OCCURRENCE OF VIRAL DNA IN PAIRED SAMPLES OF CORNEAL RIM AND CORNEA PRESERVATION FLUID

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INTRODUCTION AND PURPOSE

Cornea transplantations have one of the highest success rates amongst all transplantological procedures. Transplanted cornea tissue has demonstrated improved morphological and physiological results in the postoperative period due to modern operating techniques, advanced eye banking methods as well as antiviral and immunosuppressive therapies. However, factors such as extended corneal neovascularisation, a history of graft rejections, previous infections in the affected eye (like herpes virus or other viral infections) as well as epithelial defects are amongst the primary reasons for loss of cornea transparency or complete graft failure.

Corneas intended for transplantation are stored in preservation fluid, which is tested for bacterial and fungal infections. Moreover, cornea donors are also tested for HBV, HCV and HIV. Conversely, testing corneas for viruses is not part of the routine examinations. Newly acquired herpes keratitis (HK) infections occur in 0.9% of corneal transplant patients.[1] The reactivation of the endogenous latent HSV-1 strain is considered as being the main cause of such infections in the case of herpetic etiology. A graft-to-recipient transmission of viral infections also cannot be ruled out. This does not solely apply to herpetic viruses, as adenoviruses may also be transmitted in this way. However, literature only refers to unique cases of this.

The aim of the study was to determine the frequency at which HSV-1, HSV-2 and adenoviruses occur in transplanted corneal tissue as well as in samples of the preservation fluid. The results of testing done during transplantation procedures were combined with an 18 month observation period of the recipient. The purpose of this study was to verify the hypothesis that an infection may be caused by the virus being transferred from donor to recipient along with the transplanted cornea.

METHODS

The study comprised 57 paired samples, consisting of a fragment of corneal tissue (20 – 40 mg) remaining after its trepanation for transplantation surgery and a sample of cornea preservation fluid (0.2 ml). Material for virological studies was obtained during surgery, with all aseptic precautions, at consecutive procedures of cornea transplantation, performed in the period January – September 2011, and immediately delivered to the laboratory.

Viral DNA isolation

DNA was isolated form corneal tissue using a commercially available kit for DNA isolation form tissue samples (Macherley-Nagel NucleoSpin Tissue DNA, Düren, Germany), following the manufacturer's instructions. DNA from the samples of cornea preservation fluid was isolated with a test dedicated to liquid specimens (Roche High Pure Viral Nucleic Acid Kit, Mannheim, Germany), according to the manufacturer's guidance.

Viral DNA detection

DNA of 3 viruses (human herpesvirus 1 – HHV-1, human herpesvirus 2 – HHV-2 and human adenovirus – HAdV) was searched for in paired samples of corneal tissue fragment and a sample of cornea preservation fluid, using real time PCR technique (Roche LightCycler 2.0).
Herpesvirus DNA (HHV-1 and HHV-2), was detected using TaqMan hydrolysis probes (LightCycler HSV-1/HSV-2 Qualitative Kit, Roche Diagnostics, Rotkreuz, Switzerland).
In this study detection of adenovirus DNA was done by means of probes and starters complementary to the gene encoding the hexon protein – representing a conservative region of human adenoviruses. A hydrolysis probe of the TaqMan type, labelled with fluorochrome JOE/560, was used.

RESULTS

Among 57 cornea transplant recipients there were 26 women (45.6%) and 31 men (54.4%). The mean age of recipients was 60.6 years. The presence of herpesvirus or adenovirus DNA was detected in 4 samples, including in 1 case both samples of cornea and its preservation fluid. Viral DNA was present in 3 corneas – HHV-1 DNA in 1 sample (1.8%) and adenovirus DNA in 2 samples (3.5%). In total, positive results were obtained in 3/57 (5.3%) paired samples. The HHV-1 genome was detected both in the fragment of cornea, as well as in its preservation fluid. No HHV-2 DNA was detected in the analysed samples. In the case of adenoviral DNA, it was detected only in corneal tissue samples, while the corresponding samples of preservation fluid were negative.

Analysis of medical records of 2 corneal transplant recipients – who received corneas, which were later found to contain adenoviral DNA – did not reveal any surgical site infection of viral etiology within 1 year after surgery. The cornea, in which HHV-1 DNA was detected both in the donor cornea rim as well as in its preservation fluid, was transplanted to a woman who underwent second surgery of this type due to corneal perforation 2 months after the first surgery.

Analysis of medical records of corneal transplant recipients

We performed an analysis of medical records of corneal transplant recipients – who received corneas, which were later found to contain viral DNA.
Recipient no. 23 was a 31-year old man with keratoconus, who received an adenovirus-positive cornea from a 51-year old woman who died of an intracranial haemorrhage. Analysis of this recipient's medical records did not reveal any surgical site infection of viral etiology within 18 months after surgery. Transplanted cornea remained completely transparent, with visual acuity of 0.9.

Another recipient of an adenovirus-positive cornea transplant (patient no. 44) was an 80-year old man who suffered from bullous keratopathy after extracapsular cataract extraction. The donor was a 55-year old woman who died of subarachnoid haemorrhage. In this recipient, despite intensive and appropriate parenteral and local therapy, the transplanted tissue did not maintained good function and 14 months later it was necessary to perform a re-transplantation.

The cornea, in which HHV-1 DNA was detected both in the donor cornea rim as well as in its preservation fluid, was transplanted to a 52-year old woman (recipient no. 39) who suffered from rheumatoid arthritis. She underwent a second surgical procedure of this type due to corneal perforation 2 months after the first transplantation. The donor of HHV-1-positive cornea was a 32-year old man who died of skull and brain injury. In the post-transplant period the patient suffered from many complications because of which – despite adequate antiviral treatment – a corneal re-transplantation was necessary.

Tab. 1. Positive results of HHV-1, HHV-2 and adenovirus DNA detection in paired samples of cornea rim and cornea preservation fluid

Recipient no.	Sex	Age	HHV-1 DNA	HHV-2 DNA	Adenovirus DNA
23	M	31	---	---	Cornea (+) Preservation fluid (–)
39	K	52	Cornea (+) Preservation fluid (+)	---	---
44	M	80	---	---	Cornea (+) Preservation fluid (–)

Tab. 2. Consolidated data of HHV-1, HHV-2 and adenovirus DNA detection in paired samples of cornea rim and cornea preservation fluid (n=57)

Viral DNA	Sample		Number of positive results (%)
	cornea rim	cornea preservation fluid	
HHV-1	1	1	1 (1.8%) *
HHV-2	0	0	0 (0.0%)
Adenovirus	2	0	2 (3.5%)
Total	3	1	3 (5.3%)

* results pertain to the same pair of samples for a single recipient

CONCLUSIONS

1. The presence of viral DNA was detected in 5.3% of paired samples.
2. Adenoviruses may be more prevalent in donor corneas than herpesviruses HHV-1 and HHV-2.
3. Virological testing of corneas for transplantation should be considered, particularly in the case of donors with risk factors for herpesvirus and adenovirus reactivation.

DISCUSSION

In scientific literature pertaining to infectious complications after corneal transplantation surgery, predominate publications on bacterial or fungal etiology. In contrast to this – apart from single publications– there is lack of data on presence of viruses in donor cornea tissue and risk of their transmission to the recipients. Eye and tissue banks in the USA during a 10-year period (1994 – 2003) issued 340 174 donor corneas for recipients in the USA and 109 009 corneas – for recipients abroad. There were 162 cases of endophthalmitis recorded after corneal transplantation. Analysis showed that cornea infections were more common in recipients who received transplanted cornea from a donor who was hospitalised before death and/or had malignancy. It was concluded that the donor's health status may influence the condition of his or her eye tissues.

In our study the presence of viral DNA was detected in 5.3% of paired samples. In the context of the results of our study – detection of viral DNA in the tissue of donors' corneas – we may postulate that apart from a higher risk of recipient infection caused by colonisation of the donor by hospital strains of bacteria or fungi, in hospitalised donors there might be a higher risk of reactivation of latent viral infection due to immunodeficiency in the course of severe underlying disease or immunosuppressive therapy administered to the donor.

The role of herpes simplex virus (present name: human herpesvirus type 1 or 2, HHV-1 or HHV-2) in etiology of post transplant keratitis remains unexplained. Morris *et al*. detected HSV DNA in 3 out of 80 tested corneas (3.8%), but virus cultures were negative, which may suggest latent infection of corneal tissue. Analysis of post transplant course in these 3 patients did not reveal any complications in the form of keratitis of transplanted cornea or graft disease.

Robert *et al*. described 2 cases of HSV-1 presence in a donor's cornea with negative results of viral culture. However, in one of these recipients of HSV-1-infected cornea endothelial graft failure was diagnosed 4 months after transplantation. The authors postulated that a donor's cornea may contain infectious particles of HSV-1 or HSV-2, which may be transmitted to the recipient. In the literature there are several other reports of documented transmission of HSV virus from a cornea donor to a recipient.

Human adenoviruses (HAdVs) in healthy persons may cause ophthalmic infection, which may have different clinical forms, ranging from nodular conjunctivitis to keratoconjunctivitis, in which severe keratitis develops in 80% of cases. Decreased visual acuity may persist after adenoviral infection for months or years. Adenoviruses (particularly serotypes 8, 19 and 37) may cause chronic multifocal inflammation of the subepithelial layer of the cornea. Ricci *et al*. described a case of adenoviral infection, which caused long-lasting defects in corneal epithelium after the procedure of penetrating keratoplasty. To our best knowledge, however, there are no reports in literature on the transmission of adenoviruses from donor to recipient with the transplanted cornea.

In our study virally-infected corneas were transplanted to 3 donors. In one of them no infectious complications were observed during an 18-month post-transplant period, which could be linked to the transmission of the virus from the donor to the recipient. However, it was a young 31-year old healthy man with normal function of the immune system. In the remaining 2 recipients of the cornea, post-transplant complications led to the graft failure. These recipients were older, with severe immunosuppression as a result of long-term steroidotherapy, in whom reactivation of viruses from their latent form was possible. An etiological link between virus presence in the donor's cornea and complications in the recipient therefore cannot be excluded. Interestingly, the results of our study indicate that adenoviruses may be even more prevalent in donor corneas than herpesviruses HHV-1 and HHV-2.

We postulate that virological testing of corneas for transplantation should be considered, particularly in the case of donors with risk factors for herpesvirus and adenovirus reactivation. Ideally, corneas for recipients with a compromised function of the immune system should be selected for absence of viral genetic material, to minimise the risk of infectious complications.

REFERENCES

1. Remeijer L, Duan R, van Dun JM, *et al*. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. *J Infect Dis* 2009; 200(1): 11–19.
2. Morris DJ, Cleator GM, Klapper PE, *et al*. Detection of herpes simplex virus DNA in donor cornea culture medium by polymerase chain re action. *Br J Ophthalmol* 1996; 80: 654–657.
3. Robert PY, Adenis JP, Denis F, *et al*. Herpes simplex virus DNA in corneal transplants: prospective study of 38 recipients. *J Med Virol* 2003; 71(1): 69–74.
4. Rola A, Przybylski M, Dzieciątkowski D, *et al*. Detection of human adenoviruses with real-time PCR assay using TaqMan fluorescent probes. *Med Dośw Mikrobiol* 2007; 59: 371–377.
5. Mostafaie N, Huber KR, Sebesta C, *et al*. Diagnosis of infection with human herpes viruses in routine laboratory practice. *Clin Chem Lab Med* 2009; 47(9): 1141–1145.
6. Hassan SS, Wilhelmus KR, Dahl P, *et al*. Medical Review Subcommittee of the Eye Bank Association of America: Infectious disease risk factors of corneal graft donors. *Arch Ophthalmol* 2008; 126(2): 235–239.
7. Eye Bank Association of America (EBAA). Medical standards, 2011. Available at: www.restoresight.org.
8. Cleator GM, Klapper PE, Dennett C, *et al*. Corneal donor infection by herpes simplex virus: herpes simplex virus DNA in donor corneas. *Cornea 1994; 13(4):* 294–304.
9. Biswas S, Suresh P, Bonshek RE, *et al*. Graft failure in human donor corneas due to transmission of herpes simplex virus. *Br J Ophthalmol* 2000; 84: 701–705.
10. Zheng X. Reactivation and donor-host transmission of herpes simplex virus after corneal transplantation. *Cornea* 2002; 21(7 Suppl): S90–S93.
11. Pihos AM. Epidemic keratoconjunctivitis: a review of current concepts in management. *J Optom* 2013; 6: 69–74.
12. King D, Johnson B, Miller D, *et al*. Adenovirus-associated epidemic keratoconjunctivitis outbreaks – four states, 2008-2010. *MMWR* 2013; 62(32): 637–641.
13. Chodosh J, Astley RA, Butler MG, *et al*. Adenovirus keratitis: a role for interleukin-8. *Invest Ophthalmol Vis Sci* 2000; 41: 783–789.
14. Ricci F, Missiroli F, Ciotti M, *et al*. Persistent epithelial defect after penetrating keratoplasty caused by adenoviral infectious keratitis. *New Microbiol* 2010; 33: 171–174.
15. Yousuf MA, Zhou X, Mukherjee S, *et al*. Caveolin-1 associated adenovirus entry into human corneal cells. *PLoS ONE* 2013; 8(10): e77462.
16. Nguyen P, Yiu SC. Strategies for local gene therapy of corneal allograft rejection. *Middle East Afr J Ophthalmol* 2013; 20(1): 11–25.
17. Fagerholm P, Lagali NS, Merrett K, *et al*. A biosynthetic alternative to human donor tissue for inducing corneal regeneration: 24-month follow-up of a phase 1 clinical study. *Sci Transl Med* 2010; 2(46): 46–61.