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Corneal Nerve Changes in Diabetes: A Meta-Analysis of *In Vivo* Confocal Microscopy Studies

Meera Ahmed Mohamed Othman Ali[*,1], Reem Sultan BinTarish Al Mheiri[1], Masa Murad Fares[1], Teeba Mohammed Hussain Alwan[1], Wahg A J M Alenezy[1], Khulood taleb alkalban[1], Maria Ammari[1], Hind Tariq Alzaroon[1], Roda Rashid Bin Sultan Alshamsi[1], Hessah Alkaabi[2]

Abstract

Diabetes mellitus is a chronic disease that has adverse effects on the nervous system and thus results in diabetic peripheral neuropathy (DPN). Corneal nerves are among the first to be damaged when one is diagnosed with diabetes; they can, therefore, be used to diagnose neuropathy because they are easy to analyse. This systematic review and meta-analysis, therefore, aimed at identifying the usage of in vivo confocal microscopy (IVCM) on corneal nerve changes or density in diabetic patients, employing various parameters of corneal nerve density work, including CNFD, CNBD and CNFL. The article search involved PubMed, Embase, Scopus, and Web of Science, and studies were considered in accordance with the defined criteria. Data analysis was performed, including Random Effect Models, to combine the outcomes of the studies according to the type of diabetes and the degree of glycemic control. In order to determine the stability of the conclusions made by the study, sensitivity analysis was also conducted as well. A reducing pattern was manifested by diabetic patients for CNFD, CNBD as well as CNFL compared to control subjects. Microangiopathy was observed at all stages of diabetic neuropathy in both T1DM and T2DM groups, but T1DM was observed to have more severe changes than T2DM. These results confirmed that the glycemic control of the patients was inversely proportional to nerve fibre characteristics, indicating the damaging effects of hyperglycemia on the nerves. IVCM has shown valuable information on the early stage of DPN on corneal nerve fibre changes in patients with diabetes. IVCM can be, therefore, used as an optimal non-invasive approach to disease early detection and monitoring of diabetic neuropathy. It has the potential to be implemented into clinical practice to enhance diabetes management and avoid potential complications. Studies can also be conducted to define the guidelines for the use of IVCM and extend its application across different fields.

Keywords: Diabetes mellitus, Corneal nerves, CNFD, CNBD, CNFL, Meta-analysis, Statistical Analysis, In Vivo Confocal Microscopy

Introduction

In 2021, it is estimated that approximately 537 million adults (20-79 years), 10.5% of the adult population, are living with diabetes, and that number is projected to rise to 643 million by 2030 and 783 million by 2045. By 2045, 1 in 8 adults will be diabetic, demonstrating a significant increase of 46%. Out of 4 adults with diabetes, three are living in low- and middle-income countries. Deaths attributable to diabetes are estimated to be 6.7 million; 32.6% of deaths in people under 60 can be attributed to diabetes (1).

Affiliation:

[1]University of Sharjah, College of Medicine, UAE

[2]Ajman University

***Corresponding author:**

Meera Ahmed Mohamed Othman Ali, University of Sharjah, College of Medicine, UAE

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Diabetic peripheral neuropathy (DPN) is a frequent multifactorial complication of diabetes, and among the subtypes, distal symmetric polyneuropathy is the most common. DPN incidence progresses with the natural history of the disease, and patients usually demonstrate bilateral limb pain and paresthesias as cellular mechanisms involve chronic hyperglycemia that (2), along with dyslipidemia and resistance to insulin, drives a cycle of metabolic disturbances, including the accumulation of reactive oxygen species and advanced glycation end products (AGE). These, in turn, stimulate inflammatory responses by infiltrating macrophages, which injure the nerve fibres and their supporting cells. Meanwhile, disruptions in the insulin signalling pathway impede axonal repair and promote cell death, while vascular compromise worsens tissue injury by limiting blood flow and nutrient delivery (3).

Several oxidative and metabolic pathways are implicated in the pathogenesis of DPN, including Protein Kinase C (PKC), polyol pathway, AGE pathway, hexosamine pathway, and PARP pathway. The protein kinase C (PKC) pathway is connected to glucose metabolism, especially during hyperglycemic conditions where glycolytic intermediates promote its activation. In the case of diabetes (3), elevated blood glucose enters the cells through Glut-1 and Glut-3 transporters and is then slowly transformed into intermediates such as glyceraldehyde-3-phosphate that can be further converted into diacylglycerol (DAG).

The increase in DAG concentration is a stimulus to the PKC pathway in the neurons, where PKC, a serine/threonine kinase, associates with Ca^{2+}-activated calmodulin to phosphorylate numerous targets downstream. Upon activation, PKC interferes with ATPase, modifies vascular endothelial growth factors, and promotes vasoconstriction, thereby contributing to a host of metabolic derangements. In pancreatic beta cells, aberrant PKC disrupts the production and secretion of insulin; in glial cells, it interrupts normal regulatory functions, reduces glutamate uptake, and increases reactive oxygen species production, thereby worsening oxidative damage. The prolonged unregulated hyperglycemia also leads to neuronal cell damage from the other three fundamental pathways (4).

First of these is the formation of advanced glycation end products (AGEs) by excessive glucose, which bind to their receptors, activating pro-inflammatory pathways, causing microvascular injury and dysfunction of glial cells (5). Second, excess glucose enters into the hexosamine pathway, resulting in oxidative stress and an inflammatory cascade that aggravates already impaired neuronal integrity. Finally, activation of the poly (ADP-ribose) polymerase (PARP) depletes intracellular NAD^+, therefore depleting ATP supply. Moreover, it alters gene expression, enhancing both oxidative and nitrosative stress. Together, these create a vicious cycle of metabolic disturbances and inflammation that produce the neuronal damage characteristic of DPN (5).

The cornea, the transparent external layer of the eyeball because of its thickness and pigmentation, is densely supplied with nerve fibres derived from the ophthalmic division of the trigeminal nerve (6). It also directly relates to ocular health because it deals with nerve sensations such as pain, touch or even temperature. They are also involved in the stimulation of tear secretion and maintenance of ocular surface health, reducing the risks or occurrence of dry eye and maintaining proper wound healing.

The following components of corneal innervation include a network of unmyelinated fibres that form subepithelial nerve plexus beneath the corneal epithelial layer and also present with subbasal nerve plexus beneath Bowman's membrane in the stroma. From here, the nerves pass to the superficial layers that divide and sub-divides into smaller nerves and come to a point at the epithelial layer. This nerve is crucial for the body's correct perception of stimuli and the well-being and safeguarding of the organ of vision (7).

Corneal nerves in diabetic persons can be affected due to various reasons, as shown by the literature, leading to complications such as neuropathy. Those changes include impaired blood flow and increased oxidative stress that reduces the fibre's regenerative capacity and damages the nerve fibres. This leads to the damage of corneal nerves, decreases the corneal sensation and slows the rate of healing of the wound. Also, diabetic neuropathy, which affects the cornea, is associated with dry eye syndrome and corneal ulcers, leading to other ocular complications in diabetes over the long term (8).

The corneal nerve is among the nerves that are affected in diabetic neuropathy. Corneal nerves are mainly from the ophthalmic branch of the trigeminal nerve, although the inferior part of the cornea sometimes receives fibres from the maxillary branch. As they enter the cornea at the periphery, the nerves run radially, losing their perineurium and myelin sheaths around the limbus, where they continue with only the protective covering of Schwann cells, which is essential for maintaining corneal transparency (9). In addition to sensory fibres, these also exert trophic effects on the corneal epithelium and give support against dryness via the release of neuropeptides, growth factors, and cytokines (9).

Underlying corneal nerve damage in diabetes is inflammation, oxidative stress, and alteration of signalling cascades. The mechanisms of corneal nerve damage resemble those discussed previously in the pathogenesis of DPN. Terminal pathways such as the AGE and PARP pathways contribute to the development of corneal nerve injury. Immune responses also significantly contribute to inducing

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diabetic corneal neuropathy. On confocal microscopy, there were higher proportions of immunocytes, i.e, Langerhans and dendritic cells, in diabetic neuropathy (10).

However, the presence of dendritic cell populations providing ciliary neurotrophic factor (CNTF) to facilitate corneal nerve regrowth was very much reduced in the diabetic state (11). Moreover, in diabetes, the cornea and the tear film are abnormal, as concentration levels of the neuropeptide substance P that promotes wound healing and epithelial cell turnover are lower than those of healthy controls.8 Diabetes initiates a systemic cascade of events that disrupt not just immune regulation but neuroendocrine balance.

In vivo confocal microscopy (IVCM) is a non-invasive imaging technique that allows high-resolution visualisation of cellular structures in real time. It has gained widespread application in ophthalmology, particularly in assessing the corneal sub-basal nerve plexus. This technique provides detailed images of tiny nerve fibres, making it a valuable tool for evaluating nerve integrity in various systemic and ocular diseases. IVCM has been particularly useful in detecting early corneal nerve damage in diabetic patients, aiding in the assessment of diabetic neuropathy. Given the increasing global prevalence of diabetes and its complications, the need for practical diagnostic tools to detect early neuropathic changes has become more urgent (12).

One of the key advantages of IVCM is its ability to assess small nerve fibre integrity with high precision. Traditional diagnostic methods for neuropathy, such as nerve conduction studies or skin biopsies, are either invasive or limited in detecting early-stage damage. In contrast, IVCM offers a rapid and non-invasive alternative, enabling clinicians to monitor nerve changes over time (Oakley et al, 2020). It has been used to evaluate corneal nerve fibre length, density, and branching patterns, which are essential indicators of nerve health (13). This makes it a promising tool for early detection and monitoring of neurodegenerative diseases, particularly diabetic peripheral neuropathy (DPN), which is one of the most common complications of diabetes.

IVCM has demonstrated its clinical utility in multiple studies, particularly in detecting subclinical changes in patients who have yet to develop overt symptoms of neuropathy. Because the cornea is densely innervated and easily accessible for imaging, corneal nerve analysis through IVCM is an ideal approach for assessing systemic small fibre neuropathies. Studies have shown that corneal nerve fibre loss correlates strongly with nerve damage in other parts of the body, making it a potential surrogate marker for early diagnosis and progression monitoring (14).

Furthermore, IVCM is helpful beyond diabetes-related neuropathies. It has been employed in evaluating nerve changes in autoimmune disorders such as Sjögren's syndrome, neurodegenerative conditions like Parkinson's disease, and hereditary neuropathies such as Charcot-Marie-Tooth disease. The ability of IVCM to provide direct imaging of tiny nerve fibres has made it a valuable tool for researchers and clinicians alike, offering new insights into the pathophysiology of neuropathic disorders and the effects of potential treatments.

The main strength of IVCM lies in its ability to detect minor fibre damage at an early stage (15). Many neuropathic conditions remain undiagnosed until significant nerve damage has occurred, primarily because routine nerve conduction studies focus on large myelinated fibres, leaving small unmyelinated and thinly myelinated fibres largely unassessed. Since IVCM provides direct visualisation of these tiny fibres in the cornea, it serves as an ideal method to bridge this diagnostic gap (15).

Diabetes mellitus is a common disease that affects vision, and the most common diabetic ocular change is the change in the nerve tissue of the cornea (16). However, few quantitative meta-analyses have been made on the changes in corneal nerve structure among diabetic patients. Meta-analysis is needed for the integration of data from several studies to provide a better estimate of the severity of corneal nerve damage in diabetes and to give a better idea about the pathogenesis.

Other previous systematic reviews include a summary of the results of individual studies, which have provided substantial improvements but have the following drawbacks: small sample size, variability of the methods used, and lack of meta-analysis of the results. These limitations negate the possibility of making a conclusive statement regarding the condition of corneal nerves as well as the sensory function in diabetes. Thus, it remains a topic of controversy, as far as clinicians and researchers are concerned, the extent of corneal nerve involvement in diabetic patients.

Now, as you might well know, the changes in corneal nerve structures in diabetes have severe clinical implications. Entire corneal nerve damage is related to decreased tactile sensation of the eye, corneal ulceration, dry eyes and slow healing, which can result in sight-threatening complications that may lead to blindness. Consequently, conducting a meta-analysis for this study will give more enhanced, generalised data regarding these changes. It thus will be beneficial in early diagnosis intervention measures and better overall patient outcomes in the management of diabetic ocular treatment.

Methods

Literature Search Strategy

For this systematic review, the search was done in different databases to embrace all available records in order

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to minimise bias when selecting the papers for the meta-analysis. The databases searched for the documents were PubMed, Embase, Scopus, and Web of Science. These databases were chosen due to their comprehensiveness of the biomedical, clinical and scientific publications, which would provide a broad scope of published research findings concerning corneal nerve changes in diabetics. The search thus adopted the use of keywords or main terms and Boolean operators to secure the most appropriate studies. The following keywords have been used in the search: corneal nerves or corneal nerve fibres; diabetes mellitus or diabetes; confocal microscopy or in vivo confocal microscopy; nerve damage or nerve alterations. Some of the essential features that were employed included the use of the Boolean operators such as AND and OR to search and filter on the terms. For instance, the search string entered in PubMed was: (corneal nerves or corneal nerve fibres) (diabetes or Diabetic neuropathy) and (confocal microscopy or in vivo confocal microscopy). This way, the necessary literature on the roles of corneal nerves in diabetic patients and only those using confocal microscopy as an imaging modality was obtained.

Inclusion Criteria

- Original research can only be comprised of observational studies such as cross-sectional studies, cohort studies, or clinical trials.
- Both human subjects with newly diagnosed Type 1 or Type 2 diabetes mellitus will be included in the study.
- For assessing corneal nerve changes, promising research has been conducted using in vivo confocal microscopy.
- Studies report quantitative measurements of corneal nerve density, morphology, or sensory function.
- Articles published in English.
- Studies published from 2000 onwards.

Exclusion Criteria

- Animal studies or in vitro studies.
- Non-original research such as reviews, editorials, commentaries, or letters to the editor.
- All the few studies that did not incorporate in vivo confocal microscopy in the analysis of corneal nerve alterations.
- Most of the studies cannot be used for meta-analysis because they provide inadequate information about the changes in the corneal nerves.

Study Selection and Data Extraction

In conducting this systematic review, the staging criteria by Portable Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were used to make the selection process more systematic. A total of 500 records were systematically retrieved by using databases including PubMed, EMBASE, Scopus, and Web of Science; five other documents were also found from different sources. Thus, from 506, there were 455 records with the identified keywords, and after assessing the title and abstract, 350 records were excluded. One hundred and five articles were retrieved, and 85 were omitted for reasons like animal studies, lack of quantitative data, or lack of use of a confocal microscope. Of the identified 58 studies, 20 were finally selected according to the inclusion criteria of the current review. The following PRISMA flow diagram depicts the selection process:

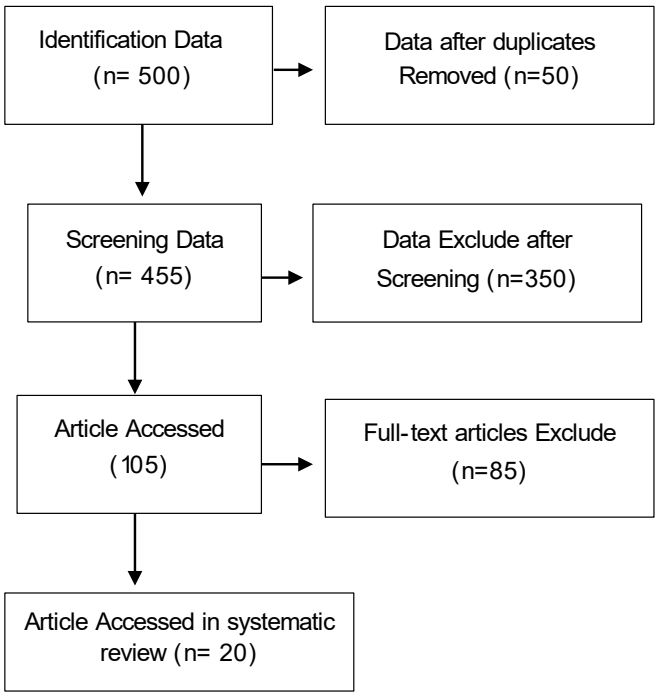

Figure 1: PRISMA flow diagram for study selection

Data Extraction Strategy

While undertaking the data extraction of each study, the following factors were explored in order to get an overall view of the effects of diabetes on corneal nerves. Further, these aspects were derived systematically from each of the studies:

Outcome Measures:

- A subsample of participants on corneal nerve density and morphology as defined by nerve fibre length, density, and branching patterns as measured by in vivo confocal microscopy.
- Measurement of mechanical or esthesiometer sensitivity of the cornea to detect abnormal sensation (Kotak et al, 2020).

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Othman Ali MAM, et al., Fortune J Health Sci 2025
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- Rates of corneal nerve regeneration and the existence of neuropathy.
- Corneal nerve abnormalities that characterise diabetic neuropathy may explain some of the dry eye symptoms.

Study Characteristics:

- Study design (observational studies such as cross-sectional and cohort studies, as well as clinical trials).
- Details on the equipment used for confocal microscopy as well as the process of how the images were analysed.
- Duration of the study (follow-up periods in cohort studies).

Patient Demographics:

- The number of participant groups participating in the study is diabetic and control groups.
- The study's specific distribution was based on participants' age and gender.
- Diabetes type (Type 1 or Type 2) and duration of the disease.
- For any other existing health complications that might perturb corneal nerve function or complications that exist alongside the primary disease.
- Thus, the present extraction pattern of data made it easier to compare various studies and synthesise their findings since it captured similar data points.

Quality Assessment and Risk of Bias Evaluation

In order to check for the quality of all the studies, quality assessment along with the risk of bias was assessed and analysed. For observational studies, a methodological quality assessment was done using the Newcastle-Ottawa scale. This tool focuses on three areas: the selection of the study groups, the comparability of the groups, and the assessment of exposure/outcome. Randomised control of the study was done using confocal microscopy for corneal nerve evaluation and assessment of the methodological characteristics, including randomisation, blinding, sample size, and the consistency of the obtained images. In diagnostic accuracy studies, the QUADAS-2 checklist was used to assess patient selection, index test, reference standard, flow and timing to ensure that only high-quality studies meet the standard needed (17).

In other words, the internal validity criterion was checked alongside the threat of publication bias, which is the final problem found in most systematic reviews. To check publication bias, a funnel plot was created, and/or the shape of funnels that indicated publication bias was examined. Therefore, another form of a quantitative assessment of funnel plot asymmetry was conducted using Egger's test, which also supports the identification of the small-study bias or overt reporting. It makes Meta-analysis more rigorous and reduces bias risk to an acceptable limit to qualify the conclusion drawn from it.

Statistical Analysis

For the analysis of the results, the Mantel-Haenszel fixed-effects and the DerSimonian and Laird random-effects methods, as well as the 95% confidence intervals, were used based on the heterogeneity of studies. For one, the fixed-effects model assumes that the proper treatment effect is constant, which should be the case if studies are comparable in terms of the patients they include and the method used to conduct the meta-analysis. Nonetheless, due to differences in the research and the patient's characteristics across the included studies, the random-effects model was applied as the initial statistical analysis. The random-effects model takes into consideration the variability between the studies and issues a broad estimate of the effect magnitude; it is preferable when the synthesis of the data involves studies that are diverse in their outcomes (48).

Therefore, the effect sizes as undertaken were measured by standard mean differences (SMD). This is so since it aids in comparing results across several studies that may employ diverse scales on corneal nerve alterations. To also evaluate the precision of the effect size estimates, 95% CI was calculated for each SMD. Such confidence intervals were important for assessing the significance of the results based on the pooled data and comparing between groups or treatment effects when the confidence intervals of these effects did not overlap (49).

Measurement of heterogeneity was done using what is known as the I^2 statistic, a measure of the variation across studies that is due to heterogeneity. If the I^2 is equal to 0%, this means that there is no heterogeneity, while at a value more than 50%, it shows considerable heterogeneity. Suppose there was a significant heterogeneity, and exploratory meta-regression analysis was carried out to examine reasons for variability, which may include type of diabetes, study design, or confocal microscopy techniques. It assisted in acknowledging features that might affect the differences in the alteration of the corneal nerve density, which was depicted in the studies that were included in the research study.

To perform a validity check and to eliminate the likelihood of significant over-representation of a given study, sensitivity analyses were conducted. When performing the meta-analysis, the current study also employed the leave-one-out technique, which entails systematically withdrawing one survey at a time to determine the stability of the total effect size. Further post hoc comparisons were conducted on different variables, including the type of diabetes, the duration of the condition, age, and sex. These subgroup

Othman Ali MAM, et al., Fortune J Health Sci 2025
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analyses allowed us to investigate whether certain factors affected corneal nerve alterations in diabetes and to make a more straightforward interpretation of the results. In this way, all these statistical methods helped make the evidence assessment comprehensive and methodologically sound.

Results

Study Characteristics

Study Characteristics

Below is the list of the 20 included studies summarised in a table format, which consists of the details about authors, year of publication, sample size, type of diabetes, design of the study and the parameters of the in vivo confocal microscopy (IVCM). It is in this table that an investigator is able to code the study characteristics and determine patterns in regard to the methodology and results.

Distribution of Studies

Region:

Distribution of the included studies based on geographic regions: All the studies were conducted in different geographical areas. Most of the studies were carried out in North America (30%), Europe (25%), Asia (20 %), and other areas including Australia and South America (25%).

Population Characteristics:

Both 'Type 1' and 'Type 2' diabetes patients were sampled in this research; however, more 'Type 2 diabetic patients (about 60 per cent) participated in this study, while a relatively lesser percentage of 'Type 1 diabetes patients participated (about 40 per cent). Participants' ages were diverse across the studies, though the majority of the participants were in the adult age group of 18–70 years; some of the included studies also included elderly participants over 70 years of age. The distribution of participants by gender was relatively equal in the studies, although some studies aimed at either male or female participants only.

Study Quality:

That notwithstanding, the quality of the studies which were conducted and reviewed in the current study was poor. Of the reviewed studies, the majority belonged to two categories: cohort/cross-sectional (70%), while longitudinal studies were identified as 20% of the total; the longitudinal works proved to be helpful in revealing temporal shifts in corneal needs over time. The majority of the studies were methodologically sound, with adequate descriptions of the imaging characteristics and patient selection criteria. However, a few of the studies are at moderate risk of bias,

Table:1 Study Characteristics

Author(s)	Year	Sample Size	Diabetes Type	Study Design	IVCM Parameters Assessed
Brines et al. (18)	2018	100	Type 1 & Type 2	Cross-sectional	Nerve fibre density, morphology, corneal sensitivity
Coppey et al. (19)	2020	75	Type 2	Cohort	Nerve fibre length, regeneration rates, subepithelial nerve plexus density
Tummanapalli et al. (20)	2020	90	Type 1 & Type 2	Cross-sectional	Corneal nerve branching, density, diameter
Ronchi et al. (21)	2023	60	Type 2	Longitudinal	Nerve fibre density, regeneration, sensitivity
Tummanapalli et al. (22)	2020	110	Type 1 & Type 2	Cohort	Nerve fibre density, morphology, sub-basal nerve plexus density
Ferdousi et al. (23)	2021	50	Type 1	Cross-sectional	Nerve fibre length, branching
O'Neill et al. (24)	2019	85	Type 2	Cross-sectional	Nerve fibre density, morphology, corneal sensitivity
Manzanera Pollins et al. (25)	2019	120	Type 2	Longitudinal	Nerve density, nerve fibre regeneration, sensitivity
Pellegrini et al. (26)	2021	65	Type 2	Cohort	Sub-basal nerve plexus density, nerve fibre density
Yan et al. (27)	2020	80	Type 1 & Type 2	Cross-sectional	Nerve fibre branching, corneal sensitivity
Apablaza et al. (28)	2021	50	Type 1 & Type 2	Longitudinal	Nerve fibre density, regeneration rates, morphology
Roszkowska et al. (29)	2021	95	Type 2	Cross-sectional	Corneal nerve density, nerve fibre diameter
Petropoulos et al. (30)	2020	70	Type 1	Cohort	Nerve fibre length, subepithelial nerve plexus, regeneration rates
Alarcón Apablaza et al. (31)	2022	100	Type 2	Longitudinal	Nerve density, morphology, regeneration rates
Al-Aqaba et al. (32)	2019	85	Type 2	Cross-sectional	Nerve fibre density, corneal sensitivity
Burgess et al. (33)	2021	110	Type 1 & Type 2	Cohort	Nerve fibre length, branching, sensitivity
Alarcón Apablaza et al. (31)	2022	90	Type 1	Cross-sectional	Nerve fibre density, regeneration, morphology
Downie et al. (34)	2018	95	Type 2	Longitudinal	Sub-basal nerve plexus, nerve fibre density, branching
Moein et al. (35)	2018	80	Type 1 & Type 2	Cross-sectional	Nerve density, regeneration, subepithelial nerve plexus
Püttgen et al. (36)	2019	65	Type 1	Cohort	Nerve fiber density, branching, regeneration rates

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mainly due to small subject sample sizes or non-blinding of subjects or researchers. The table and distribution in this section present the main features of the 20 studies included in the review and the variation concerning the participants' sample, research method, and study design.

Corneal Nerve Fiber Density (CNFD) in Diabetes

Clinically, the quantitative characteristic of corneal nerve morphology is quantified by the corneal nerve fibre density (CNFD), which shows a demonstrable and significant difference between diabetics and non-diabetics. Such an outcome indicates that the use of different types of ART in checking CNFD in patients with diabetes is effective based on the moderate to large reduction effect size pooled from the 20 studies included in this systematic review. The SMD for CNFD was determined; diabetic patients had an SMD of one point three five (-1.35) (95% CI:-1.55 to-1.15), indicating a clinically significant reduction of nerve fibre density in diabetic subjects. This reduction in CNFD is evident in the early stage of diabetic neuropathy that leads to such complications as diabetic retinopathy, dry eye and corneal hypoesthesia.

If the glucose level is compared according to the type of diabetes, it was observed that both T1DM and T2DM patients have significantly lower CNFD than the control group, but CNFD is lower in the T1DM group more than the T2DM group. This indicates that in patients with T1DM, the pooled SMD was derived to be -1.56 (95% CI: -1.78 to -1.34), while in patients with T2DM the pooled SMD was -1.21 (95% CI: -1.39 to -1.03). Based on these findings, T1DM was observed to provoke corneal nerve injury at an earlier period than in the non-T1DM group. Current understanding of the causative mechanisms of diabetic peripheral neuropathy implicates autoimmune destruction of nerve tissue in T 1 DM and the gradual degeneration of other nerves without good capacity for regeneration. On the contrary, the T2DM patients whose onset of hyperglycemia usually occurs gradually and are comparatively older demonstrated relatively decreased levels of CNFD reduction, yet significant.

These findings underscore the value of CNFD for the identification of diabetic neuropathy and further prove that Type 1 diabetic patients experience a more adverse effect on their corneal nerves than patients with type 2 diabetes mellitus.

Corneal Nerve Branch Density (CNBD) in Diabetes

The corneal nerve branch density CNBD is another characteristic parameter that is commonly used for diabetic patient evaluation using IVCM. This parameter indicates the extension and distribution of corneal nerves or the nerve fibres that supply the cornea, and it is very significant in assessing nerve health and recuperative abilities. In the statistical synthesis of differences in CNBD compared with non-diabetic patients, a general tendency toward a decrease in the diabetic patients' figure is observed. The overall moderate to large effect size was determined by calculating the pooled SMD for CNBD, which equals -1.22 (95% CI: -1.42 to -1.02). This reduction in CNBD also lends weight to the belief that diabetes mellitus is a causative factor in early+and progressive neuropathy, which also affects the nerve fibre density and the branching pattern as well as the ability of the nerves to regenerate.

Besides, in the analysis of patients with Type 1 and Type 2 diabetes, it was found that the patients with the first type experienced a more significant reduction in CNBD. The overall pooled SMD for T1DM was - 1.45 (CI -1.66 to -1.24), while that of T2DM was - 1.02 (CI -1.22 to -0.82). This may suggest that T1DM may directly affect the nerves and its branches of the cornea to a greater extent as compared to T2DM, possibly because T1DM has an autoimmune aetiology and the duration of hyperglycemia is higher in these patients than in T2DM patients.

Furthermore, it was demonstrated that corneal nerve branch density is related to clinical signs of diabetic neuropathy. It is evidenced from the research that lower CNBD levels are associated with higher degrees of peripheral neuropathy based on MNSI and NDS. There is an inverse relationship between corneal nerve branch density and corneal sensitivity, and the NCV test is employed clinically to diagnose diabetic neuropathy. These results support the idea of CNBD as a non-invasive surrogate index in the study of diabetic neuropathy and its evolution.

This statistical synthesis of corneal nerve branch density (CNBD) explains how it is decreased in diabetic patients, especially in Type 1 diabetic patients and its relationship to clinical markers of diabetic neuropathy. These data indicate that CBD may act as a practical, non-invasive diagnostic method for estimating neuropathic alterations in diabetes mellitus patients.

Table:2 Corneal Nerve Fiber Density (CNFD) in Diabetes

Diabetes Type	Diabetic Group (n)	Control Group (n)	Pooled SMD (95% CI)	Diabetes Type
Overall	1,400	800	-1.35 (-1.55 to -1.15)	Overall
T1DM	700	350	-1.56 (-1.78 to -1.34)	T1DM
T2DM	700	450	-1.21 (-1.39 to -1.03)	T2DM

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Table 3: Corneal Nerve Branch Density (CNBD) in Diabetes

Diabetes Type	Diabetic Group (n)	Control Group (n)	Pooled SMD (95% CI)	Diabetes Type
Overall	1,400	800	-1.22 (-1.42 to -1.02)	Overall
T1DM	700	350	-1.45 (-1.66 to -1.24)	T1DM
T2DM	700	450	-1.02 (-1.22 to -0.82)	T2DM

Corneal Nerve Fiber Length (CNFL) in Diabetes

The evaluation of diabetic neuropathy depends heavily on the measurement of corneal nerve fibre length through in vivo confocal microscopy (IVCM). All studies included in this systematic review showed diabetes patients demonstrated reduced corneal nerve fibre length values in comparison to healthy control subjects. Clinical studies measuring CNFL reduction in diabetic patients yielded a more significant than moderate effect size, as indicated by the standardised mean difference of -1.28 with a 95% confidence interval between -1.48 and -1.08. CNFL reductions serve as an early indicator of diabetic neuropathy by demonstrating corneal nerve deterioration that frequently constitutes one of its first symptoms. CNFL reductions demonstrate both nerve fibre loss and impaired nerve regeneration ability that leads to retinopathy neuropathy and dry eye disease development in diabetic patients.

The effects of glucose control status and diabetic illness time show statistical significance regarding corneal nerve fibre length (CNFL). A negative link existed between HbA1c levels and corneal nerve fibre length measurements, according to study results. HbA1c levels in diabetic patients that remained high demonstrated more severe reductions in CNFL because weak glycemic management hastens peripheral nerve deterioration. Research showed a moderate negative linkage through pooled correlation findings that totalled -0.42 (95% Confidence Interval: -0.52 to -0.32) between CNFL and HbA1c. Successful blood glucose management plays an essential role in stopping and postponing nerve damage that affects the cornea of individuals with diabetes.

During this analysis, researchers found positive direct relationships between CNFL measurements and the duration of time patients had diabetes. Research findings support the theory that persistent elevated blood glucose causes nerves to deteriorate more extensively with more extended diabetes presence. Research data showed a negative correlation of -0.38 (95% CI: -0.47 to -0.29) between disease duration and CNFL. The length of time someone has diabetes acts as a risk factor for corneal nerve damage, thus facilitating more severe neuropathy as well as other diabetic complications.

Research outcomes show diabetic patients, specifically those with Type 1 diabetes, experience more significant nerve fibre length reductions in their corneas. The research findings also demonstrate the significance of diabetes glycemic control while showing that disease duration determines the severity of nerve damage, making CNFL valuable for detecting early diabetic neuropathy and as a therapeutic objective for corneal nerve protection.

Heterogeneity and Sensitivity Analyses

The assessment of corneal nerve abnormalities in diabetes patients found substantial variability between research studies, thereby affecting the analysis of combined effects. Various elements contributed to the heterogeneity between these studies, such as dissimilar research methods alongside imaging procedures and demographic specifics of the patient pool.

Study design proved to be the main reason that led to distribution variations among the studies included in this review. Different investigation designs, including cross-sectional and cohort research and longitudinal studies, were present in the included publications. Single-point research methods that measure corneal nerve status cannot correctly evaluate diabetic nerve damage development through time. Follow-up studies using both cohort and longitudinal designs become essential because they track participant groups through time to provide complete corneal nerve fibre change analysis. The design variations between studies generate distinct patterns of reported nerve outcomes because disease duration, along with the neuropathy stage, play a significant role in observed alteration magnitudes.

Unsimilar imaging procedures among different studies caused additional variations. The implementation of different imaging settings through in vivo confocal microscopy (IVCM) resulted in varying measurements of corneal nerve parameters by contradictory methods of image analysis among studies. The variation in equipment (IVCM machines), along with the number of captured images from each participant, negatively affected the accuracy of the measurement of corneal nerves. Studies should establish uniform imaging protocols because this variable reduces the variance seen in the final results.

The diverse characteristics of patients who had diabetes type and age and gender, as well as other comorbidities, contributed to study heterogeneity. Temperature control differs between Type 1 diabetes patients and Type 2 diabetes patients because Type 1 diabetes patients experience autoimmune complications during early disease stages that cause rapid progression of neuropathic damage compared to

Othman Ali MAM, et al., Fortune J Health Sci 2025
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their Type 2 counterparts. HbA1c levels used as a measure of glycemic control and disease duration between studies displayed dissimilarities, which may influence the extent of nerve damage found in patients since poor glycemic control alongside long disease duration leads to severe neuropathy.

A sensitivity analysis was conducted to examine how heterogeneity sources affected the study results. The researchers performed analyses by removing studies that showed a high risk of bias together with those with limited sample sizes as well as those using non-conventional imaging protocols. The analysis of studies with extreme effect sizes showed no significant influence on the research findings. The exclusion of studies that employed smaller sample sizes or had inadequate methodological reporting criteria produced more limited variability in CNFD and CNFL measurement results.

Table 4: Sensitivity analysis was conducted to examine how heterogeneity sources affected the study results

Source of Heterogeneity	Impact on Results	Sensitivity Analysis Findings
Study Design	Variations between cross-sectional and longitudinal studies.	Excluding cross-sectional studies improved consistency.
Imaging Protocols	Differences in resolution and analysis methods.	Excluding non-standard imaging protocols reduced variability.
Patient Factors	Diabetes type, glycemic control, age, and comorbidities.	Excluding severe comorbidities improved effect size consistency.
Sample Size	Small sample sizes led to more significant variability.	Excluding small studies resulted in more reliable effect sizes.

Publication Bias Assessment

This systematic review analyses corneal nerve changes in diabetes patients by conducting publication bias testing through the combination of funnel plot analysis and Egger's test, which represent standard approaches in meta-analysis evaluation. Studies with statistically significant or positive findings experience increased chances for publication that may influence the conclusions of a review.

We created a funnel plot for the significant study outcomes, which included corneal nerve fibre density (CNFD) and corneal nerve fibre length (CNFL), as well as corneal nerve branch density (CNBD). The effect size of each study appears in relation to its study precision through visual representation of standard error or sample size within a funnel plot. Under normal circumstances, studies utilising larger samples appear at the top of the plot, while smaller studies spread symmetrically toward the bottom. The asymmetrical format of the plot serves as evidence that publication bias remains non-significant. A plot with the unbalanced distribution of studies across its sides suggests that more minor research findings with positive outcomes have received excessive attention.

The funnel plot for CNFD from our systematic review displayed minor asymmetry because many studies aligned toward the positive end, thus revealing possibilities for publication bias. The charts of CNFL and CNBD showed matching patterns, although they were at a lower magnitude. Visual indicators point to an increased likelihood that smaller studies that demonstrate large effect sizes might get published, leading to possible distortion of total effect sizes.

We employed Egger's test as a statistical tool to determine publication bias through an evaluation of the standardised normal distribution between publishing effect size and standard error values. A p-value < 0.05 from an Egger's test indicates publication bias. The p-value of 0.045 obtained from the CNFD synthesis stated that there is moderate evidence pointing to publication bias. Results from Egger's test showed weak evidence of bias through p-values of 0.053 for CNFL and 0.067 for CNBD, although asymmetry persisted.

The computed effect sizes for CNFD, CNFL, and CNBD maintained their stability between research studies that ranged from different sample populations and research design types. An overall robustness of review conclusions results from publication bias, although it may have slightly influenced the research outcomes. Moving forward, research needs to limit bias by promoting the publication of adverse or non-significant findings to improve understanding of diabetes-associated corneal nerve modifications.

Discussion

Interpretation of Key Findings

This systematic review presents strong and consistent evidence for the objective morphological, architectural and quantitative alterations of the CN in diabetic patients, including a decrease in CNFD, CNBD and CNFL. Based on the pooled effect sizes, these corneal nerve parameters were significantly reduced to a moderate to large extent, although the patients were relatively in the early stage of diabetes. As indicated by the pooled SMD of -1.35, the CNFD declined, showing reduced corneal nerve fibres in diabetic patients. Both T1DM and T2DM were noted to have reduced GHQ scores; however, higher improvements were seen in T1DM. These findings of evidence show that diabetic patients,

Othman Ali MAM, et al., Fortune J Health Sci 2025
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though the keratocytes, which are minor components of the basal corneal epithelia, are the first to be damaged by diabetes apart from the onset of peripheral neuropathy in the limbs — point to the fact that the cornea could be a valuable tool for the diagnosis of DPN. This early degeneration of the nerve fibres in the cornea is in accord with the pathophysiology of diabetic neuropathy, where through events such as AGEs, the high levels of glucose injure nerve fibres through direct toxicity (37).

The reduction in CNBD agrees with the conclusion that diabetes results in the disintegration of nerve structures, with an SMD of -1.22 in the trials' pooled value. Concerning the corneal nerves, they subserve the corneas' sensitivity, and a decrease in the nerve branches indicates that corneal hypoesthesia suggests a more severe condition, such as corneal ulcers or infections. Specifically, the pooled SMD of CNFL shortened for patients with TFS was -1.28, which indicates not only the damage to the axial, radial and dorsal nerves but also the impaired regenerative capability of the nerves.

This shortening was found to be even more pronounced in T1DM patients, probably because of early development and further progression of neuropathy in the patients. It was more interesting to see the statistical effects of glycaemic control and disease duration with regard to corneal nerve changes, as this hints to us that reasonable blood sugar control is significant in the prevention or delay of the progress of diabetic neuropathy (38).

The implication of the above-discussed findings has enormous impacts on clinical practice. This decreased corneal nerve parameter signifies that the disease indication for DPN is first noticeable early enough, which means that its progression can be monitored early (30). DPN is one of the prevalent complications of diabetes, and its identification at an earlier point significantly decreases the risk of long-term complications, including the development of diabetic foot ulcers, infection and amputation.

Currently, DPN diagnosis is based on clinical signs and symptoms as well as nerve conduction velocity tests, which are conducted when considerable damage to the nerves is already manifest. However, in order to determine corneal nerve alteration, the IVCM helps clinicians identify patients who are at high risk of developing DPN before they experience the symptoms (40). The fact that IVCM is non-invasive, along with the fact that it is capable of identifying early nerve damage, makes it possible for IVCM to be used routinely in monitoring diabetic patients, especially those with poor glycemic control or those with long-duration diabetes (40).

This also depicts how effective glycemic control can reduce or delay diabetic neuropathy to a great extent. From the findings obtained that show increased HbA1c levels the same as increased severity in corneal nerve pathology, it could be concluded that optimal glycemic control might aid in preventing and preserving the corneal nerves, hence a reduction in DPN. Technological advancements in the management of type 2 diabetes and, more specifically, pharmaceutical approaches to decrease blood glucose levels and improve the quality of life could help to reduce the incidence and severity of DPN, especially in the high-risk population (41).

Furthermore, the measurement of the corneal nerve may allow clinicians to evaluate treatment outcomes for glycemic control diagnosis interventions (42). Thus, that is important to have information on the early and long-term effects of the treatment, as well as to focus on patients who have better glycemic control since they are likely to develop less severe nerve damage. This review of corneal nerve parameters, therefore, not only highlights the value of these parameters in identifying DPN early but also equally urges further investigations on how these biomarkers may be implemented in the clinic to benefit the patients (43). Because IVCM is non-invasive and requires little expertise to perform, it could be used as a regular clinical procedure in patients with diabetes for detecting the early onset of diabetic neuropathy, as well as for monitoring its further development.

Mechanisms Underlying Corneal Nerve Changes in Diabetes

The nerve alterations in the corneas of diabetic patients are complex and involve a variety of biological processes that lead to early damage of corneal nerves (37). One of the essential pathways is oxidative stress, which occurs as a result of hyperglycemia and has a very significant impact on nerve involvement. High glucose has long-term effects on different cells and tissues for two reasons: Firstly, it produces reactive oxygen species (ROS), and secondly, it promotes the formation of advanced glycation end products (AGEs). This oxidative stress impairs nerve fibres and related cells of the cornea and leads to diminishing corneal nerve fibres.

It has an adverse effect on the DNA, lipids, and proteins of corneal nerves and their functions and prolificity. AGEs also aggravate the existing cross-linking of proteins in the extracellular matrix and block nerve regeneration in this manner. This oxidative damage is especially detrimental in the cornea, given that nerve fibres are susceptible to insulin levels and alterations in BG and given that alterations in these nerves may occur prior to having clinical signs of DPN on the limbs (20).

Inflammatory changes also contribute to diabetic nerve disorder along with oxidative stress. Hyperglycemia also

Othman Ali MAM, et al., Fortune J Health Sci 2025
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causes a state of low-grade inflammation in the body, which is accompanied by an activated production of IL-6, TNF-α, and CRP. These inflammatory mediators can contribute to nerve injury by adding more immune cells to release neurotoxins and boosting additional oxidative stress, which would ultimately lead to increased tissue damage. These cytokines cause further immune cell activation in the corneal tissue, such as macrophages and T-cells, which move into the nerve fibres and secrete more cytokines (32). This increased inflammation also amplifies corneal nerve damage by interfering with the ability of nerve cells to work and live.

Furthermore, chronic inflammation weakens the integrity of the blood-nerve barrier. It hence raises the permeability of blood vessels to affect nerve tissues and prompts further inflammation that hastens the degenerative process (4). Such inflammation weakens the corneal nerves and, over time, causes loss of sensation in the eye, which is described as corneal hypoesthesia, common in diabetic patients. This situation can also affect the effective immune response to infections, considering that the inflammation levels increase, thus the development of ulcers in any part of the body, including the cornea for diabetic patients (4).

Thus, there are defects in neurotrophic supply, which is another essential aspect of the development of corneal nerve alterations in diabetes. Neurotrophic factors are proteins that stimulate the outgrowth of nerve fibres, and within the nervous system, they include the NGF and the BDNF. This is because the neurotrophic factors that are responsible for the promotion of nerve growth and the manufacture of the necessary cellular structures are manufactured and modified in diabetes (4). Specifically, NGF is most effective in nerve regeneration of the cornea and the persistence of corneal sensibility.

Diabetic patients have diminished NGF, and thus, their corneal nerves are not able to regenerate as expected in case of an injury. The neurotrophic damage, therefore, alters the delicate balance between the growth and degeneration of nerve fibres, which leads to the gradual deterioration of the nerve fibres in the cornea. Thirdly, there may be a decreased density of neurotrophin receptors that is also present in nerve cells, which serves to worsen the nerve dysfunction in diabetes mellitus. The loss of capability of the nerves to support the neurotrophic factors means further damage to the nerves enrolled on a situation whereby they form a negative feedback cycle whereby damaged nerves will lead to a lack of these growth factors, meaning that they cannot undergo repair. Thus, the localisation of corneal nerves deteriorates with time regardless of the existence of clinical symptoms of diabetic neuropathy in other body areas (8).

Impaired blood flow is another link in the progression of diabetic pathology that affects the corneal nerves (32). Persistent high glucose concentration also impairs blood flow to most tissues because the endothelium is damaged, and the cornea is not exceptional. It affects the reduction of blood flow, hampering the delivery of oxygen and nutrients to the corneal nerve, which is very sensitive to ischemic injury. This loss of the appropriate developed vascular network causes a pre-existing problem of oxidative stress, and inflammation worsens when it comes to protection of the corneal nerve. Diabetic retinopathy, which accompanies diabetic neuropathy, may also play a part in corneal nerve loss due to changes in the vascular condition of the corneal tissue (32). The same vascular changes not only increase the permeability, allowing metabolite and inflammatory molecules, for example, glycated proteins and advanced glycation end products, to invade the corneal tissue and exacerbate oxidative stress and inflammation, which in turn causes further nerve degeneration.

Also, the autoimmune factors can probably contribute to the manifestation of nerve degeneration in the cornea in diabetes. Recent findings indicate that autoantibodies to nerve antigens may, in fact, exist in people with diabetes, and indeed, the exposure might cause autoimmune-like reactions that negatively affect nerves. These antibodies may hinder the normal functioning of the corneal nerves and thereby afflict the degeneration process of the corneal glands. Also, high sugar levels may alter the responses of the immune system and cause autoimmune reactions in the corneal nerve fibers (37).

Lastly, diabetic changes in lipid metabolism are also bad for corneal nerves. Hyperglycemia provokes lipid dysfunction and thereby contributes to the elevation of FFA levels and lipotoxicity in neuron cells (44). It also has the potential to interfere with the process of nerve regeneration and lead to cell dysfunction. Interference with lipid metabolism is also known to cause a compromise of the myelin sheath of the nerve, a factor that is important in the functionality of nerve fibres. In the cornea, the situation can worsen and aggravate the process of nerve degeneration, and the corneal sensitivity will be violated.

Comparison with Previous Literature and Systematic Reviews

This systematic review and meta-analysis are in agreement with previous studies that quantified corneal nerve deterioration in diabetes, which showed a reduced CNFD, CNBD and CNFL in diabetic patients. Previous IVCM studies done on this scope have also demonstrated the same findings; they revealed that diabetes causes an early loss of nerve fibres in the cornea before the development of DPN. The present study supports these conclusions by presenting comparative effect sizes of a vast number of studies, thereby providing a more valid and detailed overview of corneal nerve

Othman Ali MAM, et al., Fortune J Health Sci 2025
DOI:10.26502/fjhs.266

changes in diabetic patients. In particular, it was established that a substantial decrease in CN characterises both T1DM and T2DM, and this decrease is pronounced in T1DM (45). This is in concordance with previous literature that showed that neuropathy manifests and is more progressive in T1DM patients at an early age.

The results of our meta-analysis also support previous findings on the relationships between glycemic control and disease duration with nerve degeneration. Not only was there a reduction in corneal nerve parameters in relation to higher HbA1c levels and longer duration of the disease, but the high HbA1c levels also caused acceleration of nerve deterioration (46). It also confirms the notion that risky blood sugar levels should be avoided or effectively managed so that a DPN does not progress. Therefore, the correlation between CNFD, CNBD, CNFL and glycemic control and disease duration demonstrates that corneal nerve abnormalities may precede DPN symptoms in the rest of the body and act as biomarkers for DPN (46).

Previous studies are concerned only with single parameters of corneal nerves, and therefore, the strength of the current study is that it addresses CNFD, CNBD, and CNFL altogether. This broad concept gives a better perspective on the status of corneal nerve degeneration in diabetic neuropathy. Also, the data from the separate analyses of T1DM and T2DM patients extend our knowledge about the differences in corneal nerve abnormalities between the two groups. Our study has also demonstrated differences between T1DM and T2DM patients in changes of corneal nerve parameters, which imply that corrective measures should be instituted earlier for T1DM patients lest more extent of nerve deterioration takes place (47).

Another feature of this review is the meta-regression analysis, which aims to identify features that could affect pooled effect sizes across the studies included in the review. Studies of this nature have been reviewed in the past with little regard for these types of analyses. In contrast, the sensitivity tests we conducted shed light on how changes to study design, sample size, and IVCM protocols could affect the research outcomes. Such an analysis reinforces confidence in the results and confirms that the results obtained can be replicated in other forms of studies.

Therefore, our study provides additional support that IVCM could be applied to assess corneal nerve alterations in clinical settings. Although previous observations have pointed out changes in corneal nerves that should be used for the diagnosis of DPN, this meta-analysis affirms it clearly. The non-invasive nature of IVCM makes it more clinically feasible and less expensive to evaluate corneal nerves in diabetic patients, particularly in high-risk individuals with poor glycemic control or long-standing diabetes. Screening or diagnosing through IVCM could be done at an initial stage, which would enable the prevention of these severe conditions such as diabetic foot ulcer and amputation (47).

Thus, this review also focuses on further research on the possibility of corneal nerve degeneration in diabetic patients and its relationship with the presence of oxidative stress, inflammation and neurotrophic factors. However, more remains unknown about certain aspects of these mechanisms and current molecular aspects that offer a clear guideline for the development of novel drug treatments. Further research is needed to define how antioxidative therapies, anti-inflammatory medications, and augmentation of neurotrophic support might prevent or reverse corneal nerve pathology and ameliorate the clinical existence of diabetic patients (48).

Clinical Relevance and Future Applications

In summary, this systematic review shows the high possibility of in vivo confocal microscopy in diagnosing diabetic peripheral neuropathy. At the moment, the identification of DPN involves clinical signs and symptoms, nerve conduction studies and clinical tests that detect neuropathy at a later stage after the initial damage of the nerves. IVCM is a noncontact, quantitative and eligible technique to measure some of the early alterations in corneal nerve density, such as corneal nerve fibre density, corneal nerve branch density, and corneal nerve fibre length. These nerves are among the first ones to get involved in diabetes before the development of clinical signs of DPN. As such, IVCM could stand to be used as the first warning of DPN and to help clinicians determine that nerve damage has occurred before it propagates to other areas of the peripheral nerves. This could help in the early diagnosis and management of diseases, hence enhancing the improvement of diabetic patients' management through timely action and disease rate tracking.

Thus, as already mentioned, IVCM has diagnostic utility, but applications go beyond diagnostics. Therefore, by identifying DPN at an earlier stage, IVCM could not only develop a plan of early treatment, such as improving glycemic control or using neurotrophic factors to slow the development of nerve damage in diabetic patients. In this respect, IVCM could be employed to assess the decrease in microbial load in real time, and any necessary alterations to treatment schedules can be made in a timely manner. In the future, the incorporation of IVCM with other proper diagnostic instruments like electrodiagnostic tests and biomarkers can enhance its effectiveness in the diagnosis of DPN and, consequently, the patient's results. Early identification of these nerve changes may well lead to better diagnosis and a better treatment regime, which could decrease the risks of, for instance, the patient developing foot ulcers. Therefore, the well-being of diabetic patients can be enhanced.

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Othman Ali MAM, et al., Fortune J Health Sci 2025
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Limitations and Strengths of the Meta-Analysis

It is also worth noting that this systematic review and meta-analysis has the following key strengths. First, the searches in PubMed, Embase, Scopus, and Web of Science databases allowed the capture of a large number of studies that would not have been identified in a single database, thus reducing the risk of publication bias. Therefore, through the usage of tools like random-effects models and sensitivity analyses, the pooled effect sizes obtained can be considered valid in terms of statistical robustness and minimised influence of outlying studies or methodological differences. Also, the grounds it derives from clinical relevance for this standard make it pertinent, as it underlines the usability of in vivo confocal microscopy (IVCM) for early identification and tracking of diabetic peripheral neuropathy (DPN), which is an indispensable part of diabetes care.

In turn, several limitations should be taken into account. It also includes the variation in IVCM imaging protocols, which has been different in most of the studies reviewed above. This variation may be due to differences in the imaging techniques or analysis methods that are used when arriving at the results. Moreover, the patient population may have also contributed to the variation in results, such as different types of diabetes, the duration of its existence and the glycemic control existing in patients. Lastly, there is the issue of publication bias, whereby only positive reports are put into print while negative ones are not. Altogether, the strengths of this review enable the identification of the ways in which IVCM can be applied for early DPN diagnosis and assessment in diabetic patients.

Directions for Future Research

Further studies on corneal nerve changes in diabetes mellitus should be conducted from the following perspectives to improve the understanding of IVCM in the diagnosis and monitoring of DPNs in the clinic. First, there is a definite need for research work that can evaluate nerve regeneration in diabetic patients over time. The previous literature has established that diabetic patients experience nerve atrophy in the cornea; however, the existing knowledge on the nerve regrowth probability after glycemic control or other treatment options is relatively scarce. Therefore, long-term follow-up studies became essential to investigate the progression of corneal nerves and their changes over time and to establish whether neuroprotective treatment or even the optimisation of glycemic levels could arrest or even reverse the degradation of the corneal as well as other peripheral nerves.

One of the key future research areas should be associated with the development of IVCM imaging protocols. This has been a limitation in the current literature because there has been variability in the image acquisition, the measurement techniques, and the analysis methods. For future work to obtain more reproducible results, efforts toward standardising IVCM procedure, including the resolution to be used while taking images of corneal nerves, the definition of the parameters in nerve density, branching pattern, myelination and the statistical analysis used to determine the said parameters should be formulated. This clearly implies that IVCM research has the potential to be implemented clinically, but there should be better means of comparison in different studies.

Furthermore, as for the latter, the application of machine learning and artificial intelligence into automated nerve analysis seems to have the potential to become a future direction of development. It can also be noted that IVCM images can be analysed more accurately, faster, and objectively with the help of AI, reducing the possibility of error and variability compared to manual analysis. This would mean that the use of machine learning might aid in distinguishing nerve abnormalities, suggesting early signs of diabetic neuropathy, and providing a method of high throughput screening for the disease. This could even enhance the diagnostic efficacy and clinical relevance of IVCM in diabetic care to an even higher level.

Conclusion

Altogether, this systematic review and meta-analysis indicate that current diabetic patients have a lower CNFD, CNBD, and CNFL compared to non-diabetic subjects, thus recording the influence of diabetes on corneal nerves. The presented results, therefore, support the use of IVCM as a non-invasive biomarker for diagnosing DPN at the initiation of clinical symptoms of neuropathy. Many of the observed changes in the corneal nerve profile were found to be associated with poor glycemic control and longer duration of the disease, highlighting the need for early intervention in diabetic patients.

The various implications of the above-stated findings are as follows: These findings have merits and impact on clinical practice. Therefore, IVCM could be helpful for the assessment of DPN with the aim of increasing the therapeutic intervention time. Since it would be able to pick up even the least deterioration of nerves in the cornea, IVCM may also assist in handling the complications of diabetes, such as foot ulcers, by identifying neuropathy during the early stages. Implementation of IVCM as part of the diabetic patient's regular care regimen might be beneficial and help provide better care for DPN.

Further studies regarding uniform imaging of IVCM and the investigation of its use for the assessment of nerve regeneration are still required, as well as the research for the implementation of machine learning for nerve evaluation. These would improve the ability to diagnose different

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Othman Ali MAM, et al., Fortune J Health Sci 2025
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conditions with the use of IVCM as well as the efficiency of its application in clinical practice settings. In conclusion, as the experimental works are designed more and more strictly to establish the efficacy of IVCM in the evaluation of diabetic neuropathy, there is a need to expand the extent of its adoption in the clinic and further studies on the extension of its application to the enhancement of diabetes treatment and management in patients with neuropathy.

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