Acanthamoeba more commonly causes epithelial keratitis than herpes simplex in South-East England contact lens users

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Declarations
The authors declare that they have no competing interests to declare that are relevant to threcontent of this report

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Alice Milligan designed and carried out the *herpes simplex virus* keratitis audit; John Dart and Sara Sanchez designed the *Acanthamoeba* keratitis audit; Sara Sanchez, Denise Vamos and Lana Faraj carried out the *Acanthamoeba* keratitis audit; John Dart designed the study; all authors contributed to manuscript preparation.

Running head:
Herpes and *Acanthamoeba* keratitis incidence in contact lens users

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Synopsis
Acanthamoeba keratitis (AK) is more common than first presentations of herpes simplex virus keratitis (HSVK) in contact lens users in the South-East England. AK should be excluded before HSVK is diagnosed in lens users.

Subject consent to participate or publish
The Moorfields Eye Hospital Clinical Audit Committee confirmed that no ethical approval was required from subjects, either to participate in or have their data published from, this anonymised retrospective study which utilised routinely collected clinical data.

Abstract

Purpose

To compare the incidence of first episodes of herpes simplex virus keratitis (HSVK) and *Acanthamoeba* keratitis (AK) in contact lens (CL) users in London over a 3-month period.

Methods

Retrospective audits of HSVK cases attending Moorfields Eye Hospital Accident and Emergency Dept between 01/09/2019 and 30/11/2019 and AK cases attending between 01/01/2019 and 31/12/2019.

Results

Amongst CL users there were three first presentations of epithelial HSVK and four of epithelial AK in the 3-month HSVK audit period. An additional six cases of AK presented with more advanced disease stages during the same period.

Conclusion

Misdiagnosis of AK as HSVK remains common, is an established risk factor for a poor outcome following AK, and results from HSVK being a common disease in most populations whereas AK is rare except in CL users. This study is the first to compare first presentations of HSVK and AK in the CL using sub-population and shows that AK is more common that HSVK in CL users in England. In addition, critical appraisal of studies on the incidence of HSVK and AK, in other countries where CL use is the principal risk factor for AK, show that AK is likely to be as common as HSVK in CL users with a first presentation of keratitis. These findings suggest that in countries with a similar climate and demographic as in England clinicians should exclude AK before diagnosing HSVK in CL users presenting with epithelial/stromal keratitis for which our diagnostic protocol is available online.

Introduction

In the UK, and in countries where contact lenses (CL) are commonly used as alternatives to spectacles, CL wear has become the major risk factor for microbial keratitis (MK).[1] Severe keratitis in CL users results from infection by *Pseudomonas* spp., *Fusarium* spp. and *Acanthamoeba spp*.[2] *Acanthamoeba* is the least common of these causes globally although the incidence has been rising in the last decade in many countries where CL's are in widespread use and are the major risk factor for AK[1] including the USA[3], the UK and the Netherlands[4] amongst others. The outcomes of AK are poor with c. 25% of subjects having severe loss of vision or surgery, of which corneal transplant surgery is the most common procedure.[4]

Accurate and early diagnosis is critical to good MK outcomes including AK for which delayed diagnosis is an established risk factor for a poor outcome.[5] Although proven polymicrobial keratitis caused by a combination of *Acanthamoeba* and bacteria is common, occurring in 10-20% of cases in large European series[4], proven fungal and herpes coinfections have been infrequent; polymicrobial *herpes simplex virus* keratitis [HSVK] co-infection with AK was found in only 2/224 (0.9%) patients in a case series in the Netherlands from 2009-2015.[4] Despite this, HSVK has remained the most common misdiagnosis for AK; two recently reported European studies, including over 400 subjects, had HSVK/AK misdiagnosis rates above 50%.[4 5] Not only does misdiagnosis delay the effective

treatment of AK but the use of topical steroids before therapy for AK, most often as a result of treatment for misdiagnosed HSVK[4 5], is an additional established risk factor for a poor AK outcome.[4 5]

Misdiagnosis of AK as HSVK has arisen partly because HSV keratitis is common in the whole population whereas *Acanthamoeba* keratitis is rare. There have been no recent studies, to our knowledge, comparing the incidence of AK and HSV keratitis in previously unaffected patients in one location. In this study we report the results of two concurrent audits of both diseases to compare the incidence of these in a hospital serving ophthalmic emergencies South-East England. If the incidence of AK, and of a first infection with HSVK, are similar in the sub-population of CL users then best practice protocols should change to exclude AK, at the onset of an episode of keratitis, in this patient subset.

Methods

These retrospective anonymised clinical audit studies were approved by the Moorfields Audit Committee who confirmed that no ethical approval was required.

HSV keratitis

This was a retrospective review of HSVK cases attending the Moorfields Accident and Emergency Department (A&E) for the 3-month period from 01/09/2019 to 30/11/2019. The A&E department is where all acute onset cases are first seen, both self-referred or referred by their general practitioner or optometrist. The electronic record system, on which all patients are registered, was searched for all A&E attendances with a coding diagnosis of viral keratitis or herpes simplex keratitis. The full data on each of the identified subjects were then retrieved via the electronic record system to evaluate each episode; when electronic notes were incomplete, paper notes were retrieved. Inclusion criteria for this study were CL use and a diagnosis of HSVK; defined as a positive HSV PCR from a corneal swab or, when negative or not done, a clinical diagnosis in cases which responded appropriately to treatment for HSVK. Exclusion criteria were an alternative diagnosis (e.g., varicella zoster virus), missing records, or a previous diagnosis of HSVK. HSVK cases were classified as epithelial, stromal, mixed epithelial/stromal and endothelial.

Acanthamoeba keratitis

This was similarly a retrospective case note review of patients presenting to Moorfields with symptoms and signs of infectious keratitis who were ultimately diagnosed with AK from 01/01/2017 to 31/12/2019. Keratitis cases are first screened in the A&E Dept before transfer to a specialist clinic. Any patient suspected of having a non-bacterial keratitis, other than some cases of HSVK, are diagnosed using our online protocol[1] for non-bacterial keratitis or progressive keratitis (not improving after 5 days of bacterial keratitis treatment or after 2 weeks of HSVK treatment) which includes, corneal cultures,

[1] Protocol described in the Moorfields Emergency Guidelines for Corneal and External Diseases within the free to download Microguide App http://www.microguide.eu/

smears and PCR for AK (with bacterial, fungal and HSV PCR as appropriate) and *in vivo* confocal microscopy (IVCM). AK Cases were identified by retrospective audit of PCR requests for *Acanthamoeba* diagnosis, search of the pharmacy records for the patients treated with the anti-amoebic drugs PHMB and chlorhexidine (all concentrations) and a key word search of the electronic records for the terms *Acanthamoeba* and amoeba. Electronic and paper notes were reviewed. Inclusion criteria for AK were a positive PCR; a positive *Acanthamoeba* culture; histopathological confirmation of trophozoites and/or cysts; culture-negative cases shown to have *Acanthamoeba* cysts on IVCM and those who were negative for all these investigations but who had a clinical course and response to treatment consistent with that for AK excluding those for whom the outcome remained uncertain at the end of the audit. AK cases were classified using the AK staging system[4]: Stage 1 - the presence of corneal epitheliopathy only; Stage 2 - the presence of ≥1 corneal epithelial defects, perineural infiltrate(s) or stromal infiltrate in addition to Stage 1 findings; Stage 3 - a corneal ring infiltrate and 1 or more features of Stage 2 disease and Severe AK - scleritis and/or hypopyon and/or Stage 3 disease.

Contact lens use immediately before the onset of the keratitis is routinely recorded for all cases attending A&E and for all keratitis cases attending other clinics.

Results

Table 1 summarises the key outcome of this 3-month study in which the incidence of a first diagnosis of epithelial keratitis in CL users was identified as being caused by HSVK in three patients and by AK in four. During this period there were no other cases (stromal, mixed epithelial/stromal or endothelial) of a first diagnosis of HSVK in CL users although there were another 6 cases of AK in CL users, with later stages of AK at presentation (4 with stage 2, 1 with stage 3 and 1 with severe AK), for whom full data is included in the supplementary online spreadsheet https://data.mendeley.com/datasets/v3772d8vpk/draft?a=62b87e81-15fd-4926-aaee-33df3f2090e8.

HSV keratitis

During this 3-month period there were an additional 129 episodes of suspected HSVK including non-primary infections and non-CL users. Of these 27/129 had a first episode of HSVK of whom 9/27 had an HSV PCR test of which 1/9 (11.1%) was positive. Three of these 27 cases were the CL users with epithelial HSVK described in Table 1 and in the supplementary online spreadsheet. The remaining 24 cases were amongst non-CL users and were epithelial in 20/24, stromal in 3/24 and endothelial in 1/24; there were no cases of mixed epithelial/stromal HSVK.

Acanthamoeba keratitis

The Figure 1 shows that this study period had one of the higher incidences of AK for any 3-month period in 2019 for which the total of AK cases was 32. AK disease stages at presentation are shown in the Figure.

Discussion

Although, as expected, first episodes of HSVK were more common (3-fold) than AK in the whole population attending the Accident & Emergency Dept. the key finding from this audit is that in the sub-population of CL users there were similar numbers of first presentations of epithelial HSVK (n=3) as of epithelial (Stage 1) AK (n=4). Furthermore, although there were no stromal or endothelial first presentations of HSVK there were additional cases of AK (n=6), presenting with these more advanced disease stages, which could have been misdiagnosed as HSVK. Reasons for the misdiagnosis of AK as HSVK have resulted from the usually insidious onset of both conditions and the similarity of the clinical signs of epithelial and mild stromal keratitis that often accompany the early stages of both diseases[6]; the current practice of making a clinical diagnosis of HSVK without the use of the widely available investigation of HSV DNA identification by polymerase chain reaction (PCR)[7]; and because HSVK is common in the whole population, if not in the CL using sub-population, whereas *Acanthamoeba* keratitis is a rare although less so in CL users.

The role of laboratory confirmation. In the context of CL wearers in whom a provisional diagnosis of HSVK is made, the recommendation that a diagnosis be made on clinical grounds alone has not served patients well who have misdiagnosed epithelial keratitis.[7] Clinical misdiagnosis of epithelial HSVK is common, particularly when terminal bulbs and epithelial infiltrates are absent; similar lesions occur with herpes zoster and other viruses as well as with *Acanthamoeba*. HSV PCR is widely available, albeit with variable sensitivities, and provides reassuring evidence that the clinical diagnosis is correct when positive.[7] Amongst CL users presenting for the first time with a subacute onset of epithelial, or epithelial and stromal keratitis, investigations for both HSVK and AK ± fungal keratitis should be carried out including PCR for HSV, *Acanthamoeba* ± fungus, together with corneal cultures and corneal smears for histology and IVCM for *Acanthamoeba* ± fungus.[6]

The current incidence of HSVK and AK. The most recent national surveys of HSVK in whole populations are available for France (2005) showing an incidence of first presentations of 13.2 per 100,000[8] and for a county in the temperate USA (1976-2007) of 11.8 per 100,000.[9] The AK incidence, in the 2015 UK National Survey of AK, identified 124 cases; an incidence of 3.086 per 100,000 in CL users (6.6% of the population were using CL's in 2015) and 0.235 per 100,000 for the whole population (personal communication H Jasim and D Tole). This compares with the 1997-1999 England and Wales AK Survey incidence of 1.93 per 100,000 amongst CL users, rising to 8.15 per 100,000 for CL users in some regions.[10] These data suggest that amongst our sub-population of CL users with a first presentation of keratitis, as opposed to the whole population, AK is more likely to be present than HSVK; this is probably also the case in other countries with a similar climate and demographic.

A major limitation of this study is that the HSVK audit was conducted over only one 3-month period which might have been an exceptional quarter. However, there is no evidence that the incidence of first episodes of HSVK, as opposed to recurrences, is seasonal whereas AK is seasonal with a lower incidence in winter. In addition, confirmation of the diagnosis of the AK diagnosis by PCR or culture was low in this series and although it is possible that not all these subjects had AK their clinical course

was consistent with it. Misdiagnosis of some cases of either HSVK or AK in this series is unlikely to have altered our conclusions regarding the relative incidence of these diseases.

The response to this situation at Moorfields has been to adopt the protocol described in the Moorfields Emergency Guidelines for Corneal and External Diseases within the free to download Microguide App http://www.microguide.eu/. The advice given is not to make a diagnosis of HSVK in a CL user until AK has been excluded requiring culture, histopathology of a smear, PCR for both HSV and *Acanthamoeba* and IVCM. We have also audited compliance with this protocol to assist in maintenance of this standard; in the 24 month 2014 to 2015 AK was misdiagnosed as HSVK in only 8/102 (7.8%) cases. We hope these guidelines will be adopted by other ophthalmic units in the UK and elsewhere.

References

1. Ung L, Bispo PJM, Shanbhag SS, Gilmore MS, Chodosh J. The persistent dilemma of microbial keratitis: Global burden, diagnosis, and antimicrobial resistance. Survey of ophthalmology 2019;**64**(3):255-71 doi: 10.1016/j.survophthal.2018.12.003[published Online First: Epub Date]|.

2. Dart JK, Radford CF, Minassian D, Verma S, Stapleton F. Risk factors for microbial keratitis with contemporary contact lenses: a case-control study. Ophthalmology 2008;**115**(10):1647-54, 54 e1-3 doi: S0161-6420(08)00432-6 [pii]
10.1016/j.ophtha.2008.05.003[published Online First: Epub Date]|.

3. Cheung N, Nagra P, Hammersmith K. Emerging trends in contact lens-related infections. Curr Opin Ophthalmol 2016;**27**(4):327-32 doi: 10.1097/ICU.0000000000000280[published Online First: Epub Date]|.

4. Randag AC, van Rooij J, van Goor AT, et al. The rising incidence of Acanthamoeba keratitis: A 7-year nationwide survey and clinical assessment of risk factors and functional outcomes. PloS one 2019;**14**(9):e0222092 doi: 10.1371/journal.pone.0222092[published Online First: Epub Date]|.

5. Carnt N, Robaei D, Minassian DC, Dart JKG. Acanthamoeba keratitis in 194 patients: risk factors for bad outcomes and severe inflammatory complications. Br J Ophthalmol 2018 (Oct);**102** (10):1431-35 doi: 10.1136/bjophthalmol-2017-310806[published Online First: Epub Date]|.

6. Alkatan HM, Al-Essa RS. Challenges in the diagnosis of microbial keratitis: A detailed review with update and general guidelines. Saudi journal of ophthalmology : official journal of the Saudi Ophthalmological Society 2019;**33**(3):268-76 doi: 10.1016/j.sjopt.2019.09.002[published Online First: Epub Date]|.

7. Poon SHL, Wong WHL, Lo ACY, et al. A systematic review on advances in diagnostics for herpes simplex keratitis. Survey of ophthalmology 2020 doi: 10.1016/j.survophthal.2020.09.008[published Online First: Epub Date]|.

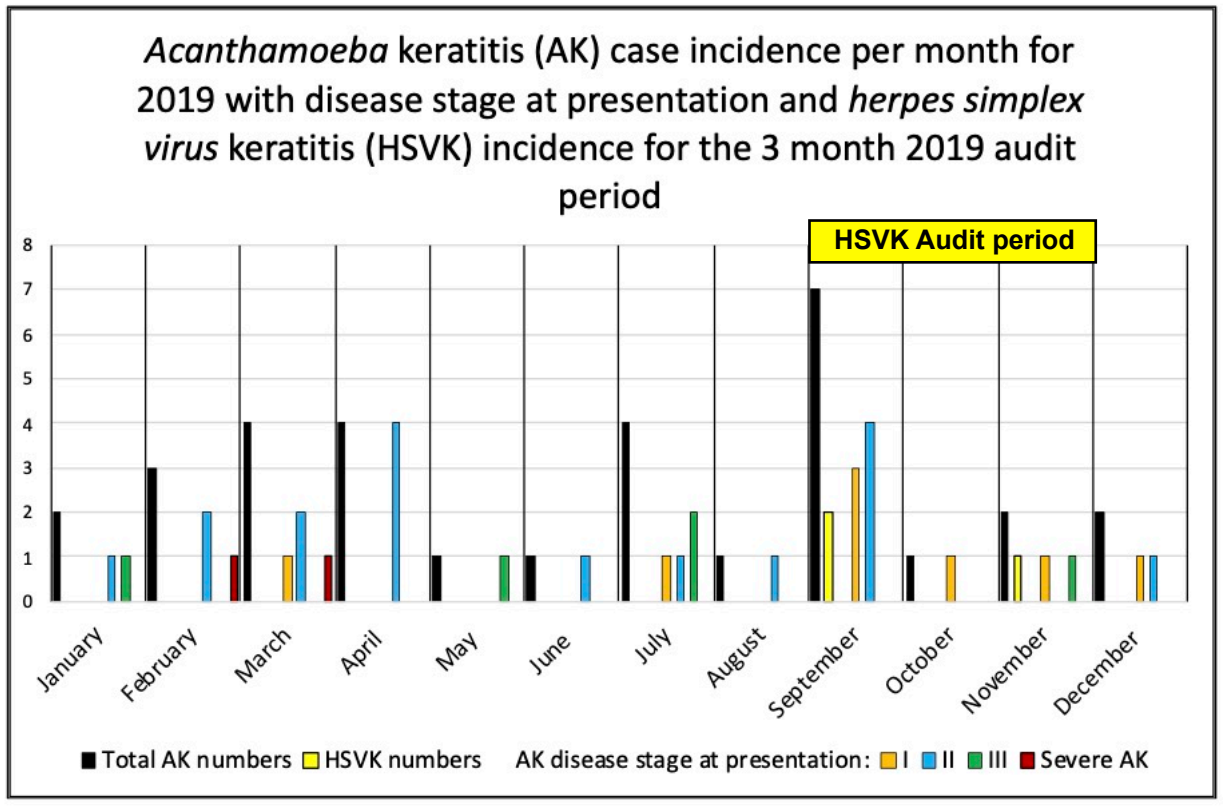
8. Labetoulle M, Auquier P, Conrad H, et al. Incidence of herpes simplex virus keratitis in France. Ophthalmology 2005;**112**(5):888-95 doi: 10.1016/j.ophtha.2004.11.052[published Online First: Epub Date]|.

9. Young RC, Hodge DO, Liesegang TJ, Baratz KH. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976-2007: the effect of oral antiviral prophylaxis. Archives of ophthalmology 2010;**128**(9):1178-83 doi: 10.1001/archophthalmol.2010.187[published Online First: Epub Date]|.

10. Radford CF, Minassian DC, Dart JK. Acanthamoeba keratitis in England and Wales: incidence, outcome, and risk factors. Br J Ophthalmol 2002;**86**(5):536-42 doi: 10.1136/bjo.86.5.536[published Online First: Epub Date]|.

Table 1
Comparison of new Acanthamoeba and Herpes simplex virus corneal epithelial keratitis cases presenting to the Emergency Dept at Moorfields between 1st September and 30th Nov 2019. Age range given to maintain anonymity.

Diagnosis	ID	Age range, Sex	Eye	Date of 1st attendance	Days of symptoms before 1st attendance	Initial BCVA	Diagnostic criteria	Treatment before 1st attendance		Final BCVA	Status at last consultation
								For HSV	With steroids		
Epithelial Acanthamoeba keratitis	AK4	30-34, F	R	05/09	5	6/9	IVCM+	N	N	6/6	Cured
	AK5	25-29, M	L	11/09	13	6/5	IVCM+	N	N	6/6	Cured
	AK7	25-29, F	L	26/09	10	6/12	IVCM+, PCR+	N	N	6/6	No active disease but on treatment at last visit
	AK8	30-34, F	L	04/10	21	6/5	IVCM+	N	Y	6/4	No active disease but on treatment at last visit
Epithelial Herpes simplex virus keratitis	HSV1	50-54, F	R	02/09	7	6/18	Clinical	N/A	N	6/6	Cured
	HSV2	45-49, M	L	09/09	10	6/6	PCR+	N/A	N	6/6	Cured
	HSV3	35-39, M	L	03/11	1	6/7.5	Clinical	N/A	N	6/6	Cured


Acanthamoeba keratitis (AK) case incidence per month for 2019 with disease stage at presentation and *herpes simplex virus* keratitis (HSVK) incidence for the 3 month 2019 audit period
HSVK Audit period
8
7
6
5
4
3
2
1
0
January
February
March
April
May
June
July
August
September
October
November
December
Total AK numbers
HSVK numbers
AK disease stage at presentation:
I
II
III
Severe AK