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HSV-1 DNA in Human Corneas: What are the Virological and Clinical Implications?

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EDITORIAL COMMENTARY

The study by Remeijer et al. [1] in this issue conducted at the Rotterdam Eye Hospital in The Netherlands adds to and significantly enhances our knowledge of HSV-1 DNA in human corneas and provides important clinical information about the consequences of the frequency of HSV-1 DNA in the cornea. Patient medical records were reviewed for relevant clinical information including graft survival following penetrating keratoplasty (PKP). The detection of HSV-1 DNA as quantitated by real-time PCR was 40/83 (48%) for herpetic stromal keratitis (HSK) patients and 15/367 (4.1%) for non-HSK patients (Table 1). HSV-1 DNA copy numbers in all corneas were quantified and the HSK corneas were found to contain ~100 times greater viral load than non-HSK corneas. Furthermore, the HSV-1 DNA corneal load correlated with increased age in both HSK and non-HSK patients. Steroid use before PKP was found to increase the viral load.

Graft success was not statistically different between the HSK (56/83, 67.5%) and non-HSK (260/367, 70.8%) patients. Although the presence or absence of HSV-1 DNA was not correlated with graft failure, the viral load in the HSK patients was related to the lack of success. This finding leads us to the question, “How does a higher viral load increase graft failure?”

The graft failure rate in HSK patients with high viral loads suggests the following possibilities. First, patients harbored in their neurons a latent strain of HSV-1 that could be characterized as a high phenotypic reactivator. High phenotypic reactivators are known to undergo significantly more reactivation episodes than low phenotypic reactivators [2,3]. A second possibility is that the corneal rim in the PKP recipient contained a sufficient amount of HSV-1 DNA to initiate replication, migrate to the transplanted cornea, reactivate, and initiate an immune response, leading to graft rejection. A third possibility is a combination of these two.

Graft failure occurred in the HSK patients receiving intense antiviral and anti-inflammatory therapy both preoperatively and postoperatively [4,5]. This long-term combination therapy is often unsuccessful in patients with chronic HSK, thus highlighting the urgent need for new

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combination therapies for this blinding eye disease [4]. Preoperative steroid treatment before PKP significantly increased the viral load of the recipient corneas and decreased the success rate following PKP [1]. These findings confirmed previous reports of the hazards of steroid therapy to the corneas of HSK patients [4,5].

Good news, problems, and dilemmas abound in the chemotherapy for epithelial HSV-1 keratitis, HSK, and chronic HSK. The good news is that the majority (90-95%) of ocular herpes is unilateral. The other good news is that topical and systemic anti-herpetic drugs for ocular herpes are very effective (success rate ~ 90%) with limited toxicity (~ 2-5%). However, with recurrent episodes of ocular herpes, clinical problems arise and therapy becomes complicated. When the patient develops HSK resulting from a reactivation of a previous HSV-1 infection, he/she is placed on intense topical and systemic antiviral and anti-inflammatory drugs. Although some patients with chronic HSK can be stabilized, many do not respond to therapy. These patients have increased stromal opacity (decreased visual acuity) and increased corneal neovascularization [1,4,5].

As noted by Remeijer et al. [1], steroid pretreatment of potential PKP patients increased the corneal load of HSV-1 DNA and decreased the graft survival. Another factor is the clinical nature of the patients who required anti-inflammatory therapy. The clinician is faced with a dilemma. The immune-associated stromal opacity may be stabilized using an anti-inflammatory covered with a potent anti-herpetic [4,5]. However, the increased neovascularization and the use of steroids both increase the risk of graft failure. This clinical quandary has not been solved. These patients will have a loss of vision if the HSK is not treated aggressively. Thus, the corneal transplant has a reduced chance of success considering these high risk factors. Effective treatment is needed to block or reduce recurrent HSK episodes which would consist of 1) an antiviral that would block or significantly reduce neuronal reactivation of HSV-1; 2) a therapy to block or reduce the anterograde transport of the virus from the neuron to the cornea; 3) an anti-inflammatory to reduce the corneal immune response, while not eliciting an increase in the ocular viral load; and, 4) a drug to block or significantly reduce corneal neovascularization. However, this ideal combination therapy is not currently available.

How do the data from Remeijer et al. [1] compare with other similar studies? Table 1 is a summary of Remeijer et al. [1] and six other studies. The range of HSK corneas positive for HSV-1 DNA was 32-86% with an average of ~40%. For non-HSK corneas positive for HSV-1 DNA, the range was 0-40% with an average of ~10%. Five studies did not quantify the viral load. Remeijer et al. [1] not only quantified the HSV-1 DNA load per cornea but collected relevant clinical data on each patient and correlated that to numerous parameters related to graft survival as noted above. No other study to date has made all of these comparisons.

The detection of HSV-1 DNA in corneas allows us to speculate on the possibility that (1) the cornea is a non-neuronal site of HSV-1 latency and (2) the cornea is a depot for reactivated and transported virions from latent neurons. If the latter is true, the "dormant" nature of HSV-1 latency is in question. Perhaps the "persistent" nature of HSV-1 in neurons and cornea will evolve.

Tables 2 and 3 are summaries of the detection of infectious HSV-1 and HSV-1 DNA from tears of healthy human volunteers, as detected by cell culture and PCR. For asymptomatic shedding of infectious HSV-1 or HSV-1 DNA, two critical factors increase detection frequency: the sensitivity of the assay, especially the PCR, and the frequency of collection of tears. As early as 1967, Kaufman et al. [6] in the first study of its kind reported the detection of infectious HSV-1 in tears (Table 2). This study included 20 samples per healthy subject, and the result was 36% (4/11) which were positive at least once during the collection period [6]. Table 3 is

a summary of studies on asymptomatic shedding of HSV-1 DNA in tears of healthy humans. In a more recent study by Kaufman et al. [7], 47/50 (94% of subjects) were positive at least once for HSV-1 DNA in tears. Samples were taken twice daily for 30 consecutive days. Of the 10 studies in Table 3, only Kaufman et al. [7] determined the HSV-1 DNA copy numbers, which had a wide variation from as low as 4 copies to more than 24,000 copies per tear sample. Variation in the copy numbers suggests a significant difference in the HSV-1 reactivation phenotype. In a related study, we [8] assayed 174 human trigeminal ganglia (TG) and detected HSV-1 DNA in 90% of these neural tissues. We quantified the HSV-1 DNA and found a wide range of copy numbers from as low as 10 copies to ~3,000 copies per 100 nanograms of host DNA. This suggested that the establishment of HSV-1 latency in the TG is highly variable. The high prevalence of HSV-1 DNA in the human TG was not a function of age or gender [8]. Taken together, the data on HSV-1 DNA in tears, cornea, and TG strongly suggest that most humans harbor HSV-1. In fact in an editorial, Kaufman [9] posed the question, “Does everyone have herpes?” The answer is that the vast majority of humans harbor HSV-1.

What does HSV-1 DNA in the cornea imply? Our perspective is that HSV-1 is latent in the corneas of HSK patients. In fact, Gordon et al. [10] proposed that HSV-1 was latent in corneas. What does the presence of HSV-1 DNA in the non-HSK and eye bank corneas imply? Perhaps the virus is not latent in these non-HSK patients but rather “passing through” following neuronal reactivation. This conclusion is based in part on the high frequency (~90%) of HSV-1 DNA shedding in normal human subjects [7] as well as the finding that ~90% of human TG contain HSV-1 DNA [8]. The article by Remeijer et al. (1) will be highly cited due to its important clinical relevance and contribution to our understanding of HSK and PKP.

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References

- (1). Remeijer L, Duan R, van Dun, JM Wefers, Bettink MA, Osterhaus AD, Verjans GMGM. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. *J Infect Dis*. 2009 In press.
- (2). Toma HS, Murina AT, Areaux RG Jr. et al. Ocular HSV-1 latency, reactivation and recurrent disease. *Semin Ophthalmol* 2008;23:249–73. [PubMed: 18584563]
- (3). Hill JM, Rayfield MA, Haruta Y. Strain specificity of spontaneous and adrenergically induced HSV-1 ocular reactivation in latently infected rabbits. *Curr Eye Res* 1987;6:91–7. [PubMed: 3030660]
- (4). Kaufman, HE.; Rayfield, MA.; Gebhardt, BM. Herpes simplex viral infections. Kaufman, HE.; Barron, BA.; McDonald, MB., editors. Butterworth-Heinemann; The Cornea. Boston: 1998. p. 247-77.
- (5). Pepose JS, Keadle TL, Morrison LA. Ocular herpes simplex: changing epidemiology, emerging disease patterns, and the potential of vaccine prevention and therapy. *Am J Ophthalmol* 2006;141:547–57. [PubMed: 16490506]
- (6). Kaufman HE, Brown DC, Ellison EM. Recurrent herpes in the rabbit and man. *Science* 1967;156:1628–9. [PubMed: 4290539]
- (7). Kaufman HE, Azcuy AM, Varnell ED, Sloop GD, Thompson HW, Hill JM. HSV-1 DNA in tears and saliva of normal adults. *Invest Ophthalmol Vis Sci* 2005;46:241–7. [PubMed: 15623779]

- (8). Hill JM, Ball M, Neumann D, et al. The high prevalence of herpes simplex virus type 1 DNA in human trigeminal ganglia is not a function of age or gender. *J Virol* 2008;82:8230–4. [PubMed: 18550674]
- (9). Kaufman HE. Does everyone have herpes? *Cornea* 2004;23:111–2. [PubMed: 15075877]
- (10). Gordon YJ, Romanowski E, Araullo-Cruz T, McKnight JLC. HSV-1 corneal latency. *Invest Ophthalmol Vis Sci* 1991;32:663–5. [PubMed: 1848213]
- (11). Shimomura Y, Deai T, Fukuda M, Higaki S, Hooper LC, Hayashi K. Corneal buttons obtained from patients with HSK harbor high copy numbers of the HSV genome. *Cornea* 2007;26:190–3. [PubMed: 17251811]
- (12). Robert P-Y, Adenis J-P, Denis F, Alain S, Ranger-Rogez S. Herpes simplex virus DNA in corneal transplants: prospective study of 38 recipients. *J Med Virol* 2003;71:69–74. [PubMed: 12858411]
- (13). Kaye SB, Baker K, Bonshek R, et al. Human herpesviruses in the cornea. *Br J Ophthalmol* 2000;84:563–71. [PubMed: 10837377]
- (14). van Gelderen BE, Van der Lelij A, Treffers WF, van der Gaag R. Detection of herpes simplex virus type 1, 2 and varicella zoster virus DNA in recipient corneal buttons. *Br J Ophthalmol* 2000;84:1238–43. [PubMed: 11049947]
- (15). Openshaw H, McNeill JI, Lin XH, Niland J, Cantin EM. Herpes simplex virus DNA in normal corneas: Persistence without viral shedding from ganglia. *J Med Virol* 1995;46:75–80. [PubMed: 7623011]
- (16). Cantin EM, Chen J, McNeill J, Willey DE, Openshaw H. Detection of herpes simplex virus DNA sequences in corneal transplant recipients by polymerase chain reaction assays. *Curr Eye Res* 1991;10(Suppl):15–21. [PubMed: 1650662]
- (17). Okinaga S. Shedding of herpes simplex virus type 1 into tears and saliva in healthy Japanese adults. *Kurume Med J* 2000;47:273–7. [PubMed: 11197148]
- (18). Khodadoost MA, Sabahi F, Behroz MJ, et al. Study of a polymerase chain reaction-based method for detection of herpes simplex virus type 1 DNA among Iranian patients with ocular herpetic keratitis infection. *Jpn J Ophthalmol* 2004;48:328–32. [PubMed: 15295656]
- (19). Asbell PA, Torres MA, Kamenar T, Bottone EJ. Rapid diagnosis of ocular herpes simplex infections. *Br J Ophthalmol* 1995;79:473–5. [PubMed: 7612561]
- (20). Kaye SB, Madan N, Dowd TC, Hart CA, McCarthy K, Patterson A. Ocular shedding of herpes simplex virus. *Br J Ophthalmol* 1990;74:114–6. [PubMed: 2155653]
- (21). Abiko Y, Ikeda M, Hondo R. Secretion and dynamics of herpes simplex virus in tears and saliva of patients with Bell's palsy. *Otol Neurotol* 2002;23:779–83. [PubMed: 12218634]
- (22). Robert P-Y, Traccard I, Adenis J-P, Denis F, Ranger-Rogez S. Multiplex detection of herpesviruses in tear fluid using the "stair primers" PCR method: prospective study of 93 patients. *J Med Virol* 2002;66:506–11. [PubMed: 11857529]
- (23). Hidalgo F, Melon S, de Ona M, et al. Diagnosis of herpetic keratoconjunctivitis by nested polymerase chain reaction in human tear film. *Eur J Clin Microbiol Infect Dis* 1998;17:120–3. [PubMed: 9629979]
- (24). Kudo E, Shiota H, Kinouchi Y, Mimura Y, Itakura M. Detection of herpes simplex virus DNA in tear fluid of stromal herpetic keratitis patients by nested polymerase chain reaction. *Jpn J Ophthalmol* 1996;40:390–6. [PubMed: 8988430]
- (25). Linder T, Bossart W, Bodmer D. Bell's palsy and herpes simplex virus: fact or mystery? *Otol Neurotol* 2005;26:109–13. [PubMed: 15699730]
- (26). Fukuda M, Deai T, Hibino T, Higaki S, Hayashi K, Shimomura Y. Quantitative analysis of herpes simplex virus genome in tears from patients with herpetic keratitis. *Cornea* 2003;22(Suppl. 7):S55–S60. [PubMed: 14703708]
- (27). Koizumi N, Nishida K, Adachi W, et al. Detection of herpes simplex virus DNA in atypical epithelial keratitis using polymerase chain reaction. *Br J Ophthalmol* 1999;83:957–60. [PubMed: 10413702]
- (28). Yamamoto S, Shimomura Y, Kinoshita S, Nishida K, Yamamoto R, Tano Y. Detection of herpes simplex virus DNA in human tear film by the polymerase chain reaction. *Am J Ophthalmol* 1994;117:160–3. [PubMed: 8116743]

Table 1
HSV-1 DNA in Human Corneas

Authors, Year, Reference	Clinical Condition of Corneas	Positives/ Total	Determination Method
Remeijer et al. 2009 [1]	HSK	40/83 (48%)	Q-PCR
Remeijer et al. 2009 [1]	Non- HSK	15/367 (4.0%)	Q-PCR
Remeijer et al. 2009 [1]	Corneoscleral Rims	2/273 (0.7%)	Q-PCR
Remeijer et al. 2009 [1]	Eye Bank	0/84 (0%)	Q-PCR
Shimomura et al. 2007 [11]	HSK	6/7 (86%)	Q-PCR
Shimomura et al. 2007 [11]	Non- HSK	4/37(11%)	Q-PCR
Robert et al. 2003 [12]	HSK	2/5 (40%)	PCR
Robert et al. 2003 [12]	Non- HSK	6/33 (18.2%)	PCR
Robert et al. 2003 [12]	Eye Bank	2/38 (5.3%)	PCR
Kaye et al. 2000 [13]	HSK	42/51(82%)	PCR
Kaye et al. 2000 [13]	Non- HSK	15/55 (27%)	PCR
van Gelderen et al. 2000 [14]	HSK	10/31(32%)	PCR
van Gelderen et al. 2000 [14]	Non - HSK	13/78 (17%)	PCR
van Gelderen et al. 2000 [14]	Eye Bank	1/23 (4%)	PCR
Openshaw et al. 1995 [15]	Non-HSK	9/24 (38%)	PCR
Cantin et al. 1991 [16]	HSK	8/11(72%)	PCR
Cantin et al. 1991 [16]	Non - HSK	4/11(36%)	PCR

HSK range of positives 32%–86%; average ~40%

Non-HSK range 0–36%; average ~10%

* Quantitative PCR

Table 2
Asymptomatic shedding of infectious HSV-1 detected by cell culture from tears of healthy subjects

Author(s), Year, Reference	N	Individuals: total positives/total subjects	Shedding frequency: total positive swabs/ total swabs	Swab frequency: number of swabs/subjects
Kaufman et al. 1967 [6]	11	4/11 (36%)	4/440 (0.9%)	20
Okinaga 2000 [17]	10	1/10 (10%)	1/1742 (0.06%)	~17
Khodadoost et al. 2004 [18]	10	1/10 (10%)	1/10 (10%)	1
Asbell et al. 1995 [19]	23	1/23 (4.4%)	1/23 (4.4%)	1
Kaye et al. 1990 [20]	24	0/24 (0/0)	0/752 (0%)	~ 31
Totals	102	14/102 (13.7%)	14/3927 (0.4%)	

Range of positives 0-36%; average ~14%

Table 3
Asymptomatic shedding of HSV-1 DNA detected by PCR from tears of healthy subjects

Authors, Year, Reference	N	Individuals: total positive/total subjects	Shedding frequency: total positive swabs/total swabs taken	Swab frequency: number of swabs/subject
Kaufman et al. 2005 [7]	50	47/50 (94%)	941/2708 (34.8%)	60
Abiko et al. 2002 [21]	22	5/22 (22.7%)	5/132 (3.8%)	6
Robert et al. 2002 [22]	93	1/93 (1%)	1/186 (0.5%)	2
Hidalgo et al. 1998 [23]	17	1/17 (5.9%)	1/17 (5.9%)	1
Kudo et al. 1996 [24]	25	1/25 (4%)	1/54 (1.9%)	1
Linder et al. 2005 [25]	10	0/10 (0%)	0/10 (0%)	1
Khodadoost et al. 2004 [18]	10	0/10 (0%)	0/10 (0%)	1
Fukuda et al. 2003 [26]	50	0/50 (0%)	0/50 (0%)	1
Koizumi et al. 1999 [27]	19	0/19 (0%)	0/38 (0%)	2
Yamamoto et al. 1994 [28]	20	0/20 (0%)	0/20 (0%)	1
Totals	316	55/316 (17%)	949/3225 (29%)	

Range of positives 0–94%; average ~17%