Diabetic corneal neuropathy as a surrogate marker for diabetic peripheral neuropathy

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Abstract
Diabetic neuropathy is a prevalent microvascular complication of diabetes mellitus, affecting nerves in all parts of the body including corneal nerves and peripheral nervous system, leading to diabetic corneal neuropathy and diabetic peripheral neuropathy, respectively. Diabetic peripheral neuropathy is diagnosed in clinical practice using electrophysiological nerve conduction studies, clinical scoring, and skin biopsies. However, these diagnostic methods have limited sensitivity in detecting small-fiber disease, hence they do not accurately reflect the status of diabetic neuropathy. More recently, analysis of alterations in the corneal nerves has emerged as a promising surrogate marker for diabetic peripheral neuropathy. In this review, we will discuss the relationship between diabetic corneal neuropathy and diabetic peripheral neuropathy, elaborating on the foundational aspects of each: pathogenesis, clinical presentation, evaluation, and management. We will further discuss the relevance of diabetic corneal neuropathy in detecting the presence of diabetic peripheral neuropathy, particularly early diabetic peripheral neuropathy; the correlation between the severity of diabetic corneal neuropathy and that of diabetic peripheral neuropathy; and the role of diabetic corneal neuropathy in the stratification of complications of diabetic peripheral neuropathy.

Key Words: corneal nerve quantification; corneal nerves; diabetic cornea; diabetic corneal neuropathy; diabetic microvascular complications; diabetic peripheral neuropathy; in vivo confocal microscopy; neurotrophic keratopathy; ocular surface

Introduction
Diabetes mellitus (DM) is a chronic metabolic disease characterized by systemic hyperglycemia. The disease manifests in either of two forms: a primary insulin production deficiency in type 1 DM (T1DM) or a gradual development of insulin resistance and decreased sensitivity towards insulin secretion in type 2 DM (T2DM) (Association, 2010). In 2013, the total number of diabetics in the world was 382 million (Alaboud et al., 2016). This figure has been projected to reach 366 million by 2030 (Khalil, 2017) and 693 million by 2045 (Alwin Robert and Al Dawish, 2019). Besides adversely affecting one’s health, DM has demonstrated debilitating effects that scale beyond the individual level – enormous healthcare costs were inflicted upon the economy in 2007, amounting to a staggering US$174 billion and US$58 billion arising from loss of productivity (Khalil, 2017; Alwin Robert and Al Dawish, 2019). Meanwhile, the worldwide economic burden of DM is poised to hit US$2.1 trillion by 2030 (Bommer et al., 2018). Given the far-reaching implications of DM as an international health challenge, it is imperative for us to understand how to actively prevent or manage the complications that are associated with DM.

DM, especially if uncontrolled over a prolonged period, is linked to the development of both macrovascular and microvascular complications. Microvascular complications can be primarily categorized into diabetic nephropathy, diabetic retinopathy, and diabetic peripheral neuropathy (DPN) (Khalil, 2017). DPN presents as a length-dependent sensorimotor neuropathy commonly presents as a symmetrical sensorimotor neuropathy OR “keratopathy”, “corneal sensitivity” AND “diabetes”, “in-vivo confocal microscopy” AND “diabetes”, “corneal nerves” AND “diabetes”. Only papers written in English were incorporated in our review, and we restricted the date of publication to the most recent ten years as much as possible. Supplementary relevant articles were also extracted from the bibliographies of the existing articles. After duplicate removal, the authors independently screened the abstracts and shortlisted papers based on our inclusion criteria. We later examined the full-text version of all selected articles. Out of the 319 articles identified and screened from the preliminary database search, a result of 102 articles was included in the final manuscript.

Diabetic Microvascular Complications
Diabetic microvascular complications manifest mainly as diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy [Alaboud et al., 2016; Khalil, 2017; Khanam et al., 2017; Alwin Robert and Al Dawish, 2019]. Diabetic nephropathy commonly presents as a symmetrical sensorimotor neuropathy (Gupta and Gupta, 2014; Alwin Robert and Al Dawish, 2019), affecting nerves in all parts of the body including those on the cornea, leading to DCN. As diabetic neuropathy is a systemic nervous disorder, patients with DCN may also suffer from DPN. Therefore, the evaluation for DCN not only presents a window to diagnose DPN early, it also could serve as a surrogate marker for DPN (Zhao et al., 2019).

Diabetic Peripheral Neuropathy
With the incidence of DM increasing across the world, the incidence of complications that follows is also expected to rise accordingly (Sun et al., 2020). DPN is a well-documented complication of DM, affecting up to 50% of patients during the clinical course (Bikbova et al., 2018). It usually manifests itself late into the disease or in uncontrolled DM, with as many as 39% of patients experiencing painful DPN when left untreated (Snyder et al., 2016). An estimated 236 million persons worldwide have been diagnosed with DPN (Tesfaye and Selvarajah, 2012). DPN is also a significant contributor to morbidity and mortality in diabetic patients (Tesfaye and Selvarajah, 2012) – patients with DPN are 10 to 20 times more likely to undergo a limb amputation than patients without DPN (Sun et al., 2020), with a lower
limb lost as a result of DPN every 30 seconds (Selvarajah et al., 2019). In the United States, the annual cost per patient to visit various healthcare institutions for DPN increased by 46%, with total healthcare costs channeled to manage this complication adding up to an astonishing US$10.91 billion a year (Liu et al., 2019).

**Pathogenesis of diabetic neuropathy**

To date, the pathophysiology of diabetic neuropathy has yet to be fully elucidated—the presentation of neuropathic symptoms differs from patient to patient and is far from uniform. Though, the underlying pathophysiological mechanisms may be similarly heterogeneous, likely attributed to the metabolic and microvascular processes from chronic hyperglycemia (Figure 1). The metabolic pathways related to the pathogenesis include formation and accumulation of advanced glycation end products (AGE), activation of the aldose reductase (polyol) pathway, activation of protein kinase C (PKC) and mitogen-activated protein kinases, as well as the production of reactive oxygen species (ROS) (Singh et al., 2014). Collectively, these processes culminate in direct nerve axonal injury, ischemia, and eventual neuronal cell loss (Hicks and Selvin, 2019). The hypoxic and ischemic environment also promotes cytokine proliferation (tumor necrosis factor-α and interleukin-6) that contributes to the role of inflammation in diabetic neuropathy (Ristikj-Stomnaroska et al., 2019). However, serum nerve growth factor has been reported to be uninvolved in the development of DPN. In fact, it has been found to be negatively associated with the severity of DPN (Kim et al., 2009).

**Advanced glycation end products**

When blood glucose is chronically elevated, glucose is shunted into alternative metabolic pathways, including the non-enzymatic addition of sugar moieties onto various adducts such as arginine and lysine residues of proteins, free amino groups on lipids, or guanine nucleic acids (Peppa et al., 2009), producing a group of molecules termed as AGE. AGEs were shown to accumulate in perineurial collagen, Schwann cells, and the axoplasm of nerve fibers (Markoulli et al., 2018). Within these target cells, they alter intracellular protein function, interfere with the physiological interaction between the extracellular matrix and their receptors, and result in the production of ROS via plasma protein binding to receptors for AGE (Ryle and Donaghy, 1995). The nuclear factor kappa B (NF-kB) transactivation pathway is subsequently activated by AGE-receptor for AGE interactions, leading to pro-inflammatory gene expression and apoptosis of neuronal cells (Kim et al., 2011).

**Aldose reductase pathway (polyol pathway)**

Likewise to the formation and accumulation of AGEs, excess blood glucose could be shunted to the aldose reductase pathway, otherwise known as the polyol pathway. The enzymes aldose reductase and sorbitol dehydrogenase catalyze the conversion of the excess glucose to sorbitol and fructose (Takemori, 2002). As sorbitol and fructose are unable to pass through the nerve cell membrane, their intracellular accumulation leads to increasing osmotic stress (Markoulli et al., 2018). This is further compounded by the depleting myo-inositol levels correspondingly decrease membrane sodium-potassium adenosine triphosphate (Na+/K+ ATPase) activity, inducing abnormal structural modifications such as axonal swelling and progressive axonal atrophy (Xia et al., 1995). With impaired endoneurial blood supply, nerve perfusion deteriorates (Markoulli et al., 2018). This culminates in overall decreased nerve conduction velocity and eventual neuronal breakdown (Mansoor et al., 2020).

**Protein kinase C pathway**

Hyperglycemia induces the production of diacylglycerol, which is an activator of PKC. PKC is a serine/threonine-related protein kinase that takes on an integral role in cellular signal transduction. When it is activated, it leads to injurious effects on target cells that are involved in the microvascular complications (Hempel et al., 1997). It has been postulated that activated PKC leads to diminished Na+/K+ ATPase activity, leading to an electrolyte imbalance that affects nerve conduction velocity and neuronal regeneration (Xia et al., 1995). Clinically, this is predictive of the production and accumulation of AGEs, such as Ruboxistaurin, have been shown to restore nerve conduction rates and neuronal blood flow (Gerald and King, 2010). Such findings are evidence that PKC pathway activation is indeed a metabolic process that mediates changes in membrane potential, neuronal function, and nerve conductivity in diabetic neuropathy (Mansoor et al., 2020).

**Production of reactive oxygen species**

Hyperglycemic-induced oxidative free radical formation is considered as the coalescing principle of all the pathways of diabetic neuropathy. Elevated blood glucose levels increase the amount of sugar entry into the mitochondria, correspondingly increasing the rate of oxidative metabolism of glucose within the mitochondria (Giacco and Brownlee, 2010). This results in excessive formation of reactive oxygen species and superoxide ions, the primary oxygen free radical produced in the mitochondria (Niedzwiecki and Dalby, 2002). With a persistent increase in the concentration of ROS across the mitochondrial electron transport chain and a corresponding decrease in cellular antioxidative capacity, this imbalance puts the body in a state of oxidative stress—a characteristic picture seen in hyperglycemia (Oyenihi et al., 2015).

Nerve cells are the most susceptible to such mitochondrial oxidative injury as they possess a relatively larger mitochondrial volume (Mansoor et al., 2020). This decreases the generation of energy required for cellular processes, inhibiting nerve conduction and causing demyelination of axons (Kim et al., 2011). Hyperglycemia-induced oxidative stress is known to induce apoptosis of tissue cells by specifically activating the Bax-caspase pathway. This diminishes the electrochemical gradient across the mitochondrial membrane, allowing for the leakage of cytochrome c into the cytoplasm and herewith apoptosis to occur (Ryle and Donaghy, 2013). In addition, ROS are strong activators of mitogen-activated protein kinases that manifest themselves as signal transducers of various pro-inflammatory pathways to produce cytokines (interleukin-1, tumor necrosis factor, interleukin-6) (Du et al., 2010), contributing to the role of inflammation in the pathogenesis of diabetic neuropathy (Ristikj-Stomnaroska et al., 2019).

**Clinical manifestations of diabetic peripheral neuropathy**

DPN has been defined as a symmetrical, length-dependent sensorimotor polyneuropathy attributed to metabolic and microvascular alterations (Tesfaye and Selvarajah, 2012). Classically, affected regions of the body demonstrate a reduced sensation and autonomic neuropathy, with decreased motor coordination, progressive weakness, and distal palsies, with decreased and absent ankle reflexes (Roszkowski et al., 2020). As the onset of sensory symptoms is insidious (Callaghan et al., 2012), it is imperative to diagnose DPN early so that limb-threatening sequelae, such as foot ulceration, gangrene, can be prevented. The established risk factors are listed in Table 1 (Zhao et al., 2016; Callaghan et al., 2018; Pai et al., 2018; Liu et al., 2019; Alam et al., 2020; Kaeput et al., 2020; Lu et al., 2020).

**Evaluation and diagnosis of DPN**

The diagnosis of DPN can be attained with both subjective and objective approaches. Routine diagnosis largely remains clinical, with elaborate history taking and a standardized physical examination. Clinical assessment is done via sensory testing for changes in sensation (such as the 128-Hz tuning fork for large fiber function) and vibration. Simple screening maneuvers such as the Semmes-Weinstein monofilament examination, superficial pain sensation and vibration testing are performed in the consultation session (Pesce et al., 2001). In addition, as the 128-Hz tuning fork (large fiber function) and the 10-g monofilament are typically used to assess the risk for ulceration and amputation (Busui et al., 2017). However, these methods
Review

Table 1 | Risk factors of diabetic peripheral neuropathy

| Risk factors                  | Value |
|------------------------------|-------|
| Age > 50 years               |       |
| Duration of diabetes         |       |
| Poor glycemic control        |       |
| Cardiovascular risk factors: hypertension, hyperglycemia, hyperlipidemia, decreased high-density lipoprotein cholesterol |       |
| Concomitant microvascular complications: diabetic nephropathy, diabetic retinopathy |       |
| Concomitant macrovascular complications: cardiovascular disease, cerebrovascular disease, peripheral vascular disease |       |
| High body mass index (Obesity) |       |
| Raised thyroid-stimulating hormone levels |       |
| Raised serum uric acid levels |       |
| Vitamin D deficiency         |       |

may only identify neuropathies in the advanced and irreversible stages (Malik, 2020). Moreover, subjective clinical testing does not propose the best validity, predictive ability, and reproducibility as opposed to undertaking an objective eludication of symptoms and signs.

To circumvent this limitation, several objective screening tools have been adopted for universal usage.

Composite scoring systems

Many variations of assessment frameworks have been validated, but the most frequently accepted scores include the Michigan Neuropathy Screening Instrument, neuropathy disability score (NDS), and the neuropathy impact questionnaire score in the lower limbs. The Michigan Neuropathy Screening Instrument is a two-part screening assessment, consisting of a 15-item self-administered questionnaire and a thorough lower limb physical examination that covers inspection, vibration sensation, and ankle reflexes. 13 items of the questionnaire evaluated symptoms of DPN, 1 item assessed peripheral vascular disease and the last item assessed the presence of general anesthesia. A score of ≥ 7 for the questionnaire is considered abnormal. On the other hand, a physical examination score of ≥ 2.5 would be sufficient to diagnose a patient with DPN (Feldman et al., 1994).

Lastly, the neuropathy impairment score in the lower limbs is a quantitative neurological examination framework that assesses various components of the neurological spectrum, encompassing muscle power grading, sensory and reflex activity grading. The scale is graded on a range of 0 points (normal) to the maximum value of 88 points for the complete absence of all motor, sensory, and reflexes in the lower extremities (Brii, 1999).

For symptomatic assessment of neuropathic pain, the Leeds assessment of symmetry of pain questionnaire-6 did not demonstrate any significant effects of symptomatic neuropathic pain, and to employ pathogenesis-oriented therapy. Management methods are primarily conservative, consisting of non-pharmacological and pharmacological interventions as long-term measures for managing the patient’s underlying diabetes.

Optimizing metabolic control

Poor glycemic control, high blood glucose variability, uncontrolled hypertension, and dyslipidemia are independent risk factors of DPN (Table 1) (Jain et al., 2017; Khanam et al., 2017; Huang et al., 2019). In 1993, the Diabetes Control and Complications Trial Research Group (DCCT) conducted a prospective study to assess the outcomes of intensive insulin therapy in a large population of 3,861 diabetic patients, proving a 40% reduction in the prevalence of clinical neuropathy in the treatment group after a 6.5 year mean follow-up period (Diabetes Control and Complications Trial Research Group et al., 1993). These findings are subsequently corroborated by another randomized 5-year prospective study that evaluated the effects of enhanced glycosylated testing on 49 diabetic patients, reporting a 70% decrease in the prevalence of neuropathy at the end of the study period (Linn et al., 1996). Thus, optimizing systemic metabolic control through pharmacological and lifestyle intervention remains the cornerstone of the diagnosis, as well as the most promising approach for preventing the progression and alleviating the prognosis of DPN (Chong and Hester, 2007; Pop-Busui et al., 2017). Depending on the type of diabetes and HbA1C level, a combination of lifestyle modification and oral hypoglycemic agents or insulin-based therapy is often instituted.

Symptomatic treatment of diabetic peripheral neuropathy

Symptoms from severe DPN can be debilitating, and an effective symptomatic relief is critical in the treatment of DPN. Pharmaceutical agents such as tricyclic antidepressants, selective serotonin-norepinephrine reuptake inhibitors, and anticonvulsants have been used but with variable outcomes (Lobo et al., 2018; Khدور, 2020). Newer treatments such as oral or topical opioids can also be used as adjuvant treatment (Snyder et al., 2016).

Pathogenesis-oriented therapy

While the above-mentioned treatments are standard care administered to patients with DPN, they often are only partially effective. More effective treatments are needed, and several novel treatments directed against pathogenesis-related to DPN are being developed. This overview may be divided into the following five categories: blocking apoptosis and maintain endothelial permeability (Galgal, 2011). Nevertheless, despite the development of the aforementioned pharmacological methods to target the pathogenic processes of DPN, none of them have received official approval and clearance by the United States Food and Drug Administration (FDAS). This is likely attributable to the inconclusive findings of previous trials, leaving the current consensus for these avenues of therapy at a stalemate. A systematic review evaluated the efficacy of aldose reductase inhibitors in the management of diabetic neuropathy, subsequently reporting no significant difference in treatment outcomes when compared to placebo therapy (Chalk et al., 2007). Relatively more promising is the utilization of PCK-beta inhibitors in several randomized controlled trials associated with use in patients with painful sensory polyneuropathy, catching a 60% reduction preventing the progression and alleviating the prognosis of DPN (Chong and Hester, 2007; Pop-Busui et al., 2017). Depending on the type of diabetes and HbA1C level, a combination of lifestyle modification and oral hypoglycemic agents or insulin-based therapy is often instituted.

Nerve conduction studies and electromyography

In clinical research, nerve conduction studies (NCS) and electromyography (EMG) are regarded as the gold-standard tools for diagnosing DPN. However, these methods are not routinely adopted in clinical practice due to their time-consuming nature and the need for specialized equipment (Won and Park, 2016). Moreover, these methods detect mainly large-fiber neuropathies, while patients with DPN may be affected by the disease of the small myelinated and unmyelinated nerve fibers which are responsible for the transmission of pain from nociceptive stimuli (Chong and Hester, 2007; Chalk et al., 2007). NCS and EMG may underdiagnose DPN patients with predominantly small fiber pathology, especially in those who are asymptomatic.

Quantitative sensory testing

Unlike nerve conduction studies, quantitative sensory testing (QST) detects changes in both large and small nerve fibers (Backonja et al., 2009). QST stimulates temperature and vibration to assess the patient’s response accordingly, quantifying their sensory thresholds. Its advantages include patient comfort because of its non-invasive nature, and the operator’s relative ease of usage. However, the variability of QST results is significantly large. This may be attributed to inconsistent patient cooperation, or diverse types of equipment used leading to differing algorithms employed (Krumova et al., 2012). Essentially, this explains poor reproducibility of results due to multiple factors that influence the outcome of the test (Petropoulos et al., 2018).

Measurement of intra-epidermal nerve fiber density via skin punch biopsy

The measurement of intra-epidermal nerve fiber density (IENFD) (per length of section (IENF/mm)), alongside corneal nerve fiber density evaluation, are both considered as objective quantitative assessments to determine the extent of small nerve fiber pathology in early DPN (Himeno et al., 2020). Skin punch biopsies are used to visualize nerve fibers via a 3-mm diameter skin retrieval. This allows accurate and quantifiable evaluation of early changes in small fiber morphology and provides a greater diagnostic accuracy compared to the previously discussed tools. However, a skin punch biopsy is an invasive procedure and predisposes patients to bleeding and infection.

Management of diabetic peripheral neuropathy

Currently, there are three main principles to be followed in the treatment of DPN: to address the underlying cause of neuropathy, to relieve the debilitating effects of symptomatic neuropathic pain, and to employ pathogenesis-oriented therapy. Management methods are primarily conservative, consisting of non-pharmacological and pharmacological interventions as long-term measures for managing the patient’s underlying diabetes.
on PKC-beta inhibitors, conducted randomized controlled trials such as the SYDEMY 2 trial have also reported a reduction in symptomatic outcomes, but no significant difference in objective neuropathic assessments such as the total Neuropathy Symptom Score (Ziegler et al., 2006). This was also preceded by another trial (alpha-lipoic acid in diabetic neuropathy III) that concluded similar findings, revealing no significant difference in total symptom score after the 7-month study period. When it comes to diagnosis of DPN, one of the biggest challenges is that standard tools lack the sensitivity to detect early signs and symptoms of disease, limiting early identification, intervention, and monitoring of disease progression.

However, over the past decade, there have increasingly been studies suggesting that corneal neuronal complications from diabetes may be present before other clinical manifestations of DPN (Edwards et al., 2012; Papanas and Ziegler, 2013). As an upregulation of the subbasal corneal nerve network, the confocal microscopy evaluation and meta-analysis has reported that corneal confocal microscopy has good diagnostic utility in detecting both sub-clinical and clinical DPN (Gad et al., 2021). Thus, evaluation of corneal nerve health has been actively explored as a sensitive and non-invasive approach to diagnose early DPN.

**Diabetic Corneal Neuropathy**

**Anatomy of the corneal nerve plexus**

The cornea is the only innervated structure in the human body (Shaheen et al., 2014), with a nerve density of approximately 7000 for touch, pain, and temperature sensation of the cornea, and 2000–4000 axons in the blink reflex, wound healing, and tear production (Shaheen et al., 2014). Patients with DPN are characterized by corneal hypoesthesia, photophobia, ocular hypertension, and neurotrophic keratitis. These symptoms, however, may not always correlate clinically, as a number of patients are often asymptomatic due to corneal hypoesthesia (Zhao et al., 2019).

**Clinical presentation of diabetic corneal neuropathy**

The cornea is mainly innervated by sensory nerves, which are responsible for a variety of physiologic functions, including corneal sensation, corneal blood flow, corneal neurotrophic ulcer, and tear secretion (Al-Aqaba et al., 2019). These anterior stromal bundles lie immediately beneath the Bowman’s layer and form a flat, dense subepithelial plexus. The subepithelial nerve bundles then advance towards the corneal surface, penetrating the Bowman’s layer. Between the Bowman’s layer and basal epithelium, the subepithelial nerves branch into smaller sub-basal branches, coursing parallel to the corneal surface (Müller et al., 2003). The sub-basal nerves anastomose in a complex nervous network, forming a dense nerve plexus arrangement throughout the cornea (Müller et al., 2003). It has a characteristic clockwise whorl pattern superficially (Figure 2B), innervating all layers of the corneal epithelium. These nerve terminals end as bulbous endings either below or within superficial squamous cells (Al-Aqaba et al., 2019).

**Evaluation of diabetic corneal neuropathy**

**Clinical history, slit-lamp evaluation, and corneal sensitivity**

The goal of clinical history-taking is mainly to exclude other conditions that may cause corneal neuropathy, such as herpetic keratitis, corneal surgery, or long-term use of contact lens. Pre-ganglionic causes such as intracranial space-occupying lesions or iatrogenic injury during neurosurgical procedures also need to be ruled out. Corneal sensation, tear film osmolarity, and tear breakup time are also important tests to measure corneal sensitivity. Decreased corneal sensitivity and tear film osmolarity are frequently observed in patients with DPN (Sakai et al., 2012). Moreover, slit lamp evaluation, together with 2% w/v solution of fluorescein sodium and other vital dyes such as lissamine green or rose bengal, allows visualization of disrupted and irregular ocular surfaces, aiding disease staging and monitoring.

Findings of corneal sensitivity tests in DPN range from a diminished to a completely absent blink reflex (Shaheen et al., 2014). An upregulation of corneal confocal microscopy has reported that corneal confocal microscopy has good diagnostic utility in detecting both sub-clinical and clinical DPN (Gad et al., 2021). Thus, evaluation of corneal nerve health has been actively explored as a sensitive and non-invasive approach to diagnose early DPN.

**In vivo confocal microscopy to visualize corneal nerve plexuses**

In vivo confocal microscopy (IVCM) is a non-invasive imaging technology that has gained significant diagnostic and research importance (Sakai et al., 2003). The sub-basal nerve plexus is less affected by diabetic complications than that in the skin (Zander and Weddell, 1951). Interventions of the cornea progresses from the stroma to the epithelium, and mainly consist of somatic sensory innervation originating from the ophthalmic branch of the trigeminal nerve (V1). Sensory and autonomic nerve bundles from the long ciliary branches of the ophthalmic branch enter the cornea in a centripetal fashion through the corneoscleral limbus at the level of the mid-stroma (Müller et al., 2003), which supplies the mid-stromal plexus, which has a nerve density and complexity that increases from central to peripheral (Müller et al., 2003; Figure 2A). Most of the mid-stromal bundles enter into a narrow band of the anterior stroma. The posterior stroma, in contrast, is poorly innervated (Al-Aqaba et al., 2019). These anterior stromal nerve bundles lie immediately beneath the Bowman’s layer and form a flat, dense subepithelial plexus. The subepithelial nerve bundles then advance towards the corneal surface, penetrating the Bowman’s layer. Between the Bowman’s layer and basal epithelium, the subepithelial nerves branch into smaller sub-basal branches, coursing parallel to the corneal surface (Müller et al., 2003). The sub-basal nerves anastomose in a complex nervous network, forming a dense nerve plexus arrangement throughout the cornea (Müller et al., 2003). It has a characteristic clockwise whorl pattern superficially (Figure 2B), innervating all layers of the corneal epithelium. These nerve terminals end as bulbous endings either below or within superficial squamous cells (Al-Aqaba et al., 2019).

**Corneal nerve fractal dimension (CNFrD)**

Corneal nerve fractal dimension (CNFrD) is a metric to evaluate the complexity and organization of the corneal nerve plexus, as well as to evaluate the treatment efficacy objectively (Mansoor et al., 2020). Studies have shown that decreased CNFD, CNFL and nerve beading, and increased nerve tortuosity were observed in both T1DM and T2DM patients (Figure 4) (Mansoor et al., 2020). Reduced nerve beading frequency reflects lower metabolic activity and translates to a higher risk of neuropathy damage in diabetic patients (Rozkoszewska et al., 2020). CNFD is the most consistently reduced in both types of DM (Rozkoszewska et al., 2021), and is the most reliable marker for early diabetic sensorimotor polyneuropathy (Hertz et al., 2012). Similarly, another study revealed that CNFD, CNFB, and CNFL were significantly reduced in patients with DPN as compared to healthy subjects (Kalteniece et al., 2020). It also found a significant inverse relationship between the severity of neuropathic symptoms with CNFD (r = −0.20, P = 0.001) and CNFL (r = −0.30, P = 0.02). The findings revealed a lower CNFrD in diabetic patients compared to control subjects, and it was further lowered in patients with DPN compared to those without DPN (Chen et al., 2018). It was also reported that CNFrD had a similar diagnostic ability to identify patients with DPN when compared with existing metrics such as CNFL (Chen et al., 2018). Moreover, CNFD may also be diagnosed more accurately and in earlier stages by analyzing subbasal inferior whorl located in the inferonasal cornea (Figure 2B), which shows reduced nerve fiber length and density before the nerve plexus central cornea does, making a more optimal imaging site for early detection (Petropeulos et al., 2015). The clinical utility of analyzing the inferior whorl via IVCM has been proven; Ferdousi et al. (2020) concluded that inferior whorl analysis via IVCM has clinical significance and specificity to both QST and NC in the diagnosis of diabetic neuropathy.

The correlation between corneal nerve metrics and the chronicity or severity of diabetes has also been explored. Diabetes chronicity, quantified by duration since diagnosis, was found to have a significant inverse relationship with CNFD, CNFL, and CNFB in both T1DM and T2DM (Ahmed et al., 2012; Petropeulos et al., 2013). Diabetic chronicity evaluated by the NCSS or QST had a similar relationship. It has also been shown that corneal nerve parameters improve as glycemic control improves in both patients with T1DM and T2DM (Boucek, 2013; Azmi et al., 2015). Azmi et al. (2015) conducted a study on T1DM patients on continuous subcutaneous insulin and compared them to those on daily injections. The former group achieved lower HbA1c levels and showed significantly greater regeneration of the subbasal nerve plexus in terms of CNFD, CNFL, and CNFB (Azmi et al., 2015). The extent of improvement of corneal nerve parameters may be related to improvement of neuropathic symptoms, and was found to be more profound in patients with pain compared to painless DPN (Kalteniece et al., 2018). Other interventions relevant to DPN such as pain relief and neuronal regeneration have also been found to help improve corneal nerve status, notably CNFD, and CNFD (Petropeulos et al., 2013).
While the reliability of IVCM may be limited by inter or intra-observer variability, it has improved after the introduction of novel techniques such as post-imaging quantification and automated analysis (Dabbah et al., 2010). Examples of such software include Neuronal and ACCMeters, which enable semi-automated and fully automated quantification respectively (Dehghani et al., 2014). Studies have shown that automated quantification of CNFL not only offers similar capability in identifying DCN in diabetic patients accurately as compared to manual analysis, it was also more advantageous in terms of speed, objectivity, and reproducibility (Dehghani et al., 2014; Ostrovski et al., 2015). Small-fiber quantification in IVCM has also been found to have similar diagnostic efficiency with IENFD, suggesting a possible role as a surrogate marker for DPN (Chen et al., 2015).

Management of diabetic corneal neuropathy

The main principles of management for DCN include preventing the progression of corneal damage, achieving epithelial healing, and symptomatic relief (Bikbova et al., 2018). They can be split into systemic glucose control as discussed earlier, and local ocular surface management.

Local ocular surface management involves maintaining a healthy, smooth, and intact corneal epithelium and to minimize the frequency of visual disturbances and discomfort (Quattrini et al., 2010; Mansoor et al., 2020). Tear film quality and consequently corneal healing can be improved with preservative-free artificial tears, ointment, or punctal occlusion, which eventually promotes corneal healing. Topical anti-inflammatory drugs, such as topical preservative-free steroids, non-steroidal anti-inflammatory drugs or cyclosporin, can be considered (Mansoor et al., 2020). In moderate cases where a persistent epithelial defect is present, it is important to prevent invasion of the underlying stroma in addition to intensive lubricant therapy mentioned above. With the increased risk of secondary infection of the edematous cornea, prophylactic antibiotic eye drops are recommended to prevent further damage (Sacchetti and Lambiase, 2014; Mansoor et al., 2020). A trial of therapeutic corneal or scleral contact lenses can be used, to act not only as a protective barrier, but also help retain therapeutic medication and lubricants on the corneal surface (Dua et al., 2018). Surgical debridement of the thickened and stagnated edges of the ulcer may help improve healing in patients with corneal ulcers (Katzman and Jeng, 2014). During the re-epithelialization process, ulcers complications such as stromal melting can be avoided with inhibitors of matrix metalloproteinases and suppressors of neutrophil action such as topical or systemic tetracyclines and N-acetylcysteine (Hossain, 2012; Ogut et al., 2016). In refractory and severe cases, a surgical approach such as partial or total tarsorrhaphy, amniotic membrane graft transplantation, cyanoacrylate glue, conjunctival flaps or lamellar/penetrating keratoplasty may be indicated (Sacchetti and Lambiase, 2014).

Adjunctive treatment to promote corneal nerve recovery and function may be considered in moderate and severe cases. These include growth factor-rich therapy such as autologous serum eye drops or platelet-rich plasma to reduce neurovascular damage and promote ocular surface healing, as well as neurotrophic factor-based therapy such as nerve growth factor eye drops to promote neuronal growth and its trophic effects (Mansoor et al., 2020; Mastropasqua et al., 2020).

Relationship between Diabetic Corneal Neuropathy and Diabetic Peripheral Neuropathy

Corneal innervation and its capacity in delineating the severity of DPN has been extensively compared to the conventional means of nerve testing such as NCS, QST as well as nerve and skin biopsies. Even though electrodiagnostic testing has always been regarded as the gold standard for the diagnosis of neuropathy, they only identify primarily large fiber changes (Petroopoulos et al., 2014). Yet, the earliest nerve fibers to undergo nerve fiber damage are those of small, unmyelinated nerve fibers, significantly reducing the reliability of earlier diagnosis using these methods (Malik, 2020). Small-fiber neuropathic changes are able to be picked up by IVCM, which are otherwise undetectable in QST and electrophysiological findings (Azmi et al., 2015). Thus, attention has hence been turned towards assessing DCN as an alternative diagnostic tool for DPN.

Diabetic corneal neuropathic changes in relation to diagnosis of diabetic peripheral neuropathy

In the consideration of corneal changes as surrogate markers for DPN, it was presented that 50% of DPN patients had pathological corneal subbasal nerve plexus changes before they developed clinical signs of DPN, indicating that the onset of corneal nerve alterations precedes the progression of DPN (Bitirgen et al., 2014). In addition, changes in corneal neurology, especially in the inferior whorl area, have also been observed before patients present diabetic retinopathy or microalbuminuria (Gad et al., 2020). The corneal nerve parameters, including CNFL, CNFD, and CNBD, were significantly reduced in diabetic patients with DPN compared to those without (Figure 5) (Misra et al., 2015; Xiong et al., 2018; Li et al., 2019). The Longitudinal Assessment of Neuropathy in Diabetes using novel ophthalmic markers (Pritchard et al., 2014) was a 4-year observational study investigating corneal nerve alterations in the progression to DPN in type 2 diabetes mellitus. The study was divided into three groups: T1DM patients with T1DM, T1DM patients without DPN, and a control group of patients with no diabetes or neuropathy. CNFL was found to be significantly reduced in T1DM patients with DPN compared to T1DM patients without DPN (14.0 ± 6.4 mm/mm² vs. 19.1 ± 5.8 mm/mm², P < 0.001), reaffirming the association between corneal nerve parameters and DPN.

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In addition to diagnostic value, measures that quantify functional and structural severity of DCN also correlate to the severity of DPN. Functionally, in QST and electrophysiological findings (Azmi et al., 2015). Thus, attention has hence been turned towards assessing DCN as an alternative diagnostic tool for DPN.

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similarities between corneal sensation and function scores of DPN yielded mixed results: while corneal sensitivity thresholds were largely similar to NDS and diabetic neuropathy symptom score, they were dissimilar to more objective metrics such as conduction velocities and quantitative sensory testing variables (Pritchard et al., 2011).

Structurally, IVCM quantifies small fiber damage rapidly, non-invasively, and is able to quantify the degree of nerve damage and to track longitudinal changes (Quattrini et al., 2007). Corneal nerve metrics have been shown to worsen as DPN progresses, helping to determine the course and severity of DPN. CNFD showed a significant increase with increasing neuropathic symptom scores as measured by NDS, diabetic neuropathy symptom score, and QST (P < 0.001). The correlation was consistent when the diabetic subjects were further stratified into mild, moderate, and severe neuropathy (Quattrini et al., 2007). Similarly, Petropoulos et al. found that CNFD, CNFL, and CNFD were significantly lower between controls and diabetic patients with worsening severity of neuropathy assessed via the NDS, vibration perception threshold, and nerve conduction studies (P < 0.001). In addition, a cross-sectional study reported a significant correlation between CNFD and nerve conduction studies (r = 0.78, P < 0.01), as well as between CNFD and motor nerve axonal hyperelectricity measurements (r = 0.44, P < 0.01) (Tummanapalli et al., 2020). Coupled with the recent development of an artificial intelligence-driven deep-learning algorithm for IVCM imaging in evaluating DPN, assessment of corneal nerve status can be a promising alternative to the conventional diagnostic and monitoring methods for DPN (Salahouddin et al., 2021).

The role of DCN in the stratification of complications of DPN

IVCM not only detects subclinical and clinical DPN, but is also able to monitor the decline in corneal nerve parameters in time to identify the development of complications (Dehghani et al., 2016). It was shown that CNFD yielded 84% specificity for early stage small fiber neuropathy, 86% sensitivity for severe small fiber neuropathy, 75% specificity for the diagnosis of DPN, and 72% specificity for the diagnosis of foot ulceration (Quattrini et al., 2007). In patients with developed diabetic neuropathoarthropathy, CNFL, CNFD, CNBD, and corneal nerve connecting points were significantly reduced compared to non-DM controls (Herlyn et al., 2018). Corneal nerve changes allow for not only the detection of the disease of neuropathic pain from foot ulcerations (Kalteniece et al., 2020). Assessment of the corneal nerve plexus is helpful but not only identifying initial and significant changes across the course of DPN, but can also risk-stratify and determine the optimal time for intervention directed towards the mitigation of complications.

Conclusions

DCN and DPN affect 46–64% and 50% of diabetic patients, respectively, during the clinical course, resulting in a significant economic burden. Early detection of DPN is paramount to halt the progression of debilitating symptoms, such as pain and sensory deficits, and late limb-threatening sequelae. At present, the gold standard for the diagnosis of DPN remains clinical assessment via careful history and sensory testing. However, neuronal damage occurs before clinical functionality changes, and DPN is often advanced and irreversible by the time it is symptomatic or clinically detectable. Many studies have validated the use of IVCM as a DSG marker for severe DPN. Corneal nerve parameters such as CNFL, CNFD, and CNBD are not only significantly associated with the severity of DPN, but also observed to be altered prior to nerve conduction studies and clinical manifestations of DPN. Several tools and existing diagnostic tests for early DPN such as EIFND, IVCM showed higher sensitivity in diagnosing DPN. Future studies may extend to the use of artificial intelligence-based evaluation of DCN in relation to natural progression and treatment efficacy of DPN.

Author contributions: WZS and NSOW contributed to the literature search and manuscript writing. MYTL and IYXL provided the supplementary data and figures. YCL provided the overall supervision of this review. YCL, HCT, and JSM reviewed the manuscript. All authors contributed to the conceptualization, design, definition of intellectual content, critical appraisal, editing, and review of the manuscript, and approved the final version of this manuscript.

Conflicts of interest: There are no conflicts of interest.

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