A prospective analysis of infectious keratitis at a tertiary facility in South Africa

M Putter MBChB, MMed(Ophth), FCOphth(SA); *Consultant Ophthalmologist, Division of Ophthalmology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa*
ORCID: http://orcid.org/0000-0001-5890-369X

DP Smit MMed(Ophth), FCOphth(SA), PhD; *Associate Professor, Division of Ophthalmology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa*
ORCID: http://orcid.org/0000-0003-3206-8184

J Maritz FCPath(SA)Viro, MMed(Virol Path); *Senior Lecturer in Medical Virology, Division of Medical Virology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University and the National Health Laboratory Service, Cape Town, South Africa*
ORCID: http://orcid.org/0000-0001-5311-9134

AW Haarhoff MMed(Ophth), FC Ophth(SA); *Consultant Ophthalmologist, 1 Military Hospital and Centurion Eye Hospital, Pretoria, South Africa*

Corresponding author: Dr M Putter, Division of Ophthalmology, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town, 8000, South Africa; tel: +27827873685; email: putter.magdel@gmail.com

Abstract

Background: The aim of the study was to determine the microbial profile of infectious keratitis and the frequency of viral and bacterial co-infection in these cases.

Materials and methods: A prospective study included 57 patients with microbial keratitis. Corneal scrapings were sent for microscopy, culture and sensitivity (MCS) and herpes virus polymerase chain reaction (PCR) testing.

Results: Males (64.9%) were predominantly affected by infectious keratitis. A positive microbial culture was obtained in 24 of 57 cases (42.1%). Sixteen patients had positive herpes virus PCR results (28.1%) with 21 viruses identified from the 16 samples. Five patients with a positive viral PCR result had more than one virus identified on PCR testing (31.3%). A viral cause of infectious keratitis was suspected clinically before corneal scraping in 11 of 16 patients (68.7%) with a positive viral PCR result.

Conclusion: In our setting, polymicrobial infection is common in patients presenting with infectious keratitis. Viral and bacterial co-infection can be predicted clinically and should prompt clinicians to perform additional sampling for laboratory diagnosis of herpes viruses.

Keywords: infectious keratitis, viral PCR, mixed bacterial and viral infections

Funding: No funding received for this work.

Conflict of interest: The authors declare that they have no competing interests to declare with regard to this study.

Introduction

Infectious keratitis is an infection of the cornea that may be associated with an epithelial defect and signs of inflammation. It can potentially lead to severe visual dysfunction[1] and is considered a leading cause of monocular blindness in the developing world. Infectious keratitis can be suppurative or non-suppurative. Suppurative keratitis is frequently caused by bacteria and fungi, while the aetiology of non-suppurative infectious keratitis could be viral, spirochaetal, parasitic or immune-related stromal necrosis.[2]

Trauma is a common cause of corneal ulceration in adults in the developing world and accounts for up to 60% of cases developing a corneal ulcer.[3-5] Superficial corneal trauma frequently leads to corneal abrasions that can rapidly progress to corneal ulceration which mostly heals with scarring and may result in permanent vision loss.[2,4] Polymicrobial keratitis has been widely reported and occurs quite commonly.[6] Effective treatment requires a prompt laboratory diagnosis to accurately identify the causative pathogen/s and minimise complications that may arise from misdiagnosis and inappropriate treatment.

The epidemiology of infectious keratitis is influenced by predisposing risk factors, climatic and geographic factors as well as seasonal variations.[7-9] In developing countries, most patients with infectious keratitis do not receive medical care due to poor access to medical facilities, lack of awareness about the gravity of their condition and poverty.[10]

Herpes viruses are known to cause recurrent and devastating keratitis in developed countries.[11] Limited data is available from African countries about herpes keratitis and diagnosis is often solely based on clinical presentation. In Africa, severe geographic or stromal ulceration presumed to be due to herpes simplex virus (HSV) on account of its morphological appearance and response to specific antiviral therapy, appears to be a common cause for ocular morbidity.[12]

Limited data is available regarding the aetiology of infectious keratitis in South Africa as well as the rest of Africa.[13]

Materials and methods

Patients who presented to the eye clinic at Tygerberg Hospital were prospectively included in the study if they met the

inclusion criteria and provided informed consent. The inclusion criteria were: 1) corneal ulceration clinically considered to be infectious in origin; 2) epithelial defects measuring at least 1 mm at their greatest width; 3) some portion of the infiltrate involving the cornea; and 4) participants had to be 16 years or older. Clinical signs that were considered indicative of an infectious origin included large epithelial defects, stromal infiltrates, corneal thinning, anterior chamber reaction and hypopyon. Patients with clinically presumed isolated viral keratitis were not included in the study.

Clinical evaluation consisted of taking a complete history and slit-lamp evaluation which included measuring the size of both the epithelial defect and stromal infiltrate horizontally and vertically. Examination was followed by preliminary clinical classification of infectious keratitis as bacterial (Gram positive, Gram negative or mixed), viral, fungal or protozoan.

After instillation of topical anaesthetic (oxybuprocaine 0.4%), separate corneal scrape biopsies were placed on a glass slide for microscopy as well as on growth media including Saboraud, blood and chocolate agar plates. An additional scrape was performed and placed in a sterile saline specimen holder for herpes virus polymerase chain reaction (PCR) testing. Corneal scrapes were performed using a new calcium alginate swab for every scrape.

The slide and plates were evaluated in the laboratory and underwent microscopy, culture and sensitivity (MCS) testing as per routine investigation. PCR testing was carried out on all samples to evaluate for the presence of human herpes viruses 1–6. Samples were processed by the National Health Laboratory Service Medical Microbiology and Virology laboratories, Tygerberg Hospital. Herpes virus testing was performed by pulse vortexing corneal swabs in saline for 30 seconds, followed by nucleic acid extraction on the NucliSENS EasyMag platform (bioMérieux, Marcy l'Etoile, France) and multiplex PCR for six human herpes viruses (HSV-1, HSV-2, VZV, EBV, CMV and HHV-6) with the Seeplex Meningitis ACE Detection assay (Seegene Inc., Seoul, Korea).

Patient care followed our standard protocol including antimicrobial therapy modified according to response to therapy, clinical opinion and test results. Informed consent was obtained from all patients. The study adhered to the principles of the Declaration of Helsinki and was approved by the Health Research Ethics Committee (IRB0005239) at Stellenbosch University.

Table I: Organisms isolated and relative frequencies

Organism	No. of isolates (n=27)	%
Gram-negative organism		
Pseudomonas species	4	14.82
Moraxella species	4	14.82
Staphylococcus haemolyticus	2	7.41
Stenotrophomonas maltophilia	2	7.41
Haemophilus influenzae	1	3.70
Gram-positive organism		
Staphylococcus aureus	6	22.22
Streptococcus pneumonia	3	11.11
Corynebacterium species	2	7.41
Streptococcus group G	1	3.70
Yeast		
Candida albicans	1	3.70
Filaments		
Penicillium species	1	3.70
Total	27	100

Results

Laboratory analyses

A total of 57 patients with a clinical diagnosis of infectious keratitis were included in the trial, of which 37 were male (64.9%). The mean age at presentation was 44.4 years (range 17–79).

MCS results

Samples for MCS and PCR were collected from all 57 participants in the study. A positive culture was obtained in 24 cases (42.1%) while the remaining 33 cases (57.9%) were culture negative. In the 24 culture positive cases, a total of 27 organisms were cultured (*Table I*). *Pseudomonas* species (14.82%) and *Moraxella* species (14.82%) were the most frequently isolated Gram-negative organisms while *Staphylococcus aureus* (22.2%) was the most frequently isolated Gram-positive organism. *Candida albicans* and a *Penicillium* fungus were isolated in one case each. In three of the culture positive cases two different organisms were isolated as illustrated in *Table II*.

Seven of 24 culture-positive patients (29.2%) were found to have a herpes virus

Table II: Summary of patient characteristics and combination of organisms in the polymicrobial infection group

Case	Age in years/Sex	Organisms cultured	HIV status	Risk factor
1	39/F	*Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*	Negative	Contact lens wear
2	18/M	*Streptococcus* group G *Staphylococcus aureus*	Negative	Allergic keratoconjunctivitis
3	49/F	*Haemophillus influenzae* *Staphylococcus aureus*	Negative	Entropion

Table III: Summary of patient characteristics and combination of organisms in the bacterial and viral co-infection group

Case	Age in years/Sex	Organism cultured	Virus	HIV status	Risk factor
1	53/M	*Staphylococcus aureus*	CMV	Positive	Previous HSV keratitis
2	18/M	*Streptococcus* group G *Staphylococcus aureus*	EBV	Negative	Allergic keratoconjunctivitis
3	26/M	*Corynebacterium*	EBV	Negative	Trauma
4	34/F	*Corynebacterium*	VZV	Positive	Previous herpes zoster ophthalmicus
5	42/F	*Staphylococcus aureus*	VZV EBV	Negative	Neurotrophic cornea
6	74/M	*Streptococcus pneumoniae*	CMV EBV	Negative	Corneal exposure
7	36/M	*Staphylococcus haemolyticus*	HSV1	Positive	Nil

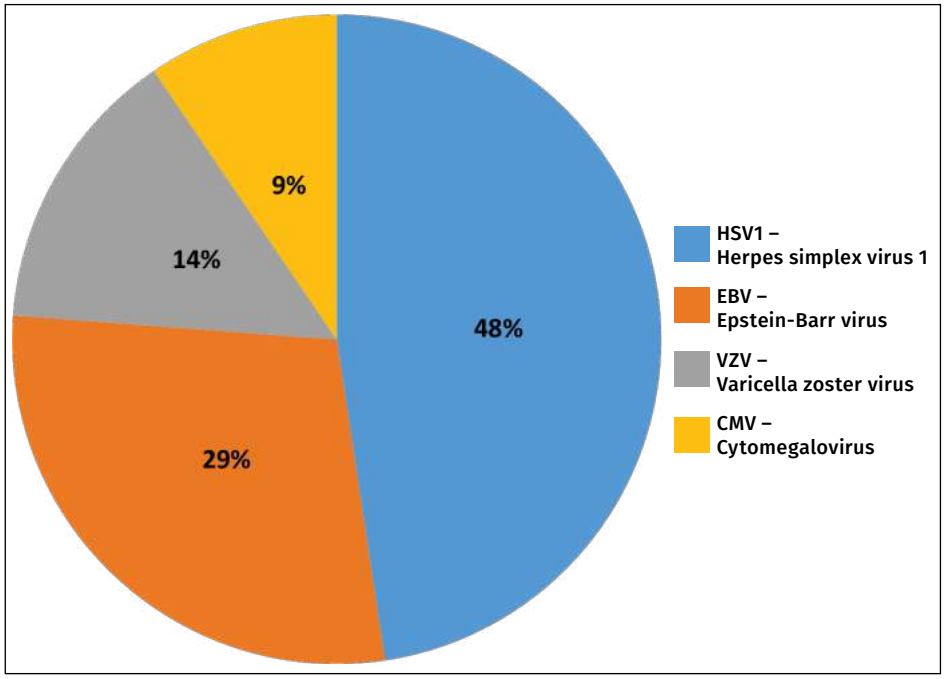

Figure I. Herpes viruses identified by PCR testing

present based on a positive viral PCR test result (*Table III*). Of the 33 MCS-negative patients, nine (27.3%) had a positive PCR test for one or more of the herpes viruses. Epstein-Barr virus (EBV) was detected in four samples, Cytomegalovirus (CMV) and Varicella-Zoster virus (VZV) were each detected in two samples and herpes simplex virus 1 (HSV-1) was present in one sample.

Viral PCR results

Sixteen patients (28.1%) had a positive herpes virus PCR result; the remaining 41 (71.9%) were PCR negative.

In total there were 21 viruses identified from the 16 samples. HSV1 accounted for almost half of the viruses identified as ten of 21 (47.6%) PCRs were HSV1 positive. VZV was only responsible for three (14.3%) of the positive PCR results, while EBV accounted for six (28.6%) and CMV for two (9.5%) of the positive PCR results (*Figure 1*). Five (31.3%) of the 16 patients with a positive viral PCR result had more than one virus identified on PCR testing (*Table IV*). Based on our clinical evaluation at initial presentation, a viral component contributing to infectious keratitis was suspected in 11 of 16 patients (68.7%) with a positive viral PCR result. In only nine of 41 patients (21.9%) with a negative viral PCR, viral infection was suspected clinically.

Discussion

In our study, a positive bacterial culture for at least one organism occurred in 42.1% of corneal scrapes. Positive culture results have been reported in 38–86% of cases in similar studies.[1,6,13,14] The most likely explanation for the lower positive culture rate in our study is that a large proportion of patients presenting to our tertiary facility are partially treated by the time they presented to our clinic. Antibiotic use prior to presentation to an ophthalmologist in developing countries has been reported in up to 58% of patients presenting to tertiary institutions.[15]

Gram-negative organisms were slightly more prevalent than Gram-positive organisms as a cause of infectious keratitis accounting for 48.2% of positive cultures. Schaftenaar *et al.* reported predominantly Gram-positive organisms (68%) to be the causative organism in a similar study recently conducted elsewhere in South Africa.[13]

Stenotrophomonas maltophilia is rarely implicated in microbial keratitis but has been reported to be the causative organism in 1.4% of cases presenting with microbial keratitis by Wu *et al.*[16] A high rate (57%) of polymicrobial infection has been demonstrated in patients with *Stenotrophomonas maltophilia*[16] keratitis, making it hard to determine the clinical significance of the organism. In our study, it was identified as the causative organism in two (8.3%) of 24 patients with a positive culture, one of which was a case of polymicrobial keratitis.

Fungi were responsible for 7.4% of all culture-proven samples, corresponding to findings from a similar study recently reported from South Africa that cultured fungi in 3.4% of cases.[13] However, this is in sharp contrast to studies from Ghana and Tanzania that reported[14,17] fungi as the causative pathogen in up to 50% of keratitis cases.

The most likely explanation for the markedly lower rate of fungal keratitis in South Africa compared to other regions in Africa is the difference in climate as it is well known that fungal keratitis is much more prevalent in tropical or subtropical areas.

More than one organism was isolated in 12.5% of culture-positive samples. Polymicrobial infection has been reported to occur in 33% of positive culture results in a similar study.[6] Lim *et al.* found that older age and larger sized corneal infiltrates were commonly associated with polymicrobial keratitis. In our study, all three patients with polymicrobial infection were younger than 50 years of age and, contrary to what one would expect, also HIV negative. It was also found that polymicrobial keratitis more often has a prolonged course of disease and decreased antibiotic sensitivity.[18] Combination therapy with fortified antibiotics covering both Gram-positive and Gram-negative organisms, which is the

Table IV: Summary of patient characteristics and combination of herpes viruses positive on PCR

Case	Age in years/Sex	Virus	HIV status	Risk factor
1	46/F	HSV1 EBV	Negative	Previous HSV keratitis
2	39/M	VZV HSV1	Positive	Nil
3	53/M	HSV1 EBV	Positive	Foreign body
4	42/F	VZV EBV	Negative	Neurotrophic cornea
5	74/M	CMV EBV	Negative	Cornea exposure

standard of treatment for corneal ulcers at our facility, covers a wide spectrum of organisms and should effectively treat a polymicrobial infection under most circumstances.

Our study focused on patients with presumed bacterial infection or suspected bacterial and viral co-infection. We therefore did not perform corneal scrapes when patients were clinically presumed to have isolated viral keratitis. Viral keratitis was proven by PCR testing in 29.2% of the 24 patients with proven microbial keratitis. Data on viral and bacterial co-infection in patients with infectious keratitis is limited. Twenty-eight per cent of the total sample size had a positive herpes viral PCR result, and 31.3% of these samples showed more than one herpes virus present on PCR testing. HSV keratitis occurs commonly and is known to recur in up to 64% of previously infected individuals.[11,19]

Our results suggest that the contributing effect of viral keratitis can be predicted clinically and should potentially lead the clinician to performing an additional sampling for laboratory diagnosis of herpetic viruses. This is not done routinely on all patients presenting to our facility with presumed microbial keratitis but should be considered in cases where a positive result will alter the clinical management of a patient.

Similar frequencies of viral PCR positive results were found in samples that yielded MCS positive (29.2%) compared to MCS negative (27.3%) results.

The fact that our sample size was small and that all patients were not tested for HIV are limiting factors. Very limited data exists regarding infectious keratitis in HIV-positive patients.[13,20] Polymicrobial infections and especially microbial and herpetic virus[13,20] co-infection have been implicated in HIV-positive patients. The existing studies have small sample sizes, and larger prospective studies are needed to gain more clarity regarding infectious keratitis in the HIV-positive population.

Microbial keratitis is a potentially blinding condition, especially in the developing world. Polymicrobial keratitis and cases with viral and bacterial co-infection require further investigation to improve the diagnostic accuracy, management and outcome of these conditions.

Conclusion

Our study has demonstrated that polymicrobial keratitis is not uncommon in our setting. One may encounter either co-infection by different bacteria or co-infection by bacteria and herpes viruses and the latter has important implications for successful therapeutic outcomes.

Take-home messages

- Roughly 40% of corneal scrapes had positive culture results.
- Gram-positive and Gram-negative infections were evenly distributed.
- Fungi causes <10% of corneal infections.
- Polymicrobial infections may occur in HIV-negative patients.
- 29% of patients with culture-positive microbial keratitis had viral co-infection.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Whitcher JP, Srinivasan M, Upadhyay MP. Prevention of corneal ulceration in the developing world. *Int Ophthalmol Clin*. 2002;**2**(1):71-77.
2. Srinivasan M, Mascarenhas J, Prashanth CN. Distinguishing infectious versus noninfectious keratitis. *Indian J Ophthalmol*. 2008;**56**(3):203-207.
3. Somabhai Katara R, Dhanjibhai Patel N, Sinha M. A clinical microbiological study of corneal ulcer patients at western Gujarat, India. *Acta Med Iran*. 2013;**51**(6):399-403.
4. Sirikul T, Prabriputaloong T, Smathivat A. Predisposing factors and etiologic diagnosis of ulcerative keratitis. *Cornea*. 2008;**27**(3):283-87.
5. Oladigbolu K, Rafindadi A, Samaila E. Corneal ulcers in a tertiary hospital in Northern Nigeria. *Ann Afr Med*. 2013;**12**(3):165.
6. Wong T, Ormonde S, Gamble G. Severe infective keratitis leading to hospital admission in New Zealand. *Br J Ophthalmol*. 2003;**87**(9):1103-108.
7. Vajpayee RB, Dada T, Saxena R. Study of the first contact management profile of cases of infectious keratitis: a hospital-based study. *Cornea*. 2000;**19**(1):52-56.
8. Ibrahim YW, Boase DL, Cree I. Factors affecting the epidemiology of acanthamoeba keratitis. *Ophthalmic Epidemiol*. 2007;**14**(2):53-60.
9. Otri AM, Fares U, Al-Aqaba M. Profile of sight-threatening infectious keratitis: A prospective study. *Acta Ophthalmol*. 2013;**91**(7):643-51.
10. Cao J, Yang Y, Yang W. Prevalence of infectious keratitis in Central China. *BMC Ophthalmol*. 2014;**14**(43):1-6.
11. Tj L. Herpes simplex virus epidemiology and ocular importance. *Cornea*. 2001;**20**(1):1-13.
12. Yorston D, Foster A. Herpetic keratitis in Tanzania: association with malaria. *Br J Ophthalmol*. 1995;**76**(10):582-85.
13. Schaftenaar E, Peters RPH, Baarsma GS. Clinical and corneal microbial profile of infectious keratitis in a high HIV prevalence setting in rural South Africa. *Eur J Clin Microbiol Infect Dis*. 2016;**35**(9):1403-409.
14. Hagan M, Wright E, Newman M. Causes of suppurative keratitis in Ghana. *Br J Ophthalmol*. 1995;**79**(11):1024-28.
15. Rahimi F, Hashemian MN, Khosravi A. bacterial keratitis in a tertiary eye centre in Iran: a retrospective study. *Middle East Afr J Ophthalmol*. 2015;**22**(2):238-44.
16. Wu A-L, Yeh L-K, Ma DHK. Clinical characteristics of *Stenotrophomonas maltophilia* keratitis. *Cornea*. 2016;**35**(6):795-800.
17. Poole TRG, Hunter DL, Maliwa EMK. Aetiology of microbial keratitis in northern Tanzania. *Br J Ophthalmol*. 2002;**86**(8):941-42.
18. Lim NC, Lim DK, Ray M. Polymicrobial versus monomicrobial keratitis: a retrospective comparative study. *Eye Contact Lens*. 2013 Sep;**39**(5):348-54.
19. The Herpetic Eye Disease Study Group. Acyclovir for the prevention of recurrent herpes simplex virus eye disease. *New Engl J Med*. 1998;**339**(5):300-306.
20. Hemady RK. Microbial keratitis in patients infected with the human immunodeficiency virus. *Ophthalmology*. 1995;**102**(7):1026-30.