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Original Research Article

Assessment of the Changes of Corneal Nerve-Morphology and Tear Indices in Patients with Neurotrophic Keratitis (NK)

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Abstract:

Aim: The aim of the present study was to characterize changes of corneal nerve-morphology and tear indices in patients with neurotrophic keratitis (NK) treated with recombinant human nerve growth factor (rhNGF).

Material & Methods: This study was conducted in Department of Regional Institute of Ophthalmology for one year, and followed the tenets of the Declaration of Helsinki. The diagnosis and treatment processes were described in detail to the patients and their families, and informed consent was signed with the consent of the patient's families. 30 patients with NK diagnosed in Department of Regional Institute of Ophthalmology were collected.

Results: Corneal fluorescence staining using a slit lamp showed that all patients had corneal epithelial defects. After 2wk of treatment, complete corneal healing was observed in two eyes of one patient; after 4wk of treatment, corneal ulcers were completely healed in four eyes of three patients; and after 8wk of treatment, all corneal ulcers of patients were completely healed. After 8wk of rhNGF treatment, the score of corneal fluorescent staining was significantly lower than that of the baseline, and the difference was statistically significant. There was no significant difference in the height of the tear river before and after rhNGF treatment (P=0.212), with the same height of the tear river after stimulation (P=0.089). After 8wk of treatment, there was a significant increase in the thickness of the lipid layer (P=0.048). The density of subbasal nerve fibers significantly increased from the second week to the eighth week after treatment, which was statistically significant (P<0.01). At the same time, the corneal nerve fiber density increased gradually with the progression of treatment. When the density of nerve fibers increased, the number of ramification and bifurcation points of subbasal nerve fibers significantly increased (P=0.002, P=0.004).

Conclusion: RhNGF can increase the density of corneal sub-basal nerve fibers, promote the healing of persistent corneal epithelial defects and corneal ulcers in patients with NK, also improving tear function partially.

Keywords: Recombinant Human Nerve Growth Factor; Neurotrophic Keratitis; Corneal Sub-basal Nerve.

Introduction

Neurotrophic keratitis (NK) is a degenerative disease of the cornea caused by trigeminal nerve injury, including virus infection, trauma, surgery, inflammation and other factors, decreased corneal perception and nutritional disorder after denervation, weakened defenses against external harmful factors, dry eye, corneal epithelial defects and corneal ulcers, and corneal stroma melting and perforation. [1] Epithelial breakdown can result in ulceration, infection, melting, and perforation secondary to poor healing. [2] Any condition or disorder that affects corneal nerves can cause NK. Since the cornea has no blood vessels, it relies on nutrients in tears and neuromediators released by corneal nerves for trophic support. Neurotrophic keratitis causes a decrease in reflex tears, and as corneal sensation decreases, less tears are produced in response to corneal stimuli. This can lead to the corneal epithelium drying out and eventually breaking down. [3]

An estimated prevalence of less than 5/10,000, affecting 6% of herpetic keratitis cases, 12.8% of herpes zoster keratitis cases, and 2.8% of patients

who underwent surgical procedures for trigeminal neuralgia. [4] However, in patients with corneal ulcers, the percentage of cases of NK is relatively high, between 13% and 27%. [5] The disease can result in serious loss of visual acuity (VA) or even blindness and poses substantial diagnostic and treatment challenges to ophthalmologists. Alterations in corneal nerves lead to impairment of sensory and trophic function, with breakdown of the corneal epithelium, affecting health and integrity of the tear film, epithelium, and stroma. [6,7] Damage at any level of the trigeminal nerve, from the trigeminal nucleus to the corneal nerve endings, may cause the development of NK. Common causes identified include herpetic infection, intracranial lesion, neurosurgery procedures, trauma, corneal dystrophy, diabetes, dry eye disease, or anterior eye surgery involving nerve transection. However, the relationship between the underlying etiology and the severity of clinical outcome of NK in terms of restoration of corneal sensitivity remains unclear. [4]

The principle of NK treatment is to prevent the progression of corneal injury and improve corneal trigeminal innervation, to restore the nutritional supply of corneal nerves, and promote corneal renewal and healing. [8] Current treatments for NK include autologous serum, preservative-free artificial tears, a corneal bandage lens, and an amniotic membrane transplantation. [9] Recently, a novel recombinant human NGF (rhNGF) produced in Escherichia coli for the treatment of ocular diseases has been introduced. RhNGF eye-drop formulation has been successfully tested in a phase I clinical trial showing a good safety and tolerability profile. [10] In July 2017, European Medicine Agency licensed full market authorisation for the use of rhNGF (Cenegermin) eye drops for the treatment of moderate and severe neurotrophic keratitis, a rare ocular disease characterised by impairment of corneal sensitivity, healing and tear production. RhNGF (cenegermin, 20 μg/mL of rhNGF) has been clinically used in Europe, United States, etc, and has been shown to aid in the recovery of corneal nerve function. [7,11,12]

Hence the purpose of this study was explored the clinical efficacy of rhNGF for corneal nerve regeneration and corneal epithelial healing in NK patients.

Material & Methods

This study was conducted in department of Regional Institute of Ophthalmology, IGIMS, Patna, Bihar, India for one year,, and followed the tenets of the Declaration of Helsinki. The diagnosis and treatment processes were described in detail to the patients and their families, and informed consent was signed with the consent of the patients' families. 30 patients with NK diagnosed in Department of Regional Institute of Ophthalmology, IGIMS, Patna, Bihar, India were collected.

Inclusion criteria:

Patients diagnosed as moderate or severe NK at our hospital. And after more than 4week of traditional treatment, including antibiotics, artificial tears, autologous sera, corneal bandage lenses etc, the corneal lesion area still was not healed, the depth of keratopathy was less than two-thirds, and there was no perforation and/or stromal melting.

Exclusion criteria:

Infectious corneal ulcers, peripheral corneal ulcers associated with autoimmune diseases, endophthalmitis, corneal dystrophies, glaucoma patients who needed to change the treatment plan midway according to the specific conditions of their eyes, and patients with loss of follow-up and incomplete data records.

Methodology

Visual acuity examination was conducted using the international standard visual acuity chart, and the best-corrected visual acuity (BCVA) at baseline and 8wk after treatment were recorded. For the corneal fluorescence staining score, sodium fluorescein was dropped into the conjunctival sac of the affected eye, the staining of the corneal epithelium was observed with cobalt blue light under a slit lamp, and the corneal staining results were recorded using a 0-12 point scale. First, the cornea was divided into four quadrants involving the supranasal, infranasal, supratemporal, and infratemporal quadrants. According to the degree of staining and staining area, it was divided into 0-3 scores (0: no staining, 1 scores: less than 5 points, 3 scores: flake staining, and 2 scores: between 1 and 3 scores).

Confocal microscopy was used to track the density and the number of nerve branches of the corneal sub-basal nerves. The images were processed anonymously before analysis to avoid statistician bias. The nerve fiber density was calculated in $\mu m/mm^2$. In each picture, the number of sub basal nerve fiber branches and nerve fiber bifurcation. In each picture, the number of sub basal nerve fiber branches and nerve fiber bifurcation points were counted and reported as numbers. Ten different IVCM images were selected to calculate the average corneal nerve density and number of nerve branches and bifurcations. For the corneal sensation test, cotton was twisted at the end of the disinfection cotton swab into a slender cotton yarn and bent to 45° with the cotton stick. The approach involved the side of the tested eye with the tip of cotton filament, which gently touched the cornea.

The resulting judgments involved those with normal corneal sensation who immediately had a reflex blink or perception response. The absence of a blinking reflex indicated a decrease or disappearance of corneal perception.

The tear index used the Keratograph 5M (Oculus, Wetzlar, Germany), which automatically measured the height of the tear river before and after stimulation during non-contact conditions. A LipiView II interferometer (Tear Science Inc, Morrisville, NC, USA) used the white light interference principle and specular reflection method to directly evaluate the color of the tear film interference light, indirect measurement of lipid layer thickness (LLT).

For the conjunctival impression cytology (CIC) examination, a prepared cellulose acetate filter paper was taken from the conjunctiva of the lower eyelid after ocular surface anesthesia and placed on a slide. A total of 1-2 drops of 100 mg/L acridine orange saline solution was added to the filter membrane. After 3-5min, the fluorescence was observed using a fluorescence microscope. According to the number and morphology of cells attached to the filter paper, the cells were graded according to the Tseng classification [13] (grade V).

For the tear ferning (TF) test, tears from the fornix of the affected eye were collected with a capillary glass tube and coated on clean slides. After drying, according to the appearance of the crystal-like branched ferns, it was roughly divided into four grades: grade I was a large and compact continuous dendritic distribution; grade II was a small, sparse, and scattered dendritic distribution; grade III was rarely a dendritic distribution, accompanied by a large number of vacuoles; and grade IV was a complete loss of fern-like appearance, with only the clustered mucus.

Statistical Analysis

The data were analyzed by SPSS statistical software for Windows, version 23.0 (SPSS, Chicago, IL, USA). The Shapiro-Wilke test was used to test the normal distribution, and the mean and standard deviation (SD) were used to represent mean±SD. The NEI scores were averaged for all NK affected eyes and a two tailed paired T test was run using the Microsoft Excel program comparing baseline (pre) and the longest post treatment duration (last visit; post) NEI scores. The pre- and post-treatment mean NEI scores were graphed using the Excel program.

Results

Table 1: Changes of corneal fluorescence staining score, conjunctival imprinting cytology, and the tear fern test at baseline and after rhNGF treatment

Ocular surface related score items	Baseline	2wk	4wk	6wk	8wk	*P* (Friedman)
Corneal fluorescein staining score	5.75±2.48	4.36±3.08	2.72±2.84	1.00±2.55	0.00±0.0	<0.01
Conjunctival Impression cytology grading	2.00±0.79	2.38±0.49	2.36±0.48	2.52±0.81	2.33±0.77	0.512
Tear ferning test grading	2.70±0.52	2.76±0.43	2.69±0.41	2.75±0.42	2.92±0.58	0.550

Corneal fluorescence staining using a slit lamp showed that all patients had corneal epithelial defects. After 2wk of treatment, complete corneal healing was observed in two eyes of one patient; after 4wk of treatment, corneal ulcers were completely healed in four eyes of three patients; and after 8wk of treatment, all corneal ulcers of patients were completely healed. After 8wk of rhNGF treatment, the score of corneal fluorescent staining was significantly lower than that of the baseline, and the difference was statistically significant.

Table 2: Changes of the tear indices in patients at baseline and after rhNGF treatment

Tear indices	Baseline	2wk	4wk	6wk	8wk	*F*	*P*
Tear river height (mm)	0.15±0.05	0.16±0.02	0.18±0.05	0.19±0.09	0.22±0.11	1.62	0.212
Tear river height after stimulation (mm)	0.26±0.07	0.28±0.10	0.23±0.07	0.24±0.06	0.38±0.21	3.80	0.089
LLT (nm)	62.18±17.83	48.32±13.7	49.51±13.73	64.36±24.26	68.32±15.95	3.14	0.048

There was no significant difference in the height of the tear river before and after rhNGF treatment (P=0.212), with the same height of the tear river after stimulation (P=0.089). After 8wk of treatment, there was a significant increase in the thickness of the lipid layer (P=0.048).

Table 3: Changes in the density, branching, and bifurcation points of corneal nerve fibers at baseline and after rhNGF treatment

Corneal nerve fibers	Baseline	2wk	4wk	6wk	8wk	P
Density (μm/mm2)	4844.76± 3176.84	10514.06 ±6240.84	12314.97 ±5420.24	14845.13 ±3879.13	15812.13±3 465.82	<0.01
Ramification (n)	2.88	7.53	9.31	11.19	12.28	0.002
Bifurcation point (n)	2.16	5.14	6.36	7.43	8.02	0.004

The density of sub basal nerve fibers significantly increased from the second week to the eighth week after treatment, which was statistically significant (P<0.01). At the same time, the corneal nerve fiber density increased gradually with the progression of treatment. When the density of nerve fibers increased, the number of ramification and bifurcation points of sub basal nerve fibers significantly increased (P=0.002, P=0.004).

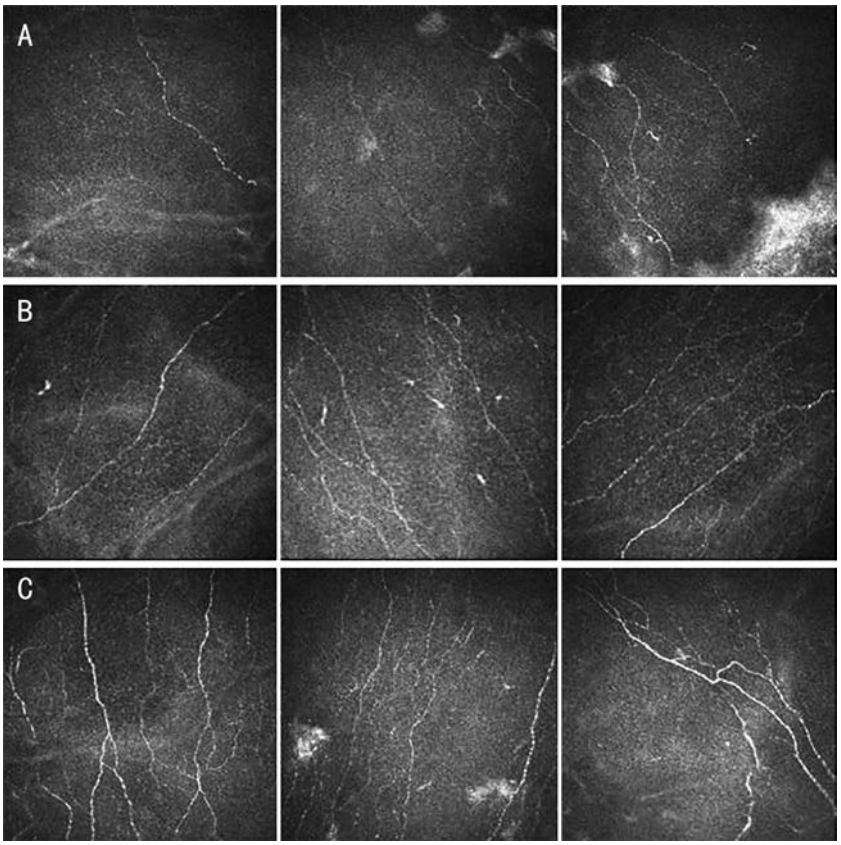

Figure 1: In vivo confocal microscopy analysis Representative images of a case of NK, male, 40-year-old, affected by chemical burn (acid). A: The nerve fibers evaluated at baseline were sparse, uneven in thickness; B: Nerve fiber density, ramification and bifurcation point showed a significant increase at week 4 of treatment with recombinant human nerve growth factor; C: The improvement was maintained at the 8-week follow-up.

Discussion

Neurotrophic keratitis (NK), a rare eye disorder that affects the cornea, is a degenerative disease of the corneal epithelium characterized by decreased corneal sensitivity and poor corneal healing. Having NK can make the cornea more vulnerable to damage and decreases reflex tearing. Epithelial breakdown can result in ulceration, infection, melting, and perforation secondary to poor healing. [2,14,15] Any condition or disorder that affects corneal nerves can cause NK. Since the cornea has no blood vessels, it relies on nutrients in tears and neuromediators released by corneal nerves for trophic support. Neurotrophic keratitis causes a decrease in reflex tears, and as corneal sensation decreases, less tears are produced in response to corneal stimuli. This can lead to the corneal epithelium drying out and eventually breaking down. [16] Multiple ocular and systemic diseases may lead to NK, however there is one common cause: damage to the trigeminal nerve (Cranial nerve V), which is the main nerve that innervates the cornea. When the nerves in the cornea are damaged this causes reduced sensitivity of the cornea and can lead to NK. [17] The most common triggers include herpetic keratitis, chemical burns, corneal/ocular surgery, chronic contact lens use and

prolonged use of topical medications. Intracranial anomalies such as acoustic neuroma, meningioma and aneurysms may compress the trigeminal nerve or ganglion causing corneal sensitivity to be compromised. Another common etiology of NK is diabetes mellitus. Chronic uncontrolled diabetes patients can develop neuropathy involving the lower extremities, which similarly can occur in the cornea leading to sensory loss. [18]

Corneal fluorescence staining using a slit lamp showed that all patients had corneal epithelial defects. After 2wk of treatment, complete corneal healing was observed in two eyes of one patient; after 4wk of treatment, corneal ulcers were completely healed in four eyes of three patients; and after 8wk of treatment, all corneal ulcers of patients were completely healed. After 8wk of rhNGF treatment, the score of corneal fluorescent staining was significantly lower than that of the baseline, and the difference was statistically significant. There was no significant difference in the height of the tear river before and after rhNGF treatment (P=0.212), with the same height of the tear river after stimulation (P=0.089). After 8wk of treatment, there was a significant increase in the thickness of the lipid layer (P=0.048). The density of sub basal nerve fibers significantly increased from the second week to the eighth week after treatment, which was statistically significant (P<0.01). At the same time, the corneal nerve fiber density increased gradually with the progression of treatment. When the density of nerve fibers increased, the number of ramification and bifurcation points of sub basal nerve fibers significantly increased (P=0.002, P=0.004). Sensory innervation injury leads to the decrease or complete loss of corneal sensitivity, resulting in refractory corneal ulcers, including NK. Regarding the relationship between corneal nerve and epithelial cell integrity [19], the corneal nerve promotes corneal epithelial cell proliferation, migration, and differentiation, as well as nerve development and survival by releasing a variety of nerve mediators, which involve substance P, calcitonin gene-related peptide, acetylcholine, norepinephrine etc. In addition, corneal epithelial cells release a variety of neurotrophic growth factors, including NGF, ciliary neurotrophic factor, and glial cell line derived neurotrophic factor, which are the basis of ocular surface balance and wound healing. [20]

Mastropasqua et al [21] evaluated the healing of the corneal nerve and epithelium after treatment of NK with rhNGF. The results showed that corneal ulcer healing was associated with corneal subbasal nerve regeneration, and the average density of sub basal nerve fibers was significantly higher than the baseline at the 4th and 8th weeks after treatment. The density of nerve fibers, the number of nerve branches, and the diameter of nerve fibers significantly increased, and the differences were statistically significant. These morphological changes were observed as early as 4wk after treatment, but the rate of improvement decreased after 4wk. However, the corneal nerve density, nerve fiber branches, and diameter did not reach values observed in the healthy control group. Our study showed that the density of subbasal nerve fibers and the number of nerves in NK patients significantly increased after treatment. The difference before and after treatment was statistically significant but did not reach the corneal nerve fiber density of healthy patients reported in previous studies.

Conclusion

In conclusion, we found that rhNGF can stimulate the healing of corneal epithelial cells, contribute to the repair and regeneration of injured nerves with NK, and obtain a good clinical effect. However, this study failed to obtain the changes in the sensory function of corneal nerve fibers, which needs further study. In short, rhNGF is a welcome non-surgical treatment option for this challenging degenerative disease. RhNGF can increase the density of corneal sub-basal nerve fibers, promote the healing of persistent corneal epithelial defects and corneal ulcers in patients with NK, also improving tear function partially.

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