding was in line with previous study [11].

It is a well-established fact that, the proportion of fungal keratitis caused by filamentous fungi increases towards tropical regions, whereas in many temperate regions fungal keratitis appears to be rare and the most frequent etiologic agents are *Candida* species. This was evident by the present study in which, all of fungal etiological agents were mycelia fungi. Of a total of 29 mold isolates *Aspergillus* and *Fusarium* species accounted for 41.4% and 24.1% of the total fungal isolates respectively, together comprising 65.2% of the total isolates. More or less similar findings of *Fusarium* species and *Aspergillus* species as the leading filamentous fungal pathogens have been described in, Southeast Brazil, North China, South Florida, Ghana and Malaysia [16,24-26] respectively, where the climate is warm and humid like Ethiopia. Comparisons of direct microscopy with culture revealed that culture technique was better in the identification of bacterial and fungi implicated in causing infectious keratitis and establish this procedures appears in ophthalmology clinics appears to be essential.

Limitations

- Small sample size (60 patients).
- No antibiotic susceptibility test was done due to lack of resources.

Conclusion

High prevalence of infectious keratitis recorded in the present study, warrants the need for nationwide study on infectious keratitis and precise identification of the causative agents for efficient treatment.

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Conflict of Interest

The work does not have financial and/or non-financial competing interest. The author declares that there is no conflict of interest regarding the publication of this paper.

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