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Diagnostic value of optic disc retinal nerve fiber layer thickness for diabetic peripheral neuropathy*#

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Abstract: Objective: To investigate the value of optic disc retinal nerve fiber layer (RNFL) thickness in the diagnosis of diabetic peripheral neuropathy (DPN). Methods: Ninety patients with type 2 diabetes, including 60 patients without DPN (NDPN group) and 30 patients with DPN (DPN group), and 30 healthy participants (normal group) were enrolled. Optical coherence tomography (OCT) was used to measure the four quadrants and the overall average RNFL thickness of the optic disc. The receiver operator characteristic curve was drawn and the area under the curve (AUC) was calculated to evaluate the diagnostic value of RNFL thickness in the optic disc area for DPN. Results: The RNFL thickness of the DPN group was thinner than those of the normal and NDPN groups in the overall average ((101.07±12.40) μm vs. (111.07±6.99) μm and (109.25±6.90) μm), superior quadrant ((123.00±19.04) μm vs. (138.93±14.16) μm and (134.47±14.34) μm), and inferior quadrant ((129.37±17.50) μm vs. (143.60±12.22) μm and (144.48±14.10) μm), and the differences were statistically significant. The diagnostic efficiencies of the overall average, superior quadrant, and inferior quadrant RNFL thicknesses, and a combined index of superior and inferior quadrant RNFL thicknesses were similar, and the AUCs were 0.739 (95% confidence interval (CI) 0.635–0.826), 0.683 (95% CI 0.576–0.778), 0.755 (95% CI 0.652–0.840), and 0.773 (95% CI 0.672–0.854), respectively. The diagnostic sensitivity of RNFL thickness in the superior quadrant reached 93.33%. Conclusions: The thickness of the RNFL in the optic disc can be used as a diagnostic method for DPN.

Key words: Type 2 diabetes; Peripheral neuropathy; Retinal nerve fiber layer thickness; Optical coherence tomography; Diagnosis

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1 Introduction

The prevalence of diabetes mellitus (DM) has increased sharply and has become a major public health problem worldwide. According to the latest data from the International Diabetes Federation (2019), the number of people with diabetes in the world reached 463 million in 2019 and is expected to reach 700 million by 2045. Diabetes-related medical expenditure will reach 958 billion US dollars by 2045 (Cho et al., 2018). Chronic complications of diabetes are the main causes of mortality, morbidity, and

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increased expenses for patients. Diabetic peripheral neuropathy (DPN) is one of the most common chronic complications of diabetes (Pirart, 1977). DPN not only severely affects the quality of life and increases the risk of amputation, but also increases mortality (Forsblom et al., 1988; Gordois et al., 2003; Seferovic et al., 2018). However, there has generally been insufficient screening and early diagnosis of DPN in clinical work (Liu et al., 2010). At present, according to the consensus of the international expert group of DPN and Chinese guidelines for the prevention and treatment of diabetes, five forms of physical examination for peripheral neuropathy are recommended as screening methods, but each has defects related to repeatability and poor sensitivity (Dyck et al., 2010). Finding a simple, convenient, and sensitive strategy for screening and diagnosing DPN is a clinical problem needing to be solved. Binns-Hall et al. (2018) explored the application of DPN-CHECK (a hand-held device that measures sural nerve conduction velocity and amplitude) combined with SUDOSCAN (a device that measures sudomotor function) for one-stop screening when patients are waiting for fundus examinations. This method could significantly improve the screening efficiency of early DPN, and the sensitivity of screening can reach 93.2%. SUDOSCAN and DPN-CHECK have been confirmed as effective tools for screening and diagnosing DPN in several studies (Smith et al., 2014; Chatzikosma et al., 2016), but the costs of equipment and examination using SUDOSCAN have become an obstacle to clinical application. According to the global guidelines for the prevention and treatment of diabetes, chronic diabetic complications must be screened regularly, and diabetic fundus examination should include optical coherence tomography (OCT) for macular degeneration or edema assessment. Modern OCT technology is widely used in clinical practice, and can quantitatively measure the thicknesses of the retinal ganglion cell-inner plexiform layer (GCIPL) and retinal nerve fiber layer (RNFL) in the macular area (Feng et al., 2018; Lakshmikantha et al., 2018; Xie et al., 2018; Wang et al., 2018). Some studies have found that retinal neuropathy is closely related to peripheral neuropathy and can occur in peripheral neuropathy diseases such as fibular muscular atrophy (Botsford et al., 2017). Retinal neurodegeneration has been reported to occur long before the appearance of diabetic retinal microvascular injury (Verma et al., 2009), and can lead to functional defects such as an abnormal electroretinogram (ERG) (Luu et al., 2010). Therefore, the application of OCT in the detection of fundus neuropathy may have reference value for the diagnosis of DPN. OCT can be carried out during fundus evaluation in clinical practice, which can significantly improve the clinical efficiency. In this study, OCT was used to measure the thickness of the RNFL in the optic disc during diabetic fundus screening, and its diagnostic value for DPN was evaluated. Our findings provide a new and efficient strategy for the clinical screening and diagnosis of DPN.

2 Subjects and methods

2.1 Subjects

Patients with type 2 diabetes who were hospitalized in the Department of Endocrinology of the Second Affiliated Hospital of Fujian Medical University (Quanzhou, China) from May 2018 to January 2019 were recruited. According to the inclusion and exclusion criteria, 90 patients were included in the study, including 30 patients with DPN (DPN group) and 60 patients without DPN (NDPN group). In the same period, 30 age-matched healthy participants who were examined in the outpatient department of our hospital were taken as the normal controls (normal group). All the subjects were Chinese, 30 to 70 years old, with 63 males and 57 females. Type 2 diabetes was diagnosed according to the diagnostic criteria of the World Health Organization (WHO, 1999). DPN was diagnosed according to the diagnostic criteria of the American Diabetes Association (ADA) position statement "Diabetic Neuropathy" (Pop-Busui et al., 2017).

The inclusion criteria were as follows: (1) patients with type 2 diabetes who met the diagnostic criteria of diabetes proposed by the WHO in 1999; (2) corrected visual acuity of ≥4.6 (standard logarithmic visual acuity table); (3) diopter of ≤±3.0 D; (4) binocular intraocular pressure range of ≤21 mmHg (1 mmHg=0.133 kPa), no history of ocular hypertension; (5) no history of internal eye surgery, laser, or trauma; (6) no obvious abnormality in the anterior segment of the eye examined by slit lamp; (7) selection of the eye on the dominant hand side, and if it did not meet the standard, the other side was selected. The

exclusion criteria were: (1) fundus diseases caused by non-diabetes; (2) fundus examination with an obvious opacity of refractive media and diseases that could not be fixed; (3) acute or severe chronic complications of diabetes; (4) inflammatory or immune diseases, tumors, thyroid diseases, vitamin B deficiency, history of exposure to poison, or hereditary peripheral neuropathy.

2.2 Collection of clinical data

Clinical data and biochemical parameters were collected, including a detailed inquiry and recording of clinical symptoms of DPN and examination of the ankle reflex, and sensitivity to acupuncture pain, vibration, pressure, and temperature. Glycosylated hemoglobin (HbA1c) was determined by high-performance liquid chromatography (HPLC; Model: D10, Bole Co., Ltd., USA).

2.3 Evaluation of DPN

Toronto clinical scoring system (TCSS) score and grading: clinical symptoms (including foot pain, numbness, tingling, fatigue, ataxia, and upper limb symptoms), 1 point for each item; tendon reflex examination (bilateral knee reflex and ankle reflex), 2 points for no tendon reflex, 1 point for weakened tendon reflex, 0 point for normal tendon reflex; five physical examinations (acupuncture sensation, temperature sensation, 10 g nylon filament pressure sensation, vibration sensation, and position sensation), 1 point for each item. The total possible score was 19 points. TCSS of ≤5 points indicates no neuropathy, TCSS of 6–8 points indicates mild neuropathy, TCSS of 9–11 points indicates moderate neuropathy, and TCSS of ≥12 points indicates severe neuropathy (Perkins et al., 2001).

Measurement of nerve conduction velocity (NCV): NCV was detected by the same physician, and the double-blind method was adopted. An electromyography/evoked potential instrument (No. Keypoint 9033A07, Focus Company, Denmark) was used for examination. In a quiet room with a constant temperature of 25 °C, the patient was in the supine position with limbs fully exposed and relaxed, and limb temperature was maintained at 32–36 °C. The motor conduction velocities of the median nerve, ulnar nerve, tibial nerve, and peroneal nerve, and sensory conduction velocities of the median nerve, ulnar nerve, superficial peroneal nerve, and sural nerve were measured.

2.4 Ophthalmic examination

The examination was carried out using the double-blind method.

Best corrected visual acuity (BCVA): all patients enrolled in the group were tested using the international standard visual acuity chart. The diopter of the patients was measured using an autorefractor (Topcon KR 800, Topcon Medical Systems Inc., Japan) and corrected. The visual acuity was converted to the logarithm of minimal angle resolution (logMAR).

Intraocular pressure: the intraocular pressure of patients was measured with a noncontact tonometer (Canon TX-20, Canon Inc., Japan), and the average value was recorded automatically three times in each eye.

Slit lamp and front lens: a slit lamp (Topcon SL-3G, Topcon Medical Systems Inc., Japan) was used to observe the conjunctiva, cornea, anterior chamber, lens, and vitreous body in turn, excluding the corneal scar, keratitis, severe lens opacity, vitreous opacity, and other fundus diseases that may have affected the results of this study. The pupil was dilated with compound tropicamide eye drops, and then the fundus was examined with slit lamp and Volk 90D front lens (Volk Inc., USA) to rule out retinal diseases caused by other fundus diseases, such as glaucoma, high myopia, and age-related macular degeneration.

Fundus photography: all patients in the group were examined using a fundus camera (Canon CR-2, Canon Inc., Japan). The pupil was coaxial with the light, and the patients were told to move the eye in different directions. The physician then adjusted the focal length and definition of the image, and selected the same ideal image as the object of study. The degree of retinopathy was further evaluated by comparison to the results examined using an anterior endoscope.

OCT: spectral domain optical coherence tomography (SD-OCT, Spectralis®, Heidelberg Engineering Inc., Heidelberg, Germany) was used to scan the whole macular area of the retina. The following procedures were used. The subject adopted a sitting position and placed their mandible on the jaw support at a suitable height. The subject was told to look at the green indicator light, and the lens was aimed at the pupil. The scanning centerline was adjusted to pass through the fovea center of the macula; the scanning mode was 768×496 and the scanning range was scanned with a macular fovea of 30°×25° volume. The scanning speed was 4000 A/s, and the resolution

was 5 μm longitudinally and 6 μm horizontally. A total of 61 B-scans were performed, and the mode of automatic real-time noise reduction was turned on to ensure that the quality of each scan was above 20 dB. The follow-up mode was selected and a total of three scans performed; the first scan was set as the reference scan. RNFL measurement: we performed circular scanning of the optic disc with a diameter of 3.46 mm centered on the optic disc. The RNFL thickness was measured around the whole optic disc including four quadrants (superior, nasal, inferior, and temporal) (Fig. 1a). Scanning was performed three times and the clearest image with the strongest signal was selected (Fig. 1b). Four-quadrant RNFL thicknesses and the overall average RNFL thickness were automatically analyzed using the analysis software supplied with the system. This operation was performed by the same experienced technician.

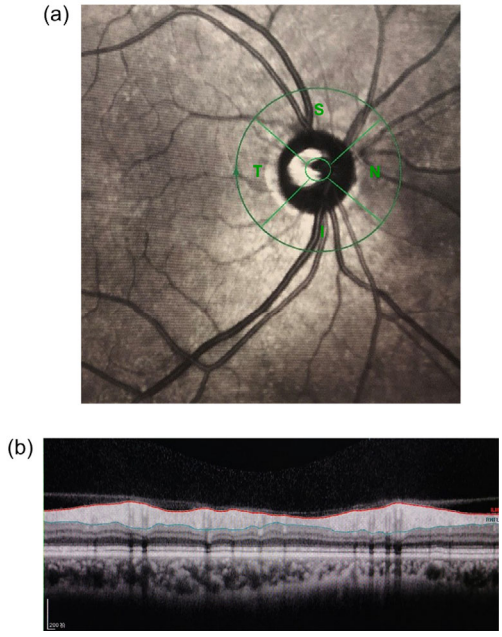

Fig. 1 Measurement of the RNFL thickness of the optic disc

(a) Measurement range. S: superior quadrant; N: nasal quadrant; I: inferior quadrant; T: temporal quadrant. (b) Measurement of the RNFL thickness of the optic disc by OCT. RNFL: retinal nerve fiber layer; OCT: optical coherence tomography

2.5 Statistical analysis

SPSS 23.0 statistical software was used. The data were tested for normality, and those with a normal distribution were expressed as mean±standard deviation, while those with a non-normal distribution were expressed as median (quartile intervals). For quantitative data with a normal distribution, Student's *t*-test was used to test the significance of differences between two groups, analysis of variance (ANOVA) was used among multiple groups, and the least significant difference (LSD) test was used for pairwise comparisons between groups. To measure data with a non-normal distribution, the Mann-Whitney *U* nonparametric test was used between two groups, the Kruskal-Wallis *H* nonparametric test was used among multiple groups, and the Nemenyi test was used for pairwise comparisons between groups. The qualitative data were tested by the chi-square test. The receiver operator characteristic (ROC) curve was drawn using MedCalc software (Version 19.5.3, MedCalc Software Ltd., Belgium) to calculate the area under the curve (AUC). The efficacies of the overall average, superior quadrant, and inferior quadrant RNFL thicknesses, and combined index of superior and inferior quadrant RNFL thicknesses in the diagnosis of DPN were compared. $P<0.05$ was considered to indicate a statistically significant difference.

3 Results

3.1 Clinical characteristics of the subjects

There were 30 cases in the normal group with an average age of (54.27±8.17) years, 60 cases in the NDPN group with an average age of (51.62±9.87) years, and 30 cases in the DPN group with an average age of (55.93±12.46) years (Tables 1 and S1). There were no significant differences in gender composition, age, or intraocular pressure among the three groups. The body mass indexes (BMIs) in the NDPN and DPN groups were higher than that in the normal group. The duration of diabetes was longer in the DPN group than in the NDPN group ((9.37±7.36) years vs. (5.70±5.29) years, $P=0.008$). HbA1c was higher in the DPN group than in the NDPN group ((8.56±2.60)% vs. (8.08±2.30)%, $P=0.001$), and there was no significant difference in BMI between these groups. The TCSS score was higher in the DPN group than in the NDPN group ((6.00±3.66) points vs. (1.00±1.64) points, $P<0.001$). The conduction velocity velocities of the motor nerve and sensory nerve were significantly lower in the DPN group than in the NDPN group, and most were lower than the normal value.

3.2 Comparison of the RNFL thickness in the optic disc area of the retina

There were no significant differences in temporal quadrant or nasal quadrant RNFL thickness among the normal, NDPN, and DPN groups (Table 2). There were significant differences in the overall average, superior quadrant, and inferior quadrant RNFL thicknesses. In pairwise comparisons, there were no significant differences in the overall average, superior quadrant, or inferior quadrant RNFL thickness between the NDPN group and the normal group. The overall average, superior quadrant, and inferior quadrant RNFL thicknesses were lower in the DPN group than in the normal group (all $P<0.001$) and NDPN group ($P<0.001$, $P=0.001$, $P<0.001$, respectively). Moreover, the DM patients were re-divided into the TCSS≤5 group and the TCSS>5 group. The RNFL thicknesses

Table 1 Clinical characteristics of the subjects in the NDPN, DPN, and normal groups

Clinical characteristics	Normal group (n=30)	NDPN group (n=60)	DPN group (n=30)	P
Male/female	13/17	31/29	19/11	0.295
Age (year)	54.27±8.17	51.62±9.87	55.93±12.46	0.147
Duration of DM (year)		5.70±5.29	9.37±7.36[c]	0.008
BMI (kg/m^2)	22.08±2.00	24.80±3.76[a]	24.25±3.86[b]	0.002
HbA1c (%)	4.99±0.60	8.08±2.30[a]	8.56±2.60[bc]	0.001
DR (%)	0	13.33[a]	43.33[bc]	<0.001
logMAR	0.000 (0000, 0.100)	0.000 (0.100, 0.300)	0.050 (0.045, 0.400)	0.025
IOP (mmHg)	12.15 (10.24, 17.73)	13.70 (9.41, 17.40)	13.55 (9.90, 18.61)	0.401
TCSS		1.00±1.64	6.00±3.66[c]	<0.001
MNCV				
Median nerve (m/s)		55.96±3.13	50.48±3.98[c]	<0.001
Proximal ulnar nerve (m/s)		59.08±5.05	53.61±5.98[c]	<0.001
Distal ulnar nerve (m/s)		61.14±7.06	52.14±8.41[c]	<0.001
Tibial nerve (m/s)		47.09±5.09	40.44±5.78[c]	<0.001
Proximal peroneal nerve (m/s)		45.42±2.98	40.93±5.21[c]	<0.001
Distal peroneal nerve (m/s)		49.83±7.64	43.29±9.18[c]	0.001
SNCV				
Median nerve (m/s)		56.07±6.43	45.84±11.25[c]	<0.001
Ulnar nerve (m/s)		56.73±4.03	50.23±5.26[c]	<0.001
Superficial peroneal nerve (m/s)		49.71±5.43	41.71±5.11[c]	<0.001
Sural nerve (m/s)		52.60±6.94	39.21±4.80[c]	<0.001

DPN: diabetic peripheral neuropathy; NDPN: non-DPN; DM: diabetes mellitus; BMI: body mass index; HbA1c: glycosylated hemoglobin; DR: diabetic retinopathy; logMAR: logarithm of minimal angle resolution; IOP: intraocular pressure; TCSS: Toronto clinical scoring system; MNCV: motor nerve conduction velocity; SNCV: sensory nerve conduction velocity. [a] NDPN group vs. normal group, $P<0.05$; [b] DPN group vs. normal group, $P<0.05$; [c] DPN group vs. NDPN group, $P<0.05$. Complete nerve electromyography data of the DPN and NDPN groups are shown in Table S1. Data are expressed as number, percentage, mean±standard deviation, or median (quartile intervals)

Table 2 Comparison of RNFL thickness of the optic disc in the normal, NDPN, and DPN groups

Group	RNFL thickness (μm)				
	Overall average	Superior quadrant	Inferior quadrant	Nasal quadrant	Temporal quadrant
Normal (n=30)	111.07±6.99	138.93±14.16	143.60±12.22	81.03±13.00	81.37±11.90
NDPN (n=60)	109.25±6.90	134.47±14.34	144.48±14.10	80.62±16.46	78.30±12.03
DPN (n=30)	101.07±12.40[cb]	123.00±19.04[cb]	129.37±17.50[cb]	73.33±18.86	78.40±14.30
P	<0.001	<0.001	<0.001	0.102	0.443
TCSS≤5 (n=73)	108.04±9.08	131.95±16.56[d]	142.10±15.52	80.48±17.18	78.30±11.40
TCSS>5 (n=17)	100.00±10.62[ef]	125.06±17.48[e]	128.06±17.93[ef]	68.35±15.95[ef]	78.47±17.87
P	<0.001	0.017	0.002	0.016	0.443

RNFL: retinal nerve fiber layer; DPN: diabetic peripheral neuropathy; NDPN: non-DPN; TCSS: Toronto clinical scoring system. [a] NDPN group vs. normal group, $P<0.05$; [b] DPN group vs. normal group, $P<0.05$; [c] DPN group vs. NDPN group, $P<0.05$; [d] TCSS≤5 group vs. normal group, $P<0.05$; [e] TCSS>5 group vs. normal group, $P<0.05$; [f] TCSS>5 group vs. TCSS≤5 group, $P<0.05$. Data are expressed as mean±standard deviation

of the normal, TCSS≤5, and TCSS>5 groups were compared. There were no significant differences in the temporal quadrant among the three groups (P=0.443), but there were significant differences in the overall average, nasal quadrant, superior quadrant, and inferior quadrant. In pairwise comparison, the overall average, nasal quadrant, and inferior quadrant RNFL thicknesses were thinner in the TCSS>5 group than in the normal group (P=0.001, P=0.005, P=0.001, respectively) and TCSS≤5 group (P=0.001, P=0.006, P=0.001, respectively), but there was no significant difference in the superior quadrant between the TCSS>5 and TCSS≤5 groups (P=0.116).

3.3 Diagnostic value of optic disc RNFL thickness for diabetic peripheral neuropathy

The diagnostic values of the overall average, superior quadrant, and inferior quadrant RNFL thicknesses and the combined index of the superior and inferior quadrant RNFL thicknesses were evaluated by calculating the AUC of the ROC curve (Fig. 2). A comparison of the four indexes is shown in Table 3. All four indexes showed good diagnostic efficiency, and the AUC of the overall average RNFL thickness for diagnosing DPN was 0.739 (95% confidence interval (CI) 0.635–0.826, P<0.001); the sensitivity was 71.67% and the specificity 75.86%; and the best cut-off value was 105 μm. The AUC of the superior quadrant RNFL thickness in the diagnosis of DPN was 0.683 (95% CI 0.576–0.778, P=0.004); the sensitivity was 93.33% and the specificity 44.83%; the best cut-off value was 116 μm. The AUC of RNFL thickness in the inferior quadrant for diagnosing DPN was 0.755 (95% CI 0.652–0.840, P<0.001); the sensitivity was 81.67% and the specificity 68.97%; and the best cut-off value was 131 μm. The AUC of the combined index of

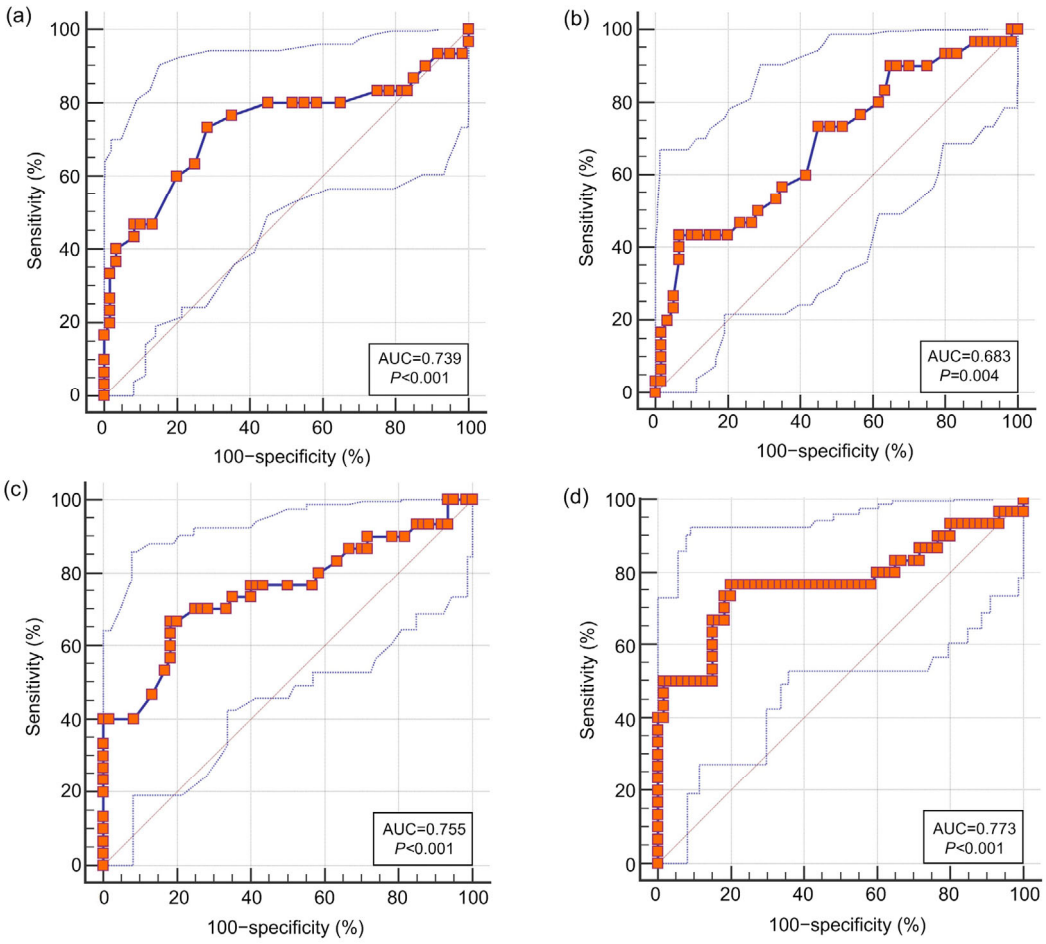

Fig. 2 ROC curve of DPN diagnosed by RNFL thickness of the optic disc

(a) Overall average RNFL thickness; (b) Superior quadrant RNFL thickness; (c) Inferior quadrant RNFL thickness; (d) Combined index of the superior and inferior quadrant RNFL thicknesses. AUC: area under the curve; ROC: receiver operator characteristic; DPN: diabetic peripheral neuropathy; RNFL: retinal nerve fiber layer

Table 3 Comparison of the overall average, superior quadrant and inferior quadrant RNFL thicknesses, and the combined index of the superior and inferior quadrant RNFL thicknesses in the diagnosis of DPN

RNFL	AUC	95% CI	Sensitivity (%)	Specificity (%)
Overall average	0.739[abc]	0.635–0.826	71.67	75.86
Superior quadrant	0.683[bc]	0.576–0.778	93.33	44.83
Inferior quadrant	0.755[c]	0.652–0.840	81.67	68.97
Combined index of the superior and inferior quadrants	0.773	0.672–0.854	76.67	80.00

RNFL: retinal nerve fiber layer; DPN: diabetic peripheral neuropathy; AUC: area under the curve; CI: confidence interval. [a] Compared with superior quadrant RNFL thickness, $P>0.05$; [b] Compared with inferior quadrant RNFL thickness, $P>0.05$; [c] Compared with combined index of the superior and inferior quadrant RNFL thicknesses, $P>0.05$

superior and inferior quadrant RNFL thickness in the diagnosis of DPN was 0.773 (95% CI 0.672–0.854, $P<0.001$). The four indexes had the same diagnostic efficacy, RNFL had better sensitivity in the superior quadrant (up to 93.33%), and the combined superior and inferior indexes had a specificity of 80.00%.

4 Discussion

This was a cross-sectional study of the diagnostic efficacy of RNFL thickness in the optic disc for DPN. The results showed that the RNFL thickness of the optic disc was lower in patients of the DPN group than in those of the NDPN group and normal controls. The overall average, superior quadrant, and inferior quadrant RNFL thicknesses of the optic disc could be used as indicators for screening and diagnosing DPN. The superior quadrant RNFL thickness had better sensitivity (up to 93.33%). The diagnostic efficiency of the combined index of the superior and inferior quadrant RNFL thicknesses was similar to those of the three separate indexes, and had a specificity of 80.00%. The RNFL thickness had a high sensitivity, but a relatively lower specificity for diagnosing DPN. The diagnosis of DPN in this study was based on NCV, which can detect injury to myelinated nerve fibers such as Aα and Aβ nerve fibers. In contrast, the retinal nerve fiber is a small unmyelinated nerve fiber. Most studies have demonstrated that small fiber neuropathy usually occurs during the early stage of DPN (Boulton and Malik, 2010; Breiner et al., 2014).

It is generally believed that the optic nerve is a part of the central nervous system, and the retinal nerve is closely related to central neuropathy. Retinal neurodegeneration often occurs in central degenerative diseases such as Parkinson's disease, Alzheimer's disease, and multiple sclerosis (Yu et al., 2014). Recent studies have found that retinal neuropathy is closely related to peripheral neuropathy. Some studies have indicated that in diabetes, retinal neurodegenerative lesion occurs and leads to functional defects and abnormal ERGs before the occurrence of retinal microvascular injury (Verma et al., 2009; Luu et al., 2010). Diabetic retinal neuropathy is associated with peripheral neuropathy (Shahidi et al., 2012; Srinivasan et al., 2016), including visceral autonomic neuropathy (Kim et al., 2016, 2017), although the mechanisms for their injury are different (Lin et al., 2018; Ni et al., 2020a, 2020b). Therefore, diabetic retinal neuropathy may be used as a basis for the screening and diagnosis of DPN. Because screening and early diagnosis of DPN are generally insufficient in clinical practice, the development of simple and efficient screening methods is highly desirable. At present, it is recommended that the clinical symptoms and physical examination serve as the main screening method for DPN. This has the advantage of being practical and relatively easy, but has the disadvantage of poor sensitivity and repeatability (Dyck et al., 2010). Binns-Hall et al. (2018) applied DPN-CHECK and SUDOSCAN as a one-stop screening strategy for DPN when patients were waiting for fundus examinations. This significantly improved the efficiency of DPN screening. However, the equipment cost hampers its application in clinical practice. Our study suggests that OCT, as a routine clinical method for the evaluation of maculopathy, can be used to evaluate retinal neuropathy and serve as an efficient method for DPN screening.

This study revealed that the overall average, superior quadrant, and inferior quadrant RNFL thicknesses were obviously abnormal in the DPN group. All three indexes and the combination of the superior

and inferior quadrant RNFL thicknesses had good diagnostic efficacy for DPN. Some similar results have been reported in recent years. Shahidi et al. (2012) found that RNFL thickness was related to the neuropathy disability score (NDS) in DPN. Srinivasan et al. (2016) also found that the overall average, superior quadrant, and inferior quadrant RNFL thicknesses in DPN patients with NDS≥3 points were significantly thinner than those in patients without DPN. Some studies have indicated that the RNFL in the inferior quadrant was significantly thinner in a subclinical DPN group (Li et al., 2016), suggesting that it might be an early diagnostic index for DPN. Srinivasan et al. (2017) suggested that the diagnostic sensitivity of the inferior quadrant RNFL thickness was 69%, the specificity was 80%, and the best cut-off value was 97 μm. The cut-off value was quite different from that in the present study, and was considered to be related to the race and age of the subjects, and the design of the research scheme (Alasil et al., 2013).

The RNFL in the temporal and nasal quadrants tended to be thinner in the DPN group, but there was no significant difference among the three groups. Previous study has shown that the shapes of the four quadrants of the posterior sieve plate of the human sclera are significantly different, and the number and area of the ethmoidal foramen are significantly smaller in the nasal and temporal quadrants than in the superior and inferior quadrants, so there are significantly fewer nerve fiber bundles in the nasal or temporal quadrant than in the superior or inferior quadrant (Jonas et al., 1991). A reduced penetration of nerve fiber bundles indicates that the dynamic range of measurable RNFL thickness is small, which may explain the absence of a difference in RNFL thickness in the temporal and nasal quadrants among the three groups. In contrast, why multiple sclerosis affects RNFL mainly in the temporal quadrant is unclear (Zhang et al., 2018). Whether different pathophysiological mechanisms can lead to morphological changes in the RNFL in different quadrants at different time remains to be further studied. The degenerative lesion of the RNFL in the inferior quadrant was the most obvious. This was probably because this quadrant has the densest nerve fibers and most oxygen and blood flow, and therefore is more likely to be affected by retinal ischemia and hypoxia caused by microcirculatory disorders, metabolic abnormalities, and other factors (Jonas et al., 1992). Some studies have also suggested that the RNFL thickness of the macular fovea is related to DPN, and could predict its occurrence (Kim et al., 2018). Taking into account that diabetic, macular lesions are more obvious and have a higher diversity, and detection of the optic disc RNFL thickness is more suitable for clinical application. However, there are some limitations of this study. The number of participants is small and the RNFL thickness is affected by many factors such as race, age, duration of diabetes, and the degree of retinal microangiopathy. Consequently, these findings must be verified by multi-center, large-sample, prospective observation studies.

5 Conclusions

This study explored the use of OCT technology, a routine clinical method for diabetic fundus screening, as a means of screening and diagnosing DPN, to improve the efficiency of clinical DPN screening.

Contributors

Xia-hong LIN designed the study. Jing-wen FANG, Xue-feng BAI, Yong ZHUANG, and Xiao-yu CHEN collected the data. Xiao-hong WU, Jing-wen FANG, and Ying-qiong HUANG performed the data processing and data analysis. Xiao-hong WU and Jing-wen FANG wrote and edited the manuscript. All authors edited and approved the final manuscript, and they have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xiao-hong WU, Jing-wen FANG, Yin-qiong HUANG, Xue-feng BAI, Yong ZHUANG, Xiao-yu CHEN, and Xia-hong LIN declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). This study was approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical University, Quanzhou, China. Informed consent was obtained from all patients for being included in the study.

References

Alasil T, Wang KD, Keane PA, et al., 2013. Analysis of normal retinal nerve fiber layer thickness by age, sex, and race using spectral domain optical coherence tomography. *J Glaucoma*, 22(7):532-541.

https://doi.org/10.1097/IJG.0b013e318255bb4a

Binns-Hall O, Selvarajah D, Sanger D, et al., 2018. One-stop microvascular screening service: an effective model for the early detection of diabetic peripheral neuropathy and the high-risk foot. *Diabet Med*, 35(7):887-894. https://doi.org/10.1111/dme.13630

Botsford B, Vuong LN, Hedges TR III, et al., 2017. Characterization of Charcot-Marie-Tooth optic neuropathy. *J Neurol*, 264(12):2431-2435. https://doi.org/10.1007/s00415-017-8645-2

Boulton AJM, Malik RA, 2010. Neuropathy of impaired glucose tolerance and its measurement. *Diabetes Care*, 33(1): 207-209. https://doi.org/10.2337/dc09-1728

Breiner A, Lovblom LE, Perkins BA, et al., 2014. Does the prevailing hypothesis that small-fiber dysfunction precedes large-fiber dysfunction apply to type 1 diabetic patients? *Diabetes Care*, 37(5):1418-1424. https://doi.org/10.2337/dc13-2005

Chatzikosma G, Pafili K, Demetriou M, et al., 2016. Evaluation of sural nerve automated nerve conduction study in the diagnosis of peripheral neuropathy in patients with type 2 diabetes mellitus. *Arch Med Sci*, 12(2):390-393. https://doi.org/10.5114/aoms.2016.59265

Cho NH, Shaw JE, Karuranga S, et al., 2018. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*, 138: 271-281. https://doi.org/10.1016/j.diabres.2018.02.023

Dyck PJ, Overland CJ, Low PA, et al., 2010. Signs and symptoms versus nerve conduction studies to diagnose diabetic sensorimotor polyneuropathy: Cl vs. NPhys trial. *Muscle Nerve*, 42(2):157-164. https://doi.org/10.1002/mus.21661

Feng L, Hu JH, Chen J, et al., 2018. An efficacy analysis of anti-vascular endothelial growth factor therapy for choroidal neovascularization secondary to multifocal choroiditis and comparison with wet age-related macular degeneration. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(4):327-332. https://doi.org/10.1631/jzus.B1700535

Forsblom CM, Sane T, Groop PH, et al., 1988. Risk factors for mortality in Type II (non-insulin-dependent) diabetes: evidence of a role for neuropathy and a protective effect of HLA-DR4. *Diabetologia*, 41(11):1253-1262. https://doi.org/10.1007/s001250051062

Gordois A, Scuffham P, Shearer A, et al., 2003. The health care costs of diabetic peripheral neuropathy in the U.S. *Diabetes Care*, 26(6):1790-1795. https://doi.org/10.2337/diacare.26.6.1790

International Diabetes Federation, 2019. IDF Diabetes Atlas, 9th Ed. International Diabetes Federation, Brussels, Belgium.

Jonas JB, Mardin CY, Schlötzer-Schrehardt U, et al., 1991. Morphometry of the human lamina cribrosa surface. *Invest Ophthalmol Vis Sci*, 32(2):401-405.

Jonas JB, Schmidt AM, Müller-Bergh JA, et al., 1992. Human optic nerve fiber count and optic disc size. *Invest Ophthalmol Vis Sci*, 33(6):2012-2018.

Kim JH, Lee MW, Byeon SH, et al., 2018. Associations between individual retinal layer thicknesses and diabetic peripheral neuropathy using retinal layer segmentation analysis. *Retina*, 38(11):2190-2196. https://doi.org/10.1097/IAE.0000000000001835

Kim K, Yu SY, Kwak HW, et al., 2016. Retinal neurodegeneration associated with peripheral nerve conduction and autonomic nerve function in diabetic patients. *Am J Ophthalmol*, 170:15-24. https://doi.org/10.1016/j.ajo.2016.06.038

Kim K, Kim ES, Rhee SY, et al., 2017. Clinical characteristics and risk factors for retinal diabetic neurodegeneration in type 2 diabetes. *Acta Diabetol*, 54(11):993-999. https://doi.org/10.1007/s00592-017-1043-5

Lakshmikantha HT, Ravichandran NK, Jeon M, et al., 2018. Assessment of cortical bone microdamage following insertion of microimplants using optical coherence tomography: a preliminary study. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(11):818-828. https://doi.org/10.1631/jzus.B1700612

Li H, Yu XB, Chen Q, et al., 2016. Relationship between retinal nerve fibre layer thickness and peripheral neuropathy in patients with type 2 diabetes. *Chin J Diabetes Mellitus*, 8(7):418-421 (in Chinese). https://doi.org/10.3760/cma.j.issn.1674-5809.2016.07.009

Lin YC, Shen ZR, Song XH, et al., 2018. Comparative transcriptomic analysis reveals adriamycin-induced apoptosis via p53 signaling pathway in retinal pigment epithelial cells. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(12):895-909. https://doi.org/10.1631/jzus.B1800408

Liu F, Bao YQ, Hu RM, et al., 2010. Screening and prevalence of peripheral neuropathy in type 2 diabetic outpatients: a randomized multicentre survey in 12 city hospitals of China. *Diabetes Metab Res Rev*, 26(6):481-489. https://doi.org/10.1002/dmrr.1107

Luu CD, Szental JA, Lee SY, et al., 2010. Correlation between retinal oscillatory potentials and retinal vascular caliber in type 2 diabetes. *Invest Ophthalmol Vis Sci*, 51(1):482-486. https://doi.org/10.1167/iovs.09-4069

Ni CM, Ling BY, Xu X, et al., 2020a. CX3CR1 contributes to streptozotocin-induced mechanical allodynia in the mouse spinal cord. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 21(2):166-171. https://doi.org/10.1631/jzus.B1900439

Ni CM, Sun HP, Xu X, et al., 2020b. Spinal P2X7R contributes to streptozotocin-induced mechanical allodynia in mice. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 21(2):155-165. https://doi.org/10.1631/jzus.B1900456

Perkins BA, Olaleye D, Zinman B, et al., 2001. Simple screening tests for peripheral neuropathy in the diabetes clinic. *Diabetes Care*, 24(2):250-256. https://doi.org/10.2337/diacare.24.2.250

Pirart J, 1977. Diabetes mellitus and its degenerative complications:

a prospective study of 4400 patients observed between 1947 and 1973 (author's transl). *Diabète Métab*, 3(2):97-107 (in French).

Pop-Busui R, Boulton AJ, Feldman EL, et al., 2017. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diab Care*, 40(1):136-154. https://doi.org/10.2337/dc16-2042

Seferovic JP, Pfeffer MA, Claggett B, et al., 2018. Three-question set from Michigan Neuropathy Screening Instrument adds independent prognostic information on cardiovascular outcomes: analysis of ALTITUDE trial. *Diabetologia*, 61(3):581-588. https://doi.org/10.1007/s00125-017-4485-y

Shahidi AM, Sampson GP, Pritchard N, et al., 2012. Retinal nerve fibre layer thinning associated with diabetic peripheral neuropathy. *Diabetic Med*, 29(7):e106-e111. https://doi.org/10.1111/j.1464-5491.2012.03588.x

Smith AG, Lessard M, Reyna S, et al., 2014. The diagnostic utility of Sudoscan for distal symmetric peripheral neuropathy. *J Diabetes Complications*, 28(4):511-516. https://doi.org/10.1016/j.jdiacomp.2014.02.013

Srinivasan S, Pritchard N, Vagenas D, et al., 2016. Retinal tissue thickness is reduced in diabetic peripheral neuropathy. *Curr Eye Res*, 41(10):1359-1366. https://doi.org/10.3109/02713683.2015.1119855

Srinivasan S, Pritchard N, Sampson GP, et al., 2017. Diagnostic capability of retinal thickness measures in diabetic peripheral neuropathy. *J Optom*, 10(4):215-225. https://doi.org/10.1016/j.optom.2016.05.003

Verma A, Rani PK, Raman R, et al., 2009. Is neuronal dysfunction an early sign of diabetic retinopathy? Microperimetry and spectral domain optical coherence tomography (SD-OCT) study in individuals with diabetes, but no diabetic retinopathy. *Eye (London)*, 23(9):1824-1830. https://doi.org/10.1038/eye.2009.184

Wang BH, Xu YS, Xie WJ, et al., 2018. Effects of corneal thickness distribution and apex position on postoperative refractive status after full-bed deep anterior lamellar keratoplasty. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(11):863-870. https://doi.org/10.1631/jzus.B1800230

WHO (World Health Organization), 1999. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus. WHO, Geneva. Available from https://apps.who.int/iris/handle/10665/66040

Xie WJ, Xu YS, Zhang X, et al., 2018. Assessments of tear meniscus height, tear film thickness, and corneal epithelial thickness after deep anterior lamellar keratoplasty. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(3):218-226. https://doi.org/10.1631/jzus.B1700095

Yu JG, Feng YF, Xiang Y, et al., 2014. Retinal nerve fiber layer thickness changes in Parkinson disease: a meta-analysis. *PLoS ONE*, 9(1):e85718. https://doi.org/10.1371/journal.pone.0085718

Zhang X, Yu H, Zhang Y, et al., 2018. The application of RNFL thickness detection in early differential diagnosis among various types of idiopathic optic neuritis. *J Clin Neurosci*, 55:82-85. https://doi.org/10.1016/j.jocn.2018.06.052

List of electronic supplementary materials

Table S1 Complete nerve electromyography data of the DPN and NDPN groups

中文概要

题　目：视盘区视网膜神经纤维层厚度对糖尿病周围神经病变的诊断价值

目　的：本文探讨视盘区视网膜神经纤维层（RNFL）厚度对糖尿病周围神经病变的诊断价值。

创新点：在进行糖尿病眼底检查的同时进行糖尿病周围神经病变的筛查，可以提高糖尿病周围神经病变的筛查效率。

方　法：本文选择 2 型糖尿病患者无合并周围神经病变、2 型糖尿病合并周围神经病变和健康对照组为研究对象，应用光学相干断层成像技术测定视盘区四个象限和整体平均 RNFL 厚度并进行比较，评估视盘区 RNFL 厚度对糖尿病周围神经病变的诊断价值。

结　论：视盘区 RNFL 厚度可以作为糖尿病周围神经病变的一种诊断手段。

关键词：2 型糖尿病；周围神经病变；视网膜神经纤维层厚度；光学相干断层成像技术；诊断