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Corneal Confocal Microscopy as an Early Diagnostic Tool for Diabetic Neuropathy: A Systematic Review

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Objective To evaluate the effectiveness of corneal confocal microscopy, its parameters, and threshold as an early diagnostic method for diabetic neuropathy.

Method A systematic review was conducted through PubMed, CENTRAL, and Scopus databases, searching for studies implementing corneal confocal microscopy (CCM) in patients with diabetes for detecting neuropathy in the early stage. Quality assessment of studies selected were performed using selected risk-of-bias assessment tool.

Results The search yielded 9 studies with a total of 2027 subjects. From the 9 studies reviewed, CCM proved to be a reliable method of diagnosis for diabetic neuropathy with consistent sensitivity and specificity. Fiber length, density, and bead size are the most reliable parameters for diagnosis.

Conclusion Significant correlation between CCM parameters and diabetic neuropathy were found. Therefore, corneal confocal microscopy showed promising potentials as an early diagnostic tool for diabetic neuropathy.

Keywords *Corneal confocal microscopy, diabetic neuropathy, early diagnosis*

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Introduction

Diabetes is a significant metabolic condition characterized by inappropriately elevated blood glucose levels. About 422 million people are affected by diabetes, 1.6 million of which died because of the disease.[1] In Indonesia alone, about 7% of the population are affected by diabetes.[2] This is a cause of concern as diabetes can lead to various complications, including blindness, kidney failure, limb amputations, and cardiovascular diseases.[3] One common complication of diabetes is diabetic peripheral neuropathy, a condition characterized by loss of sensation, tingling, and other types of neuropathic pain on the lower limbs.[4]

Diabetic neuropathy occurs when there is damage to sensory nerve fibers and cell death resulted from oxidative stress and inflammation. Hyperglycemia, insulin resistance, and overall dysregulation of metabolic pathways are the main causes of excessive reactive oxygen species that result in axonal injury. Consequently, patients with diabetic neuropathy could develop foot ulcers, experience pain, and even progress to the extent of the need for lower limb amputation. It is almost definite that the quality of life will be severely affected by this condition. Currently, treatment for diabetic neuropathy is limited to supportive care and glycemic control, which might be limited in terms of prognosis. However, early diagnosis could be vital in preventing further complications, such as foot ulcers and amputation, as high-risk patients would receive more intensive glycemic control and foot care to reduce risk of foot ulcers. Thus, prevention and early diagnosis are vital in implementing management as early as possible, in order to prevent other complications and ensure that the highest quality of life possible can be achieved.[5,6]

Currently, diagnosing diabetic neuropathy is still a challenge, proven by many cases where it is often diagnosed late where severe consequences, such as foot ulceration, have already manifested. In addition, half of the patients with diabetic neuropathy are asymptomatic. Even when symptoms are present, many patients have trouble describing their symptoms clearly, making early and accurate diagnosis difficult. Consequently, a valid and quantifiable method of diagnosis is needed to detect diabetic neuropathy at its earliest stage.[7]

There are several instruments that can be used to diagnose diabetic neuropathy, such as Michigan Neuropathy Screening Instrument questionnaire and Physical Assessment, and Diabetic Neuropathy Symptom. Despite that, currently there is no consensus about which questionnaires are best used for diagnosis or evaluating the degree of the disease, and the evaluation relies solely on clinical expertise and judgement, which might be unreliable in some cases.[4]

The gold standard for early diagnosis is the nerve conduction velocity test. This method exhibits decrease in nerve conduction velocity and reduction in amplitude of muscle action potential in patients with diabetic neuropathy. Its strength relies on the fact that it is quantifiable, repeatable, and sensitive

enough to detect sensory and motor losses when symptoms have not been apparent, as well as its ability to predict ulceration and mortality. However, this method is invasive and painful. Moreover, its utilization requires highly trained specialists, such that this diagnostic tool is not widely available in public health units. It is also limited to detecting large nerve fiber dysfunction, and not small sensory fiber damage, even though this small fiber damage is the earliest manifestation of diabetic neuropathy.[4,8] Hence, there is an opportunity in developing a better diagnostic method.

Recent studies have shown that corneal confocal microscopy (CCM) can be used to detect early diabetic neuropathy. CCM detects morphology of corneal nerve fibers in sub-basal corneal plexus by illuminating a single point of tissue and reconstructing it into a high resolution, magnified image. Parameters commonly measured are nerve fiber density, nerve fiber length, nerve fiber branching, beading, and tortuosity.[9] It can be used to quantify small nerve fiber function, thus detecting early diabetic neuropathy. In addition, it is non-invasive compared to the current gold standard of diagnosis.[10]

Therefore, this systematic review aims to evaluate the diagnostic value of CCM for early detection of diabetic neuropathy, along with its parameters and thresholds. Through the results of this review, the authors hope to improve guidelines of current diabetic neuropathy diagnosis, thereby improving early diagnosis and risk management of diabetic patients, and consequently, reducing complications and improving the quality of life of diabetic patients.

Methods

Search strategy

This systematic review of clinical trials is conducted based on the PRISMA statement. We explored PudMed, Cochrane Controlled Register of Trials (CENTRAL), Scopus, CINAHL, and Science Direct databases from the last 5 years up to 17th October 2020 with the following keywords: (cornea*) AND ("Microscopy, Confocal"[Mesh]) AND (Diabetes Mellitus) AND (("Diabetic Neuropathies"[Mesh]) OR (“Small Fiber Neuropathy”[Mesh])). The search was limited to human participants and the language was restricted to Bahasa Indonesia and English, which were the only languages readable by the authors. Details of the literature search strategy are shown in Figure 1.

Inclusion and exclusion criteria

Studies were screened according to the inclusion criteria as follows: (1) observational studies; (2) study population, involving patients of type II diabetes mellitus with or without neuropathy; and (3) studies within the last 5 years. Conversely, exclusion criteria were also set: (1) irretrievable full-text articles, (2) unknown and/or inappropriate study types and settings, (3) incompatible language (articles not in English or Bahasa Indonesia).

Data extraction and risk of bias assessment

We extracted data from selected studies, which included: author and year of publication, location, study design, study population, intervention, and outcome. These were assessed using Newcastle-Ottawa Scale, which consists of 9 domains with scores of 7-9 classified as good quality, 4-6 as having high risk of bias, and 0-3 as very high risk of bias. Risk of bias assessment was conducted by the three reviewers (AUTHOR, AUTHOR, and AUTHOR) and any discrepancies were resolved by consensus between reviewers. Appendix 1 provides details of risk of bias assessment of included studies.

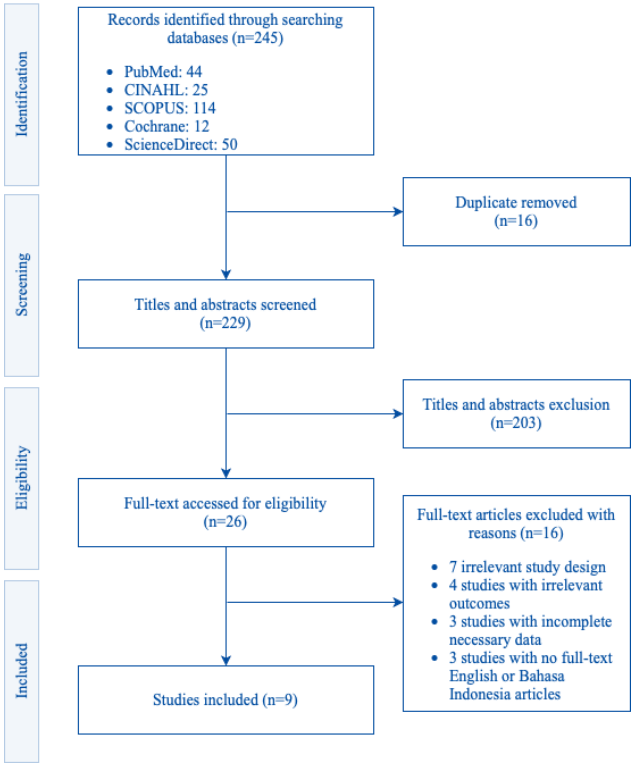

Figure 1 Diagram flow of literature search strategy.

Results

Study selection

The main characteristics of included studies in this systematic review are shown in Table 1. The initial search yielded 242 studies from PubMed, Scopus, Cochrane Controlled Register of Trials (CENTRAL), CINAHL, and ScienceDirect databases. Duplicates were removed, titles and abstracts were screened, and finally full-text articles were assessed for eligibility. Twenty-one were further excluded due to irrelevant study designs, language restrictions, and irrelevant outcomes. This resulted in a final of 9 studies to be included in qualitative synthesis, comprising of 2 cohort studies and 7 cross-sectional studies.

Study characteristics and outcomes

Study characteristics included in this review are shown in Table 1. Overall, this review included a total of 2027 patients. Studies were conducted in 6 countries across the world. Outcomes were associated with relevance of confocal microscopy assessment in terms of nerve fiber density (NFD), nerve branch density (NBD), nerve fiber length (NFL), and nerve fiber tortuosity (NFT) to severity of neuropathy with their corresponding p-values. These also show relevant thresholds for which diabetic neuropathy can be diagnosed.

Based on quality assessment, the majority of the studies fulfilled more than 7 of the criteria, indicating that the studies were of low bias risk and therefore relatively good qualities.

Table 1. Characteristics of studies.

No	Author; Year of Publication	Location	Study design	Study population	Assessment	Results
1	Hafner J et al; 2019[17]	Vienna, Austria	Prospective, cross-sectional study	• 94 type 2 DM • 68 no DR • 48 NPDR • 41 PDR	• Imaging with Heidelberg Spectralis OCT for macular and peripapillary neuroretinal layer thicknesses • Confocal microscopy for evaluating NFL, NFD, and NBD • Skin punch biopsy and lower limb inspection using 2 validated scores: Utah Early Neuropathy Scale (UENS) and Michigan Neuropathy Screening Instrument (MNSI)	• ↓NFL and NFD in NPDR and PDR compared to no DR; and PDR compared to NPDR • Similar NFD and NFL for eyes with PDR and no DR • ↓IENFD in NPDR ($p<0.001$) and PDR ($p<0.001$) compared to no DR • Loss of statistical significance for the differences in NFD between PDR and no DR during secondary sensitivity analysis • Low negative correlation of intra-epidermal and corneal fiber loss with UENS and MNSI ($p<0.05$) • Moderately significant positive correlation between stage of DR and UENS ($p<0.001$) and MNSI ($p<0.001$) score
2	Andersen ST et al; 2018[18]	Denmark	Cross-sectional study	• 144 type 2 DM • 25 controls	• DPN defined according to Toronto criteria for confirmed DPN	• ↓NFD in patients with confirmed DPN ($p=0.04$) and without DPN ($p=0.01$) compared to controls

						• No significant difference between NFL (p=0.06) and NBD (p=0.29) between groups • NFD associated with age, height, total- and LDL cholesterol
3	Yan A et al; 2019[22]	Manchester, UK	Cross-sectional	• 57 type 2 DM patients • 26 healthy controls • 54 type 1 DM as disease control group	• DPN assessed by Toronto consensus criteria • Neuropathy severity assessed by TNS, vibration sensibility (128 Hz tuning fork), pinprick sensibility (Neurotip) • *In vivo* CCM conducted bilaterally and image analysis were conducted using the fully automated nerve analysis software ACCMetrics. • 8 images representing the central cornea dan 5 images representing inferior whorl were identified • Corneal nerve variables quantified were NFD, NFL, NBD, and IWL	• ↓NFD in diabetic patients compared to healthy controls (P<0.001) • ↓NBD and NFL in type 2 DM patients (NBD: P<0.001; NFL: P<0.001) • ↓IWL in type 2 DM patients (P<0.001) • 28% difference in IWL (P=0.009); 22% difference in NFL (P=0.02); NBD was 29% lower in DPN+ (P=0.02); NFD was 18% lower (P=0.03)
4	Xiong Q et al; 2017[25]	Shanghai, China	Cross-sectional, observational study	• 128 type 2 DM patients: • 49 no DSPN • 43 mild DSPN • 36 moderate-to-severe DSPN • 24 age-matched controls	• DSPN assessed using Diabetic Neuropathy Study Group of the European Association for the Study of Diabetes in 2009 dan Consensus Statement of the joint 8th International Symposium • CCM performed using HRT II microscope with RCM	• ↓NFL in type 2 DM patients compared to healthy control (no, mild, and moderate-to-severe DSPN) (P=0.012, P=0.003, and P<0.001 respectively) • ↓NFL in patients with moderate-to-severe DSPN compared to patients with no DSPN

					• Examination sites were the central cornea dan part of the cornea 2 mm inferior to the limbus • From 100 singles images captured during each examination, 5 nerve fiber images were selected were selected to be processed using Fiji imaging analysis software • Parameters assessed were NFL, NBD, and NFD	(P<0.001) or mild DSPN (P=0.004) • ↓NBD in type 2 DM patients compared to healthy control (P=0.036, P=0.016, and P<0.001 respectively) • ↓NBD in patients with moderate-to-severe DSPN compared to patients with no DSPN (P<0.001 for both) • Neither NFL nor NBD decreased significantly between patients with no or mild DSPN. • NFD was similar among the four groups (Healthy control, no, mild, or moderate-to-severe DSPN)
5	Ishibashi F, 2016[27]	Japan	Cross-sectional	• 162 type 2 DM patients, with or without clinical evidence of diabetic neuropathy • 45 healthy control subjects	• DPN assessed with electrophysiology and nerve conduction velocity (NCV) studies • Clinical evaluation of neuropathy using the Neuropathy Disability Score (NDS) • CCM for evaluating NFD, NFL, NBD, tortuosity, and bead size	• ↓NFD, NFL, NBD, and BF in diabetic patients (P<0.0001) • Further reduction in patients with moderate and severe neuropathy • Significant difference of CNFD and BS between patients with and without neuropathy (P=0.02, P=0.03) • ↑TG and BS in diabetic patients • Significant correlation between HbA1c and all CNF parameters except TG and BF. NDS was associated with CNFD

						and CNFL inversely and with BS positively
6	Ishibashi F, 2017[28]	Hiroshima, Japan	Cross-sectional	• 103 subjects with diabetes type 2, with or without clinical evidence of diabetic neuropathy • 42 age-matched control	• CCM to measure NFD, NFL, NBD, NBL, frequency of beading, and bead size • Pupillary light reflex • Clinical evaluation of neuropathy with DNSGJ criteria • Electrophysiology and nerve conduction velocity studies at left medial malleoulus to assess DPN	• ↓NFD and BF in diabetic patients without clinical signs of neuropathy • ↑TG and BS compared with those of the control subjects • Neurophysiological test results in the patients without neuropathy were not different from those of the control subjects
7	Dehghani C, 2016[29]	Queensland, Australia	Prospective cohort	• 55-year-old Caucasian male with type 2 DM	• Annual assessments comprised of HbA1c, lipid profile and blood pressure • DPN assessed using neuropathy disability score (NDS), quantitative sensory testing (QST) of thermal and vibration perception, nerve conduction studies (NCS) • Corneal nerve parameters measured using IVCCM	• Rapid decline in NFD, NBD and fiber length (NFL) before development of foot ulcer • No significant deterioration of other measures (NDS, QST, NCS)
8	Perkins BA et al; 2018[30]	Manchester, UK	Multicenter cohort study	• 998 from 5 centers • 516 type 1 DM • 482 type 2 DM	• NFL quantification and electrophysiological examination • AUC and diagnostic thresholds derived and validated in randomly	• Derivation AUC for NFL was 0.77 in type 1 DM ($p<0.001$) and 0.68 in type 2 DM ($p<0.001$), and reproduced in validation set

					selected samples using ROC analysis	• Optimal threshold for automated NFL was 12.5 mm/mm^2 in type 1 and 12.3 mm/mm^2 in type 2 • In total cohort, lower threshold value below 8.6 mm/mm^2 to rule in DSP and upper value of 15.3 mm/mm^2 to rule out DSP associated with 88% specificity and 88% sensitivity
9	Tummanapalli SS et al; 2019[31]	Sydney, Australia	Prospective cross-sectional	• 38 type 1 DM and • 32 type 2 DM patients	• DPN assessed using Toronto consensus criteria • Neuropathy severity assessed using TNS, vibration sensibility (128 Hz tuning fork), pinprick sensibility (Neurotip) • Bilateral CCM examination • 8 central and 3 to 4 IW images were selected and quantified for CNFD, CNFL, CNBD, CTBD, CNFrD, IWL, and IWNFrD • Images analyzed using fully automated analysis software ACCMetrics	• ↓ all corneal nerve parameters in DPN+ patients compared to DPN- patients (P<0.050), except IWL (P=0.190) • CNFL had the highest AUC (0.809, P<0.003) with an optimum diagnostic threshold ≤ 13.64 mm/mm^2 (81% sensitivity, 81% specificity), followed by CNFrD (0.777, P=0.007), CNBD (0.764, P=0.011), CNFD (0.762, P=0.012), and IWNFrD (0.734, P-0.024) • AUC for IWL was not significantly different from the reference line (0.617, P=0.258)

Discussion

Gold standard for diagnosing diabetic neuropathy

It has long been established that the gold standard for the diagnosis of diabetic neuropathy is nerve conduction studies (NCS). NCS is able to quantify conduction velocity and amplitude of nerve action potential. Both velocity and amplitude correlate strongly with neuropathy, as reduced amplitude implies axonal loss and slowing of conduction velocity implies axon injury and demyelination. Meanwhile, EMG is able to record muscle electrical activity by using an insertional electrode. Abnormal spontaneous activity such as sharp waves and fibrillation potential could suggest active denervation, while chronic axonal neuropathies can be characterized by long duration and large amplitude of motor unit potentials, as uninjured motor axons innervate denervated muscle fibers. As a result, EMG is not only valuable in determining location of nerve lesion, but also in determining the chronicity of neuropathy.[11]

However, there are various limitations to these approaches. Aside from being known to be painful and invasive, due to their nature, these approaches can only detect large nerve fibers dysfunction, even though most of the peripheral nerves (70-90%) are classified as small. This means that diabetic neuropathy can only be detected in its severe form, despite the need for early detection that is a prerequisite for improving early prevention of progression and enhancing quality of life. This is a great disadvantage as late diagnosis prevents early risk factor management, which could impact neuropathic sequelae.[12] NCS and EMG also require specialized doctors and equipment which are not typically available in public settings, further highlighting the need for a better approach to diagnose diabetic neuropathy in its early stage.[11,12]

Corneal confocal microscopy and diabetes

As the most densely innervated tissue in the human body and being mostly transparent, the eye provides a non-invasive visual access to nerve fibers beneath, thus becoming a unique potential marker of neurodegenerative changes. Recent studies show that there is a strong relationship between corneal nerve structure and morphology with neuropathy.[13] Corneal confocal microscopy (CCM) is an in vivo, non-invasive, novel technique to study the internal structure of cornea cellular structure. It is able to provide imaging comparable to in vitro histochemical technique. As a result, this technique has the potential as a marker for peripheral nerve damage.[14]

CCM is able to provide images for various layers of the cornea, from the epithelium, the Bowman's membrane, the stroma, to finally the corneal endothelium. In studying nerve structure and morphology, the Bowman's layer becomes the focus of study as it shows nerve bundles of the sub-basal nerve plexus.[14] Parameters commonly observed are corneal nerve fiber length (NFL), corneal nerve fiber density (NFD), corneal nerve branch density (NBD), and corneal nerve fiber

tortuosity (NFT), although recent studies show other parameters that are also sensitive in diagnosing neuropathy.[13,14]

CCM also provides objective, quantifiable, and reproducible results. With the advancement of technology, there are already several automated softwares to quantify the results of corneal nerve fibers, so operators' expertise would not cause bias or interfere with results. This further supports CCM's advantages over current methods of diagnosis, which are either invasive, painful, and require specialized expertise, such as NCV and EMG, or simply not objective enough, such as questionnaires.[15]

Nerve fiber density, nerve fiber length, and nerve branch density

One of the most common microvascular complications of type 2 diabetes is diabetic neuropathy. In its early development, manifestation of neurodegeneration may be present before any visible microvasculopathy occurs. As diabetes is a systemic disease, progression of ocular neurodegenerative change could be associated with neuropathic changes in other organs. Hyperglycemia results in decreased oxygen and nutrient supply to small corneal fibers, leading to changes in NFD, NFL, and NBD. Similarly, due to high blood sugar levels, vascularity of the eyes may become altered, leading to proliferative ocular neurodegeneration.[16]

Hafner et al discovered that NFL and NFD were significantly decreased in eyes with proliferative and non-proliferative diabetic retinopathy (PDR and NPDR) in comparison to those without retinopathy. The threshold values in this case were 14,7/mm^2 for NFD and 14,6mm/mm^2 for NFL. Similarly, intraepidermal nerve fiber density (IENFD) in NPDR and PDR also decreased compared to no DR. However, NBD is deemed not as reliable as NFL and NFD in diagnosing neuropathy. Despite having the ability to measure diabetic retinopathy, there is also strong correlation with diabetic peripheral neuropathy as both have similar microvascular complications and pathomechanism.[17]

In line with that, Andersen et al's study revealed significantly lower NFD in patients with both confirmed diabetic polyneuropathy and without diabetic polyneuropathy compared to controls.[17] This discovery is in line with previous studies which evaluate NFL and NFD as the most reliable markers for early diabetic polyneuropathy.[19-21] Similarly, this study also found that NBD is less reliable in diagnosing neuropathy compared to other parameters.[18]

Another study by Yan et al also proved that NFL showed the strongest negative correlation with the severity of neuropathy, which is in line with previous studies conducted by Ahmed A et al and Petropoulos et al.[22,23] This study also shows significant decline in corneal nerve parameters in type 2 diabetes patients without clinical neuropathy. This finding supports the use of CCM for the early diagnosis of neuropathic in diabetic patients.[22] It has also been found that Aδ and C fibers were susceptible to injury in patients with diabetes due to the lack of protection and nutrition supply usually

provided by Schwann cells.[24] Similar results were also found in a study by Xiong Q in which NFL, along with NBD and NFD, were reduced in type 2 DM patients with neuropathic symptoms compared to those without neuropathic symptoms.[25]

Similar studies by Ishibashi et al support CCM diagnostic value.[26,27] In one study, it is observed that there is strong correlation between both NFL and NFD and the severity of neuropathy. Although there is no difference in neurophysiological tests between control and the subgroup without neuropathy, there is a significant difference in NFL, NFD, and NBD.[26] A different study by Ishibashi et al supported this, as there is steady decrease of NFL, NFD, and NBD with increasing severity of diabetic neuropathy.[27] However, compared to NCV as gold standard of testing, NFL and NFD had the strongest correlation, while NBD shows weak, although still statistically significant, correlation.[26]

In a cohort study following a type 2 diabetic patient over a 7-year period, it is further proven that NFL, NFD, and NBD is a reliable marker of diabetic neuropathy. These parameters rapidly decline in relation to the severity of neuropathy, and especially before the development of further complications such as foot ulcer. In comparison, other non-corneal parameters such as blood pressure, lipid profile, neuropathy disability score (NDS), and quantitative sensory testing (QST) showed no deterioration, and even showed improvement in QST. Therefore, it can be concluded that CCM is a reliable diagnostic tool for neuropathy, its progression, and severity.[28]

Other parameters for diagnosis

Aside from previously stated parameters, a study has also shown that corneal nerve fiber bead size and nerve fiber tortuosity (NFT) are a good indication of diabetic neuropathy. Unlike other parameters, bead size and NFT correlate negatively with diabetic neuropathy, which means that with increasing severity of neuropathy, these parameters increase. Altered beading structures have strong correlation with loss of nerve fiber density and branches, while tortuosity is related to nerve fiber length, therefore verifying further that NFL, NFD, and NFB are altered in diabetic neuropathy patients.[26,27]

While the exact mechanism has not been elucidated, it is predicted that mitochondria dysfunction plays a role in altering the size of beads. In diabetic rats, it is observed that motor protein involved in axonal transport of mitochondria is changed, resulting in changes in mitochondria distribution. In addition, patients with diabetic neuropathy usually have high mitochondrial accumulation of glycogen particles. Alterations of these components might contribute to the changes in density and size of bead.[26]

From a diagnostic point of view, corneal nerve bead size seems to be the most sensitive and specific compared to other parameters of CCM. Expansion of bead size can be observed in patients without clinical evidence of DPN,and gets larger with the severity of the neuropathy.

It also has a good association with NCV of the median nerve, thus increasing potential for CCM as a predictive tool for neuropathy in type 2 diabetes patients.[26]

Threshold, specificity, and specificity

On a larger scale, a multicenter study by Perkins et al analyzed the receiver operating characteristic (ROC) curves generated from CCM of type 2 diabetic patients. Both manual and automated corneal nerve quantification revealed that CCM had diagnostic validity for diabetic sensory polyneuropathy, particularly through NFL as the most optimal variable. In fact, the value of area under the curve was 0.68. These were reproduced in the validation set. In the total cohort, a lower threshold value of below 8.6 mm/mm2 to rule in polyneuropathy and an upper value of 15.3 mm/mm2 to rule out polyneuropathy was associated with 88% specificity and 88% sensitivity.[29]

Another study by Tummanapali et al which analyzed the ROC curve also proved that in the diagnosis of DPN in type 2, NFL was the most optimal parameters with a value of 0.809 ($P < 0.003$) for the AUC and an optimum diagnostic threshold value of ≤ 13.64 mm/mm2, which is associated with 81% sensitivity and 81% specificity. This was followed by NFrD (nerve fractal dimension) with an AUC value of 0.777 ($P = 0.007$), NBD with 0.764 ($P = 0.011$), NFD with 0.762 ($P = 0.012$), TBD (total branch density) with 0.762 ($P = 0.012$), and IWNFrD (inferior whorl nerve fractal dimension) with 0.734 ($P = 0.024$). This study also showed that IWL (inferior whorl length) was not a reliable parameter to discriminate patients with diabetic neuropathy from those without, as the AUC value of 0.617 ($P = 0.258$) is not significantly different from the reference line of 0.500. This may be because in type 2 diabetic patients without DPN, the IWL value was already reduced compared to the healthy controls of similar ages.[30]

Similarly, in a study by Ishibashi et al, NFL and NFD have high sensitivity and specificity, at 63% and 65% for NFL ($p=0.071$), and 66% and 54% for NFD ($p=0.02$). The threshold value between healthy subjects and diabetic patients is 11.6mm/mm^2 for NFL and 23.1/mm^2 for NFD. Bead size also show consistent results at 65% sensitivity and 53% specificity, with threshold at 9.76μm^2 ($p=0.031$). In addition, there is strong correlation between NFL and NFD to NCV as gold standard of testing.[26]

In comparison to neurophysiological assessment, CCM also shows strong correlation. CCM findings were compared to polyneuropathy assessment via the Utah Early Neuropathy Scale (UENS) and Michigan Neuropathy Screening Instrument (MNSI). Low negative correlation of intra-epidermal and corneal fiber loss with UENS and MNSI ($p<0.05$) was found, while moderately significant positive correlations between stage of DR and UENS ($p<0.001$) and MNSI ($p<0.001$) score were also revealed. These showed the applicability of CCM in evaluation of polyneuropathy severity.[17]

Therefore, in comparison to other markers for neuropathy, the specificity and sensitivity of CCM can be considered

adequate or even superior. In fact, diagnosis via clinical signs revealed poor accuracy, ranging from 25-85%.[30] This particularly leads to large proportions of underdiagnosis and misdiagnosis of diabetic neuropathy in clinical practice, such that CCM is a better reproducible option for diagnosis. CCM also has superior specificity to the gold standard NCS, which has a value of 62.1%. The sensitivity of CCM is lower than NCS (94%); however, since NCS can only detect large fiber neuropathies in the later stage, this difference can be ignored.[31,32]

Future application and research

This review provides further evidence of the use of CCM as a marker of patients with diabetic neuropathy and that there is a possibility that this method can be used as one of the main diagnostic tools for diabetic neuropathy in the future. CCM has shown a superior specificity and an adequate sensitivity compared to nerve conduction studies as the current gold standard in diagnosing diabetic neuropathy. Moreover, the ability of CCM to detect early polyneuropathy, along with its established feasibility and reproducibility, means that CCM can potentially be one of the tools to diagnose diabetic neuropathy. Future researches are needed to confirm the possible implementation of CCM in the official guideline for diagnosis of diabetic neuropathy.

Limitations

This study is not without limitation due to the exclusion of the studies not written in English or Indonesian, which are the languages readable by the authors. Another limitation is that most studies in this review were conducted outside Asia. This indicates that more research on the use of CCM for the diagnosis of diabetic neuropathy in Asia, specifically Indonesia, is needed in order to develop the possibility of using this method as one of the diagnostic tools for diabetic neuropathy in this region in particular.

Conclusion

Diabetes is one of the most common metabolic conditions with possible severe complications including diabetic neuropathy, commonly diagnosed at a later stage. The invasive nature and late-stage detection by nerve conduction study as the current gold standard prompts the need for another method which is as effective but less invasive. This review has shown that the non-invasive CCM can be a more suitable method in order to diagnose diabetic neuropathy, with evidence from various studies that prove the high sensitivity and specificity of CCM. With increasing severity of neuropathy, NFD, NBD, and NFL decrease in value, while NFT and bead size increases with consistency, even in early stages. This means that these parameters can be reliable diagnostic markers for early diabetic neuropathy, although for NBD, the consistency is still controversial. Furthermore, these parameters can be used to gauge the severity of neuropathy. Further studies, especially in Asia, are needed to investigate the possibility of implementing this method in the guideline for diagnosis of diabetic neuropathy and to further

confirm which parameters are the most reliable to be used for diagnosis.

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