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Methods for evaluation of corneal nerve fibres in diabetes mellitus by in vivo confocal microscopy: a scoping review protocol

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ABSTRACT

Introduction Globally, 422 million people have diabetes. Late complications of diabetes are blindness, kidney failure, heart attack, stroke and lower limb amputation. The prevalence of diabetic peripheral neuropathy and diabetic retinopathy is 50% and 35%, respectively. In vivo confocal microscopy (IVCM) is a rapid, non-invasive method to evaluate subbasal corneal nerve fibres, which are small fibres of the peripheral nervous system. Corneal nerve fibre changes can be a marker of diabetic peripheral neuropathy. There is currently no gold-standard procedure for IVCM imaging, image processing or quantitative analysis of the corneal nerve fibres in the subbasal plexus. This protocol describes a scoping review to map, summarise and critically evaluate current methods used with IVCM evaluation in people with diabetes mellitus.

Methods The scoping review will follow Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for scoping review. A comprehensive search of the literature will be conducted in MEDLINE, Embase, Cochrane, Scopus and Web of Science. The search strategy will include terms related to IVCM, diabetes and corneal nerve fibres. We will set inclusion and exclusion criteria prior to the search, and two reviewers will screen titles and abstracts independently. One reviewer will full text read eligible articles and chart data from the studies. A descriptive summary of the methods used in imaging, image processing and quantitative analysis of peripheral corneal nerve fibres by IVCM will be written.

Ethics and dissemination Ethical approval is not required since this is a scoping review based on previously published articles. The findings will be published in a scientific peer-reviewed journal.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Protocol guidelines and the updated JBI guidelines for scoping review by Peters *et al*.
⇒ Report the results according to the PRISMA extension for Scoping Reviews checklist.
⇒ Professional librarian conducted the search.
⇒ Not meta-analysis.

BACKGROUND

According to the WHO, about 422 million people had diabetes in 2014. Late complications of diabetes are blindness, kidney failure, heart attack, stroke and lower limb amputation.[1] Diabetic peripheral neuropathy (DPN) is a common complication of diabetes, represented in half of the patients with diabetes.[2 3] Globally, as many as 35% (93 million people) have some degree of diabetic retinopathy (DR) and 5%–10% of people with diabetes has sight-threatening stages of retinopathy and diabetic macular oedema (DME) (28 million people).[4]

DR and DME are leading causes of blindness and visual impairment in the working-age population.[5 6] Reduced vision leads to everyday challenges related to tasks such as reading and moving around outside and is associated with psychosocial outcomes such as higher frequency of depression and depressive symptoms.[7] DPN is also associated with sensory loss and weakness in muscles.[3] It leads to posture, gait and sensation loss challenges,[8] as well as foot ulcers.[9] DPN in its late stages can even lead to amputation,[10] reduced sleep quality and depression.[11]

The eye provides unique opportunity to evaluate neurodegenerative changes in the cornea and retina. In vivo confocal microscopy (IVCM) has the ability to examine the corneal nerves with high resolution and extreme precision.[12] It is a rapid, non-invasive method to analyse corneal morphology and quantify corneal nerve density, nerve length, tortuosity and thickness.[13] However, studies are performed with three different types of confocal microscopy based on different light scanning principles. Tandem scanning uses white light and a rotating disc, slit scanning uses white light and a moving linear slit, and laser scanning uses a focused laser spot that

is raster scanned, to illuminate and collect light from the structure in the cornea.[12 14] The light source used and illumination area has impact on the quality of the images regarding contrast and resolution.[15 16]

Stem *et al* suggest that pathology in corneal nerve fibres seems to manifest before peripheral neuropathy,[17] while others have reported that corneal nerve loss may predict incident neuropathy and progresses with DPN severity.[18]

There is evidence that corneal nerve fibre changes also manifest before visible DR, and may worsen progressively with increasing severity of DR.[19] In more severe DR stages, DPN is also more pronounced.[20] Several studies have explored the associations between corneal nerve fibres and DR[19 21–24] and between corneal nerve fibres and DPN.[18 25 26] Bitirgren (2014),[19] Petropoulos (2015),[22] and Schiano Lomoriello (2019)[23] found reduced corneal nerve fibre length (CNFL), corneal nerve fibre density (CNFD) and corneal nerve branch density (CNBD) in those with diabetes without DR compared with healthy controls. Nitoda (2012)[21] and Srinivasan (2017)[24] found differences in CNFL with different degrees and classifications of DR compared with healthy controls. The meta-analysis by Jiang *et al*,[25] evaluating 1680 participants, concluded that CNFL, CNFD and CNBD were significantly reduced in patients with DPN compared with healthy controls.[25] However, that analysis also concluded that comparing studies is challenging based on the different instrumentation and analysis methods used across studies.

CNFL is currently considered to be the most reliable surrogate parameter for quantifying corneal small fibre loss and hence early DPN.[20 27–31] An upper and lower value of 15.3 mm/mm^2 and 8.6 mm/mm^2, respectively, have been found to rule in and out diabetic sensorimotor polyneuropathy (DSPN), with an 88% specificity and 88% sensitivity in a total cohort of 998 people with diabetes mellitus (DM), from five centres.[31] However, defining thresholds is limited based on the sample, type of diabetes, sample size and measurement errors that limit the precision.[27] Hafner *et al* suggest that ophthalmologists have the opportunity to play a critical role in the early diagnosis of DPN. They also point out the need for further understanding of the complex and divergent pathophysiological processes of diabetes to facilitate early identification to prevent vision-threatening retinal disease and advanced neuropathy.[20]

Changes in the corneal nerves may proceed reduced corneal sensitivity in people with diabetes.[32 33] A reduction in CNFD[32] and CNFL has been found in people with DM.[34]

There are some challenges to overcome before IVCM can be used for broader clinical use. First, the corneal nerve parameters may differ depending on the corneal region imaged and used for analysis; therefore, a sampling bias exists. The corneal nerve density is significantly higher in the inferior whorl region, located 1–1.5 mm inferior nasal to corneal apex, compared with the central cornea,[35]. Also, there is a higher nerve fibre density in central cornea compared with the periphery.[36]

Second, each image represents a small field of view, where less than 1% (400×400 µm) of the total subbasal nerve plexus is included.[37] Studies have tried to overcome this by collecting multiple images for analysis. Vagenas *et al* stated that five images, not overlapping more than 20%, give an average that was within 13% of the true mean 80% of the time. By increasing to eight randomly chosen images, still not overlapping more than 20%, this gives an average that was within 30% of the true mean 95% of the time.[38] Badian *et al* stated that the CNFL was underestimated by 34% in the central region compared with a wide-field mosaic generation of the subbasal nerve plexus (SBNP) for 90% of eyes with type 2 DM.[39] They suggest larger areas of the corneal SBNP should be evaluated to improve the diagnostic sensitivity and specificity and to confirm other studies that found a significant reduction in people with type 2 DM.[39] Therefore, it is essential to consider the image acquisition area used in different studies when sampling the raw material to evaluate sampling biases when comparing studies.

Third, the quality of the images are factors to consider: the instrument used has an impact on the quality, image depth, enhancement in software used to influence the visibility of the nerves, and repeatability and reliability of measurement methods.[37] The experience level of the clinician will also affect the image quality.

Fourth, the methods of image analysis are also essential. Different studies report the use of different software for image analysis. Both software-associated wide-field imaging and algorithms based on artificial intelligence have been explored to achieve efficient screening.[18] Manual analysis is more time-consuming and subject to reduced inter and intrarater variability.[40] When manually analysing the sample, it is also essential to mask the observer to the groups with and without diabetes to avoid performance biases.

Automated analysis reduces the time for analysis and interobserver/intraobserver variability. According to Herrera-Pereda *et al* computerised analysis is still not efficient and validated for clinical practice, however, Lagali *et al* showed in 2017 that automated nerve detection and tracing algorithms were fast and could be used in the clinic.[34 40]

There is currently no gold-standard procedure for IVCM for imaging, image processing and quantitative analysis of the corneal nerve fibres in the subbasal plexus. However, Petropoulos *et al* state that generally 5–8 non-overlapping images from the apical cornea and two from the inferior whorl should be used to assess DPN.[18] Given, however, the widely varying methodologies and practices inherent in the literature, we believe that it is useful for future research and clinical care to perform a detailed scoping review to map and summarise methods used in IVCM evaluation of corneal nerve fibres in DM.

METHODS

The review will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Protocol guidelines and the updated Joanna Briggs Institute's(JBI) guidelines for scoping review by Peters *et al*[41] and report the results according to the PRISMA extension for Scoping Reviews checklist.[42] This paper is the protocol for the scoping review.

Identifying the research question

The scoping review aims to map and summarise methods used in IVCM evaluation of corneal nerve fibres in people with DM, and critically review the methodology for imaging acquisition, image processing and quantitative analyses of peripheral corneal nerve fibres by IVCM in people with DM and relevant control groups. The goal is to objectively identify low bias, reproducible, and appropriate imaging techniques, image processing and image analysis methods for research and clinical practice.

A preliminary search for existing reviews in PubMed, Cochrane database for systematic review and in PROSPERO (January 2022) did not identify any published review on the methodology using IVCM to evaluate corneal nerve fibres in the SBNP in the context of diabetes.

We suggest evidence of multiple limitations and heterogeneity in the sampling procedure, selection of images and statistical analysis reported in previous studies. We aim to further explore these aspects by addressing the following underlying research questions:

1. What type of diabetes has been evaluated and using which diagnostic criteria?
2. What are the methodology and image techniques used in IVCM examination of nerves in people with DM?
3. What data reduction methods are used to obtain a relevant and representative raw image dataset?
4. What methods are used to process images and extract quantitative nerve parameter data from the raw image dataset?
5. How are the extracted images analysed? Based on single image, multiple single images or merged images?
6. What outcome measures and quantitative outcome values (SD, mean, median, IQR) are reported for corneal nerve parameters in subjects with diabetes and healthy controls?
7. What statistical analyses are performed to compare corneal peripheral nerves across subject groups, and are these appropriate and do the studies have adequate statistical power?
8. How can we improve the quality and reproducibility of methodology and reduce bias in future studies investigating the peripheral corneal nerves with IVCM?

Identifying relevant studies

A three-step search strategy will be used to identify evidence in the scientific literature.

First, the databases (1) MEDLINE, (2) Embase, (3) Cochrane, (4) Scopus and (5) Web of Science will be used to search for studies involving IVCM and corneal nerve fibres in people with DM. These are databases with references in (1) health sciences and biomedical research, (2) pharmacology, general public health, substance abuse, environmental and occupational medicine, (3) research results from health research, (4) a wide variety of disciplines and (5) all academic fields of the world's most cited scholarly journals in the Sciences, Social Sciences and Arts and Humanities. We will develop a full search strategy for each database together with an academic librarian who will perform the search. The search will be conducted using the keywords (Thesaurus/Medical subject headings (MESH) and text words (keywords)) for "in vivo confocal microscopy" AND cornea AND ("diabetes mellitus"). The entire search term is provided in online supplemental file 1. We will search the databases from inception, including papers published in English, German and the Scandinavian languages. Where relevant, authors will be contacted to identify additional sources. Second, additional text words in the title and abstract and index terms used to describe the articles will be included in the second search in MEDLINE, Embase, Cochrane, Scopus and Web of Science using all identified keywords and index terms. Third, we will search the reference list of papers, including full-text reading, for additional sources.

Inclusion criteria: Empirical and research articles based on humans >18 years of age and articles published in peer-reviewed journals will be included. We will include randomised controlled trials, controlled trials, intervention studies, experimental studies, quasi-experimental studies, observational studies with a control group, within-subject/repeated measures studies and case–control studies, study protocols and observational studies without a control group. Qualitative studies, literature reviews commentaries, letters, editorials, conference papers or proceedings, opinion or discussion papers, dissertations/thesis, abstracts/presentations, grey literature and brief reports will be excluded.

Study selection

The search result will be imported to Endnote for removal of duplicate studies and then exported to Rayyan[43] for screening of titles, keywords and abstracts. We will complete the screening and select studies in two steps.

First, two independent reviewers will screen the title and abstracts to identify eligible studies. A sample of 25 studies will be screened regarding title and abstract to determine the inter-rater agreement, and a 75% agreement should be met before working further. In cases of disagreement about inclusion, the review authors will discuss. If a consensus is not achieved, a third reviewer will be consulted to make the decision.

In the next step, one reviewer will screen the full text of the potentially eligible studies. If questions arise on inclusion criteria is met, a second author will be consulted to discuss and make a decision. A PRISMA flow chart will document the study selection procedure.

Charting the data

Data will be extracted, and a descriptive or tabular summary of the methods used in imaging acquisition, image processing and quantitative analysis of peripheral corneal nerve fibres by IVCM will be written. Extracted data will fall within the following main domains: publication details, demographics of the participants and outcome variables. In addition, summary tables will include essential information about the source; author(s), title, year of publication, journal, study design, aim, demographics and sample size. Furthermore, we will chart the studies' inclusion and exclusion criteria, imaging instrumentation, imaging procedures, image sampling and extraction procedures, outcome variables and outcome findings and the study's conclusion and implications. If necessary, the data extraction form will be updated during the review process as this is seen as a process of improvement. Two reviewers have piloted the data extraction form (online supplemental file 2) according to the recommendations from Peters *et al*.[41]

Collating, summarising and reporting the results

We will collate the results and summarise IVCM imaging and image analysis methods, provide an overview of the quality of imaging techniques and image analysis methods, and identify potential best practices, limitations and areas for further improvements. From the included studies and through the use of standardised tools such as AXIS, ROBBIN and ROB2, the authors will provide a descriptive analysis of the bias that may be presented in the different studies.

Patient and public involvement statement

No patients or public were directly involved in this study's concept, design and planning.

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Contributors The authors confirm contribution to the paper as follows: study conceptualisation; VS and NL. SAS wrote the original draft, and SAS, VS and NL reviewed and edited the draft, and VS was the primary investigator and supervised SAS in writing the protocol. All authors approved the protocol.

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Competing interests None declared.

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Supplementaryfile 1

Patient/problem	**Intervention / Exposure (Hvilke tiltak vurderes)** **Undersøkelsesmetoder**	**Outcome (Hvilke resultat/utfall av interesse)**
Diabetes Mellitus Diabetes Mellitus, Type 2 Diabetes Mellitus, Type 1 Diabetes mellitus type 2 (DM2) Diabetes mellitus, noninsulin-dependent Diabetes mellitus, noninsulin dependent Diabetes mellitus, non insulin dependent Diabetes mellitus, ketosis-resistant Diabetes mellitus, slow-onset Diabetes mellitus, slow onset Slow onset diabetes mellitus Type 1 diabetes Type 2 diabetes mellitus Type 2 diabetes Non-insulin dependent diabetes mellitus Noninsulin dependent diabetes mellitus Non insulin dependent diabetes mellitus Insulin dependent diabetes mellitus Diabetes mellitus, adult onset Diabetes mellitus, adult-onset Adult-onset diabetes mellitus Adult onset diabetes mellitus IDDM T1DM NIDDM T2D DM1 DM2 Diabet*	Microscopy, Confocal confocal laser scanning microscopy confocal microscopies confocal microscopy confocal microscopy, scanning laser laser microscopies laser microscopy laser scanning confocal microscopy laser scanning microscopies laser scanning microscopy microscopies, confocal microscopies, laser microscopies, laser scanning microscopy, confocal microscopy, confocal, laser scanning microscopy, laser microscopy, laser scanning scanning microscopies, laser scanning microscopy, laser In vivo confocal microscopy (IVCM) Corneal confocal microscopy (CCM) In vivo corneal confocal microscopy (IVCCM) Microscopy confocal Confocal microscopy Laser scanning confocal microscopy (LSCM) Heidelberg Retina Tomograph Heidelberg Retina Tomograph 2 Heidelberg Retina Tomograph 3 Heidelberg Retina Tomograph II Heidelberg Retina Tomograph III Heidelberg Retinal Tomograph Heidelberg Retinal Tomograph 3 HRT	Cornea Bowman Membrane Corneal Stroma Cornea Stroma Cornea cell Descemet Membrane Endothelium, Corneal Cornea Endothelium Epithelium, Corneal Cornea Epithelium Limbus Corneae Cornea Limbus Nerve Fibers Nerve fiber Nerve plexus nervous plexus nervus plexus neural plexus Corneal nerve morphology Corneal morphology Corneal nerve fiber morphology Corneal nerve fibre length Corneal nerve fibre thickness Corneal nerve fibre tortuosity Corneal nerve fibre density Corneal nerve branch density Subbasal nerve plexus Subbasal nerve density Sub-basal nerve plexus Sub?basal nerve* plexus Corneal sub basal nerve Corneal subbasal nerve plexus Subbasal nerve plexus Sub basal corneal nerve bundle Small Fiber Neuropathy Corneal nerve plexus Corneal nerve fractal dimension Corneal nerve fiber structure Corneal nerve fiber damage Corneal nerve fiber pathology

	HRT 3 HRT II Rostock Cornea Module HRT III Rostock Cornea Module HRT Rostock Cornea Module HRT3 Heidelberg Retinal Tomograph with Rostock Corneal Module HRT3-RCM HRT2-RCM HRT-RCM	Corneal microstructural changes Corneal structure Small fiber pathology Corneal nerve loss Inferior whorl Inferocentral whorl Morphological changes Corneal nerve * Corne* nerve* Corneal nerve assessment

Supplementary file 2

List over preliminary data to be extracted:

Author(s), title, year of publication, journal, study design, aim, type of diabetes mellitus, descriptive data of target population(s) such as sample size, age, sex, diabetes duration, examination methodology diabetic neuropathy, exclusion criteria, corneal sensitivity, optical coherence tomography, tear proteomics/analysis, diabetic retinopathy, IVCM methodology; instrument, number of images, randomly selected images, sampling and selection criteria, region of interest, quantitative variables from the sub basal nerve plexus, analysis tools, statistical methodology whether comparing groups, evaluating associations between variables, and or sensitivity and specificity analysis and which statistical tools, and the study's conclusion and implications.