

Corneal esthesiometry and sub-basal nerves morphological changes in herpes simplex virus keratitis/uveitis patients

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Abstract

• **AIM:** To describe and compare corneal sensation and morphological changes of sub-basal corneal nerves by *in vivo* laser scanning confocal microscopy (LSCM) in herpes simplex virus (HSV) keratitis/uveitis and contralateral, clinically unaffected eyes.

• **METHODS:** A prospective clinical study included 30 HSV eyes and 30 contralateral eyes of 30 patients, diagnosed with unilateral HSV keratitis/uveitis. Both eyes underwent a complete ophthalmological examination, Cochet-Bonnet aesthesiometry and LSCM of the central cornea, using the Heidelberg Retina Tomograph III Rostock Cornea Module. After 6mo, the same examination of the HSV affected and contralateral, clinically unaffected eyes was performed.

• **RESULTS:** HSV eyes, as compared to contralateral eyes, demonstrated a significant decrease in mean corneal sensation (3.1 ± 1.6 vs 5.3 ± 0.8 cm), total nerve fibres number (5.7 ± 4.4 vs 15.1 ± 5.4), nerve branches (3.4 ± 3.0 vs 8.4 ± 4.7), main nerve trunks (2.3 ± 1.6 vs 5.8 ± 2.2), and nerve fibres density (7.5 ± 5.6 vs 18.1 ± 5.3 mm/mm², $P < 0.05$). There was no significant difference between keratitis and uveitis eyes in mean corneal sensation and nerve fibres parameters. After 6mo, corneal sensation and sub-basal nerve fibres parameters were increased significantly, but did not reach the parameters of contralateral, clinically unaffected eyes.

• **CONCLUSION:** Corneal aesthesiometry and LSCM in HSV affected eyes reveals a significant decrease of corneal sensation and sub-basal nerve fibres which recovers at

6mo but does not reach the normal level.

• **KEYWORDS:** aesthesiometry; confocal microscopy; herpes simplex virus; keratitis; uveitis

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INTRODUCTION

Herpetic eye disease caused by herpes simplex virus (HSV) is a condition that usually develops as an epithelial, stromal or endothelial keratitis, or uveitis. The disease is a major cause of corneal blindness. The incidence of HSV keratitis is approximately 1.5 million, worldwide, including 40 000 new cases of related blindness each year^[1-3]. In most cases, HSV keratitis/uveitis manifests as a unilateral disease^[4]. After the first episode of infection during the first year, the disease recurs in 10% of patients, within 2y-23%, 5y-36% and 20y-60% of patients^[5].

HSV keratitis/uveitis is a common cause of corneal hypoesthesia, which is associated with a reduced number of sensory nerves plexus at the corneal sub-epithelium layer, and tends to progress with HSV keratitis recurrences^[6-9]. Recurrent episodes of the infection may lead to corneal scarring, opacities or irregular astigmatism^[10]. The nerve loss in HSV-1 infected corneas includes the nerve endings in the epithelium, the nerve plexus at the subepithelium interface, and the large nerve stalks that enter the corneal stroma at the limbus^[11]. Corneal hypoesthesia presents an increased risk for neurotrophic corneal damage^[9].

Mouse models show that after loss of sensory nerves, the corneal stroma gradually reinervates. The innervating nerves sprout and hyperinnervate the corneal stroma, but do not form the nerve plexus at the subepithelium layer or extend neurites into the epithelium, remaining the cornea less sensitive^[9,11]. Hyperinnervation of corneal stroma by sympathetic nerves determines HSV keratitis severity and disturbance of the sensory nerve plexus and epithelial nerve endings regeneration^[9].

Laser scanning confocal microscopy (LSCM) is a non-invasive method of examining the cornea at the cellular level. This method is becoming as an ancillary for slit-lamp biomicroscopy, which has been the gold standard for detecting epithelial defects, stromal oedema and infiltration, keratic precipitates, and iritis due to HSV^[12-13]. Corneal sub-basal nerves, which are reduced due to HSV keratitis/uveitis, can also be clearly visible by LSCM^[14].

Recently, there has been studies analyzing the morphology changes of corneal cells and their associations with changes in corneal innervation and sensation and investigating alterations of sub-basal corneal nerves for patients with HSV keratitis^[12,15-17]. To our knowledge, so far, longitudinal studies that have analyzed this data, and studies that have compared the results between HSV keratitis and uveitis patients have not been performed.

Our research is a longitudinal study with the main purpose to describe and correlate corneal sensation and sub-basal nerve fibres decrease in HSV epithelial, stromal, endothelial keratitis and uveitis compared with contralateral eye; after then, to compare the data of primary and repeated tests after 6mo.

SUBJECTS AND METHODS

Ethical Approval The study was approved by the Biomedical Research Ethics Committee 2015-07-09 No. BE-2-26 and 2017-01-26 No. P1-BE-2-26/2015. Written informed consent was obtained from all subjects who participated in the study.

Subjects A prospective clinical study included 30 HSV eyes and 30 contralateral eyes of 30 patients (16 men and 14 women, mean age 57.5±18.6y, range 33-85). Inclusion criterion was diagnosis of active unilateral HSV keratitis/uveitis, according to the clinical examination. Epithelial keratitis was diagnosed in patients with characterized multiple epithelial blisters filled with fluid, isolated or merged dendritic infiltrates. Herpes stromal keratitis was diagnosed in patients who had stromal opacities or destruction of corneal stroma, ulcerative infiltrates and/or neovascularization. Patients who presented with characterized swelling of the central part of stroma with the ring infiltrate and precipitate on corneal endothelium had endothelial keratitis. Patients with unilateral keratic precipitates, high intraocular pressure and patchy iris atrophy had anterior herpetic uveitis. For all patients, serology tests to detect anti-HSV antibodies were performed. Exclusion criteria were a previous history of other ocular infection, contact lens wearing, diabetes mellitus, glaucoma and previous trauma, intraocular or refractive surgery. Both eyes underwent an ophthalmological examination, Cochet-Bonnet aesthesiometry (Cochet-Bonnet; Luneau Ophthalmologie, Chartres, France) and LSCM (Heidelberg Retina Tomograph 3 with the Rostock Cornea Module, Germany) of the central cornea. The most representative image (typically at a depth of 50 to 70 µm) of

Table 1 Demographic data of the patients with HSV keratitis/ uveitis

Parameters	HSV keratitis			HSV uveitis	Total
	Epithelial	Stromal	Endothelial		
Patients (n)	7	7	9	7	30
Age (y)	50.2±21	70.4±8.8	64±15.7	46.5±19.3	57.5±18.6
Sex (M/F)	5/2	2/5	5/4	4/3	16/14

HSV: Herpes simplex virus.

the sub-basal nerve plexus was selected for analysis of each eye. The corneal nerve analysis was performed using the semi-automated tracing program NeuronJ^[18]. The sub-basal nerves were categorized as main nerves; branches, which branched from the main nerves and the total nerves as main nerves with nerve branches. Nerve density was assessed by measuring the total length of the nerve fibres (main nerves and nerve branches) in mm/mm². Main nerve trunks and nerve branching were defined as the total number in one image. The number of total nerves measured was defined as the number of all nerves, including main nerve trunks and branches in one image and were reported as number per image as described in previous studies^[15,17].

Statistical Analysis The results were analyzed by Kruskal-Wallis and Mann-Whitney tests. The Kruskal-Wallis test was applied to compare the scores for more than 2 independent groups and the Mann-Whitney for the scores of two independent groups. Differences on dependent variables were analyzed by the Wilcoxon signed-rank test. The Pearson correlation coefficient was calculated to determine relationships between nerve density parameters and aesthesiometry results in HSV affected eyes. Differences were considered statistically significant, when *P* values<0.05. After 6mo from the first clinical examination, the same investigation was repeated and the data were compared with the primary test results.

RESULTS

Thirty HSV keratitis/uveitis eyes, including epithelial (*n*=7), stromal (*n*=7), endothelial keratitis (*n*=9) and uveitis (*n*=7), were analyzed and compared with contralateral, clinically unaffected eyes. Demographic data of all subgroups are presented in Table 1.

Although the patients in the subgroup of the stromal keratitis were older, we did not find a correlation between the age and corneal sensation or sub-basal nerve fibres parameters (*P*>0.05). HSV affected eyes showed a significant reduction in the mean corneal sensation and sub-basal nerve plexus parameters when compared with contralateral eyes. In particular, the mean corneal sensation (3.1±1.6 vs 5.3±0.8 cm), mean nerve density (7.5±5.6 vs 18.1±5.3 mm/mm²), the total number of nerve fibres (5.7±4.4 vs 15.1±5.4), the number of main nerve trunks (2.3±1.6 vs 5.8±2.2), and the number of

Table 2 Quantitative analysis of the corneal sensation and sub-basal nerve fibres parameters in HSV keratitis/uveitis subgroups and contralateral eyes mean±SD

Parameters	HSV unaffected eyes	HSV keratitis eyes			HSV keratitis (total) eyes	HSV uveitis eyes
		Epithelial	Stromal	Endothelial		
Eyes (n)	30	7	7	9	23	7
Corneal sensation (cm)	5.3±0.8	4.1±0.7 ^b	1.7±1.4 ^{ab}	2.9±1.9 ^a	2.9±1.7 ^a	3.8±1.2
Nerve fibres density (mm/mm ²)	18.1±5.3	8.4±2.7 ^a	3.8±4.0 ^a	8.5±6.7 ^a	7.0±5.1 ^a	7.7±6.1 ^a
Total nerve fibres number	15.1±5.4	7.2±3.3 ^a	3.6±4.1 ^a	6.5±4.7 ^a	5.8±4.2 ^a	5.5±5.3 ^a
Main nerve trunks	5.8±2.2	2.8±1.3 ^a	1.2±0.8 ^a	3.2±2.3 ^a	2.4±1.8 ^a	2.2±1.3 ^a
Nerve branches	8.4±4.7	4.4±2.2	2.4±3.4 ^a	3.3±2.7 ^a	3.4±2.7 ^a	3.5±4.1 ^a

HSV: Herpes simplex virus. ^aDifference between HSV keratitis and contralateral eyes ($P<0.05$); ^bDifference of corneal sensation between epithelial and stromal keratitis ($P<0.05$).

branches (3.4±3.0 vs 8.4±4.7) were all found to be significantly lower ($P<0.05$).

Subgroup analysis of the HSV keratitis/uveitis eyes demonstrated that the eyes with epithelial, stromal, endothelial keratitis and uveitis had a reduced corneal sensation and sub-basal nerve plexus parameters, as compared with the contralateral eye parameters, including the mean corneal sensation, nerve fibres density, the total number of nerves, the number of main nerve trunks, and the number of branches, some of which reached statistical significance (Table 2).

A comparison between keratitis subgroups demonstrated that the stromal keratitis group showed a more profound decrease in sensation and sub-basal nerve parameters as compared with the epithelial and endothelial subgroups, however only the corneal sensation results between stromal and epithelial keratitis reached statistical significance ($P<0.05$; Table 2). HSV keratitis eyes, as compared with HSV uveitis eyes, showed a decrease of corneal sensation (2.9±1.7 vs 3.8±1.2 cm), sub-basal nerve fibres density (7.0±5.1 vs 7.7±6.1 mm/mm²) and nerve branches (3.4±2.7 vs 3.5±4.1) parameters, but did not reach statistical significance ($P>0.05$). The decrease in corneal sensation significantly correlated to the diminishment of the density of sub-basal corneal nerve fibres in patients with HSV keratitis/uveitis ($r=0.6$; $P<0.05$).

After 6mo, the same examination of HSV, affected and contralateral eyes was performed, and compared with the primary examination data. Corneal sensation and the parameters of sub-basal nerve fibres were increased: corneal sensation (4.6±1.3 vs 3.1±1.6 cm; $P<0.05$), nerve density (12.2±4.7 vs 7.5±5.6 mm/mm²; $P<0.05$), the total number of nerves (8.6±3.5 vs 5.7±4.4; $P<0.05$), the number of main nerve trunks (3.2±1.3 vs 2.3±1.6; $P<0.05$), and the number of branches (5.6±3.1 vs 3.4±3.0; $P<0.05$), however the mean nerve density, the total number of nerves, the number of main nerve trunks and the number of branches still did not reach the contralateral eyes parameters ($P<0.05$).

DISCUSSION

Cornea is the most densely innervated tissue in the human

body, supplied by the terminal branches of the ophthalmic division of the trigeminal nerve. Small nerve branches from the anterior stroma penetrate Bowman's layer and run between Bowman's layer and the basal epithelium, configuring the sub-basal nerve plexus. Corneal innervation provides not only corneal sensation and a protective function, but also plays an important role on the regulation of epithelial integrity, proliferation, and wound healing^[19-21]. During our study we compared the parameters of sub-basal nerve fibres in HSV keratitis and uveitis patients and presented a decrease of sub-basal nerve fibre parameters by LSCM in HSV affected eyes, and correlated LSCM findings with corneal sensation.

The diminishment of the corneal sensation and sub-basal nerve plexus was noted in all subgroups, this was more severe in the stromal keratitis group, however only the corneal sensation decrease between stromal and epithelial keratitis reached statistical significance. Nagasato *et al*^[17] found that sub-basal nerve changes were more severe in epithelial and stromal types of HSV keratitis than in the endothelial type compared with controls. The study of Hamrah *et al*^[15] showed a significant correlation between corneal sensation and sub-basal nerve fibre parameters.

In addition to the examination of the eyes with HSV, we compared the results of diseased and contralateral eyes. We analyzed the results of each HSV keratitis/uveitis subgroups (epithelial, stromal, endothelial keratitis and uveitis) and found a significant decrease of corneal sensation and sub-basal nerve fibre parameters as compared to contralateral eyes. The sub-basal nerve density in normal eyes is about 21.7 mm/mm²^[22]. In our study, we found that the sub-basal nerve density of contralateral eyes was 18.1±5.3 mm/mm². These findings suggest that HSV corneal disease could potentially lead to a loss of corneal innervation not only in HSV affected, but also in contralateral, clinically unaffected eyes. Similar results were presented in Moein *et al*^[23] study.

Corneal nerves may be damaged due to many ocular and systemic pathological conditions, such as acute ocular infection, herpetic eye disease, diabetes, *etc*. Corneal nerve

dysfunction causes a partial or complete neurotrophic keratopathy, which can result in persistent epithelial defects, stromal thinning and perforation, vision loss or blindness^[24]. HSV keratitis is usually unilateral (the incidence of bilateral is 3%-11.9%), even though bilateral corneal nerve changes have been found in our and previous studies^[15,17,25]. The exact mechanisms of contralateral eye changes are still unclear. It could be explained by the central nervous system, mediated contralateral effects, where central nervous system pathways are responsible for affecting of contralateral undamaged neurons^[26-27].

For the first time entering in the human body HSV virus through the retrograde axonal path enters the sensory ganglia, where remains lifelong in a latent state. HSV might travel from trigeminal ganglia between nerve anastomosis to the contralateral mesencephalic trigeminal nucleus. It allows primary trigeminal fibres to cross the pontine tegmentum to reach the contralateral principal nucleus and to cause contralateral damage to the distal nerve plexus without contralateral clinical manifestations^[25,28]. These anatomic variations make it possible that unilateral nerve damage may result in bilateral changes, although corneal sensory innervation is mediated by unilateral ophthalmic nerve pathway. In addition, biochemical mechanisms may also play a role by releasing inflammatory mediators from neurons^[26-27,29].

We found that after six months corneal sensation and sub-basal nerve fibre parameters were significantly increased, as compared with the data of the previous examination; however the mean nerve density, the total number of nerves, the number of main nerve trunks and the number of branches still did not reach the parameters of the clinically unaffected eyes. The similar results were presented in Moein *et al*^[23] study, where corneal sensation and sub-basal nerve parameters were measured after 37mo of the disease manifestation.

Although regeneration of corneal nerves is still not fully explained process, it showed that during corneal damage increased immune response correlates with decreased innervations^[30]. Cruzat *et al*^[30] demonstrated a strong and significant correlation between the increase in dendritiform cell density and the decrease in sub-basal corneal nerves in patients with infection keratitis, suggesting a connection between corneal immune response and nerve alterations.

Chucair-Elliott *et al*^[31] in mouse model showed that HSV infected corneas significantly retain both structure and sensitivity of the corneal nerve network after levels of pro-inflammatory cytokines and influx of macrophages and CD8⁺ T cells into the cornea are reduced. The same researcher demonstrated that semaphorins, which are axonal growth cone guidance molecules, also play a role in corneal reinnervation^[11].

Injured corneal stromal fibroblasts release semaphorins,

neurotrophins, immune regulatory factors and matricellular proteins and play a role in nerve regeneration growth, differentiation and wound healing^[32]. Nerve regeneration and inflammation processes might share common pathways in tissue repair. Inflammation management could be a strategy for improving corneal nerves regeneration.

A limitation of the present study may be related to the small sample of tested patients, the evaluation of only central part of the cornea, Cochet-Bonnet aesthesiometer, which only measures mechanical nociceptors, and LSCM testing method. LSCM is a contact diagnostic tool, thus it may cause an ocular discomfort to a patient leading to increasing eye movements that may blur the images. In this study, we compared the results between HSV keratitis/uveitis and contralateral, clinically unaffected eyes, but we did not have a healthy control group. This is the main flaw of this study. Therefore, a larger clinical research must be performed to provide more detailed results.

The results of our study could be informative for clinical practice as a part of diagnostic procedures, which help to diagnose HSV keratitis and uveitis, to determine exactly the depth of keratitis, to value the severity of infection, and corneal cells damage. Furthermore, the obtained results could aid clinical practitioners in defining of prognostic factors for the disease outcomes.

LSCM (HRTII-RCM) is a relatively new, non-invasive, painless technique that does not carry any risk of complications^[33]. LSCM and corneal Cochet-Bonnet aesthesiometry allows to observe corneal sub-basal nerves morphology, density and function. The study of corneal sensory innervation and sensation in HSV affected corneas demonstrates a significant decrease of analyzed parameters and a positive correlation between corneal sensation and sub-basal nerve plexus density. The examination of the same patients after 6mo reveals the significant increase of corneal sensation and sub-basal nerve parameters; however the parameters of corneal sub-basal nerves still do not reach the clinically unaffected eyes.

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