Ocular surface changes in type II diabetic patients with proliferative diabetic retinopathy

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Abstract

• **AIM:** To detect and analyze the changes on ocular surface and tear function in type II diabetic patients with proliferative diabetic retinopathy (PDR), an advanced stage of diabetic retinopathy (DR), using conventional ophthalmic tests and the high-resolution laser scanning confocal microscopy.

• **METHODS:** Fifty-eight patients with type II diabetes were selected. Based on the diagnostic criteria and stage classification of DR, the patients were divided into the non-DR (NDR) group and the PDR group. Thirty-six patients with cataract but no other ocular and systemic disease were included as non-diabetic controls. All the patients were subjected to the conventional clinical tests of corneal sensitivity, Schirmer I test, and corneal fluorescein staining. The non-invasive tear film break-up time (NIBUT) and tear interferometry were conducted by a Tearscope Plus. The morphology of corneal epithelia and nerve fibers was examined using the high-resolution confocal microscopy.

• **RESULTS:** The NDR group exhibited significantly declined corneal sensitivity and Schirmer I test value, as compared to the non-diabetic controls ($P<0.001$). The PDR group showed significantly reduced corneal sensitivity, Schirmer I test value, and NIBUT in comparison to the non-diabetic controls ($P<0.001$). Corneal fluorescein staining revealed the progressively injured corneal epithelia in the PDR patients. Moreover, significant decrease in the corneal epithelial density and morphological abnormalities in the corneal epithelia and nerve fibers were also observed in the PDR patients.

• **CONCLUSION:** Ocular surface changes, including blunted corneal sensitivity, reduced tear secretion, tear film dysfunction, progressive loss of corneal epithelia and degeneration of nerve fibers, are common in type II diabetic patients, particularly in the diabetic patients with PDR. The corneal sensitivity, fluorescein staining scores, and the density of corneal epithelial cells and nerve fibers in the diabetic patients correlate with the duration of diabetes. Therefore, ocular surface of the patients with PDR should be examined regularly by conventional approaches and confocal microscopy to facilitate early diagnosis and treatment of keratopathy.

• **KEYWORDS:** type II diabetes; proliferative diabetic retinopathy; ocular surface; corneal sensitivity; confocal microscopy; tear film break-up time

INTRODUCTION

C urrently, an estimate of 347 million people in the world has diabetes, making it one of the most common medical conditions worldwide [1]. Diabetes has received widespread attention in that it causes life-threatening or debilitating complications in heart, kidney, brain, and eye [2]. In the eye, diabetic retinopathy (DR), cataract, glaucoma, keratopathy, chronic dry eye, and refractive abnormalities are the diseases associated with diabetes [3]. Among these diseases, DR, characterized by exudates, microaneurysms, and hemorrhages, is the major ocular complication of diabetes [4]. Besides the disorders in retina, the incidence of corneal abnormalities is also high in diabetes. Literatures have shown that the diabetics have an increased risk of developing corneal epithelial fragility and defects, decreased corneal sensitivity and thickness, abnormal wound healing, and increased susceptibility to infected corneal ulceration [5-9]. For example, Schultz *et al* [10] reported that 47%-64% of diabetic patients suffered from keratopathies. Didenko *et al* [11] showed that as high as 73.6% of the patients with diabetes

had corneal complications, including punctate keratopathy, pannus, endothelial dystrophy, and corneal ulcer.

The prevalence and interventions of DR have been studied extensively [12-15]. However, the ocular surface disorders in the diabetic patients with DR have been rarely studied, and the correlation between the severity of these disorders and that of DR remains unknown, despite that the incidence of the ocular surface disorders is as high as retinal disorders in the patients with DR [16,17]. Therefore, this study aims to examine the functional and pathological changes on ocular surface in the diabetic patients with proliferative DR (PDR), an advanced stage of DR and the diabetic patients without DR. The corneal sensitivity, tear secretion, tear film function, corneal epithelia and nerve fibers were examined by conventional and considered as sensitive approaches. The results of this study demonstrated the incidence and severity of ocular surface disorders in the diabetics with PDR, indicating the importance of early diagnosis and treatment of keratopathy in these patients.

SUBJECTS AND METHODS

Subjects Fifty-eight patients (74 eyes) were diagnosed with non-insulin-dependent diabetes mellitus [(NIDDM) 27 men and 31 women] according to the diagnostic criteria for NIDDM approved by World Health Organization [18]. The mean age of the diabetic patients was 50 ± 8y (ranging from 32 to 62y) with the duration of diabetes ranging from 5 to 15y (mean 9 ± 3y). In addition, 36 cataract patients (21 males and 15 females) between the age of 34 and 61y (mean 52 ± 8y) without any other ocular and systemic disease were recruited as control subjects. The cataract patients were enrolled for the control subjects because of their abundance and availability in our hospital. Although the changes in corneal optical properties, such as astigmatism and high order aberrations, have been reported in the pre-operative cataract patients, the changes in the parameters to be measured in this study have rarely been reported in such patients [19,20]. All procedures of this study were approved by the ethical committee in Shanxi Eye Hospital and in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants.

There was no significant difference in age (F=0.358, P=0.701) or gender (χ^2 =0.217, P=0.897) between the diabetic and control groups. Based on the results of binocular indirect ophthalmoscope, fundus fluorescein angiography, as well as the classification criteria of DR, the diabetics were divided into two groups. The patients without detectable fundus changes were included as non-DR (NDR) group, and those with PDR changes in fundus were included as PDR group. The NDR group was composed of 28 patients (34 eyes), 13 of whom were male (16 eyes) and 15 female (18 eyes), aging from 32 to 59y (mean 49 ± 8 y). The PDR group contained 30 patients (40 eyes), including 14 males (18 eyes) and 16 females (22 eyes), the age of the PDR patients ranged from 34 to 62y (mean 50 ± 9y).

The diabetic patients and the controls were recruited from December 2011 to May 2013 in our hospital. The HbA1C of all the diabetic patients was maintained between 6% and 7% by either medicinal treatment or dietary control and exercise. None of the patients had a history of ophthalmic trauma or operation, and none wore contact lenses. The corneas appeared transparent by slip-lamp examination, and the intraocular pressure was normal for all the patients.

Corneal Sensitivity Test Corneal sensitivity was examined using a Cochet-Bonnet esthesiometer (Luneau SA, France). The patient sat and faced forward. A fully extended nylon filament (length: 60 mm; diameter: 0.12 mm) was used for the examination. The tip of the nylon filament was applied perpendicularly to the surface of central cornea and progressed steadily. The length of the filament was decreased 5 mm every time till the patient reported the sensation of foreign body on the cornea. The length of the nylon filament was then recorded. The tests of all the patients were repeated three times by the same experienced doctor who had been masked for patient grouping. The test results were confirmed by another doctor.

Schirmer I Test A piece of filter paper (5×35-mm^2) was folded in one end. The folded end was placed into the accus conjunctivae at 1/3 mid-exterior of the lower eyelid without topical anesthesia. The patient was asked to close the eye gently. The filter paper was withdrawn 5min later, and the length of the wetted crease was measured.

Corneal Fluorescein Staining The staining strip coated with fluorescein was placed in the conjunctiva sac of the patient's lower eye lid. The patient was asked to close eyes for 5s and blink several times. Cornea staining was then examined using a slit lamp under the cobalt blue light and scored 0-3 according to the area and intensity of the staining. 0: no staining; 1: sporadic punctuated staining; 2: dense punctuated staining; and 3: intense patchy staining.

Non –invasive Tear Film Break –up Time The non-invasive tear film break-up time (NIBUT) was measured by a Tearscope Plus (Keeler, UK) as previously described[21]. Briefly, the image of the patient's cornea was monitored by the doctor from the monitoring hole in the mirror at 1 to 2 cm in front of the patient's eye. The NIBUT was calculated from the time when patient started gazing ahead after several blinks to when the cornea angular image appeared distorted.

Tear Interferometry Test The tear interferometry test was conducted using a DR-II Tearscope Plus (Kowa, Japan). Under illumination of a white light beam, the interference patterns of the tear film lipid layer were analyzed and graded from I to V according to the Yokoi standards of grading[22]. Grade I or grade II was considered normal, whereas grade III or above abnormal.

Corneal Confocal Microscopy The corneal endothelia and nerve fibers were examined by a laser scanning confocal microscope (Heidelberg Retina Tomography II, HRT II, Germany) equipped with a Rostock Corneal Module. Briefly, the patient's head was positioned anteroposterior, with the mandible resting on the check bracket and the forehead in contact with the headband bracket. The objective lens covered with a disposable sterile contact cap for cornea was positioned at 5-10 mm in front of the patient's cornea. The laser beam was adjusted to focus on the center of the cornea. The objective lens was then moved forward till the contact cap slightly touched the cornea. The images of cornea epithelia and nerve fibers with different depths and angles were captured and archived.

Statistical Analysis Data were expressed as mean±SD. The differences of cornea sensitivity, Schirmer I test, NIBUT, and corneal nerve axonal density between the study groups were analyzed by one-way ANOVA followed by Tukey t-test. The correlations between the examined parameters and the diabetic duration were examined by Spearman's rank correlation analysis. The differences in the percentage of the patients graded more than III in tear interferometry test, and in the percentage of the patients positive for cornea fluorescein staining were analyzed by Chi-square test. The differences in gender composition and cornea fluorescein staining score were analyzed by rank sum test. All the statistical analyses were performed using the software SPSS13.0 (SPSS Inc., San Diego, CA, USA). A $P<0.05$ was considered statistically significant.

RESULTS

Corneal Sensitivity Test Corneal sensitivity was decreased in both diabetic groups (NDR: 44± 9 mm, PDR: 34±18 mm) as compared to that in the control group (53±7 mm), and there was statistical significance between either diabetic group and the control group (Figure 1, both $P<0.001$, NDR *vs* control, PDR *vs* control). Moreover, the corneal sensitivity measured in the PDR group was also significantly lower than the NDR counterpart (Figure 1, $P< 0.001$, NDR *vs* PDR), suggesting that corneal sensitivity was more severely affected in the patients with the advanced stage of DR. A negative correlation was found between corneal sensitivity and the diabetic duration by Spearman's rank correlation analysis (Spearman's correlation coefficient= -0.657, P =0.02). These findings suggest the reduced corneal sensitivity along with the course of diabetes.

Schirmer I Test The volume of tear secretion was examined by the conventional Schirmer I test. The average value of Schirmer I test in the control group was 20±5 mm, whereas the value was reduced to 12±6 mm in the NDR group, and even to 9±6 mm in the PDR group (Figure 2). Highly significant difference was found among the groups (F=19.2, $P< 0.001$) and also between the NDR or PDR and
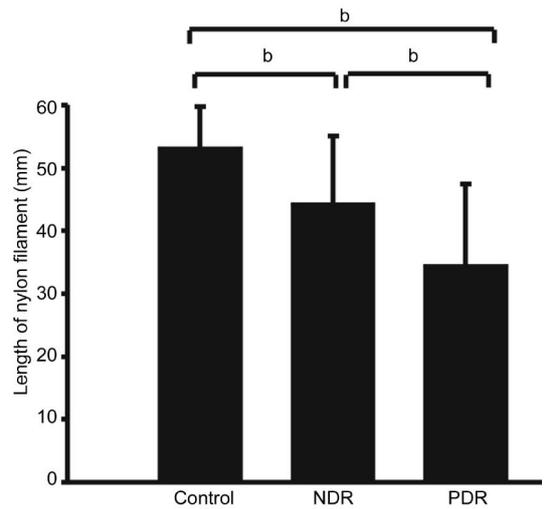
Figure 1 Corneal sensitivity was measured by a Cochet – Bonnet esthesiometer Corneal sensitivity was significantly reduced in both NDR and PDR group in comparison to the non-diabetic controls; corneal sensitivity in the PDR group was also significantly lower than that in the NDR group. [b] $P< 0.001$.
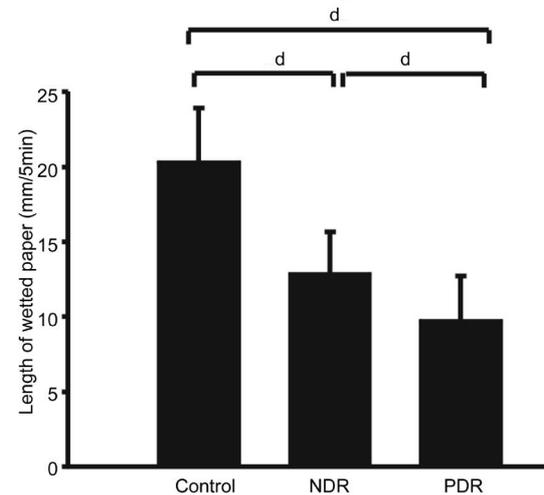
Figure 2 Basic tear secretion was measured by Schirmer I test Basic tear secretion in the NDR and PDR group was both significantly lower than that in the non-diabetic controls. The tear section in the PDR group was also significantly diminished as compared to that in the NDR group. [d] $P< 0.001$.

the control group (Figure 2, both $P< 0.001$, NDR *vs* control, PDR *vs* control). The percentage of the patients with Schirmer I test value less than 5 mm was 8.82% (3/34 eyes) in the NDR group, 15% (6/40 eyes) in the PDR group, and only 2.78% (1/36 eyes) in the control group. However, Spearman's rank correlation analysis showed that the Schirmer I test value did not correlate with the duration of diabetes (Spearman's correlation coefficient=-0.164, $P=0.122$). These results suggest that significant reduction in tear secretion might occur even before pathologies of DR were detected in fundus, and this tear deficiency might be exacerbated when DR progressed into an advanced stage, although it did not correlate with the duration of diabetes.

Corneal Epithelial Fluorescein Staining The corneal epithelial condition was examined by the conventional

Table 1 Percentage of the patients positive for corneal fluorescein staining or graded higher than III in tear interferometry test

Group	Percentage of the patients positive for cornea fluorescein staining	Percentage of the patients graded > III in tear interferometry test
NDR	52.9% (18/34 eyes)	29.4% (10/34 eyes)
PDR	85% (34/40 eyes)	52.5% (21/40 eyes)
Control	38.9% (14/36 eyes)	16.7% (6/36 eyes)
X^2	10.73	11.29
P	<0.005	<0.004

fluorescein staining. Positive staining was detected in 52.9% (18/34 eyes) of the NDR group, 85% (34/40 eyes) of the PDR group, and 38.9% (14/36 eyes) of the control group (Table 1). The corneal staining score in the PDR group was significantly higher than that in the control group (P< 0.001, PDR vs control) and the NDR group (P< 0.05, PDR vs NDR; Table 1), and the staining score in all the diabetic patients was positively correlated with the disease duration (Spearman's correlation coefficient = 0.46, P<0.05). These results suggest that the damage to the intactness of corneal epithelia may start in diabetic patients without retinopathy, and the damage becomes more severe as diabetes progresses.

Non-invasive Tear Film Break-up Time The tear film stability was examined by NIBUT. The average NIBUT in the PDR group was 8 ±3s, being only 53.3% and 57.1% of that in the control and the NDR group (both P< 0.001, PDR vs control, PDR vs NDR), respectively (Figure 3). The NIBUT measured in the NDR group was similar to that in the controls (Figure 3, P>0.05, NDR vs control). The correlation was not found between the NIBUT and the duration of diabetes (Spearman's correlation coefficient = -0.163, P = 0.142). These results suggest that tear film stability has not been affected in the type II diabetic patients without DR, but is profoundly compromised in the patients with PDR.

Tear Interferometry Test This test is designed to analyze the lipid layer of the tear film, and has been widely used for the diagnosis of dry eye. The percentage of patients whose tear film was graded more than III was 29.4% (10/34 eyes) in the NDR group, 52.5% (21/40 eyes) in the PDR group, and 16.7% (6/36 eyes) in the control group (Table 1). The Chi-square analysis revealed a significant difference among the groups (χ^2=38.46, P< 0.001), and the percentages of the patients graded higher than III in the NDR and PDR group were both significantly greater than that in the control group (Table 1, P< 0.05, NDR vs control; P< 0.001, PDR vs control). These results indicate that the quality of tear film lipid layer was significantly compromised in patients with diabetes, and dry eye may be more prevalent in the diabetics than the non-diabetic population.
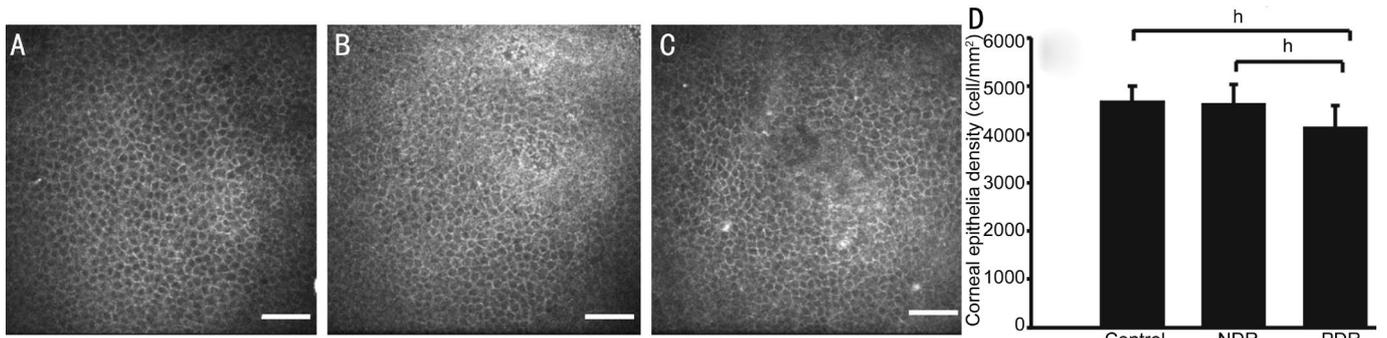
Corneal Confocal Microscopy Corneal confocal microscopy revealed that the thickness of the epithelial layer averaged at 17±5 μm in all the participants. Corneal epithelia of the control patients exhibited regular cell shape with a
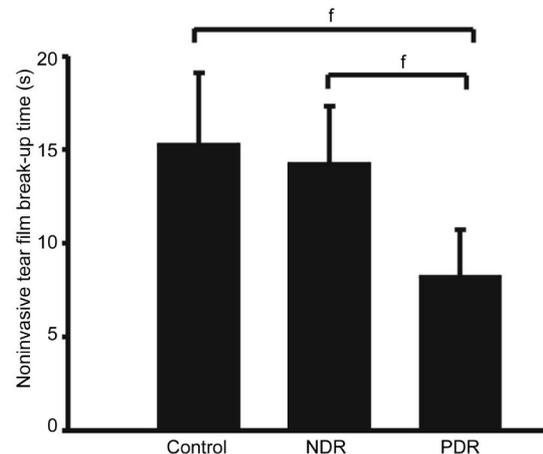

Figure 3 NIBUT was measured by a Tearscope Plus The NIBUT in the PDR group was significantly shorter than that in the NDR group and the control group. The NIBUT in the NDR group was not significantly different from that in the controls. ^{f}P< 0.001.

density of 4670 ±522 cells/mm² (Figures 4A, 4D). A few swelled cells with irregular shape were observed in the epithelia of the NDR group (Figure 4B), and the density of the epithelial cells in this group (4612±601 cells/mm²) was comparable to that in the controls (Figure 4D). In contrast, many exfoliated and edematous epithelial cells were observed in the cornea of the PDR patients (Figure 4C), and the cell density (4121±474 cells/mm²) was significantly lower than both the NDR group (Figure 4D, P< 0.05, PDR vs NDR) and the controls (Figure 4D, P< 0.05, PDR vs controls). Moreover, the cell density of the corneal epithelia in diabetic patients was negatively correlated with the duration of diabetes (Spearman's correlation coefficient = -0.311, P<0.05), indicating a progressive loss of epithelia over the course of the disease.

Corneal confocal microscopy also showed that corneal nerve fibers in the PDR patients had slim axons. These axons branched less and were more torturous than those in the non-diabetic controls and NDR group (Figure 5A, 5B, 5C). The axonal density of the nerve fibers in the PDR group (802 ±264 μm/field) reduced 33.8% as compared to that in the controls (1212±223 μm/field, P<0.001, PDR vs control), but was not significantly different from that in the NDR group (1118±234 μm/field, P=0.283, PDR vs NDR) (Figure 5D). The density of nerve fibers in the diabetic patients was negatively correlated with the duration of the disease

Figure 4 Corneal epithelial cells were examined by a high–resolution confocal microscope A: Corneal epithelia from the controls were in a regular shape and with a discerned contour; B: The irregular and swelled corneal epithelia were observed sporadically in the NDR group; C: Corneal epithelial exfoliation and edema were seen more frequently, and the epithelial cell edges were irregular and blurred in the patients with PDR; D: Scale bar = 25 μm. Density of corneal epithelial cells was measured by a HRT-II confocal microscope. The epithelial cell density in the PDR group was significantly lower than the controls and the NDR group. [h] $P < 0.001$.
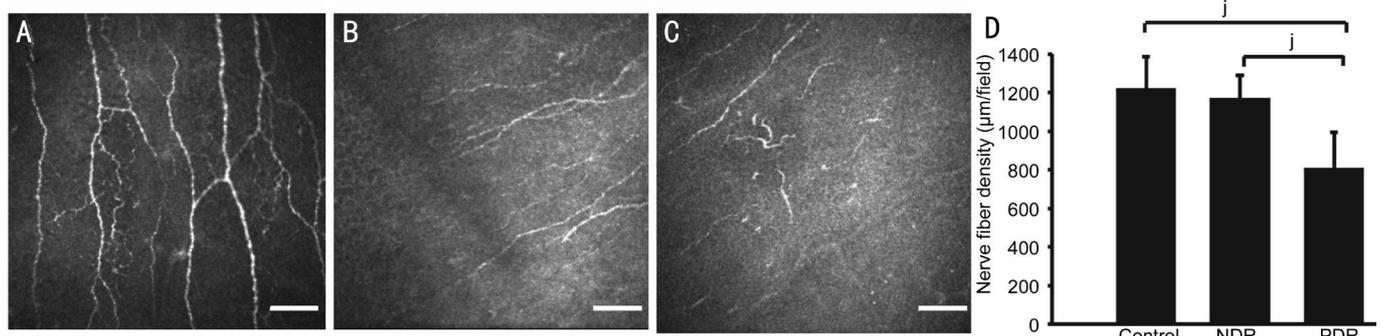
Figure 5 Corneal nerve fibers were examined by confocal microscopy A: In the control group, nerve fibers were straight and thick. The axonal fibers branched at acute angles; B: In the NDR group, nerve fibers were more tortuous and branched less than those in the control group; C: The nerve fibers in the PDR patients were sparse, thin and discontinued. The fibers had even fewer branches and exhibited more tortuous axonal trajectories than the NDR group. Scale bar = 25 μm; D: The density of corneal nerve fibers was examined by a HRT-II confocal microscope. The nerve fiber density in the PDR patients was significantly decreased as compared to that in the NDR and the control group. [j] $P < 0.001$.

(Spearman's correlation coefficient=-0.473, $P = 0.010$). These confocal microscopic observations reflect the progressive diabetic neuropathy on the cornea, which might be the neuroanatomical basis of the blunted corneal sensitivity in the diabetic patients with PDR (Figure 1).

DISCUSSION

Although DR is one of the major diabetic complications and the leading cause of blindness in the working age population worldwide, the ocular surface disorders in the patients with DR have not acquired as much attention [23]. Therefore, the ocular surface changes were examined in the current study in the type II diabetic patients with PDR and those without DR. The conventional clinical tests, such as corneal sensitivity, Schirmer I test, and corneal fluorescein staining, the non-invasive approaches using the Tearscope Plus, and the considered more sensitive confocal microscopy were employed in the examinations. As a result, the ocular surface changes, including the deteriorated corneal sensitivity, reduced tear secretion, compromised corneal epithelial intactness and tear film quality, as well as the progressive loss of corneal epithelia and degenerated corneal nerve fibers, were found in these patients. These findings highlight the

importance of examining and treating ocular surface disorders in the diabetic patients, particularly the patients with PDR.

Tear film provides nutrition to the cornea and prevents the cornea from dryness and keratinization [24]. Abnormalities in tear amount or tear film function lead to ocular surface disorders, such as chronic dry eye and keratitis [25]. The Schrimer's test and tear film break-up time are conventional methods for measurement of tear quantity and tear film stability, respectively; whereas the DR-II Tearscope Plus device employed in this study objectively and non-invasively quantifies the amount of tear production and analyzes the tear film lipid layer [26,27]. To the best of our knowledge, this is the first dynamic observation of tear film in type II diabetic patients using this device. The results of our study showed that the stability of the tear film, as indicated by NIBUT, in the NDR patients was not significantly different from the non-diabetic controls (Figure 2), however, the tear film function detected by interferometry test in this group was significantly compromised (Table 1). These results are consistent with those from the previous studies using conventional Schirmer I test and tear film break-up time in

diabetic patients [6,28,29]. More importantly, the results measured by the DR-II Tearscope Plus could be abnormal even if the conventional Schirmer I test value was normal. This is probably due to an increased tear reflectivity [30] and suggests that the DR-II Tearscope Plus could be more sensitive and objective than the Schirmer I test, supporting the further clinical usage of this device.

Due to the instability and malfunction of tear film, diabetic patients are susceptible to dry eye. It has been demonstrated that the incidence of dry eye is 70% in type II and 57% in type I diabetic population[31]. Indeed, the results of our corneal interferometry test showed that dry eye syndrome was more prevalent in diabetic patients than in the controls (Table 1), which is consistent with the previous report that there is an association between dry eye syndrome and duration of type II diabetes [32]. The mechanisms underlying higher incidence of dry eye in diabetic patients remain to be investigated. Grus *et al* [33] have speculated that the diminished stimulating signals from ocular surface to lachrymal gland caused by the blunted corneal sensitivity and the weakened lachrymal gland regulation may account for the higher incidence of dry eye in the diabetics. In addition, the impaired main lachrymal gland secretion together with the decreased mucin production in diabetic patients may also provide an explanation [34].

The quality of tear lipid layer and the stability of tear film are correlated with the index of superficial punctate keratopathy[35]. Therefore, the decreased production of tears and the impaired tear film function in diabetic patients render them susceptible to corneal epithelial exfoliation and edema during cataract and fundus surgery. Moreover, these patients are also afflicted by persistent defects and slow regeneration of corneal epithelia, as well as bullous keratopathy [6,9]. In fact, although the corneas of the PDR patients in the current study appeared transparent and undamaged under a slit lamp, irregular shape and edema of the corneal epithelial cells were revealed under a high-resolution confocal microscope (Figure 4A). These results are consistent with previous findings [36]. However, this study identified a significant reduction in the corneal epithelial density by confocal microscopy in the PDR patients, the phenomenon that was not observed before (Figure 4B) [36,37]. This is probably due to the strict criteria used for subject inclusion in this study. Moreover, the corneal confocal microscopy used in this study examines and compares corneas at the same precise location, which could unveil the site-specific differences between groups that might be missed in the previous studies [36,37].

Peripheral neuropathy is a common diabetic complication[38]. Corneal sensory fibers are derived from the ophthalmic branch of trigeminal nerve with the input from autonomic nerve, therefore, they may be affected by diabetic neuropathy[39]. Indeed, the corneal nerve fibers with reduced density, scattered distribution and tortuous axonal trajectory were observed in the PDR patients by confocal microscopy (Figure 5). These results are consistent with the previous reports from basic and clinical research. For example, Li *et al* [40] observed sparse nerve plexuses, thin axon fibers, irregular fibril distribution and degeneration of mitochondria in alloxan-induced diabetic rabbits. Moreover, Chang *et al* [41] showed that the patients with type II diabetes exhibited significantly reduced corneal nerve fiber and nerve branch density, as well as elevated nerve tortuosity coefficient as compared to the healthy control subjects. Furthermore, taken together with the results of corneal sensitivity measured in this study (Figure 1), the progressive damage in the corneal nerve fibers caused by diabetic neuropathy may, at least in part, account for the reduced corneal sensitivity that correlates with the severity of DR and the duration of diabetes (Figures 1 and 5). On the other hand, corneal confocal microscopy is considered as a sensitive approach that detects morphological changes in corneal nerve fibers before a significant reduction in corneal sensitivity occurs, hence, it allows for earlier detection of subtle diabetic neuropathy in the cornea than conventional test of corneal sensitivity.

In general, ocular surface changes are associated with the type II diabetic patients with PDR that manifest as reduced tear production, compromised tear film quality, progressive loss and damage of corneal epithelia, and degeneration of corneal nerve fibers. Correlation analyses further suggest that corneal changes are correlated with the duration and severity of diabetes. Therefore, clinical examinations of diabetic patients should include the tests performed in this study, particularly the cornea sensitivity and tear film function tests in the patients with a long history of diabetes and PDR. Confocal microscopy provides a non-invasive and precise examination of corneal epithelia and nerve fibers, and facilitates early diagnosis of diabetic keratopathy and neuropathy, thus may be used in the ocular surface examination of the diabetic patients with PDR.

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