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Diabetic Peripheral Neuropathy Triggers Herpes Simplex Keratitis Recurrence: A Novel Molecular Mechanism Study and Hypothesis

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Abstract

Diabetes Mellitus (DM) most commonly affects the retina, irides and crystalline lens. However, never is known about the Diabetic Peripheral Neuropathy (DPN) triggered Herpes Simplex Keratitis (HSK). The interaction between HSK and DPN was also investigated to attempt to provide an assistant for clinical diagnosis. A 62-year-old Chinese man, with a history of diabetes for many years, was recruited in this study. Laboratory Examinations were employed to diagnose DM. Routine examination, slit-lamp examination, cornea confocal microscope and indirect Immunofluorescence assay (IFA) were employed to examine Ophthalmological disease. Electromyogram (EMG) and Michigan Neuropathy Screening Instrument (MNSI) were utilized to diagnose DPN. By ophthalmological examination and IFA assay, patient was diagnosed as epithelial HSK. However, anti-viral treatment could not significantly improve corneal symptoms. Meanwhile, by laboratory examinations and neurological analysis, this patient was diagnosed as DPN combined with epithelial HSK finally. By treating with anti-viral drugs and anti-DPN drugs, the symptoms of cornea were significantly improved. In conclusion, we reported and investigated the DPN combined with epithelial HSK for the first time. DPN might trigger HSK in patient with history of diabetes mellitus. Anti-viral drugs together with anti-DPN drugs would be a promising program for this kind of patient.

Keywords: Herpes simplex keratitis; Diabetic peripheral neuropathy; Trigeminal ganglion; Neuron injury

Introduction

Diabetic peripheral neuropathy (DPN) is a kind of nerve damage caused by diabetes mellitus (DM). DPN leads to numbness, loss of sensation, and sometimes pain in the patient's feet, legs, hands, or face [1,18]. Normally, having diabetes for several years may increase the likelihood of having DPN. HSK mainly caused by the herpes simplex virus 1 (HSV1), and both of the morbidity and blindness are document among the keratitis diseases. The characteristic of HSK is the acute or chronic inflammation induced by HSV, and the recurrence [7]. Although more commonly reported that DPN could affect the sensory nerves, motor nerves, or autonomic nerves, the affection of the optic nerves has never been reported.

In clinical, we discovered a rare case of epithelial HSK in patient with diabetes, which has never been reported and investigated in the previously published studies. Thus, in the present study, we studied this patient through detailed experiment and clinical therapy.

Materials and Methods

Clinical description

A 62-year-old Chinese man had a history of diabetes for many years, who was recruited for this study after the symptoms of redness, tearing, blurred vision in right eye for one month. Furthermore, the patient reported that he often unable to put pressure on his feet and would have to sit or lay down to relieve the pain.

The ethics committee of the No.1 Hospital of Xi'an approved this study. The patient has given his consent for this study.

Ophthalmological examination

Cornea confocal microscope observation:

A minimum of 3 representative images of the subbasal nerve plexus were selected for analysis for each eye. Two masked observers evaluated the confocal images for corneal nerve morphology and analyzed the subbasal nerve plexus as described previously [3]. Briefly, nerve density was assessed by measuring the total length of the nerve fibers in micrometers per frame (158, 700 μm^2). Main nerve trunks were defined as the total number of main nerves in one image. Nerve branching was defined as the total number of nerve branches in one image. The number of total nerves was defined as the number of all nerves, including main nerve trunks and branches in one image [15].

Slit lamp biomicroscopy:

The level of corneal haze in patient was gauged by slit lamp microscope (BX 900 Slit Lamp, Haag-Streit, USA) examination before therapy with only anti-viral drugs or combining treatment, as described early [16]. Grade 0 was a completely clear cornea; grade 0.5 had trace haze seen with careful oblique illumination with the slit lamp biomicroscope; grade 1 was more prominent haze not interfering with the visibility of fine iris details; grade 2 was mild obscuration of iris details; grade 3 was moderate obscuration of the iris and lens; and

grade 4 was complete opacification of the stroma in the area of ablation. The Grade of the haze in patient was also calculated by the haze area size, with the unit of "mm^2". Haze grading was performed by two observers (RRM, FGR, AS, AT and/or JTR) in a masked manner.

Indirect immunofluorescence assay (IFA):

Corneal scraping smears were obtained by pressing the surface of one end of the slide firmly, but gently on the corneal lesion. Corneal scraping smears were collected for the detection of viral antigen. Scraping smears were air dried for 30 minutes at room temperature and fixed in acetone for 30 minutes at -20°C. Scraping smears were stained by IFA for the detection of HSV1 antigen using a polyclonal antibody to HSV1, as described elsewhere [17]. The entire smear was screened for positively stained cells or tissues (infected cells) with green staining.

Laboratory examinations:

Blood samples were obtained at 8 a.m. after 12 hours of fasting. Blood glucose, including fasting plasma glucose (FPG) and 2-hour post-loading plasma glucose after 75 g oral glucose tolerance test (2 h OGTT PPG), was measured by using glucose oxidase method on an autoanalyzer (Modular P800; Roche, Basel, Switzerland). Type 2 diabetes was diagnosed according to the 1999 World Health Organization (WHO) criteria (FPG ≥ 7.0 mmol/l and/or 2 h OGTT PPG ≥ 11.1 mmol/l). Impaired glucose regulation (IGR) was defined as impaired fasting glucose (IFG, FPG ≥ 6.1 and<7.0 mmol/l). Glycosylated hemoglobin (HbA1c) was measured by immunoturbidimetric method (C8000 device, Abbott Company, USA). HbA1c value more than 6.5% is defined as diabetes. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and low-density lipoprotein cholesterol (LDL) were measured using chemiluminescence methods on the autoanalyzer (Modular E170; Roche).

Neurological examination

Electromyogram (EMG) analysis:

EMG was performed in the patient involved in this study. Nerve conductions were studied using Nihon Kohden MEB-9104K neuropack μ device (Tokyo, Japan). Nerve conduction studies were as follows: ulnar nerve sensory and motor conductions in upper extremity and deep peroneal motor, posterior tibial motor, and sural nerve conductions in both lower extremities. Based on the results of a study of the Chinese population, the normal limits for nerve conduction evaluations were determined as follows: distal latency for ulnar nerve motor conduction was 3.3 ms; Compound Muscle Action Potential (CMAP) amplitude was 7 mV, motor conduction velocity was 39.6 m/s; ulnar nerve distal sensory conduction speed was 37.3 m/s, amplitude was 7 μV; deep peroneal nerve motor conduction distal latency was 5.8 ms, amplitude was 3.6 mV, conduction speed was 40.9 m/s, F response latency was 52 ms; sural nerve conduction speed was 33.8 m/s, amplitude was 5 μV. At least two pathological nerve conductions, one of which was in the sural nerve, led to symmetric polyneuropathy diagnosis [9,13].

Michigan Neuropathy Screening Instrument (MNSI):

The MNSI questionnaire is self-administered. Responses were added to obtain a total score. 'Yes' responses to questions 1-3, 5-6, 8-9, 11-12, 14-15 are each counted as one point. 'No' responses to questions 7 and 13 each count as one point. Question 4 was considered to be a measurement of impaired circulation and question 10 a measure of general asthenia and were not included in the published scoring algorithm [4]. A score more than 7 was considered as abnormal [4]. All 15 questions were included in the new scoring algorithms.

During the MNSI examination, a health professional inspects each foot for deformities, dry skin, calluses, infections and fissures. Each foot with any abnormality receives a score of 1. Each foot is also inspected for ulcers and each foot with an ulcer receives a score of 1. The ankle reflexes are also elicited. If the reflex is absent, the patient is asked to perform the Jendrassic manoeuver and, if present, the reflex is designated as present with reinforcement and is scored as 0.5. If the reflex is absent with the Jendrassic manoeuver, the reflex is designated as absent and is scored as 1. Vibration sensation is then tested in the great toe using a 128 Hz tuning fork. In general, the examiner should be able to feel vibration in his or her hand for 5 s longer than a normal subject can at the great toe. Vibration is scored as present if the examiner senses the vibration on his or her finger for<10 s longer than the subject feels it in the great toe, decreased if sensed for ≥ 10 s (scored as 0.5) or absent (scored as 1). The total possible score is 8 points and, in the published scoring algorithm, a score ≥ 2.5 is considered as abnormal [4].

Statistic analysis:

All data are analyzed by SPSS 20.0, and presented as the mean ± SD. Statistical analysis was performed using the *t*-test.

Results

Ophthalmological routine examination

For the ophthalmological routine examination, vision in the left eye was 0.2 and 0.1 in right eye. Slit-lamp examination indicated the ciliary body congestion, corneal bedewing, anterior chamber flare (-), keratic precipitates (KP) (-) and no descemet membrane folds and hypopyon. Furthermore, a geographic epithelial lesion with the size about 3×3 mm2 was also discovered (Figure 1A).

Laboratory characteristics

Laboratory data of the present patient in this study is given in Table 1. Mean values for LDL, TG, HDL and TC were 105.7 mg/dL, 171.8 mg/dL, 41.5 mg/dL and 243.9 mg/dL, respectively. Mean HbAc1 value was 7.9 (%). The FPG level and OPPG level were 9.6 mmol/L and 14.6 mmol/L, respectively.

Neuropathy characteristics

For the neuropathy assessment, the score of the patient obtained in the MNSI questionnaire was 9 points. After the questionnaire, physical examination part of MNSI was applied to the patients. According to MNSI, diabetic peripheral neuropathy score detected in the patient was 3.5 points. Together with the result of EMG evaluation, neuropathy was diagnosed in this patient. Furthermore, this patient had both of sensory and motor neuropathies. The above results were also indicated in Table 1.

Index	Total
FPG (mmol/L)	9.6
OPGG (mmol/L)	14.6
HbAc1 value (%)	7.9
LDL (mg/dL)	105.7
TG (mg/dL)	171.8
HDL (mg/dL)	41.5
TC (mg/dL)	243.9
MNSI questionnaire score	9
Mean examination score	2
Diabetic peripheral neuropathy with MNSI (MNSI ≥ 2.5)	3.5
Diabetic peripheral neuropathy with EMG	Yes

Table 1: Laboratory and neurological characteristics of the patient. FPG: plasma glucose; OGTT: Oral Glucose Tolerance Test; TG: Triglycerides; TC: Total Cholesterol; HDL: High-Density Lipoprotein cholesterol; LDL: Low-Density Lipoprotein cholesterol; HbAc1: **Hemoglobin A1c; MNSI: Michigan Neuropathy Screening Instrument; EMG: Electromyogram.**

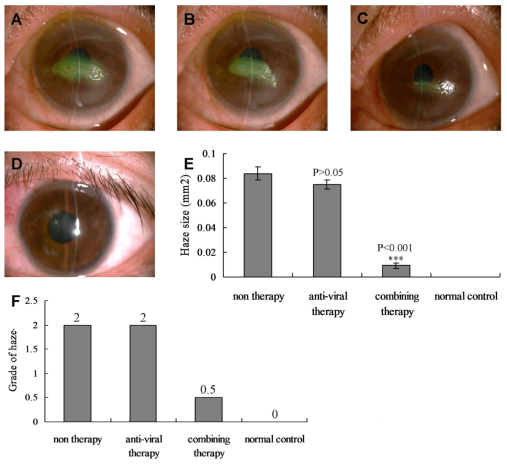

Figure 1: Slit-lamp biomicroscopic images and haze grade of the right eye at presentation. A. Geographic-like epithelial lesion of the cornea when un-treated with drugs; B. Epithelial lesion of the cornea when treated with only anti-viral drug; C. Combining therapy effects for the epithelial lesion; D. Images of the slit-lamp of the normal individual; E. Haze size of cornea in the patient's right eye and normal individual; F. Grade of haze in patient's right eye and normal individual. The green light slit-lamp image showing the fluorescein staining of the lesion in the right eye at presentation. Statistical differences of the data of B and C group compared with A group were illustrated as P>0.05 and *** P<0.001, respectively.

Corneal examination characteristics

According to the cornea confocal microscope observation, the corneal branch nerve fiber density of subbasal nerve plexus were decreased, the tumid and dendritic nerves increased, and the curving nerves increased under the basal epithelium in the right eye (Figure 2A). The IFA showed obvious fluorescence-positive cells in the cornea scraping smear (Figure 2E). Combining the results of above ophthalmological routine and IFA examination, this patient was diagnosed as epithelial HSK.

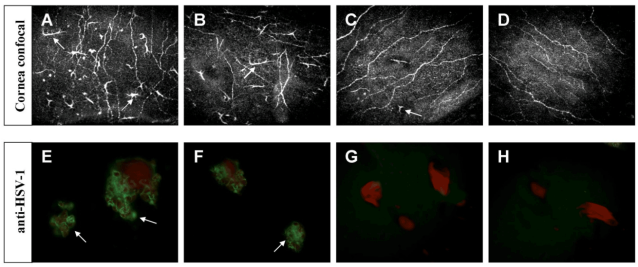

Figure 2: **Ophthalmological** examination images of the right eye in patient and normal individual. Cornea confocal microscope (A to D) and IFA (E to H) analysis were employed to diagnose the HSK. A and E: The images when the patient un-treated with drugs; B and F: The images when the patient treated with only anti-viral drug; C and G: The images when the patient undergoes combining therapy; D and H: The images of the normal individual without any therapy. The arrows in the figure shows the dendritic, tumid and curving nerves in A to D, and HSV positive staining tissues in E to H.

Anti-viral drug interference for epithelial HSK

From the ophthalmological routine examination results, the patient was diagnosed as epithelial HSK. So the patient was treated with topical Ganciclovir ointment 3%, 4 times a day, for two weeks, but no significant improvement regarding the corneal symptoms, visions and ulcers (Figure 1B). For the cornea confocal microscope and IFA analysis, there were not obvious changes of the corneal hazes (Figure 2B) and positive staining cells (Figure 2F). The anti-viral treatment has not improved the corneal haze size (Figure 1E) and grade of corneal haze (Figure 1F), compared with the non drug treatment (patients treated without any drugs for the HSK) (P>0.05). This phenomenon is very rare in the cornea clinical practice. In clinical, Ganciclovir could perform a satisfied effect for the inhibition of HSV, and the symptom would be alleviated after one week treatment.

Combining drug interference for DPN combined with epithelial HSK

According to the MNSI and EMG analysis results, and the diabetes diagnosis, the patient was diagnosed as DPN combined with epithelial HSK finally. We performed a therapeutic method that treat the patient with anti-HSV1 drug and anti-DPN drugs at the same time. For the HSK therapy, continue using the Ganciclovir ointment 3%, and the ancitabine for subconjunctival injection two times one week. For the DPN therapy, we employed the Aldose Reductase Inhibitor (ARI), Epalrestat, take 50mg orally, and three times one day. Meantime, 500 μg Mecobalamin was also intramuscular injected, three times one week, to improve the microcirculation and antioxidation.

Through one week combining therapy, there was a significant improvement for the corneal symptoms, compared with pro-therapy. At the same time, the cornea became clear and light, congestion and

edema disappeared, haze size diminished (Figure 1C). The combining drug interference significantly decreased the corneal haze size (Figure 1E) and grade of corneal haze (Figure 1F), compared with the non drug treatment ($P>0.05$). Furthermore, the corneal symptoms even achieved the condition of the normal cornea (Figure 1D, Figure 2D and 2H). The Cornea confocal microscope examination also indicated that the dendritic, tumid and curving nerves were decreased significantly, compared with the non drug treatment (Figure 2C). No positive HSV1 staining cells were detected by the IFA analysis (Figure 2G). Following up this patient for 3 months, the HSK has not been recurrent.

Discussion

In clinical, the anti-viral drugs can only inhibit the virus replication in the cornea, but can't effectively damage the latent virus in the trigeminal ganglia or cornea. Many inducible risk factors could activate the latent virus, and lead to the recurrence. In the present study, a new risk factor (DPN) which triggers the HSK occurrence was discovered and explored. Our data strongly suggested that combining the anti-DPN and anti-viral drugs could therapy the HSK by inhibiting the occurrence of DPN, so as to receive a satisfied improvement for the epithelial HSK patient with DPN.

Diabetes mellitus most commonly affects the retina [8], irides, crystalline lens which could lead to the occurrence of diabetic retinopathy, diabetic cataract, iridocyclitis. HSK involvement has rarely been reported, and the pathogenesis of the diabetes mellitus is unknown but likely results from a DPN-mediated trigeminal ganglion-injury, leading to the latent HSV1 transmit to the cornea epithelial cells, so as to trigger the occurrence of HSK.

In the present study, the patient was firstly diagnosed as HSK, and treated with anti-viral drugs. Unfortunately, no significant improvements were discovered. According to the history of diabetes of the patient, and the symptoms that who unable to put pressure on his feet and would have to sit or lay down to relieve the pain, the patent was performed the neurological examination, including MNSI and EMG. The neurological examination results and cornea confocal microscope findings obviously showed that the patient underwent the disturbance of DPN. So the patient was finally diagnosed as DPN combined with epithelial HSK. For the diagnosed DPN combined with HSK, we selected the Epalrestat, which is a kind of ARI and plays a role in enhancing the motor nerve conduction and improving the autonomic nerve function [10,14]. Moreover, Mecobalamin was also utilized to repair injured nerve by improving the synthesis of the cellular nucleic acid, protein and phosphatidylcholine [12]. Interestingly, when adopted the combination of anti-DPN drugs and anti-viral drugs, the symptoms were improved obviously. This result indicated that the anti-DPN drugs could repair the damaged neurons and inhibit the transmission of latent HSV1 to the corneal epithelial cells continuously.

Corneal infection by HSV is a common condition that usually develops as an acute or chronic corneal inflammation [2]. HSK is most often due to reactivation of a latent infection of trigeminal sensory neurons innervating the cornea and possibly also of corneal epithelial cells by the neurotropic HSV1 [6,7]. In the present study, the cornea confocal microscope observation showed that the corneal branch nerve fiber density of subbasal nerve plexus decreased, the neurons tumid, and dendritic particles appeared, which indicated that the DPN might induce the injury of the corneal nerves. The damaged trigeminal ganglion cells may be end up with death via apoptosis or the other death pathways. An important characteristic of the virus is that the virus would be released or infect the other cells when the present host cells were dead or necrotic. So the latent HSV1 virus would be released form the damaged trigeminal ganglion cells. What's important is that the HSV1 virus performs a significantly neurotropic characteristic, which allows the HSV1 infect the target cells following the trigeminal ganglion [5]. Thus, the HSV1 virus may be re-activated and migrated to the cornea via the nerve branches, and invade the corneal epithelial cells and induce the HSK.

Furthermore, when the nerves damaged, some neurons such as astrocyte, microglia in the trigeminal ganglion could release a series of pro-inflammatory cytokine, including TNF-α, IL-6 [11,19]. The TNF-α and IL-6 could aggravate the HSV1 virus mediated inflammatory reaction in the HSK, so as to cause the cornea injury in a further step. Thus, may be the impaired cell-mediated immunity associated with long-standing and poorly controlled diabetes, which may also play an important role in the pathogenic process of HSK.

In summary, the DPN caused neurons damage may result in the transmission and reactivation of HSV1 virus, and trigger the herpes simplex keratitis. In the present study, we proposed a hypothesis that DPN damages the optic nerve, activates the latent HSV, and triggers the HSK finally (Figure 3). The DPN should be cautioned, especially in patients with a history of diabetes mellitus, which would activate the latent HSV1 virus in the peripheral neurons and cause the HSK. Anti-viral therapy assistant with anti-DPN therapy may be warranted in an effort to prevent the recurrence or activation of HSK in such patient.

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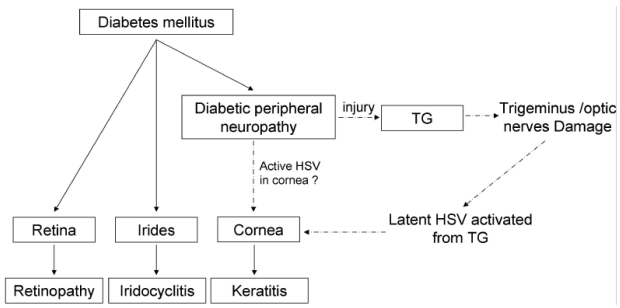

Figure 3: Hypothesized pathogenic pathway for the DPN triggered latent HSV activation and HSK. The solid represents the certain signal pathway, and dotted arrow represents the hypothetical signal pathway. TG: **trigeminal ganglia**.

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