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Corneal Neuropathy: An Underrated Manifestation of Diabetes Mellitus

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Abstract

Diabetes Mellitus (DM) is widely recognized as a major cause of blindness, with Diabetic Retinopathy (DR) being the most frequently cited underlying pathophysiologic mechanism. This makes DR, among all ocular manifestations of diabetes, the focus of most diagnostic as well as therapeutic interventions. However, diabetic neurotrophic keratopathy, a common ocular complication of diabetes that is caused by corneal nerve fiber damage, is a diagnostic entity that generated a lot of attention recently in the ophthalmology community for being largely unrecognized, underdiagnosed and generally not treated, rendering a large number of the growing population susceptible to this serious ocular complication. This is largely because of the challenge in diagnosis and management of diabetic neurotrophic keratopathy.

In this manuscript, we discuss the epidemiology, pathophysiologic mechanisms, diagnostic challenges and the innovative therapeutic interventions of diabetic neurotrophic keratopathy, an entity with potentially serious implications for diabetic patients, both type 1 as well as type 2 alike.

Introduction

The World Health Organization Global Burden of Disease Study described the global prevalence of Type 2 Diabetes Mellitus (DM) to be 220 million in 2010, foreshadowing an increase to 366 million by 2030 [1,2]. Regardless of the exact global indices, it is undeniable that DM is an important public health concern. Its prevalence is increasing due to poor lifestyle choices and an aging population, especially with a catastrophic increase in the prevalence of obesity [3]. In the USA, the overall cost of diabetes was approximately 245 billion dollars in 2012, made up of 176 billion dollars in medical expenditures and 69 billion dollars in decreased productivity [4]. Although type 2 diabetes is more common than type 1 diabetes in developed countries, type 1 diabetes has also demonstrated a noteworthy increase over the last 30 years and makes up a significant portion of the cost burden of health systems worldwide.

The microvascular consequences of DM include blindness, nephropathy that may result in end-stage renal failure, peripheral neuropathy, and autonomic neuropathy. In addition, these patients are at increased risk of cardiovascular disease, particularly stroke and myocardial infarction [5]. The main risk factor for the occurrence of microvascular complications in DM is poor glycemic control, reflected by an increased HbA1c [6].

In fact, the Diabetes Control and Complications Trial demonstrated that HbA1c concentration (as a reflection of glycemic control) is an independent risk factor for developing diabetic retinopathy and nephropathy [7]. The UK Prospective Diabetes Study also supported these findings by showing that improved glycemic control decreases the incidence of retinopathy [8].

Diabetes significantly damages the ocular tissue, with harm to this organ noted even at the earliest phases of the disease. In fact, diabetic retinopathy is the most frequent microvascular complication of diabetes and is the main cause of blindness among working-age adults in Westernized societies [9].

However, recent developments in ocular surface imaging technology have led to the study of the microstructural effects of DM on the ocular surface, particularly the cornea and the tear film. In fact, even if the ocular surface seems smooth on slit lamp examination in individuals with DM, subclinical changes within the cornea may exist [10,11]. Thus, even though diabetic retinopathy is the most critical ocular complication associated with

diabetes, diabetic keratopathy (or the effects of diabetes on the cornea) is also a common complication, the visual consequences of which are often underestimated. In fact, it has been estimated to be present in 47-64% of diabetic patients throughout the course of this lifelong disease [12].

Therefore, given how common diabetic keratopathy is, and how early it is seen in the course of the disease, it is important to be aware of its presence. From the well-known corneal changes due to diabetes, nerve function and epithelial wound healing have been most thoroughly analyzed, especially because they cause serious vision-threatening manifestations of diabetic keratopathy, including neurotrophic ulcers, recurrent corneal erosions, stromal opacification, surface irregularities and microbial keratitis [13,14].

Clinical experience has also shown that patients with DM have a damaged ocular surface with a resulting increased prevalence of punctate keratopathy, persistent epithelial defects, decreased corneal sensitivity and epithelial fragility; even though recurrent corneal erosions, microbial keratitis, and neurotrophic ulcers are potentially more visually devastating, chronic epithelial defects are more frequently seen in these patients [15-17]. A number of these irregularities are believed to occur concurrently with diabetic peripheral neuropathy [18-20].

In Vivo Confocal Microscopy (IVCM) is now the standard instrument for evaluating the living cornea at a cellular level in healthy and diseased corneas [21-24]. In recent years, it has been employed to discover and oversee the evolution of DM and its complications [25-28]. This tool demonstrates consistently repeatable results in the evaluation of the corneal epithelium and sub-basal nerve plexus in both healthy and diabetic corneas [27-30]. Not only have alterations in the corneal epithelium and sub-basal nerve plexus been seen in these patients, but studies have persistently demonstrated a reduction in corneal endothelial cell density in DM patients when compared with healthy controls. In fact, corneal endothelial pleomorphism and polymegathism have been exhibited in both type 1 and type 2 DM [31]. The pathogenesis of these alterations are unclear; however, they may be osmotic with eventual changes in morphology and endothelial pump function.

The review below provides an overview of corneal anatomy and physiology and focuses on various aspects of diabetic keratopathy, including its pathogenesis, diagnostic and treatment options. The authors hope to highlight its vision-threatening potential and raise awareness of this often underestimated ophthalmic ailment associated with DM.

Anatomy and Physiology of the Cornea

The cornea is a clear avascular connective tissue that is the main structural barricade of the eye, protecting the eye from intraocular infection. In combination with the tear film that overlies it, it is the main anterior refractive surface for the eye [32].

The horizontal diameter of the cornea is 11.5 to 12 mm [33] and 1.0 mm greater than the vertical diameter. It is 0.5 mm thick at the center and its thickness slowly increases toward the periphery. The cornea has a prolate shape – flatter in the periphery and steeper centrally - which forms an aspheric optical system [32].

The human cornea is made up of 5 layers: epithelium, Bowman's layer, stroma, Descemet membrane, and endothelium. The two membranes are interface layers, while the other 3 are cellular (Figure 1) [32].

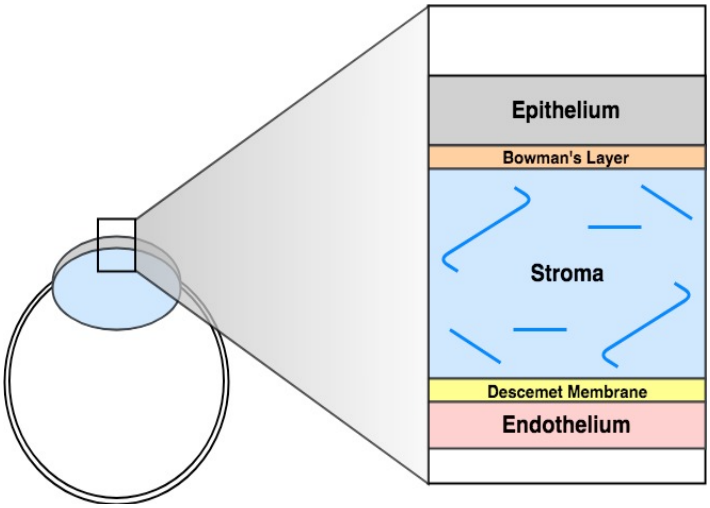

Figure 1: The human cornea is made up of 5 layers: epithelium, Bowman's membrane, stroma, Descemet membrane, and endothelium. The two membranes are interface layers, while the other 3 are cellular.

Epithelium

This is a stratified, non-keratinizing squamous layer characterized by extreme uniformity from limbus to limbus. It is bathed with a tear film, which contributes to optical clarity by removing micro-irregularities of the anterior epithelial surface. The cornea, together with the air-tear film interface on top of it, forms 2/3 of the overall refractive power of the eye [33].

The corneal epithelium and its tear film are anatomically and physiologically related. The conjunctival goblet cells produce the mucinous layer of the tear film, which interacts directly with the corneal epithelium, and cooperates with the corneal epithelial cell glycocalyx so as to create hydrophilic dissemination of the tear film with every eyelid blink. Breakdown of the glycocalyx from illness causes tear film instability. Not only is the tear film the main armor of the cornea from infection or toxic damage, the tear film also provides immunological and growth factors necessary for epithelial wellbeing, development, and reparation after injury [34]. The epithelial basement membrane is made up of Type 4 collagen and laminin released by basal cells; it is estimated to be 0.05 micrometers thick. If diseased, fibronectin levels grow, starting a cycle of healing that can last up to 6 weeks. Throughout these 6 weeks, the epithelial connection to the underlying, freshly laid basement membrane is fragile.

Bowman's Membrane

Bowman's membrane is not a true membrane. It is the acellular component of the most anterior part of the stroma; in fact, it is just anterior to the stroma. It is estimated to be 15 micrometers thick and helps the cornea sustain its contour. When damaged, it won't redevelop and can scar [32].

Corneal Stroma

The corneal stroma makes up 80-85% of the corneal thickness. Its embryological origin is the second wave of neural crest migration during the 7th gestational week, after the formation of the primitive endothelium. The stroma is transparent due to its exact organization of the stromal fibers and extracellular matrix (ECM). The collagen fibers are set in parallel bundles called fibrils, which are then packed in layers or lamellae arranged in parallel. The stroma consists of 200-250 separate lamella, and each layer is placed at right angles relative to fibers in nearby lamellae [35].

The central stroma is thinner than the peripheral stroma, and the collagen fibrils may alter their orientation to pass circumferentially as they approach the limbus [36]. This arrangement decreases forward light scatter and adds to the transparent quality and mechanical force of the cornea.

In addition, the organization of the lamellae changes based on the depth within the stroma. Superficial layers are less precisely organized than deeper layers, and this explains the increased facility of surgical dissection closer to the posterior layers of the cornea. In addition, these alterations explain variations in response to corneal edema. Descemet membrane folds are due to disproportionate edema of the posterior stroma enforced by the stiffer anterior cornea and structural constraint inflicted by the limbus [37]. Stromal swelling is thus guided posteriorly and causes relative leveling of the posterior surface, which can cause Descemet membrane to form folds seen as striae. Stromal collagen fibrils consist of type 1 collagen in a heterodimeric complex with type 5 collagen, which forms their distinctive and slender diameter [38]. These are then encircled by specialized proteoglycans, made up of keratan sulfate or chondroitin sulfate/dermatan sulfate side chains, which permit maintenance of their structural properties and hydration.

The main cell type of the stroma is the keratocyte, which aids in maintaining the ECM environment. These cells create collagen, glycosaminoglycans, and matrix metalloproteases (MMPs) - all of which are critical to this layer's homeostasis. Many of these keratocytes exist in the anterior stroma and include "crystallines," which make up 25-30% of soluble protein in the cells. These crystallines decrease backscatter of light from the keratocyte and uphold corneal clarity [39].

Descemet Membrane

Starting at the 8-week stage in utero, endothelial cells, essentially made up of a single layer of squamous epithelial cells, secrete Descemet membrane; thus, Descemet membrane is the basement membrane of the corneal endothelium. The anterior portion has a banded façade when looked at by electron microscopy and is secreted prior to birth; the posterior portion is unbanded and produced after birth. This layer can grow to 10 micrometers in thickness with age [32].

Endothelium

The endothelium of the cornea contributes to corneal transparency by maintaining the cornea in a dehydrated state. It itself is a monolayer, which looks like a honeycomb-like mosaic when observed from the posterior angle. In the beginning of embryogenesis, a monolayer of well-ordered arranged cuboidal cells derived from the neural crest line the posterior cornea [40]. These cells then flatten and become firmly adherent to one another. A discontinuous acellular layer lies immediately before the flattened layer, which then becomes Descemet membrane [41].

The endothelial layer is 10 micrometers thick when a patient is born and made up of a uniform thickness layer of cells that goes across the entire posterior corneal surface and blends with the cells of the trabecular meshwork [41]. In addition, Descemet membrane becomes uniform and continuous, joining peripherally with the trabecular beams at a site called Schwalbe line [41]. This is an area that can only be visualized by gonioscopy and signifies the end of the Descemet membrane and the beginning of the trabecular meshwork.

The cells then flatten over time until they reach 4 micrometers of thickness in adulthood. Nearby cells share lateral interdigitations and include gap and tight junctions laterally along their boundaries. These lateral boundaries include a large number of Na+, K+-ATPase pump sites [42]. The basal surface of the endothelium includes many hemidesmosomes that foster linkage to Descemet membrane.

Even though endothelial cells have no mitotic activity in vivo, humans are born with a substantial reserve. From a patient's 20s to their 80s, the cell density decreases from 3000-4000 cells/mm^2 to approximately 2600 cells/mm^2 and the percentage of hexagonal cells lessens from 75% to an estimated 60% [43]. In addition, the central endothelial cell density declines at a mean rate of 0.6% per year in normal corneas [44]. Furthermore, it has been shown that eyes with endothelial cell counts below 500 cells/mm^2 may have a greater chance of developing corneal edema. The morphology (size and shape) of endothelial cells also seems to have an association with pump function.

An increase in cell size (polymegathism) and variation of cell shape (pleomorphism) are also linked to a decreased capability of the endothelial cells to dehydrate the cornea [45]. Certain processes, including age, trauma, inflammation, and other disease processes (i.e. Fuchs endothelial dystrophy) lessen the number of endothelial cells with age, but the lingering cells are able to "extend" and take over the space of the degenerated endothelial cells. When this happens, these cells lose their hexagonality (pleomorphism) and increase in size (polymegathism).

As noted prior, the stroma is upkept in a relatively dehydrated

state (78% water content) by the endothelial cells [46].

This deturgescence is maintained by a pump-leak method as fluid moves from the corneal stroma down the osmotic gradient from a hypo-osmotic stroma to a hypertonic aqueous humor. This passive bulk fluid motion requires little energy but is driven by energy-necessitating processes of transporting ions to create the osmotic gradient. The two most crucial ion transport systems are the membrane-bound Na+ and K+-ATPase site and the intracellular carbonic anhydrase pathway. Both of these systems create a net flux of ions from the stroma to the aqueous humor. The endothelium is distinctive in that it is permeable to an extent, allowing the ion flux needed to generate the osmotic gradient [41].

Blood Supply of the Cornea

Even though the normal human cornea is avascular, it utilizes certain constituents of the blood for maintenance; these are delivered by end branches of the ophthalmic and facial arteries transported through the tear film and the aqueous humor as well as minute blood vessels at the outermost edge of the cornea at the limbus [32].

Nerve Supply of the Cornea

The cornea is one of the most innervated and sensitive organs in the body. Corneal nerves branch from the nasociliary branch of the first (ophthalmic) division of the trigeminal nerve. In the anterior layers of the cornea, the nerves penetrate the stroma radially in thick trunks creating plexiform arrangements, which ultimately puncture Bowman membrane to create a plexus below the basal epithelial layer [47]. The cornea also has autonomic sympathetic nerve fibers [32].

Symptoms of Diabetic Keratopathy

The symptoms of diabetic keratopathy are analogous to those of dry eye syndrome; these include blurry vision, reflex tearing, burning, photosensitivity, and foreign body sensation [48,49]. In mild instances of diabetic keratopathy, the cornea may look disease-free on slit-lamp biomicroscopy; still, patients will complain of these symptoms, likely due to apoptosis and mild inflammation [50].

Pathogenesis

Overview

Hyperglycemia and the creation of advanced glycation end products have specific effects on various parts of the cornea, causing three main types of tissue dysfunction with physiological effects that are evaluable (Figure 2): malfunctioning wound healing in the corneal epithelium, abnormalities of the sub-basal nerve plexus, and loss of corneal endothelial pump function.

Hyperglycemia causes IGFBP3 (Insulin Growth Factor Binding Protein 3) release, which competitively inhibits IGF-1 (Insulin Growth Factor-1); on the other hand, TGFb3 (Transforming Growth Factor Beta 3), EGFR (Epidermal Growth Factor Receptor), and CNTF (Ciliary Neurotrophic Factor) are decreased in hypoglycemic states. The final outcome is one of decreased epithelial cell proliferation and amplified apoptosis during the healing process of epithelial defects.

Damage to neurons is also critical. Extended periods of hyperglycemia cause the collection of advanced glycation end products which activate inflammation and oxidative stress. In this state, NGF (Nerve Growth Factor) and sphingolipid production is hindered and thus, so is their positive impact on neuronal health and myelin creation.

Hyperglycemia also causes endothelial cell damage and loss of pump function. Corneal stromal swelling may be due to this as well as loss of the epithelial barrier, crosslinking of stromal collagen and matrix, and endothelial pump loss [51].

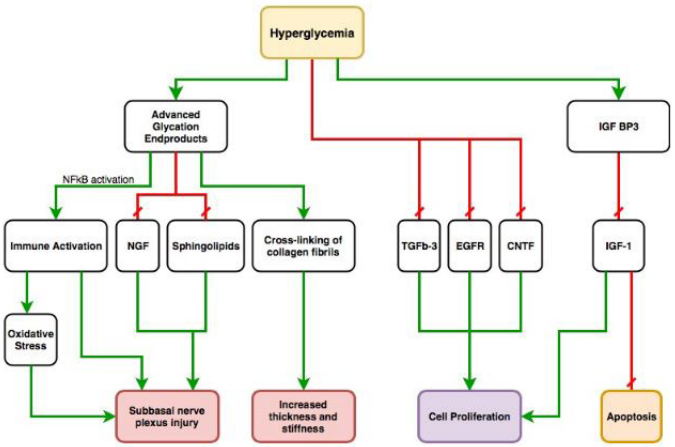

Figure 2: Representation of the pathological events contributing to disease in diabetes mellitus. Increased blood glucose affects various parts of the cornea in 3 distinct ways: defective corneal endothelial pump function, poor wound healing in the corneal epithelium, damage of sub-basal nerves. CNTF, ciliary neurotrophic factor; EGFR, epithelial growth factor receptor; IGF-1, insulin-like growth factor 1; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells transcription factor; NGF, nerve growth factor; TGFb3, transforming growth factor Beta-3. Red stop arrows indicate inhibition; green arrows show activation.

Corneal Sensitivity

Various articles detail the presence of lessened corneal sensitivity and increased corneal sensitivity threshold in patients with DM [52-54]. This rise in sensitivity threshold and consequent irregular neural regulation in the cornea causes recurrent corneal erosions and delayed epithelial wound healing [55]. Patients with DM exhibit dwindling corneal sensitivities when measured with contact or noncontact aesthesiometry; this finding is age-independent and implies the potential for corneal sensitivity to become an ophthalmic indicator of diabetic polyneuropathy [53,56].

The importance of this measure in the ophthalmic evaluation of patients with DM is further supported by the substantial difference in corneal sensitivity thresholds in patients with DM as

compared to those without DM. Tavakoli et al. conducted a study which demonstrated a noteworthy positive association between decreased corneal sensitivity, increasing age, and deteriorating metabolic control of DM [57].

Neurotrophic Keratopathy

This entity has been classified into three stages [58].

Stage 1 is described by rose Bengal staining of the inferior palpebral conjunctiva, punctate keratopathy, and lessened tear breakup time. If these manifestations become persistent, stromal scarring, superficial vascularization, and epithelial hyperplasia may be seen.

Stage 2 is characterized by epithelial breakdown where cells form an oval or circular contour that is frequently concentrated in the superior half of the cornea. This epithelial defect is encircled by loose epithelium that becomes hazy with a defective healing process. Eventually, the edges of the defect become smooth and rolled as the defect ages without epithelial growth [59].

Stage 3 is manifested by stromal involvement with a corneal ulcer that may evolve to melting and perforation. Superimposed infection or topical treatment with corticosteroids further augments the chance of perforation [60].

Corneal Thickness and Biomechanical Properties

The biomechanical aspects of the cornea are due to a combination of the structure and makeup of the extracellular matrix and the extent of corneal hydration. In DM, hyperglycemia-induced glycosylation of collagen may negatively affect corneal biomechanics [61]. A study from 1995 investigated collagen fluorescence in diabetic and control human corneas, demonstrating increased glycosylation in the corneas of patients with DM (2.88+/- 0.48 and 2.41 +/- 0.24 units/mg collagen, respectively). Furthermore, the increased units of collagen glycosylation were correlated with the extent of glycemia, length of diabetes, and advanced stages of retinopathy. These authors also noted elevated pentosidine levels in the corneas of patients with DM, implying oxidative stress on the cornea [61].

These modifications may augment corneal stiffness, although clear correlation with clinical practice has yet to be shown. An ex-vivo study of rabbit corneas that attempted to mimic the modifications in biomechanical properties of the cornea in diabetes with increased glucose concentrations reported greater corneal thickness and stiffness compared with that in normal corneas [62]. These authors also placed corneas in settings imitating increased glucose in the aqueous, and these corneas were then placed under cycles of posterior pressure mimicking intraocular pressure (IOP) [62]. The stiffness was shown to increase from 20% in normal to 37% in glucose-laden corneas [62]. Still, further studies are necessary to clearly elucidate the effects of biomechanical modifications on the human diabetic cornea.

In the past 10 years, instruments that attempt to evaluate corneal biomechanical aspects in vivo have become commercially available. The ocular response analyzer (Reichert Ophthalmic Instruments, Depew, NY, USA) is one such device that measures corneal biomechanical properties by applying a controlled pulse of air to the cornea to temporarily deform it. An infrared-based detector discovers the two points at which the cornea is applanated [63]. The alteration between the inward and outward pressure values calculated during the dynamic bidirectional applanation procedure is called corneal hysteresis, and it is due to the viscous damping within the cornea. Corneal resistance factor, on the other hand, is an indicator of the general resistance of the cornea [63,64]. However, some studies report lower [65] and others higher [66,67], values of these factors in the corneas of patients with DM; unfortunately, there is no consensus regarding the effects these properties have on the corneas of DM patients. This may be due to the subpar repeatability of ocular response analyzer measurements. In fact, a study of 49 normal patients showed a significant level of variability in corneal hysteresis measurements with a repeatability coefficient of variation 12.3% and coefficient value of 2.6% [68].

The Corvis ST (Oculus, Wetzlar, Germany) combines Scheimpflug imaging technology to examine corneal deformation in response to a perfectly metered air puff. By scanning the cornea, the Scheimpflug camera records 4330 images per second during applanation of the air pulse [69]. This is a reproducible method, both in normal and keratoconic corneas [70]. Corneal deformation breadth and thus, elasticity, are lower and inversely correlated with HbA1c and blood glucose concentration in persons with DM [71]. Of course, length and gravity of disease (measured by increased HbA1c) may also have a significant role; for this reason, additional prospective studies using this new imaging method would be helpful. Corneal biomechanics and central corneal thickness play a role in the correctness of IOP measurement. In DM, the increased thickness and stiffness of the cornea may lead to an overestimation of IOP measured by Goldman tonometry and could thus possibly affect glaucoma management in these patients [65,72].

Precorneal Tear Film

A reduction of the trophic effect of trigeminal sensory nerves on the cornea leads to a decrease of the constancy, emission and quality of the lipid layer of the tear film in persons with DM when compared to healthy controls [18,49]. Therefore, these patients often endorse dry eye symptoms, especially burning and foreign body sensation. In more advanced instances, a tolerance to dryness and epitheliopathy may be seen due to lowered corneal sensitivity correlated with the progression to diabetic neurotrophic keratopathy [48]. Certain characteristics of the tear fluid also contribute to this; its higher glucose concentration, due to conjunctival vessel leakage [73], changes the wound healing ability of the corneal epithelium and injures the microvascular source to the lacrimal gland resulting in lowered lacrimation [16].

A faster tear breakup time has been noted in DM patients, showing a decrease in tear film stability [74]. This debility is associated with lack of control of DM and peripheral neuropathy [49]. In addition, lowered goblet cell density is thought to affect tear film stability in these patients [75]. Goblet cells are the major

suppliers of the tear film mucins that guard the cornea and lead to a stable periocular tear film. Not only does trigeminal nerve damage affect tear film stability, but neuropathic alterations are also thought to impair lacrimal gland function, causing a decrease in basal tear formation in patients with DM. In fact, evaluation of lacrimal gland function with the Schirmer test has demonstrated decreased tear production rates in patients with diabetes when compared to those without diabetes [49]. Theoretically, retinopathy and eventual treatment with panretinal photocoagulation may amplify the probability of dry eye in patients with DM, possibly due to impairment of long ciliary nerves [74,75]. With that said, no statistically significant difference was found in corneal sub-basal nerve density in a prior study of DM patients with and without panretinal photocoagulation [76].

Increased serum glucose levels and oxidative stress can cause advanced glycation end products (AGEs) to form and change the structure of the ocular surface protein matrix. Also, the cellular reaction to stress in the eye leads to the formation of nuclear factor Kappa-B which causes widespread tissue destruction, manifesting as diabetic keratopathy [77]. In fact, increased levels of AGEs and nuclear factor Kappa-B have caused inflammatory modifications in the lacrimal glands of diabetic rats [78,79].

Corneal Epithelium

Epithelial basement membrane modifications have been noted in the skin, kidney, central and peripheral nervous systems, retina, and cornea in patients with DM [80-83]. The corneal epithelial basement membrane undergoes thickening, resulting in multilaminated layers [81,84]. This causes scattering of light in the cornea that is not seen on routine clinical examination, but is detectable on IVCM [85]. Patients with retinal vascular hyperpermeability also exhibit increased light scattering in the cornea [86]. In addition, the formation of advanced glycation end-products on the epithelial basement membrane also contributes significantly to the development of epithelial disorders in DM [87].

Diabetic changes of the corneal epithelium lead to the following manifestations: superficial punctate keratitis, microcystic edema, the creation of abnormal epithelial basement membrane, and full thickness breaks [88]. Corneal basement membrane modifications cause recurrent epithelial defects, which decrease the capacity of the cornea to protect against infection [12,49,55,75,89]. A number of studies have reported an association between higher HbA1c levels and decreased corneal barrier function.

The upkeep of an adequate corneal epithelial cell density is contingent on the necessary balance between differentiation, migration, cell proliferation, and cell death. Various studies have highlighted the vulnerability of the cornea in patients with diabetes to infections and recurrent erosions. A study by Tsubota et al. showed a modified maturation of epithelial cells in the corneas of DM patients [90].

A different study reported the collection of glycogen granules and focal epithelial degeneration [10]. Frueh et al. noted decreased, though statistically insignificant, basal epithelial cell density in the cornea of patients with DM compared to controls [91]. Two more studies also demonstrated decreased basal epithelial cell density in DM patients and implied that this may be a result of decreased corneal innervation causing basal cell diminution [92,93].

Sub-Basal Nerve Plexus

The corneal sub-basal nerve plexus has a number of functions; these include maintaining a healthy epithelial surface by upholding corneal sensitivity and epithelial metabolism and by releasing neuropeptides and growth factors [94]. DM affects corneal nerves [30,55,95,96]. In fact, a decrease in the sub-basal nerve density in the cornea of individuals with DM is thought to cause abnormal epithelial and endothelial cell densities [92].

The following alterations of corneal nerves are noted: reduction in nerve branching and sub-basal nerve density, and amplified nerve tortuosity; this increase in nerve tortuosity may represent nerve regeneration in diabetic keratopathy [26,96,97].

The past 10 years have seen the advancement of IVCM, allowing us to better understand the morphology of corneal sub-basal nerves in DM. Rosenberg et al. showed an association between corneal sensation and corneal sub-basal nerve density in 23 patients with type 1 DM; he used IVCM to demonstrate this association [55]. Tandem or slit-scanning confocal microscopes are the primary tools used in these investigations. The development of laser scanning confocal microscopes has allowed repeatable, qualitative and quantitative analysis of the corneal sub-basal nerve plexus. Even though a few measurements such as nerve branch density, nerve length, nerve tortuosity, and corneal nerve density have been evaluated in various studies, the most reliable measure has been found to be nerve density measured as total nerve length. A decrease in this measure by as much as 50% in patients with DM as compared with normal controls, has been exhibited [29,30,98-102].

Ten years' prior, Patel and McGhee demonstrated the two-dimensional architecture of the corneal sub-basal nerve plexus in the human eye; they showed a radiating outline of nerve fiber bundles heading towards an area 1-2 mm inferior to the corneal apex, in the configuration of a whorl [103]. After, Edwards et al. utilized a new automated method for imaging and montaging the sub-basal nerve plexus in control and DM patients. Due to the decreased sub-basal nerve density and increase in fragmented nerves noted in patients with DM, it is challenging to create confluent maps in these patients [104].

Studies that utilized tandem or slit-scanning microscopes showed a clear association between corneal sensitivity and corneal nerve density [55], implying a relationship between corneal nerve alterations and the extent of diabetic peripheral neuropathy [25]. In fact, nerve regeneration in the cornea has been shown to occur within half a year of diabetes-related pancreas and kidney transplantation (which led to remedying of the diabetes), even though significant corneal nerve damage was noted before surgery. In addition, researchers have shown associations between

improvements in sub-basal nerve density, nerve branch density and tortuosity and decreased HbA1c [28].

In recent years, it has been implied that corneal alterations may occur before typical clinical and electrophysiological tests of neuropathy; this further emphasizes the possible use of IVCM of the cornea and corneal sensitivity testing as measures in the evaluation of diabetic peripheral and autonomic neuropathy [99,105]. Thus, it seems that corneal IVCM may not only be utilized as a marker for peripheral neuropathy but may also be a tool used routinely to assess the development and further advancement of neuropathy, possibly as retinal photos to assess for the progression of diabetic retinopathy.

As mentioned previously, when diabetes-related corneal neuropathy presents as keratopathy, patients often don't experience symptoms until later stages of the disease. However, initial signs on exam may be seen very early in the disease course as decreased corneal sensation presents with few symptoms and is often subclinical [99,106]. In later stages, these patients may necessitate topical growth factors and even surgical treatment, as discussed in later sections of this overview [107].

Corneal Neuropathy before Peripheral Neuropathy?

In the past, sural/peroneal nerve biopsies and skin punch biopsies were used (and continue to be used) to investigate peripheral nerve fibers; however, this only evaluates nerve morphology, not function [108]. In addition, it is an invasive technique that is not practical because it is difficult to perform repeated analysis at the same location for evaluation of disease progression or in longitudinal research studies. A number of studies have shown a correlation between modifications in corneal sub-basal nerve plexus and peripheral neuropathy [28,30,76,109]. Interestingly, decreased sub-basal nerve density in the cornea can be seen in patients with almost no, if any, signs of peripheral neuropathy. Therefore, corneal nerve changes may manifest with or even before symptoms or clinical evidence of peripheral neuropathy is present. Moreover, studies in patients with DM have shown abnormal corneal nerve density, as confirmed by IVCM, but with normal intraepidermal peripheral nerve fiber density, as shown by skin punch biopsies [100,110].

The area under the receiver operator characteristics curves (AUC) is a reliable method of diagnostic performance. The AUC values for corneal nerve density are 70% for automated measurement and 76% for manual; this demonstrates corneal sub-basal nerve density as a strong diagnostic value of the advancement of diabetes-related peripheral neuropathy [110]. Furthermore, Misra et al. recently demonstrated an inverse correlation between corneal sensitivity and cardiac autonomic neuropathy, which is a diabetic complication with a significant mortality rate [99]. Thus, corneal sensitivity may also be a possible parameter for autonomic cardiac neuropathy.

Endothelium

Diabetes may cause changes to corneal endothelial cells in many ways: cell density and morphology (including polymegathism and pleomorphism). One study of 220 patients with Type 2 DM showed augmented polymegathism and pleomorphism of the corneal endothelial cells [111]. Some studies have shown that these manifestations occur before diabetic retinopathy in early phases of the disease [112]. While an ex vivo study did imply noteworthy morphologic changes of the cornea between patients with type 1 and type 2 DM compared with control patients [113], an appropriately age-matched, controlled, longitudinal clinical study would improve our understanding of malfunctions of the corneal endothelium, as certain abnormalities have been noted simply with age, independent of the presence of DM.

Diagnosis

Confocal Imaging

One of the most exciting and innovative aspects of imaging in recent years is the development and use of modern scanning laser ophthalmoscopy. The most frequently utilized form of this in vivo corneal microscopy is the Heidelberg Retinal Tomography (Heidelberg, Germany), which is executed with a corneal modular lens [114-117]. Images obtained are processed by imaging software for measurements of nerve fiber length, nerve branch density, nerve tortuosity, and nerve fiber density [118] in the sub-basal nerve plexus; this is because modifications in this layer (as opposed to the intrastromal nerve layer) are more pertinent in DM. DeMill et al. utilized NeuronJ, a plug-in for the NIH freeware Image J, for analysis [119].

Particular nerve indicators are possibly useful in a specific area of the cornea for certain clinical situations. The sub-basal nerve plexus can be analyzed [30,101,120-123] in two areas of the cornea: central cornea and the inferior whorl [124]. Petropoulos et al. has reported that the nerve fiber density at the inferior whorl is more prone to early nerve fiber damage than the central corneal area in DM patients prior to the development of peripheral neuropathy [125].

Scans can be analyzed manually (CCModule), in a semi-automated way (NeuronJ) or in a completely automated (ACCModule) method [126,127]. All 3 methods have been shown to have high repeatability, which cannot be enhanced with increasing magnification, but can be enhanced with experience [128-134]. The rate of image analysis can be augmented with the utilization of automated quantification methods and wide-field imaging [135-137]. Furthermore, calculations from manual analysis are well-matched to calculations from full automation [138].

Treatment of Diabetic Ocular Disease

Systemic Treatment

Systemic treatment in DM is the core treatment of any diabetic manifestation. Tight blood glucose control, especially together with an endocrinologist, can stop further continuation of corneal neuropathy and epithelial disease [28,139].

New Systemic Therapies

Below is a brief description of recent systemic therapies that have been investigated in the treatment of diabetic corneal disease. Beta carotene, an antioxidant, was demonstrated to decrease diabetes-related ultrastructural alterations to the cornea in a rat model [140]. This consequence of beta carotene was correlated with a decrease in average blood glucose in treated groups.

Enalapril, an ACE inhibitor, together with alpha lipoic acid (antioxidant) and menhaden oil reversed diabetic corneal and peripheral neuropathy in streptozotocin-induced diabetic rats when given orally [141].

Resolvin-D1, an anti-inflammatory eicosanoid, decreased corneal and peripheral nerve deterioration in diabetic rats when given as an oral supplement with menhaden (fish) oil. This manifestation was exclusive of effects on blood glucose levels [142].

Ilepatril, a vasopeptidase inhibitor and antihypertensive medication, can damage vaso- and neuo-active peptides as well as angiotensin converting enzyme (ACE). In rats with streptozotocin-induced diabetes, oral ilepatril guards against corneal nerve degeneration [143].

KIOM-79 (a mixture of 80% ethanol extracts of parched Puerariae radix, gingered Magnoliae cortex, Glycyrrhizae radix and Euphorbiae radix) can be utilized as an oral therapeutic medication by decreasing advanced glycation end products (AGE) in tissues such as the cornea. In a rat model of DM, it also decreased downstream oxidative destruction, nuclear factor Kappa-B activation and Bax overexpression in the cornea [144].

Local Treatment

The goal of local treatment in diabetic keratopathy is to uphold a moisturized and even ocular surface with an intact epithelium and sufficient blink response. This decreases visual symptoms and improves comfort. The precise treatment necessary depends on the extent of the damage and the particular structures injured. Mild disease will present as dry eye or recurrent erosions, while more serious manifestations may present as secondary infections and neurotrophic ulcers. A step-wise approach is often helpful with the goal of preventing further injury, helping re-epithelialization, and hindering infection while upholding lubrication of the ocular surface [107]. The typical treatment of neurotrophic corneal ulcers is made up of the following: use of topical antibiotics, increasing the use of preservative-free topical lubricants, decreasing evaporative tear loss, shielding the corneal surface with a bandage contact lens (to decrease further trauma if the ocular surface is irregular), guarding the corneal surface through patch closure of the eyelid, tarsorrhaphy or induced ptosis and more permanent resolutions, including conjunctival flap construction. Unfortunately, even when combined, these treatments may be futile, and the final result is frequently significant damage or lack of vision [145].

Growth Factors

A multitude of growth factors have been noted in the corneal epithelium and their gene expression investigated [146-148]. The responsibility of growth factors in supporting the structure and function of the cornea and in aiding with epithelial corneal healing has been unearthed in recent years [148,149], For example, autologous serum has demonstrated benefit in epithelial wound healing both in clinical studies and in vitro [150]. The following paragraphs provide a summary of the main growth factors involved in corneal epithelial healing.

Insulin-like Growth Factor-1

Insulin-like growth factor-1 is a regulatory peptide whose structural homology is similar to proinsulin. It has been demonstrated to facilitate proliferation, differentiation, and survival, contingent on the target cell and the presence of other hormones and growth factors. IGF-1 is released by the liver; similar to other growth factors, it is created locally, is at high concentrations in serum, and may act either in an autocrine or paracrine fashion [151]. Much of the literature reports that IGF-1 stimulates cell motility in a number of benign and malignant cell types. Still, its role in corneal epithelial cell motility and migration is debated [152].

It was demonstrated that substance P (SP) and IGF-1 synergistically stimulated corneal epithelial migration in an organ culture of the cornea. It was also shown that these two factors separately did not stimulate epithelial cell migration; however, co-treatment together did affect epithelial cell migration considerably [153]. In addition, IGF-1 appreciably augmented migration and expression of laminin-5 in cultured human corneal epithelial cells [154]. Clinically, the use of eye drops with both SP and IGF-1 has been demonstrated to be a successful treatment in the case of a child with neurotrophic and anhidrotic keratopathy [155]. Furthermore, eye drops with peptides (based on SP and IGF-1) have been shown to be successful in the hindrance of superficial punctate keratopathy in diabetic patients after cataract surgery [156] and have also effectively stimulated quick corneal epithelial healing in patients with persistent epithelial defects [157].

Insulin

This is an anabolic peptide hormone related to IGF; while it is known to play a role in wound repair, its exact function is not well-described [151]. Insulin incites the movement of human epidermal keratocytes [158]. In addition, topical insulin has been demonstrated to help with the healing of ulcerations [159,160] and burns [161]. It has also been reported that insulin is seen in the human tear fluid, and receptors to it have been demonstrated on the cornea [162], human ocular surface [163] and neuronal and vascular tissues of the retina [164-166]. The role of these receptors in the eye have not been elucidated yet, but diabetes is the main reason for blindness in individuals of working age and is frequently correlated with diseases of the corneal epithelium [55,166].

Zagon et al. reported that rigorous systemic therapy with insulin, which causes normal serum glucose levels in rats with diabetes, hinders the interruption in wound healing of the ocular surface epithelium noted in poorly controlled diabetic animals [167]. When systemic treatment with insulin induces normoglycemia in

diabetic animals, lowered levels of DNA synthesis in the ocular surface epithelium are returned to normal values when examined 3 weeks after the inciting wound. It also been described that treatment with topical insulin hastens wound healing in diabetic rats compared with the untreated diabetic group. With that said, topical insulin showed no improvement of corneal re-epithelialization of corneal lesions in non-diabetic healthy rats [168]. The precise method for hastening corneal epithelialization in diabetics after topical insulin administration has yet to be learned. The authors hypothesized it to be due to the capability of topical insulin to replenish lowered levels of DNA synthesis in basal epithelial cells to normal values, noted 48 hours after initial wound formation.

These findings hint at a possibly innovative and encouraging therapeutic indication for topical insulin in the treatment of diabetic corneal wounds. Topical administration of insulin to the surface of the eye will likely cause some, if only mild, systemic absorption. In fact, serum glucose levels monitored up to 14 hours after topical exposure to 1 unit of insulin had no influence on plasma glucose values in healthy or diabetic rats [168].

It is important to note that the insulin utilized in this study was compounded as a simple solution. This differs from the commercially available insulin dosage forms. In fact, commercial insulin includes pharmaceutical additives that are supplemented to uphold and extend the physical and microbial constancy of the medication. These additives include sodium edetate (antioxidant and chelator) and benzalkonium chloride (preservative). These two are well-known to enhance the stability of the formulation. Furthermore, this may elevate insulin systemic absorption and thus change blood glucose levels [107].

Thus, the possible therapeutic advantages may be lessened by systemic absorption of insulin from the eye, theoretically augmenting the effects of co-administered hypoglycemic medications [169]. Of course, this may not play a noteworthy role in humans since the molecular weight of insulin (6000 Da) is comparatively high to bypass the tight ocular/blood barriers, thus ensuring that only 4-5% of the given dose is absorbed via nasolacrimal drainage [170]. In addition, the topical dose necessary for corneal healing is believed to be 2-25 times less than the dose needed to cause hypoglycemia as a consequence of systemic absorption [168].

Hepatocyte Growth Factor (HGF), EGF, Keratocyte Growth Factor (KGF), TGF-B

These factors are expressed in corneal endothelial cells and keratocytes, and have been shown to be upregulated in rabbit keratocytes after the healing of corneal defects [171,172]. With that said, contradictory outcomes have been demonstrated in human trials, including those of EGF [173,174]. Furthermore, TGF-B has been suggested as a powerful propagator of corneal scarring. Corneal scarring is a significant impediment in influencing results of photorefractive surgery, leading to symptoms of haze and a decrease in best corrected visual acuity [175,176].

Nerve Growth Factor

This is a non-covalently linked dimer made up of two 118-residue polypeptides; each of these 118-residue polypeptides includes three intramolecular disulfide bridges. This factor is an example of the neurotrophic group of growth factors that enhance expansion of sympathetic and sensory neurons and development of neurons in the central nervous system [177].

Its biological role is not only active in neuronal cells, but in immunological cells as well [178]. Lambiase et al. discovered NGF receptors (TrKA) on the human cornea; it is expressed constitutively in healthy rat and human corneas [179]. Studies imply that NGF and its receptors are active in ocular inflammation and corneal epithelial differentiation. More recently, it has been shown that NGF exists in the conjunctiva, tear fluid, lacrimal glands and cornea [180-183].

The association between NGF and the health of the ocular surface has been investigated by various studies; one such study demonstrated that NGF stimulates proliferation and differentiation of rabbit corneal epithelial cells [184]. Another study reported greater NGF plasma levels in vernal keratoconjunctivitis with an association between mast cell conjunctival permeation and NGF levels [185]. Lambiase et al. administered topical murine NGF eye drops to 14 eyes with non-infectious corneal ulcers caused by chemical burns (3 eyes), abuse of topical anesthetics (2 eyes), orbital tumor surgery (1 eye), essential neurotrophic keratitis (5 eyes), surgery of acoustic neuroma (1 eye), penetrating keratoplasty of unknown reason (1 eye) and a lamellar keratoplasty for a herpetic vascularized scar (1 eye). Despite different causes of all of these ulcers, all corneas fully healed between 10 days to 6 weeks of NGF treatment. On the other hand, all patients endorsed mild to moderate conjunctival hyperemia, pain and photophobia, and nine patients did exhibit superficial or deep corneal neovascularization on exam; these were all symptoms that vanished once the ulcers were fully healed [145].

Bonin et al. discovered that murine NGF eye drops (1 drop of 200 micrograms/mL NGF solution every two hours for two days, followed by one drop six times daily until the ulcer demonstrated signs of recovery, followed by one drop four times daily of 200 micrograms/mL) improved corneal sensitivity and enhanced corneal epithelial healing in 45 eyes with moderate and severe neurotrophic keratitis within 12-42 days of NGF treatment. Further healing of the ulcers occurred, and improvement in corneal sensitivity and visual acuity were noted in NGF-treated eyes. Only in a few cases was a relapse noted during the follow-up period. Of note, temporary side effects, including hyperemia and pain in the eye, were seen upon administration of NGF treatment [186]. Additionally, Micera et al. commented on the possibility of NGF as a future treatment in a number of diseases of both the cornea and retina [187].

For these reasons, NGF has enticed a number of pharmaceutical scientists to cultivate specialized drug delivery systems with the goal of hindering NGF's degradation, thus prolonging its biological half-life and promoting its biological capabilities [188-190]. All of the above supports the clinical attractiveness of NGF as a possible

therapeutic agent to enhance corneal wound healing. With that said, much of the experiments investigating its clinical effect have used the 2.5S murine NGF; this has a few shortcomings: it is expensive, leads to allodynia (hyperalgesia upon topical use), and there are insufficient quantities of it (obtained from adult male mouse submandibular glands, snake venom) [107].

A few attempts have been made to create recombinant human nerve growth factor (fhNGF) with various microorganisms, including *Saccharomyces cerevisiae* [191,192], *Escherichia coli* inclusion bodies [193,194], insects [195,196] and mammalian cells [197]. Still, many of these investigations have been performed in vitro, and the clinical evidence from studies in vivo show that the performance of rhNGFs in human peripheral neuropathies is nowhere near as potent as that of murine NGF [198].

The creation of rhNGF and the technology necessary for it aren't complex; nevertheless, the biological role of B-NGF demands the creation of three disulfide bonds and a cysteine knot within 2 B-chains of 120 amino acids each after cutting pro-peptide sequences from a larger precursor molecule [199-201]. Colangelo et al. recently described the creation (in a laboratory) of rhNGF that was demonstrated to be effective both in vitro and in vivo [202]. This provides hope for the future of this growth factor and its capabilities in treating the ocular surface.

Substance P

Substance P (11-amino-acid polypeptide) is part of the tachykinin family and is a neurotransmitter of the trigeminal nerve. It is present physiologically in the normal cornea [152]. Its concentrations in tears are believed to reveal neuropeptide concentrations in ocular tissues. In cases of unilateral corneal hypoesthesia, its concentrations were lower in tears from the pathological eye than those in tears from the healthier eye [203].

Its topical use has no measureable effect on the speed of corneal epithelial wound closure in rabbits [204]. Still, when used simultaneously with IGF-1, it has been shown to accelerate corneal epithelial migration in organ cultured corneas [152] and in rats with trigeminal denervation [205]. As discussed above, this amalgamation has been successful in healing corneal epithelium in humans. Even so, SP is not commercially available.

Aldose Reductase Inhibitors

Aldose reductase (AR) is the rate-limiting enzyme in the polyol pathway in which glucose is converted to sorbitol. Its activity has been investigated in diabetic corneal epithelial disease mostly because of the consequence that AR inhibitors have on diabetic epithelial pathology [206]. Cogan et al. first utilized Sorbinil (an AR inhibitor) to treat a non-healing corneal ulcer in a patient with diabetes, which significantly improved [207]. Ohashi et al. continued this trend in 1988 when they utilized topical CT-112 (an AR inhibitor) to treat two patients with diabetes with non-improving corneal epithelial lesions [207].

The lesions resolved after treatment; furthermore, they manifested again when the CT-112 therapy was terminated and improved again when CT-112 therapy was begun. The patients were injury free on long-term maintenance treatment with CT-112 [207]. Yokoi et al. used topical CT-112 on one group of galactosemic rats, vehicle alone to another group of galactosemic rats, and vehicle alone to a third group of normal rats. Three weeks after, fluorescein uptake (which was used as a parameter of corneal permeability) was evaluated by fluorophotometry in the 3 groups. The galactosemic rats that had been treated with vehicle alone exhibited a much higher sodium fluorescein uptake than did normal rats and the CT-112-treated galactosemic rats. Interestingly, there was no significant difference in the fluorescein uptake between the vehicle-treated normal rats and the CT-112-treated galactosemic rats [208]. Overall, the use of AR inhibitors has been demonstrated to decrease corneal alterations after they have progressed, and decrease the formation of these changes when compared with untreated controls in both animals and humans. However, the pathogenesis by which AR activity leads to diabetic corneal pathology remains to be learned [206].

Miscellaneous

Nicergoline is an ergoline derivative that traverses the blood-brain barrier and has been extensively and safely to treat cognitive disease from dementia and stroke. In vivo studies have demonstrated that nicergoline treatment leads to appreciable increases in NGF levels within the frontal region of the brain and aids in the maintenance of cholinergic neurons, augmenting the amount of NGF and brain-derived neurotrophic factor in the brain of aged rats [209,210]. Kim et al. showed that oral nicergoline (10 mg/kg daily) for 2 weeks amplified the speed of corneal wound healing in 50 rat eyes [211].

This consequence of nicergoline is believed to be due to increased levels of NGF in the cornea and/or lacrimal glands. Of note, the particular ocular tissue accountable for cumulative NGF levels as a response to nicergoline has yet to be unraveled.

Carnosine is an antioxidant that may be utilized to oppose the consequences of advanced glycation endproducts in the ocular surface [142]. Its topical use has been demonstrated to be successful in upholding thiol levels in the cornea of rats with diabetes. Unfortunately, this study did not assess tear function or the state of the corneal surface by imaging [212].

Sericin and Aloe vera are topical protective medications that have been shown to uphold corneal surface wound healing [213,214].

Targeting microRNA miR-146Aa can treat persistent corneal defects in diabetic corneas, but this has only been demonstrated in organ cultures [215].

Conclusion

DM is a serious public health concern, due to its increasing prevalence and significant effects on different bodily organs, including the heart, kidneys, and the eyes. While diabetic retinopathy is the most common source of blindness in these

patients, diabetic keratopathy is an underestimated and frequent cause of visual loss in diabetics. In fact, corneal epithelial changes are seen in one in every four diabetics [13]. Not only are these corneal manifestations common, but recent advances in ocular surface imaging technology have shown that corneal changes occur very early in these patients. Furthermore, they affect different layers of the cornea, including the epithelium, sub-basal nerve plexus, and endothelium. Systemic control of diabetes and local treatment, with the goal of maintaining an undamaged epithelium and adequate lubrication, are the mainstays of treatment in diabetic keratopathy. While there are still many questions to be answered regarding this condition (including the possibility of breakthrough treatment options), one fact is undeniable: with the ever-increasing number of patients diagnosed with DM, diabetic keratopathy is an underrated manifestation of diabetes that is often disregarded by both clinicians and researchers. The authors hope that this review will increase awareness of this often under-valued ophthalmic condition, recognizing diabetic keratopathy as a vision-threatening manifestation of DM.

References

1. Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414: 782-787.
2. Wild S, Roglic G, Green A, Sicree R, King H (2004) Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27: 1047-1053.
3. Abouzeid M, Philpot B, Janus ED, Coates MJ, Dunbar JA (2013) Type 2 diabetes prevalence varies by socioeconomic status within and between migrant groups: analysis and implications for Australia. BMC Public Health 13: 252.
4. Yang W, Dall TM, Halder P, Gallo P, Kowal SL, et al. (2013) American Diabetes A. Economic costs of diabetes in the US in 2012. Diabetes Care 36: 1033-1046.
5. Nathan DM (1993) Long-term complications of diabetes mellitus. New England Journal of Medicine 328: 1676-1685.
6. Lachin JM, Orchard TJ, Nathan DM (2014) Update on cardiovascular outcomes at 40 years of the diabetes control and complications trial/ epidemiology of diabetes interventions and complications study. Diabetes Care 37: 39-43.
7. Kilpatrick ES, Rigby AS, Atkin SL (2008) A1C variability and the risk of microvascular complications in type 1 diabetes data from the diabetes control and complications trial. Diabetes Care 31: 2198-2202.
8. Stratton IM, Kohner EM, Aldington SJ, Turner RC, Holman RR, et al. (2001) Group UKPDS. Risk factors for incidence and progression of retinopathy in type II diabetes over 6 years from diagnosis. UKPDS 50. Diabetologia 44: 156-163.
9. Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, et al. (2015) Diabetic retinopathy: vascular and inflammatory disease. Journal of Diabetes Research 2015: 582060.
10. Rehany U, Ishii Y, Lahav M, Rumelt S (2000) Ultrastructural changes in corneas of diabetic patients: an electron-microscopy study. Cornea 19: 534-538.
11. Scheler A, Spoerl E, Boehm AG (2012) Effect of diabetes mellitus on corneal biomechanics and measurement of intraocular pressure. Acta Ophthalmologica 90: 447-451.
12. Schultz R, Van HD, Peters M, Klewin KM, Schutten WH (1981) Diabetic keratopathy. Trans Am Ophthalmol Soc 79: 180-199.
13. Inoue K, Kato S, Ohara C, Numaga J, Amano S, et al. (2001) Ocular and systemic factors relevant to diabetic keratoepitheliopathy. Cornea 20: 798-801.
14. Pflugfelder SC (2006) Is autologous serum a tonic for the ailing corneal epithelium? American Journal of Ophthalmology 142: 316-317.
15. Hyndiuk RA, Kazarian EL, Schultz RO, Seideman S (1977) Neurotrophic corneal ulcers in diabetes mellitus. Archives of Ophthalmology 95: 2193-2196.
16. Kaiserman I, Kaiserman N, Nakar S, Shlomo V (2005) Dry eye in diabetic patients. American Journal of Ophthalmology 139: 498-503.
17. Schultz R, Van HD, Peters M, Klewin KM, Schutten WH (1981) Diabetic keratopathy. Trans Am Ophthalmol Soc 79: 180-199.
18. Misra SL, Patel DV, McGhee CN, Pradhan M, Kilfoyle D, et al. (2014) Peripheral neuropathy and tear film dysfunction in type 1 diabetes mellitus. J Diabetes Res 2014: 848659.
19. Achtsidis V, Eleftheriadou I, Kozanidou E, Voumvourakis KI, Stamboulis E, et al. (2014) Dry eye syndrome in subjects with diabetes and association with neuropathy. Diabetes Care 37: 210-211.
20. Misra S (2013) Corneal Microstructure in Diabetes Mellitus and Its Association with Peripheral Neuropathy and Cardiac Autonomic Neuropathy. Doctoral thesis, Department of Ophthalmology, The University of Auckland.
21. Efron N (2007) Contact lens-induced changes in the anterior eye as observed *in vivo* with the confocal microscope. Progress in Retinal and Eye Research 26: 398-436.
22. Grupcheva CN, Chew GS, Edwards M, Craig JP, McGhee CN (2001) Imaging posterior polymorphous corneal dystrophy by *in vivo* confocal microscopy. Clinical & Experimental Ophthalmology 29: 256-259.
23. Niederer RL, Perumal D, Sherwin T, McGhee CN (2008) Laser scanning *in vivo* confocal microscopy reveals reduced innervation and reduction in cell density in all layers of the keratoconic cornea. Investigative Ophthalmology & Visual Science 49: 2964-2970.
24. Patel DV, Grupcheva CN, McGhee CN (2005) *In vivo* confocal microscopy of posterior polymorphous dystrophy. Cornea 24: 550-554.
25. Malik RA, Kallinikos P, Abbott CA, van Schie CH, Morgan P, et al. (2003) Corneal confocal microscopy: a non-invasive surrogate of nerve fibre damage and repair in diabetic patients. Diabetologia 46: 683-688.
26. Malik RA, Veves A, Walker D, Siddique I, Lye RH, et al. (2001) Sural nerve fibre pathology in diabetic patients with mild neuropathy: relationship to pain, quantitative sensory testing and peripheral nerve electrophysiology. Acta Neuropathologica 101: 367-374.
27. Tavakoli M, Boulton AJ, Efron N, Malik RA (2011) Increased Langerhan cell density and corneal nerve damage in diabetic patients: role of immune mechanisms in human diabetic neuropathy. Contact Lens & Anterior Eye 34: 7-11.

28. Tavakoli M, Kallinikos P, Iqbal A, Herbert A, Fadavi H, et al. (2011) Corneal confocal microscopy detects improvement in corneal nerve morphology with an improvement in risk factors for diabetic neuropathy. Diabetic Medicine 28: 1261-1267.

29. Misra SL, Goh YW, Patel DV, Riley AF, McGhee CN (2014) Corneal microstructural changes in nerve fiber, endothelial and epithelial density after cataract surgery in patients with diabetes mellitus. Cornea 34: 177-181.

30. Messmer EM, Schmid-Tannwald C, Zapp D (2010) *In vivo* confocal microscopy of corneal small fiber damage in diabetes mellitus. Graefes Archive for Clinical and Experimental Ophthalmology 248: 1307-1312.

31. Roszkowska AM, Tringali CG, Colosi P, Squeri CA, Ferreri G (1999) Corneal endothelium evaluation in type I and type II diabetes mellitus. Ophthalmologica 213: 258-261.

32. Delmonte DW, Kim T (2011) Anatomy and Physiology of the Cornea. J Cataract Refract Surg 37: 588-598.

33. R€ufer F, Schr€oder A, Erb C (2005) White-to-white corneal diameter; normal values in healthy humans obtained with the Orbscan II topography system. Cornea 24: 259-261.

34. Krachmer JH, Mannis MJ, Holland EJ, Cameron JD (2005) Corneal reaction to injury. In: eds, Cornea, 2nd ed. Philadelphia 115-133.

35. Maurice DM (1970) The transparency of the corneal stroma. Vision Research 10:107-108.

36. Meek KM, Boote C (2004) The organization of collagen in the corneal stroma. Experimental Eye Research 78: 503-512.

37. Gipson IK, Joyce NC (2008) Anatomy and cell biology of the cornea, superficial limbus, and conjunctiva. In: Albert DM, Jakobiec FA, eds, Principles and Practice of Ophthalmology, 3rd ed. Philadelphia, PA, Saunders 423-420.

38. Fini ME, Stramer BM (2005) How the cornea heals; cornea-specific repair mechanisms affecting surgical outcomes. Cornea 24: 2-11.

39. Jester JV, Moller-Pedersen T, Huang J, Sax CM, Kays WT, et al. (1999) The cellular basis of corneal transparency: evidence for □corneal crystalline□. Journal of Sciencei 112: 613-622.

40. Beebe DC, Coats JM (2000) The lens organizes the anterior segment: specification of neural crest cell differentiation in the avian eye. Developmental Biology 220: 424-431.

41. Watsky MA, McDermott ML, Edelhauser HF (1989) *In vitro* corneal endothelial permeability in rabbit and human: the effects of age, cataract surgery and diabetes. Experimental Eye Research 49: 751-767.

42. Stiemke MM, Edelhauser HF, Geroski DH (1991) The developing corneal endothelium: correlation of morphology, hydration and Na/K ATPase pump site density. Current Eye Research 10: 145-156

43. Yee RW, Matsuda M, Schultz RO, Edelhauser HF (1985) Changes in the normal corneal endothelial cellular pattern as a function of age. Current Eye Research 4: 671-678.

44. Bourne WM, Nelson LR, Hodge DO (1997) Central corneal endothelial cell changes over a ten-year period. Investigative Ophthalmology & Visual Science 38: 779-782.

45. Geroski DH, Matsuda M, Yee RW, Edelhauser HF (1985) Pump function of the human corneal endothelium; effects of age and cornea guttata. Ophthalmology 92: 759-763.

46. Polse KA, Brand RJ, Cohen SR, Guillon M (1990) Hypoxic effects on corneal morphology and function. Investigative Ophthalmology & Visual Science 31: 1542-1554.

47. Oliveira-Soto L, Efron N (2001) Morphology of corneal nerves using confocal microscopy. Cornea 20: 374-384.

48. Alves Mde C, Carvalheira JB, Modulo CM, Rocha EM (2009) Tear film and ocular surface changes in diabetes mellitus. ArquivosBrasileiros De Oftalmologia 71: 96-103.

49. Dogru M, Katakami C, Inoue M (2001) Tear function and ocular surface changes in noninsulin-dependent diabetes mellitus. Ophthalmology 108: 586-592.

50. Park JH, Kang SS, Kim JY, Tchah H (2016) Nerve Growth Factor Attenuates Apoptosis and Inflammation in the Diabetic Cornea. Cornea 57: 6767-6775.

51. Shih KC, Lam KS-L, Tong L (2017) A systemic review on the impact of diabetes mellitus on the ocular surface. Nutrition & Diabetes 7: 251-261.

52. Murphy PJ, Patel S, Kong N, Ryder RE, Marshall J (2004) Noninvasive assessment of corneal sensitivity in young and elderly diabetic and nondiabetic subjects. Investigative Ophthalmology & Visual Science 45: 1737-1742.

53. Pritchard N, Edwards K, Vagenas D, Shahidi AM, Sampson GP, et al. (2010) Corneal sensitivity as an ophthalmic marker of diabetic neuropathy. Optometry and vision science: official publication of the American Academy of Optometry 87: 1003-1008.

54. Neira-Zalentein W, Holopainen JM, Tervo TM, Borrás F, Acosta MC, et al. (2011) Corneal sensitivity in diabetic patients subjected to retinal laser photocoagulation. Investigative Ophthalmology & Visual Science 52: 6043-6049.

55. Rosenberg ME, Tervo TM, Immonen IJ, Müller LJ, Grönhagen-Riska C, et al. (2000) Corneal structure and sensitivity in type 1 diabetes mellitus. Investigative Ophthalmology and Visual Science 41: 2915-2921.

56. Saito J, Enoki M, Hara M, Morishige N, Chikama T, et al. (2003) Correlation of corneal sensation, but not of basal or reflex tear secretion, with the stage of diabetic retinopathy. Cornea 22: 15-18.

57. Tavakoli M, Kallinikos PA, Efron N, Andrew BJM, Malik RA, et al. (2007) Corneal sensitivity is reduced and relates the severity of neuropathy in patients with diabetes. Diabetes Care 30: 1895-1897.

58. Mackie IA. Current ocular therapy. In: Fraunfelder F, Roy FH, eds. Current Ocular Therapy, 4th ed. Philadelphia, PA: WB Saunders 506-508.

59. Cavanagh HD, Pihlaja D, Thoft RA, Dohlman CH (1976) The pathogenesis and treatment of persistent epithelial defects. Transactions American Academy of Ophthalmology and Otolaryngology 81: 754-769.

60. Groos E, Krachmer JH, Mannis MJ, Hooland EJ (1997) Cornea: fundamentals of corneal and external disease. In: eds. Cornea: Fundamentals of Corneal and External Disease. St. Louis, MO: Mosby 1339-62.

61. Sady C, Khosrof S, Nagaraj R (1995) Advanced Maillard reaction and

crosslinking of corneal collagen in diabetes. Biochemical and Biophysical Research Communications 214: 793-797.

62. Ni S, Yu J, Bao F, Li J, Elsheikh A, et al. (2011) Effect of glucose on the stress-strain behavior of *ex-vivo* rabbit cornea. Experimental Eye Research 92: 353-360.

63. Luce DA (2005) Determining *in vivo* biomechanical properties of the cornea with an ocular response analyzer. Journal of Cataract and Refractive Surgery 31: 156-162.

64. Ocular response analyzer: understand the cornea, understand the pressure. Brochure. Depew, NY. (https://www.scribd.com/document/61032829/Ocular-Response-Analyzer-Brochure).

65. Sahin A, Bayer A, Ozge G, Mumcuoğlu T (2009) Corneal biomechanical changes in diabetes mellitus and their influence on intraocular pressure measurements. Investigative Ophthalmology & Visual Science 50: 4597-4604.

66. Scheler A, Spoerl E, Boehm AG (2012) Effect of diabetes mellitus on corneal biomechanics and measurement of intraocular pressure. Acta Ophthalmologica 90: 447-451.

67. Goldich Y, Barkana Y, Gerber Y, Rasko A, Morad Y, et al. (2009) Effect of diabetes mellitus on biomechanical parameters of the cornea. Journal of Cataract and Refractive Surgery 35: 715-719.

68. Kynigopoulos M, Schlote T, Kotecha A, Tzamalis A, Pajic B, et al. (2008) Repeatability of intraocular pressure and corneal biomechanical properties measurements by the ocular response analyser. Klinische Monatsblätterfür Augenheilkunde 225: 357-360.

69. Hon Y, Lam AK (2013) Corneal deformation measurement using Scheimpflug noncontact tonometry. Optometry & Vision Science 90: 1-8.

70. Ali NQ, Patel DV, McGhee CN (2014) Biomechanical responses of healthy and keratoconic corneas measured using a noncontact Scheimpflug-based tonometer biomechanics of healthy and keratoconic corneas. Investigative Ophthalmology & Visual Science 55: 3651-3659.

71. Pérez-Rico C, Gutiérrez-Ortíz C, González-Mesa A, Zandueta AM, Moreno-Salgueiro A, et al. (2015) Effect of diabetes mellitus on Corvis ST measurement process. Acta Ophthalmologica 93: 193-198.

72. Whitacre MM, Stein RA, Hassanein K (1993) The effect of corneal thickness on applanation tonometry. American Journal of Ophthalmology 115: 592-596.

73. Lane JD, Krumholz DM, Sack RA, Morris C (2006) Tear glucose dynamics in diabetes mellitus. Current Eye Research 31: 895-901.

74. Ozdemir M, Buyukbese MA, Cetinkaya A, Ozdemir G (2003) Risk factors for ocular surface disorders in patients with diabetes mellitus. Diabetes Research and Clinical Practice 59: 195-199.

75. Yoon KC, Im SK, Seo MS (2004) Changes of tear film and ocular surface in diabetes mellitus. Korean Journal of Ophthalmology 18: 168-174.

76. Misra S, Ahn HN, Craig JP, Pradhan M, Patel DV, et al (2013) Effect of pan retinal photocoagulation on corneal sensation and the corneal sub basal nerve plexus in diabetes mellitus. Investigative Ophthalmology & Visual Science 54: 4485-4490.

77. Kim J, Kim CS, Sohn E, Jeong IH, Kim H, et al. (2011) Involvement of advanced glycation end products, oxidative stress and nuclear factor-kappaB in the development of diabetic keratopathy. Graefe's Archive for Clinical and Experimental Ophthalmology 249: 529-536.

78. Skarbez K, Priestley Y, Hoepf M, Koevary SB (2010) Comprehensive review of the effects of diabetes on ocular health. Expert Review of Ophthalmology 5: 557-577.

79. Alves M, Calegari VC, Cunha DA, Saad MJ, Velloso LA, et al. (2005) Increased expression of advanced glycation end-products and their receptor, and activation of nuclear factor Kappa-B in lacrimal glands of diabetic rats. Diabetologia 48: 2675-2681.

80. Bradley JL, Thomas PK, King RH, Watkins PJ (1994) A comparison of perineurial and vascular basal laminal changes in diabetic neuropathy. Acta Neuropathological 88: 426-432.

81. Taylor HR, Kimsey RA (1981) Corneal epithelial basement membrane changes in diabetes. Investigative Ophthalmology & Visual Science 20: 548-553.

82. Vracko R (1974) Basal lamina layering in diabetes mellitus. Evidence for accelerated rate of cell death and cell regeneration. Diabetes 23: 94-104.

83. Ljubimov AV, Burgeson RE, Butkowski RJ, Couchman JR, Zardi L, et al. (1996) Basement membrane abnormalities in human eyes with diabetic retinopathy. The Journal of Histochemistry and Cytochemistry 44: 1469-1479.

84. Ljubimov AV, Huang ZS, Huang GH, Burgeson RE, Gullberg D, et al. (1998) Human corneal epithelial basement membrane and integrin alterations diabetes and diabetic retinopathy. The Journal of Histochemistry and Cytochemistry 46: 1033-1041.

85. Morishige N, Chikama TI, Sassa Y, Nishida T (2001) Abnormal light scattering detected by confocal biomicroscopy at the corneal epithelial basement membrane of subjects with type II diabetes. Diabetologia 44: 340-345.

86. Takahashi N, Wakuta M, Morishige N, Chikama T, Nishida T, et al. (2007) Development of an instrument for measurement of light scattering at the corneal epithelial basement membrane in diabetic patients. Japanese Journal of Ophthalmology 51: 185-190.

87. Kaji Y, Usui T, Oshika T, Matsubara M, Yamashita H, et al. (2000) Advanced glycation end products in diabetic corneas. Investigative Ophthalmology & Visual Science 41: 362-368.

88. Schultz RO, Peters MA, Sobocinski K, Nassif K, Schultz KJ (1983) Diabetic keratopathy as a manifestation of peripheral neuropathy. American Journal of Ophthalmology 96: 368-371.

89. Gekka M, Miyata K, Nagai Y, Nemoto S, Sameshima T, et al. (2004) Corneal epithelial barrier function in diabetic patients. Cornea 23: 35-37.

90. Tsubota K, Chiba K, Shimazaki J (1991) Corneal epithelium in diabetic patients. Cornea 10: 156-160.

91. Frueh BE, Korner U, Bohnke M (1995) Confocal microscopy of the cornea in patients with diabetes. Klin Monbl Augenheilkd 206: 317-319.

92. Quadrado MJ, Popper M, Morgado AM, Murta JN, Van Best JA (2006) Diabetes and corneal cell densities in humans by *in vivo* confocal microscopy. Cornea 25: 761-768.

93. Chang PY, Carrel H, Huang JS, Wang IJ, Hou YC, et al. (2006) De-

creased density of corneal basal epithelium and subbasal corneal nerve bundle changes in patients with diabetic retinopathy. American Journal of Ophthalmology 142: 488-490.

94. Beuerman RW, Schimmelpfennig B (1980) Sensory denervation of the rabbit cornea affects epithelial properties. Experimental Neurology 69: 196-201.

95. Mehra S, Tavakoli M, Kallinikos PA, Efron N, Boulton AJ, et al. (2007) Corneal confocal microscopy detects early nerve regeneration after pancreas transplantation in patients with type 1 diabetes. Diabetes Care 30: 2608-2612.

96. Kallinikos P, Berhanu M, O'Donnell C, Boulton AJM, Efrone N, et al. (2004) Corneal nerve tortuosity in diabetic patients with neuropathy. Investigative Ophthalmology and Visual Science 45: 418-422.

97. Mocan MC, Durukan I, Irkec M, Orhan M (2006) Morphologic alterations of both the stromal and subbasal nerves in the corneas of patients with diabetes. Cornea 25: 769-773.

98. Tavakoli M, Mitu-Pretorian M, Petropoulos IN, Fadavi H, Asghar O, et al. (2013) Corneal confocal microscopy detects early nerve regeneration in diabetic neuropathy after simultaneous pancreas and kidney transplantation. Diabetes 62: 254-260.

99. Misra SL, Craig JP, Patel DV, McGhee CN, Pradhan M, et al. (2015) *In vivo* confocal microscopy of corneal nerves: an ocular biomarker for peripheral and cardiac autonomic neuropathy in type 1 diabetes mellitus an ocular biomarker for diabetic neuropathy. Investigative Ophthalmology and Visual Science 56: 5060-5065.

100. Ziegler D, Papanas N, Zhivov A, Allgeier S, Winter K, et al. (2014) Early detection of nerve fiber loss by corneal confocal microscopy and skin biopsy in recently diagnosed type 2 diabetes. Diabetes 63: 2454-2463

101. Pritchard N, Edwards K, Dehghani C, Fadavi H, Jeziorska M, et al. (2014) Longitudinal assessment of neuropathy in type 1 diabetes using novel ophthalmic markers (LANDMark): study design and baseline characteristics. Diabetes Research and Clinical Practice 104: 248-256.

102. Zhivov A, Winter K, Hovakimyan M, Peschel S, Harder V, et al. (2013) Imaging and quantification of subbasal nerve plexus in healthy volunteers and diabetic patients with or without retinopathy 8: 52157.

103. Patel DV, McGhee CN (2005) Mapping of the normal human corneal sub-basal nerve plexus by *in vivo* laser scanning confocal microscopy. Investigative Ophthalmology & Visual Science 46: 4485-4488.

104. Hossain P, Sachdev A, Malik RA (2005) Early detection of diabetic peripheral neuropathy with corneal confocal microscopy. Lancet 366: 1340-1343.

105. Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, et al. (2010) Corneal confocal microscopy: a novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes Care 33: 1792-1797.

106. Lambiase A, Rama P, Aloe L, Stefano B (1999) Management of neurotrophic keratopathy. Current Opinion in Ophthalmology 10: 270-276.

107. Abdelkader H, Patel DV, McGhee CNj, Alany RG (2011) New therapeutic approaches in the treatment of diabetic keratopathy: a review. Clinical & Experimental Ophthalmology 39: 259-270.

108. England JD, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, et al. (2009) Practice parameter: evaluation of distal symmetric polyneuropathy: role of autonomic testing, nerve biopsy, and skin biopsy (an evidence-based review). Report of the American Academy of Neurology, American Association of Neuromuscular and Electro Diagnostic Medicine, and American Academy of Physical Medicine and Rehabilitation. Neurology 72: 177-184.

109. Hertz P, Bril V, Orszag A, Ahmed A, Ng E, et al. (2011) Reproducibility of *in vivo* corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. Diabetic Medicine 28: 1253-1260.

110. Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, et al. (2015) Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. Diabetes Care 38: 1138-1144.

111. Shenoy R, Khandekar R, Bialasiewicz A, Al Muniri A (2009) Corneal endothelium in patients with diabetes mellitus: a historical cohort study. European Journal of Ophthalmology 19: 369-375.

112. Matsuda M, Ohguro N, Ishimoto I, Fukuda M (1990) Relationship of corneal endothelial morphology to diabetic retinopathy, duration of diabetes and glycemic control. Japanese Journal of Ophthalmology 34: 53-56.

113. Schultz RO, Matsuda M, Yee RW, Edelhauser HF, Schultz KJ (1984) Corneal endothelial changes in type I and type II diabetes mellitus. American Journal of Ophthalmology 98: 401-410.

114. Malik RA (2014) Which test for diagnosing early human diabetic neuropathy? Diabetes 63: 2206-2208.

115. Malik RA (2014) From the bedside to the bench and back again, with corneal confocal microscopy. Investigative Ophthalmology & Visual Science 55: 1231.

116. Patel SV (2015) Corneal confocal microscopy and diabetic neuropathy. Investigative Ophthalmology & Visual Science 56: 5066.

117. Avetisov SE, Novikov IA, Makhotin SS, Surnina ZV (2015) New approach to corneal nerve fibers morphometry in diabetes mellitus on the basis of confocal biomicroscopy. Vestnikoftalmologii 131: 5-14.

118. Edwards K, Pritchard N, Vagenas D, Russell A, Malik RA, et al. (2014) Standardizing corneal nerve fibre length for nerve tortuosity increases its association with measures of diabetic neuropathy. Diabet Med 31: 1205-1209.

119. DeMill DL, Hussain M, Pop-Busui R, Shtein MR (2016) Ocular surface disease in patients with diabetic peripheral neuropathy. British Journal of Ophthalmology 100: 7924-7928.

120. Pritchard N, Edwards K, Russell AW, Perkins BA, Malik RA, et al. (2015) Corneal confocal microscopy predicts 4-year incident peripheral neuropathy in type 1 diabetes. Diabetes Care 38: 671-675.

121. Misra SL, Craig JP, Patel DV, McGhee CN, Pradhan M, et al. (2015) *In vivo* confocal microscopy of corneal nerves: an ocular biomarker for peripheral and cardiac autonomic neuropathy in type 1 diabetes mellitus. Investigative Ophthalmology & Visual Science 56: 5060-5065.

122. Nitoda E, Kallinikos P, Pallikaris A, Moschandrea J, Amoiridis G, et al. (2012) Correlation of diabetic retinopathy and corneal neuropathy using confocal microscopy. Current Eye Research 37: 898-906.

123. Petropoulos IN, Alam U, Fadavi H, Asghar O, Green P, et al. (2013)

Corneal nerve loss detected with corneal confocal microscopy is symmetrical and related to the severity of diabetic polyneuropathy. Diabetes Care 36: 3646–3651.

124. Pritchard N, Dehghani C, Edwards K, Burgin E, Cheang N, et al. (2015) Utility of assessing nerve morphology in central cornea versus whorl area for diagnosing diabetic peripheral neuropathy. Cornea 34: 756-761.

125. Petropoulos IN, Ferdousi M, Marshall A, Alam U, Ponirakis G, et al. (2015) The inferior whorl for detecting diabetic peripheral neuropathy using corneal confocal microscopy. Investigative Ophthalmology & Visual Science 56: 2498-2504.

126. Dabbah MA, Graham J, Petropoulos IN, Tavakoli M, Malik RA (2011) Automatic analysis of diabetic peripheral neuropathy using multi-scale quantitative morphology of nerve fibers in corneal confocal microscopy imaging. Medical Image Analysis 15: 738-747.

127. Zhivov A, Winter K, Peschel S, Guthoff RF, Stachs O, et al. (2011) Quantitative analysis of corneal subbasal nerve plexus with *in vivo* confocal laser scanning microscopy. Klin Monbl Augenheilkd 228: 1067-1072.

128. Efron N, Edwards K, Roper N, Pritchard N, Sampson GP, et al. (2010) Repeatability of measuring corneal subbasal nerve fiber length in individuals with type 2 diabetes. Eye Contact Lens 36: 245-248.

129. Hume DA, Lovblom LE, Ahmed A, Yeung E, Orszag A, et al. (2012) Higher magnification lenses versus conventional lenses for evaluation of diabetic neuropathy by corneal *in vivo* confocal microscopy. Diabetes Research and Clinical Practice 97: 37-40.

130. Petropoulos IN, Alam U, Fadavi H, Marshall A, Asghar O, et al. (2014) Rapid automated diagnosis of diabetic peripheral neuropathy with *in vivo* corneal confocal microscopy. Investigative Ophthalmology & Visual Science 55: 2071-2078.

131. Ahmed A, Bril V, Orszag A, Paulson J, Yeung E, et al. (2012) Detection of diabetic sensorimotor polyneuropathy by corneal confocal microscopy in type 1 diabetes: a concurrent validity study. Diabetes Care 35: 821-828.

132. Hertz P, Bril V, Orszag A, Ahmed A, Ng E, et al. (2011) Reproducibility of *in vivo* corneal confocal microscopy as a novel screening test for early diabetic sensorimotor polyneuropathy. Diabetic Med 28: 1253-1260.

133. Sellers EA, Clark I, Tavakoli M, Dean HJ, McGavock J, et al. (2013) The acceptability and feasibility of corneal confocal microscopy to detect early diabetic neuropathy in children: a pilot study. Diabetic Medicine 30: 630-631.

134. Utsunomiya T, Nagaoka T, Hanada K, Omae T, Yokota H, et al. (2015) Imaging of the corneal subbasal whorl-like nerve plexus: more accurate depiction of the extent of corneal nerve damage in patients with diabetes. Investigative Ophthalmology & Visual Science 56: 5417-5423.

135. Patel DV, McGhee CN (2013) Techniques for wide-field assessment of the human corneal subbasal nerve plexus. Cornea 32: 140-141.

136. Dehghani C, Pritchard N, Edwards K, Russell AW, Malik RA, et al. (2014) Fully automated, semiautomated, and manual morphometric analysis of corneal subbasal nerve plexus in individuals with and without diabetes. Cornea 33: 696-702.

137. Edwards K, Pritchard N, Gosschalk K, Sampson GP, Russell A, et al. (2012) Wide field assessment of the human corneal subbasal nerve plexus in diabetic neuropathy using a novel mapping technique. Cornea 31: 1078-1082.

138. De Clerck EE, Schouten JS, Berendschot TT, Kessels AG, Nuijts RM, et al. (2015) New ophthalmologic imaging techniques for detection and monitoring of neurodegenerative changes in diabetes: a systematic review. The Lancet Diabetes & Endocrinology 3: 653-663.

139. IshibashiF, Okino M, Ishibashi M, Kawasaki A, Endo N, et al. (2012) Corneal nerve fiber pathology in Japanese type 1 diabetic patients and its correlation with antecedent glycemic control and blood pressure. Journal of Diabetes Investigation 3: 191-198.

140. Abdul-Hamid M, Moustafa N (2014) Amelioration of alloxan-induced diabetic keratopathy by beta-carotene. Experimental and Toxicologic Pathology 66: 49-59.

141. Davidson EP, Holmes A, Coppey LJ, Yorek MA (2015) Effect of combination therapy consisting of enalapril, alpha-lipoic acid, and menhaden oil on diabetic neuropathy in a high fat/low dose streptozotocin treated rat. European Journal of Pharmacology 765: 258-267.

142. Shevalye H, Yorek MS, Coppey LJ, Holmes A, Harper MM, et al. (2015) Effect of enriching the diet with menhaden oil or daily treatment with resolving D1 on neuropathy in a mouse model of type 2 diabetes. Journal of Neurophysiology 114: 199-208.

143. Davidson EP, Coppey LJ, Yorek MA (2012) Early loss of innervation of cornea epithelium in streptozotocin-induced type 1 diabetic rats: improvement with ilepatril treatment. Investigative Ophthalmology & Visual Science 53: 8067-8074.

144. Kim J, Kim CS, Kim H, Jeong IH, Sohn E, et al. (2011) Protection against advances glycation end products and oxidative stress during the development of diabetic keratopathy by KIOM-79. Journal of Pharmacy and Pharmacology 63: 524-530.

145. Lambiase A, Rama P, Bonini S, Caprioglio G, Aloe L (1998) Topical treatment with nerve growth factor for corneal neurotrophic ulcers. New England Journal of Medicine 338: 1174-1180.

146. Hongo M, Itoi M, Yamaguchi K, Imanishi J (1992) Distribution of epidermal growth factor (EGF) receptors in rabbit corneal epithelial cells, keratocytes and endothelial cells, and the changes induced by transforming growth factor-beta 1. Experimental Eye Research 54: 9-16.

147. Kiritoshi A, SundarRaj N, Thoft RA (1991) Differentiation in cultured limbal epithelium as defined by keratin expression. Invest Ophthalmol Vis Sci 32: 3073-3077.

148. Schultz GS, Davis JB, Eiferman RA (1988) Growth factors and corneal epithelium. Cornea 7: 96-101.

149. Tripathi BJ, Kwait JPS, Tripathi RC (1990) Corneal growth factors: a new generation of ophthalmic pharmaceuticals. Cornea 9: 2-9.

150. Schulze SD, Sekundo W, Kroll P (2006) Autologous serum for the treatment of corneal epithelial abrasions in diabetic patients undergoing vitrectomy. American Journal of Ophthalmology 142: 207-214.

151. McAvoy JW, Chamberlain CG (1990) Growth factors in the eye. Prog Growth Factor Res 2: 29-43.

152. Nishida T, Nakamura M, Ofuji K, Reid TW, Mannis MJ, et al. (1996) Synergistic effects of substance P with insulin-like growth factor-1 on epithelial migration of the cornea. Journal of Cellular Physiology 169: 159-166.

153. Nakamura M, Ofuji K, Chikama T, Nishida T (1997) The NK1 receptor and its participation in the synergistic enhancement of corneal epithelial migration by substance P and insulin-like growth factor- 1. British Journal of Pharmacology 120: 547-552.

154. Lee HK, Lee JH, Kim M, Kariya Y, Miyazaki K, et al. (2006) Insulin-like growth factor-1 induces migration and expression of laminin-5 incultutured human corneal epithelial cells. Investigative Ophthalmology & Visual Science 47: 873- 882.

155. Brown SM, Lamberts DW, Reid TW, Nishida T, Murphy CJ (1997) Neurotrophic and anhidrotic keratopathy treated with substance P and insulin like growth factor 1. Archives of Ophthalmology 115: 926-927.

156. Chikamoto N, Chikama T, Yamada N, Nishida T, Ishimitsu T, et al. (2009) Efficacy of substance P and insulin-like growth factor-1 peptides for preventing postsurgical superficial punctate keratopathy in diabetic patients. Japanese Journal of Ophthalmology 53: 464-469.

157. Yamada N, Matsuda R, Morishige N, Yanai R, Chikama TI, et al. (2008) Open clinical study of eye-drops containing tetrapeptides derived from substance P and insulin-like growth factor-1 for treatment of persistent corneal epithelial defects associated with neurotrophic keratopathy. British Journal of Ophthalmology 92: 896-900.

158. Benoliel AM, Kahn-Perles B, Imbert J, Verrando P (1987) Insulin stimulates hypotactic migration of human epidermal keratinocytes through activation of NFkappa B transcription factor. Journal of Cell Science 110: 2089-2097.

159. Rosenthal SP (1968) Acceleration of primary wound healing by insulin. Archives of Surgery 96: 53-55.

160. Van Ort SR, Gerber RM (1976) Topical application of insulin in the treatment of decubitus ulcers: a pilot study. Nursing Research 25: 9-12.

161. Pierre EJ, Barrow RE, Hawkins HK, Nguyen TT, Sakurai Y, et al. (1998) Effects of insulin on wound healing. Journal of Trauma and Acute Care Surgery 44: 342-345.

162. Naeser P (1997) Insulin receptors in human ocular tissues. Immunohistochemical demonstration in normal and diabetic eyes. Upsala Journal of Medical Sciences 102: 35-40.

163. Rocha EM, Cunha DA, Carneiro EM, VellosoL A, Boschero AC, et al. (2002) Insulin, insulin receptor and insulin-like growth Factor-I receptor on the human ocular surface. Advances in Experimental Medicine and Biology 506: 607-610.

164. Thomopoulos P, Pessac B (1979) Insulin receptors in cultured mouse retinal cells. Diabetologia 16: 275-279.

165. Im JH, Pillion DJ, Meezan E (1986) Comparison of insulin receptors from bovine retinal blood vessels and nonvascular retinal tissue. Investigative Ophthalmology & Visual Science 27: 1681-1690.

166. Zagon IS, Jenkins JB, Sassani JW, Wylie JD, Ruth TB, et al. (2002) Naltrexone, an opioid antagonist, facilitates reepithelialization of the cornea in diabetic rat. Diabetes 51: 3055-3062.

167. Zagon IS, Sassani JW, McLaughlin PJ (2006) Insulin treatment ameliorate impaired corneal reepithelization in diabetic rats. Diabetes 55: 1141-1147.

168. Zagon IS, Klocek MS, Sassani JW, McLaughlin PJ (2007) Use of topical insulin to normalize corneal epithelial healing in diabetes mellitus. Archives of Ophthalmology125: 1082-1088.

169. Lee YC, Simamora P, Pinsuwan S, Yalkowsky SH (2002) Review on the systemic delivery of insulin via the ocular route. International Journal of Pharmaceutics 233: 1-18.

170. Chiou GC (1991) Systemic delivery of polypeptide drugs through ocular route. Annual Review of Pharmacology and Toxicology 31: 457-467.

171. Wilson SE, Walker JW, Chwang EL, He YG (1993) Hepatocyte growth factor, keratinocyte growth factor, their receptors, fibroblast growth factor receptor-2, and the cells of the cornea. Investigative Ophthalmology & Visual Science 34: 2544-2561.

172. Li DQ, Tseng SC (1996) Differential regulation of cytokine and receptor transcript expression in human corneal and limbal fibroblasts by epidermal growth factor, transforming growth factor-alpha, platelet derived growth factor B, and interleukin-1 beta. Investigative Ophthalmology & Visual Science 37: 2068-2080.

173. Hosotani H, Ohashi Y, Yamada M, Tsubota K (1995) Reversal of abnormal corneal epithelial cell morphologic characteristics and reduced corneal sensitivity in diabetic patients by aldose reductase inhibitor, CT-112. American Journal of Ophthalmology 119: 288-294.

174. Daniele S, Gilbard J, Schepens C (1992) Treatment of persistent epithelial defects in neurotrophic keratitis with epidermal growth factor: a preliminary open study. Graefes Archive for Clinical and Experimental Ophthalmology 230: 314-317.

175. Jester JV, Barry-Lane PA, Petroll WM, Olsen DR, Cavanagh HD (1997) Inhibition of corneal fibrosis by topical application of blocking antibodies to TGF-beta in the rabbit. Cornea16: 177-187.

176. Mayers J, Gomes J, Siepser S, Rapuano CJ, Eagle RC, et al. (1997) Effect of transforming growth factor beta 1 on stromal haze following excimer laser photorefractive keratectomy in rabbits. Journal of Refractive Surgery 13: 356–361.

177. Levi-Montalcini R (1987) The nerve growth factor 35 years later. Science 237: 1154-1162.

178. Levi-Montalcini R, Aloe L, Alleva E (1990) A role for nerve growth factor in nervous, endocrine and immune system. Prog Neuroendocrinimuunology1: 1-10.

179. Lambiase A, Bonini S, Micera A, Rama P, Bonini S, et al. (1998) Expression of nerve growth factors on the ocular surface in healthy subjects and during manifestation of inflammatory diseases. Invest Ophthalmol Vis Sci 39: 1272-1275.

180. You L, Kruse FE, Volcker HE (2000) Neurotrophic factors in the human cornea. Investigative Ophthalmology & Visual Science 41:692-702.

181. Touhami A, Grueterich M, Tseng SC (2002) The role of NGF signaling in human limbal epithelium expanded by amniotic membrane culture. Investigative Ophthalmology & Visual Science 43: 987-994.

182. Ghinelli E, Johansson J, Ríos JD, Chen LL, Zoukhri D, et al. (2003) Presence and localization of neurotrophins and neurotrophin receptors in rat lacrimal gland. Investigative Ophthalmology & Visual Science 44: 3352-3357.

183. Ríos JD, Ghinelli E, Gu J, Hodges RR, Dartt DA, et al. (2007) Role of neurotrophins and neurotrophin receptors in rat conjunctival goblet cell secretion and proliferation. Investigative Ophthalmology & Visual Science 48:1543-1551.

184. Kruse F, Tseng S (1993) Growth factors modulate clonal growth and differentiation of cultured rabbit limbal and corneal epithelium. Investigative Ophthalmology & Visual Science 34: 1963-1976.

185. Lambiase A, Bonini S, Bonini S, Micera A, Magrini L, et al. (1995) Increased plasma levels of nerve growth factor in vernal keratoconjunctivitis and relationship to conjunctival mast cells. Investigative Ophthalmology & Visual Science 36: 2127-2132.

186. Bonini S, Lambiase A, Rama P, Caprioglio G, Aloe L, et al. (2000) Topical treatment with nerve growth factor for neurotrophic keratitis. Ophthalmology 107: 1347-1352.

187. Micera A, Lambiase A, Aloe L, Bonini S, Levi-Schaffer F, et al. (2004) Nerve growth factor involvement in the visual system: implications in allergic and neurodegenerative diseases. Cytokine Growth Factor Rev 15:411-417.

188. Sakiyama-Elbert S, Hubbell J (2000) Controlled release of nerve growth factor from a heparin-based cell ingrowth matrix. Journal of Controlled Release 69: 149-158.

189. Xie Y, Ye L, Zhang X, Cui W, Lou J, et al. (2005) Transport of nerve growth factor encapsulated into liposomes across the blood-brain barrier: *in vitro* and *in vivo* studies. Journal of Controlled Release 105: 106-119.

190. Pfister LA, Alther E, Papaloïzos M, Merkle HP, Gander B, et al. (2008) Controlled nerve growth factor release from multiply alginate/ chitosan-based nerve conduits. European Journal of Pharmaceutics and Biopharmaceutics 69: 563□572.

191. Kanaya E, Higashizaki T, Ozawa F, Hirai K, Nishizawa M, et al. (1989) Synthesis and secretion of human growth factor by *Saccharomyces cerevisiae*. Gene 83: 65–74.

192. Nishizawa M, Ozawa F, Higashizaki T, Hirai K, Hishinuma F, et al. (1993) Biologically active human and mouse nerve growth factors secreted by the yeast *Saccharomyces cerevisiae*. Applied Microbiology and Biotechnology 38: 624-630.

193. Negro A, Martini I, Bigon E, Cazzola F, Minozzi C, et al. (1992) Synthesis of the biologically active b-subunit of human nerve growth factor in *Escherichia coli*. Gene110: 251-256.

194. Rattenholl A, Lilie H, Grossmann A, Stern A, Schwarz E, et al. (2001) The pro-sequence facilitates folding of human nerve growth factor from *Escherichia coli* inclusion bodies. European Journal of Biochemistry 268: 3296-3303.

195. Barnett J, Chow J, Nguyen B, Eggers D, Osen E, et al. (1991) Physicochemical characterization of recombinant human nerve growth factor produced in insect cells with a baculovirus vector. Journal of Neurochemistry 57: 1052-1061.

196. Allen SJ, Robertson AG, Tyler SJ, Wilcock GK, Dawbarn D (2001) Recombinant human nerve growth factor for clinical trials: protein expression, purification, stability and characterisation of binding to infusion pumps. Journal of Biochemical and Biophysical Methods 47: 239-255.

197. Iwane M, Kitamura Y, Kaisho Y, Yoshimura K, Shintani A, et al. (1990) Production, purification and characterization of biologically active recombinant human nerve growth factor. Biochemical and Biophysical Research Communications 171: 116-122.

198. Apfel SC (2002) Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold? International Review of Neurobiology 50: 393-413.

199. Ullrich A, Gray A, Berman C, Dull TJ (1983) Human beta nerve growth factor gene sequence highly homologous to that of mouse. Nature 303: 821-825.

200. Edwards RH, Selby MJ, Garcia PD, Rutter WJ (1988) Processing of the native growth factor precursor to form biologically active nerve growth factor. The Journal of Biological Chemistry 263: 6810-6815.

201. Shooter EM (2001) Early days of the nerve growth factor proteins. Annual Review of Neuroscience 24: 601-629.

202. Colangelo AM, Finotti N, Ceriani M, Alberghina L, Martegani E, et al. (2005) Recombinant human nerve growth factor with a marked activity *in vitro* and *in vivo*. Proceedings of the National Academy of Sciences of the United States of America 102: 18658-18663.

203. Yamada M, Ogata M, Kawai M, Mashima Y (2000) Decreased substance P concentrations in tears from patients with corneal hypesthesia. Am J Ophthalmol129: 671-672.

204. Kingsley RE, Marfurt CF (1997) Topical substance P and corneal epithelial wound closure in the rabbit. Investigative Ophthalmology & Visual Science 38: 388-395.

205. Nagano T, Nakamura M, Nakata K, Yamaguchi T, Takase K, et al. (2003) Effects of substance P and IGF-1 in cornea epithelial barrier function and wound healing in a rat model of neurotrophic keratopathy. Investigative Ophthalmology & Visual Science 44: 3810-3815.

206. Herse PR (1988) A review of manifestations of diabetes mellitus in the anterior eye and cornea. American Journal of Optometry and Physiological Optics 65: 224-230.

207. Ohashi Y, Matsuda M, Hosotani H, Tano Y, Ishimoto I, et al. (1988) Aldose reductase inhibitor(CT-112) eye drops for diabetic corneal epitheliopathy. American Journal of Ophthalmology 105: 233-238.

208. Yokoi N, Niiya A, Komuro A, Yokogaki S, Naka H, et al. (1997) Effects of aldose reductase inhibitor CT-112 on the corneal epithelial barrier of galactose-fed rats. Current Eye Research 16:595-599.

209. Nishio T, Sunohara N, Furukawa S, Akiguchi I, Kudo Y (1998) Repeated injections of nicergoline increase the nerve growth factor level in the aged rat brain. Japanese Journal of Pharmacology 76: 321-323.

210. Giardino L, Giuliani A, Battaglia A, Carfagna N, Aloe L, et al. (2002) Neuroprotection and aging of the cholinergic system: a role for the ergoline derivative nicergoline (Sermion). Neuroscience 109: 487-497.

211. Kim SY, Choi JS, Joo CK (2009) Effects of nicergoline on corneal epithelial wound healing in rat eyes. Investigative Ophthalmology & Visual Science 50: 621-625.

212. Shi Q, Yan H (2010) Changes of the thiol levels in the corneas of the diabetic rats: effect of carnosine, aspirin and a combination eye drops. International Journal of Ophthalmology 3: 211-215.

213. Nagai N, Ito Y (2013) Therapeutic effects of sericin on diabetic keratopathy in Otsuka Long-Evans Tokushima Fatty rats. World Journal of Diabetes 4: 282-289.

214. Atiba A, Wasfy T, Abdo W, Ghoneim A, Kamal T, et al. (2015) *Aloe vera* gel facilitates re-epithelialization of corneal alkali burn in normal and diabetic rats. Clinical Ophthalmology 9: 2019-2026.

215. Winkler MA, Dib C, Ljubimov AV (2014) Targeting miR-146a to treat delayed wound healing in human diabetic organ-cultured corneas 9: 114692.