• Clinical Research •

Reduced choroidal peripapillary capillaries in thyroid-associated ophthalmopathy with early stage of dysthyroid optic neuropathy

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Abstract

● **AIM:** To investigate whether the subtle change of choroidal/retinal vessel densities and volumes in thyroid-associated ophthalmopathy (TAO) could be an early sign to detect dysthyroid optic neuropathy (DON).

● **METHODS:** This was a retrospective cross-sectional study, and a total of 98 eyes from 50 subjects were enrolled under certain criteria. Thirty-four eyes of normal controls and 64 eyes of TAO, including 39 eyes of DON and 25 eyes of TAO without DON, underwent optical coherence tomography angiography (OCTA) scanning. All the tested parameters of OCTA scanning including choroid radial peripapillary capillaries (RPC), retinal nerve fiber layer (RNFL), and macular ganglion cell complex (GCC) were compared among groups, and the correlation between OCTA parameters and visual function parameters was also investigated.

● **RESULTS:** Whole choroidal RPC was significantly reduced in DON (48.24%±0.4978%) compared to normal (50.33%±0.3173%) and TAO without DON (49.16%±0.5463%; *P*=0.0041). The reduction of whole choroidal RPC was also correlated with visual field (VF) defect in DON (*r*=0.5422, *n*=39). Although vision acuity and VF were improved in all the patients with DON after being treated with medical and surgical decompression, the reduction of RPC density were not reversed.

● **CONCLUSION:** There is a notable reduction in choroidal RPC in DON, which is correlated with VF defect. The reduction of RPC density could not be reversed immediately by medical and surgical decompression even when vision and VF were improved. These findings suggest that choroidal RPC could be a useful parameter to diagnose and monitor early stage of DON.

● **KEYWORDS:** dysthyroid optic neuropathy; thyroid-associated ophthalmopathy; choroidal radial peripapillary capillaries; optical coherence tomography angiography; optic nerve

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INTRODUCTION

Thyroid-associated ophthalmopathy (TAO), also known as Graves' ophthalmopathy, is an autoimmune disorder which has characteristic ocular manifestations, such as proptosis, eyelid retraction, eyelid lag and restrictive extraocular myopathy[1]. Among a series of TAO associated clinical findings, dysthyroid optic neuropathy (DON) is the most severe vision-threatening condition. The pathogenesis of DON is complicated, which is not fully understood by far. The most widely accepted theory is that DON is caused by the combined effects of mechanical, vascular and inflammatory process. Mostly, DON is secondary to a compartment syndrome in orbital apex, which is caused by enlargement of extraocular muscles and orbital fat resulting from orbital fibroblast deposition of hyaluronic acid[2].

There is no specific diagnosis guideline for DON due to various of clinical manifestations and limited detecting methods. Most clinicians diagnose DON by a combination of radiological findings and clinical manifestations. However, some patients may not present external signs, such as proptosis, because in some cases, DON only caused by congestion at the orbital apex[3]. The decline of visual acuity also sometimes lag behind

other clinical presentations of DON, therefore, a number of tests should be run to evaluate the function of optic nerve for diagnosis of DON, including papillary exam, automated visual field (VF) and contrast sensitivity[4].

There are around 4% to 8% TAO patients having DON[5], of which the irreversible vision loss is largely caused by delayed diagnosis due to lack of efficient detecting method at early stage of TAO. It has been reported that the axonal changes of optic nerve are detected in patients with DON[6]. The compression caused by enlarged extraocular muscles and orbital fat may stretch the optic nerve and reduce the blood flow supply of retina. A report demonstrated that blood flow volume of superior ophthalmic vein decreased in DON eyes[7]. These findings suggest that the changes of optic nerve and hemodynamic state of the eye might be valuable for early diagnosis of DON.

Optical coherence tomography angiography (OCTA) is a non-invasive imaging facility which could be applied to measure the thickness of retinal nerve fiber layer (RNFL), macular and many other parameters of the eye. OCTA is a high-speed optical coherence tomography (OCT) that could characterize a map of blood flow and vessel network of different layer and area of the retina and choroid, which is achieved by comparing the captured signals between sequential scans taken at same cross-section. OCTA assists clinicians to non-invasively visualize and assess retinal and choroidal perfusion, while at the same time the assessment is trustworthy due to its reliable reproducibility, sensitivity and specificity[8-9]. With the emergence of OCTA, it is possible to study the correlation between microvascular perfusion changes and the development of DON.

Here we demonstrated that OCTA could detect early defects in density of radial peripapillary capillaries (RPC) around optic disc in DON, which was also significantly correlated with defect of VF and vision. The findings suggested that OCTA could be used to early diagnose DON in clinics.

SUBJECTS AND METHODS

Ethical Approval This was a retrospective cross-sectional study conducted at the Department of Ophthalmology in Shanghai General Hospital, China, from January 2019 to December 2020. The study protocol and ethics were approved by the Ethics Committee of Shanghai General Hospital (ref 2020KY206). The study was conducted complied with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Study Participants A total number of 98 eyes from 50 subjects were enrolled according to the criteria of this study and imaged by RTVue-XR Avanti (Opto Vue, Inc, Fremnt, CA, USA) platform and analyzed with the split-spectrum amplitude decorrelation angiography (SSADA) algorithm. Each patient underwent a series of ophthalmological examinations, including slit-lamp clinical examination, best-corrected visual acuity test, ocular motility, severity of proptosis, VF test, clinical activity score (CAS)[10] and OCTA scanning of peripapillary RNFL, macular ganglion cell complex (GCC) and choroid RPC. There were 11 DON patients received intravenous corticosteroids treatment to relieve the clinical symptoms of optic nerve compression, while 5 of whom further received orbital decompression surgery due to the unresponsive to corticosteroids treatment. For the eyes received steroid therapy and surgery, the examinations were practiced both pre- and post- treatment/surgery. The comparison was made between pre-operation and 6-month after treatment/surgery.

Patients diagnosed with TAO were based on Barley criteria[1]. Inclusion criteria for TAO participant were: 1) At least 18 years of age; 2) No history of radioactive iodine therapy or thyroidectomy. TAO participants were further divided into two groups, DON and non-DON. The diagnosis of DON was based on clinical findings[4,10]: 1) Decreased visual acuity compared to previous medical records; 2) Apparent VF defect mean deviation (MD) <-10 dB in Humphrey test; 3) Relative afferent pupillary defect; 4) Evidence of apical crowding in computed tomography or magnetic resonance imaging.

Inclusion criteria for normal subjects were: 1) at least 18 years of age; 2) normal clinical appearance of the optic disc; 3) no RNFL loss; 4) no VF defect.

Exclusion criteria for all participants were: 1) Any retinal pathology and optic neuropathy, such as uveitis and diabetic retinopathy; 2) Any complication inducing VF loss, such as glaucoma or ocular tumor; 3) Any history of ocular trauma or intraocular surgery; 4) Vulnerable individuals or those who cannot conduct any test required in this study.

Humphrey Visual Field Test Humphrey Visual Field Analyzer II 750 (Carl Zeiss Meditec) was used to test VF for all participants, the data was calculated by Humphrey Swedish Interactive Threshold Algorithm (SITA) 30-2 test. The included results should meet the criteria that fixation loss was less than 20%, and both false-negative errors and false-positive errors were less than 15%. All tests were performed without any inappropriate operation, such as eyelid artefacts, inattention, and fatigue effects. Any defects of VF caused by other diseases was excluded as described before.

OCTA Image Acquisition and Processing The RTVue-XR Avanti could be used to visualize vascular structures of distinct layers of the retina and choroid. The scan was non-invasive and achieved by low-coherence interferometry. RTVue-XR Avanti used an 840 nm light source as the scan beam wavelength with an A-scan rate of 70 000 A-scan per second. Motion artefacts were minimized by dual orthogonal volumetric imaging of the retina. Each studied eye received four volumetric raster scans, which were composed of two horizontal priority (X-fast)

and two vertical priority (Y-fast) scans. The scans provided consecutively information outside a 6×6 mm^2 field size of observed structures and assessed the details of retina in distinct layers.

RTVue-XR Avanti system has been designed to minimize scanning time based on the SSADA algorithm. The system can also measure the variation of OCT signals among consecutive scans, therefore the motion of blood flow could be captured. In order to quantify and analyze the nerve fiber layer and macular circulation, *en face* retinal angiogram images were processed and vessel density was calculated using the Avanti trend analysis software.

Measurement of Radial Peripapillary Capillaries, Macular Ganglion Cell Complex and Peripapillary RNFL The thickness of peripapillary RNFL and macular GCC were obtained using RNFL 4.5 scanning mode and analyzed by the GCC scan algorithm installed in the RTVue-XR Avanti. The RNFL thickness was measured around the optic disc with a circle of 4.5 mm diameter using RNFL mode, while the GCC scan was examined around the fovea with a square area of 6×6 mm^2. GCC scan covers multiple layers of the retina, including the RNFL, the ganglion cell layer and the inner plexiform layer. The calculation of the scanned segmentations of the RNFL and GCC was analyzed by the SSADA algorithm. Furthermore, the SSADA algorithm can also measure and analyze separated areas of the retina including average, superior and inferior hemi-retinal RNFL and GCC.

Statistical Analysis All the data were calculated as mean and standard deviations and compared between groups. For the comparison between two groups, Student's *t* test was used to compare the average values of all the measurements. Gender frequency comparison was analyzed by the Chi-square test. The correlation analysis was performed by univariate analysis with Pearson correlation test and multivariate analysis with ANOVA testing to determine the correlation between RPC and other parameters, such as visual acuity and MD and pattern standard deviation (PSD) of VF. Statistical significance was considered as $P<0.05$. One-way ANOVA test with Tukey correction was applied for multiple comparison among groups with resultant significance level set at $P<0.01$.

RESULTS

Demographic Data and Clinical Features Table 1 summarizes the clinical characteristics of each group. According to the inclusive and exclusive criteria, 34 eyes were included in normal group, while 64 eyes were included in TAO group. TAO was further divided into two groups, DON and TAO without DON, with 39 eyes in DON and 25 eyes in non-DON respectively. There was no statistically significant difference in DON, TAO without DON and normal controls, regarding to age and gender. The visual acuity of DON was significantly lower than the other two groups, though the CAS showed no difference between non-DON and DON.

Table 1 Summary of characteristics of all subjects

Parameters	Normal	non-DON	DON	*P*
n	34	25	39	
Gender, male (%)	41.2	36	43.6	
Age, y	59.06±1.963	53.79±1.696	58.87±1.070	ns
Visual acuity	0.8±0.039	0.9±0.028	0.5±0.046	<0.0001
CAS score		4.56±0.16	4.87±0.26	ns

DON: Dysthyroid optic neuropathy; CAS: Clinical activity score. ns: No significance.

Table 2 Comparisons of parameters between normal and TAO

mean±SEM

Parameters	Normal	TAO	[a]*P*
RPC (%)			
Whole	50.33±0.3173, *n*=34	48.63±0.3707, *n*=60	0.0025
Inside disc	50.02±0.7654, *n*=34	48.02±0.8533, *n*=59	ns
Peripapillary	53.24±0.3914, *n*=34	51.83±0.3896, *n*=58	0.01
Perip-superior	53.07±0.4316, *n*=34	51.69±0.4308, *n*=58	0.03
Perip-inferior	53.45±0.4310, *n*=34	51.95±0.4029, *n*=58	0.01
Superior	53.50±0.5557, *n*=34	52.50±0.4846, *n*=58	ns
Temporal	56.18±0.6052, *n*=34	53.66±0.5692, *n*=58	0.005
Inferior	55.50±0.4800, *n*=34	54.71±0.5189, *n*=58	ns
Nasal	48.97±0.5827, *n*=34	47.90±0.5370, *n*=58	ns
GCC (μm)			
Average	99.76±1.335, *n*=34	98.30±1.005, *n*=64	ns
Superior	100.2±1.733, *n*=34	98.33±1.014, *n*=64	ns
Inferior	99.15±1.081, *n*=34	98.36±1.090, *n*=64	ns
RNFL (μm)			
Average	104.0±1.401, *n*=34	111.3±3.030, *n*=64	ns
Superior	106.1±1.530, *n*=34	115.1±3.346, *n*=64	ns
Inferior	101.9±1.433, *n*=34	107.3±2.830, *n*=64	ns
Rim area (mm^2)	1.418±0.05359, *n*=34	1.703±0.09058, *n*=64	0.03
Disc area (mm^2)	2.235±0.05719, *n*=34	2.425±0.08202, *n*=64	ns
FLV	0.7850±0.1463, *n*=34	1.225±0.1995, *n*=64	ns
GLV	1.996±0.3832, *n*=34	2.565±0.4646, *n*=64	ns
VF			
MD (dB)	-2.409±0.4732, *n*=34	-5.465±0.8455, *n*=63	0.01
PSD (dB)	2.627±0.4097, *n*=34	3.431±0.3267, *n*=63	ns

FLV: Focal loss volume; GCC: Ganglion cell complex; GLV: Global loss volume; MD: Mean deviation; PSD: Pattern standard deviation; RNFL: Retinal nerve fiber layer; RPC: Radial peripapillary capillaries; TAO: Thyroid associated ophthalmopathy; VF: Visual field; ns: No significance. [a]Statistical significance tested with Student's *t* test.

Reduction of Radial Peripapillary Capillaries in Thyroid Associated Ophthalmopathy Compared to Normal Table 2 is an overview of a comparison in a series of parameters between TAO and normal subjects. When compared to normal participants, TAO showed significant reduction in the percentage of choroidal RPC, including whole and peripapillary area of choroidal RPC, as well as in MD of VF (Table 2). The percentage of whole choroidal RPC was 50.33%±0.3173% in normal controls, but reduced to 48.63%±0.3707% in TAO

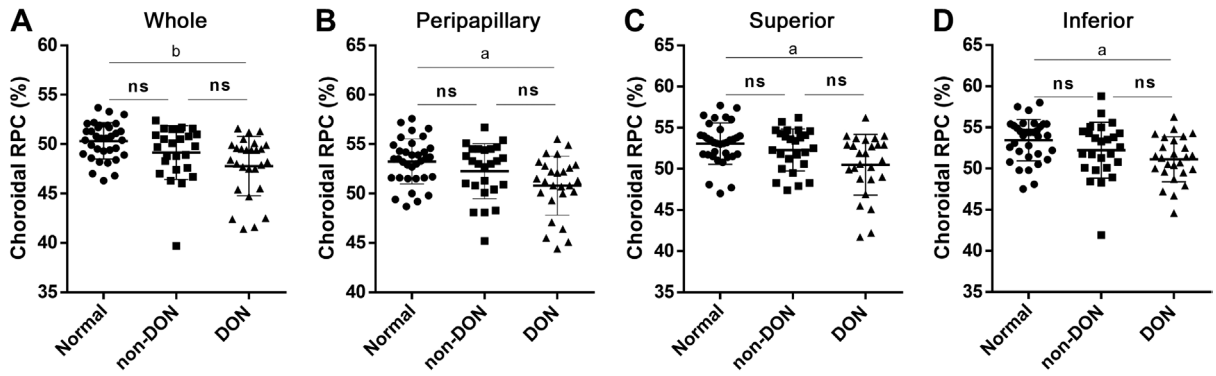

Figure 1 The comparisons of RPC among normal, non-DON and DON There was a significant reduction of choroidal RPC in DON compared to both normal and TAO without DON, including whole area, peripapillary, superior and inferior area of choroidal RPC. [a]*P*<0.005; [b]*P*<0.0005; ns: No significance. TAO: Thyroid associated ophthalmopathy; RPC: Radial peripapillary capillaries; DON: Dysthroid optic neuropathy.

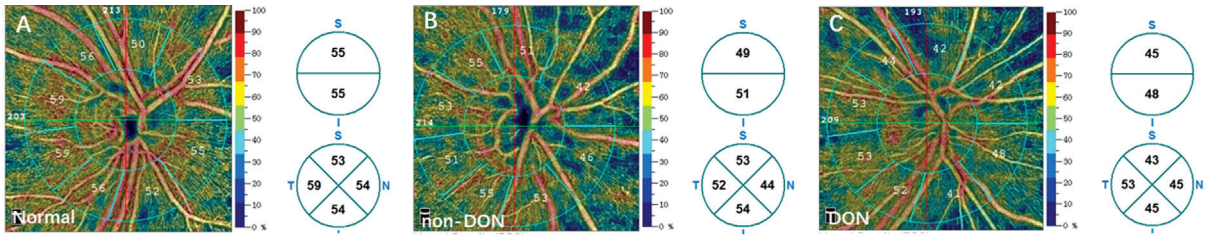

Figure 2 Reduced RPC small vessel density is observed in both non-DON and DON The RPC small vessel density is reduced in both non-DON and DON patients, and the reduction is more severe in DON. RPC: Radial peripapillary capillaries; DON: Dysthroid optic neuropathy.

(*P*=0.0025). Similarly, significant reduction was seen in peripapillary choroidal RPC (51.83%±0.3896% in TAO *vs* 53.24%±0.3914% in normal controls, *P*=0.01; Table 2). While there was no statistical difference between the two groups in thickness of GCC and RNFL, disc area and percentage of focal loss volume (FLV) and global loss volume (GLV).

Significant Reduction of Radial Peripapillary Capillaries in Dysthroid Optic Neuropathy Next, we further divided the patients of TAO into two subgroups, DON and non-DON, according to the criteria described in method section. DON had a significant reduction in both VF and the percentage of choroidal RPC, including whole and peripapillary, compared to both normal and TAO without DON (Figure 1). The percentage of whole choroidal RPC significantly reduced from 50.33%±0.3173% in normal to 49.16%±0.5463% in TAO without DON and further to 48.24%±0.4978% in DON (*P*=0.0041). The percentage of peripapillary choroidal RPC was also significant reduced from 53.24%±0.3914% in normal to 52.27%±0.5562% in TAO without DON, and further reduced to 51.50%±0.5399% in DON (*P*=0.03; Table 3). In agreement to the findings, the representative OCTA images also illustrated that the choroidal capillaries around optic disc were thinner in DON patients than that in normal and TAO without DON (Figure 2). There was no significant difference between DON, TAO without DON and normal controls in most tested parameters (Table 3). The above data suggested that change of choroidal RPC could be a specific sign in DON, and there was a possibility that the reduction of choroidal RPC in DON correlates with VF defect.

Correlations Between Radial Peripapillary Capillaries and Visual Function Parameters To further investigate if the reduction of choroidal RPC accounts for any pathological changes in DON, we ran a series analysis to detect any correlations. Data showed that the whole percentage of choroidal RPC had a significant correlation with visual defect, especially defect in VF in DON (*r*=0.5422), while such correlations were not observed in either normal (*r*=0.1529) or TAO without DON (*r*=0.07371; Table 4), which suggested that the reduction of choroidal RPC was a specific change in DON.

Radial Peripapillary Capillaries Density Could not be Reversed by Medical or Surgical Decompression To investigate if the RPC density and other parameters would improve when DON is relieved, we compare all these observed clinical data before and after treatment. There were 11 DON eyes received intravenous methylprednisolone pulse therapy due to severe symptoms of optic nerve compression such as rapidly decline of vision acuity and defect of VF, but 5 of which did not respond well to corticosteroids treatment as the deterioration of vision and VF continued. Therefore, the 5 DON eyes further received orbital decompression surgery followed with symptoms of optic nerve compression significantly improved in all cases. After medical or surgical decompression, vision acuity and VF improved in all treated eyes, but there was no significant difference of choroidal RPC

Table 3 Comparisons of papameters among groups mean±SEM

Parameters	Normal	TAO		^aP
		Non-DON	DON	
RPC (%)				
Whole	50.33±0.3173	49.16±0.5463	48.24±0.4978	0.0041
Inside disc	50.02±0.7654	48.28±1.528	47.83±0.9849	ns
Peripapillary	53.24±0.3914	52.27±0.5562	51.50±0.5399	0.03
Perip-superior	53.07±0.4316	52.29±0.5126	51.23±0.6456	0.04
Perip-inferior	53.45±0.4310	52.24±0.6808	51.73±0.4911	0.004
Superior	53.50±0.5557	52.84±0.7111	52.24±0.6658	ns
Temporal	56.18±0.6052	54.16±0.6896	53.27±0.8564	0.01
Inferior	55.50±0.4800	55.16±0.8863	54.36±0.6228	ns
Nasal	48.97±0.5827	48.52±0.7074	47.42±0.7761	ns
GCC (μm)				
Average	99.76±1.335	100.4±1.521	96.97±1.301	ns
Superior	100.2±1.733	100.4±1.386	97.00±1.378	ns
Inferior	99.15±1.081	100.4±1.887	97.05±1.295	ns
RNFL (μm)				
Average	104.0±1.401	104.3±1.459	115.7±4.771	0.02
Superior	106.1±1.530	107.3±1.939	120.2±5.219	0.01
Inferior	101.9±1.433	101.2±1.428	111.2±4.465	ns
Rim area (mm^2)	1.418±0.05359	1.434±0.05565	1.875±0.1381	0.0018
Disc area (mm^2)	2.235±0.05719	2.344±0.09332	2.477±0.1208	ns
FLV	0.7850±0.1463	0.8456±0.1709	1.469±0.3041	0.0479
GLV	1.996±0.3832	1.586±0.3159	2.069±0.3598	ns
VF				
MD (dB)	-2.409±0.4732	-1.483± 0.2778	-8.085±1.219	<0.0001
PSD (dB)	2.627±0.4097	1.980±0.1513	4.386±0.4740	0.0002

DON: Dysthroid opitc neuropathy; FLV: Focal loss volume; GCC: Ganglion cell complex; GLV: Global loss volume; MD: Mean deviation; PSD: Pattern standard deviation; RNFL: Retinal nerve fiber layer; RPC: Radial peripapillary capillaries; TAO: Thyroid associated ophthalmopathy; VF: Visual field; ns: No significance. [a]Difference between groups were tested with one way ANOVA test with Tukey correction.

Table 4 Correlation between RPC and other variables among groups

Parameters	RPC (%)[a]					
	Normal		TAO			
			Non-DON		DON	
VA	P=0.8887	r=0.0249	P=0.0574	r=0.3849	P=0.0195	r=0.3931
VF						
MD	P=0.3881	r=0.1529	P=0.7262	r=0.07371	P=0.0009	r=0.5422
PSD	P=0.1803	r=-0.2353	P=0.6311	r=-0.101	P=0.0002	r=-0.6012

MD: Mean deviation; PSD: Pattern standard deviation; RPC: Radial peripapillary capillaries; TAO: Thyroid associated ophthalmopathy; VA: Visual acuity; VF: Visual field. [a]Correlation between parameters was tested with Pearson correlation test.

Table 5 Comparisons of parameters before and after treatment mean±SEM

Parameters	Treatment		^aP
	Before	After	
RPC (%)			
Whole	48.28±1.147	47.58±1.144	ns
Inside disc	51.50±1.679	50.79±1.867	ns
Peripapillary	51.77±1.450	50.85±1.095	ns
Perip-superior	51.59±1.282	50.17±1.167	ns
Perip-inferior	51.59±1.630	50.34±1.317	ns
VA	0.42±0.0876	0.76±0.0576	0.0003
VF-MD (dB)	-9.019±2.8	-2.315±0.8782	0.0266

MD: Mean deviation; RPC: Radial peripapillary capillaries; VA: Visual acuity; VF: Visual field; ns: No significance. [a]Statistical significance tested with paired *t*-test.

density between pre-treatment (whole 48.28%±1.147%) and post-treatment (whole 47.58%±1.144%; Table 5). The data demonstrated that both medical and surgical decompression could reverse defected visual function to a certain level, but the decline of choroidal RPC still remained.

DISCUSSION

In this study, we demonstrated that the percentage of choroidal RPC was significantly reduced in the patients of DON compared to both normal controls and the patients of TAO without DON. The reduction of choroidal RPC also correlated with defects of VF, which suggested the change of choroidal RPC could be an early sign of optic nerve damage. OCTA can be used to investigate vessel volume and density of multiple layers of the eye, which makes clinicians able to observe changes undetected by other investigating methods.

So far, there is no single protocol or guideline for diagnosing DON[11-12], clinicians have to measure grades of clinical signs and symptoms of patients, and also run a series of tests, including CT or MRI scan, and VF test. Only with the combination of clinical findings and test results can DON be diagnosed. However, there are still a number of DON patients facing with delayed diagnosis due to unspecific clinical presentation[3-4]. For example, some patients with DON only caused by congestion at orbital apex without any sign of proptosis, and patients sometimes are lack of clinical manifestations of orbital inflammation, which could cause the delay use of radiology test as there is little sign of further investigations. Decreased visual acuity is also an unspecific symptom, though it is more often found in DON rather than thyroid eye diseases (TED) alone. It has been reported that there are about 47% of DON patients with visual acuity lower than 20/40, while the number is only 3% in TED patients[2]. Reduction of contrast sensitivity, colour vison change and an afferent pupillary defect are the other signs specific for DON, though all of these signs can be absent in some cases[13].

VF test can accurately detect DON, according to studies, most of the DON eyes develop a central or paracentral scotoma during the progress of disease[14]. Visual evoked potential (VEP) can also assist to detect DON, and sometimes it is even more sensitive than VF test[15]. Therefore, the retinal function is one of the diagnostic factor for DON. Our data also suggested

that there was a significant difference of VF defect between DON and non-DON.

Orbital imaging techniques, such as MRI and CT, play a vital role in diagnosing and following DON[16]. The muscle index is significantly greater in orbits with DON, and DON almost never occurs in patients when muscle index is less than 50%[17-18]. Orbital soft tissue imaging also help to diagnose DON, with up to 94% sensitivity and 91% specificity[10]. However, cost of these imaging detecting methods is high and some disable patients may find it difficult to take the scans. Therefore, there is still a demanding of a new technique which could detect subtle changes for early diagnose of DON.

Our study demonstrated that a significant reduction of choroidal RPC observed in DON patients rather than normal and TAO without DON by using OCTA. RPC is a superficial capillary layer which comprise a unique vascular plexus. There have been reports demonstrating that RPC is necessary to metabolic demands of retinal ganglion cell (RGC) axons[19-20]. As the RGC axons are vulnerable to decease of blood flow, the structural changes to RPC network could lead to pathogenesis of RGC axonal loss[21]. There is evidence also showing an correlation between RPC loss and RNFL changes in chronic glaucoma[22]. The change of peripapillary microvasculature could not be efficiently detected by OCT[23], which indicates that OCTA could be the most effective and easiest way to detect changes in retinal and/or choroidal microvasculature. Our data indicated a significant correlation between RPC reduction and VF defect. However, the decrease of choroidal RPC density could not be reversed 6mo after relieving optic nerve compression by either corticosteroid treatment or optic nerve decompression surgery though vision acuity significantly improved. However, longer term follow-up is required to further understand if the reduction of choroidal RPC could be recovered or permanently affected. Therefore, it is suggesting that choroidal RPC density could be clinically useful for early diagnose of DON.

There were studies indicating that the macular microvascular densities were significantly reduced in TAO patients[5,24]. But our data presented no significant finding observed neither between normal and TAO, nor between normal and DON. One of the possible reasons of getting different result is that the acquisition area of GCC is different, we acquired 6×6 mm^2 OCTA images for GCC rather than 3×3 mm^2 acquired in other studies. Another possibility is that the different enrolled criteria of TAO may give biased readout. There was even one study reporting that microvascular density significantly increased in active TAO[25]. Taken these findings together, the GCC density varies during different stage of TAO, which indicates that the change of GCC density could not effectively monitor development of TAO.

Our data demonstrated that the density of choroidal RPC, both whole and peripapillary area, was significantly decreased in DON compared to normal eyes and TAO without DON. The decrease of vessel density in the peripapillary area in eye with DON had also been shown by one previous study[5]. The reduction of choroidal RPC has been found to be correlated with defects of VF. Therefore, the change of choroidal RPC could be an early sign of optic nerve damage. Furthermore, our study found that the reduction of RPC density could not be reversed by medical or surgical decompression. So far, the most widely accepted mechanism is that DON is secondary to a compartment syndrome in orbital apex, and the changes of vessels in orbit may also be related to DON. The decreased blood flow in the active stage of orbitopathy, while the reversed or even absent blood flow in many advanced cases can even induce the optic nerve vasculature which further develops ischemia. Medical and surgical decompression could decrease the direct optic nerve compression in DON, however, might not benefit to restore the vasculature[4].

There are limitations in our study. First, the observation period is not very long. Longer follow-up could help to get more comprehensive understanding of the role of choroidal RPC in the development of DON. Second, the sample size of treated eyes is also small, more samples would make the conclusion stronger. Last, the diameter of analyzed area by OCTA seems to make some biases in making conclusions, which requires further investigations in future study.

In conclusion, there are advantages of using OCTA in diagnosis of DON. It improves our understanding of pathogenic relationships between optic disc circulation and VF defect. It is also non-invasive and easy to operate, which can be accepted by a wide range of patients. Therefore, choroidal RPC scan by OCTA could be an effective way to investigate and monitor DON, it is also possible to help in early diagnosis of DON.

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