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Retinal thickness and microvascular pathway in Idiopathic Rapid eye movement sleep behaviour disorder and Parkinson's disease

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ABSTRACT

Introduction: Retinal impairment has previously been described in Parkinson's Disease (PD), also in early stage of disease. Idiopathic Rapid-eye-movement sleep Behavior Disorder (iRBD) is considered the strongest marker in the diagnosis of "Prodromal PD". Thus, we evaluated the thickness of retinal layers and the microvascular retinal pattern in a group of iRBD patients compared to PD and healthy subjects (HCs).
Methods: retinal layer's thickness and microvascular pattern among PD, iRBD and HCs were assessed using Spectral-Density Optical Coherence Tomography (SD-OCT) and OCT-Angiography (OCT-A), respectively.
Results: Forty-one eyes from 21 PD, 37 eyes from 19 iRBD and 33 eyes from 17 HCs were analysed. Peripapillary Retinal Nerve Fiber Layer (RNFL) was thinner in PD and RBD compared to HCs. All macular retinal layers, except for retinal pigment epithelium, resulted to be significantly thinner in iRBD and in PD compared to HCs, also adjusting by age, sex and hypertension. Macular RNFL and ganglionic cell layer were thinner in PD compared to iRBD. Moreover, in iRBD, a peculiar microvascular pattern was found, characterized by a higher vascularization of the deep capillary plexus with respect both PD patients and HCs.
Conclusion: in PD and iRBD patients retina was thinner than HCs, and values of iRBD were between PD and HCs. Moreover, in iRBD, a peculiar microvascular pattern has been found, characterized by a higher vascularization of the deep capillary plexus. Our findings suggest that retina might be considered a biomarker of neurodegeneration in iRBD, easily estimable using non-invasive tool such as OCT and OCT-A.

1. Introduction

Idiopathic Rapid eye movement sleep Behavior Disorder (iRBD) is a condition characterized by the presence of abnormal behaviors in the REM sleep phase, such as movements and vocalizations caused by a dream enactment behavior [1]. In the last decades, increasing evidences from epidemiological studies have shown that presence of iRBD is associated with a higher risk of developing a neurodegenerative disease, especially $\alpha-$ synucleinopathies such as Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB) and Multiple System Atrophy (MSA) [1]. In a recent multicenter study of iRBD patients, up to 28% of the sample converted to an $\alpha-$ synucleinopathy with a mean time to phenoconversion of 4.6 years [2]. Previous studies with longer follow-up demonstrated that up to 90% of patients developed a neurodegenerative disease almost 14 years after RBD diagnosis [3]. On the bases of these evidences iRBD is considered the most specific risk factor for the development of PD, being the strongest marker for the diagnosis of "Prodromal PD" [4].

Although PD has been classically considered a movement disorder, several non-motor symptoms represent very common features of the disease [4]. Among NMSs, visual impairment, including colour vision, visual acuity and contrast sensitivity [5] has been also described in an

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early stage of PD [5]. Furthermore, several studies based on Optical Coherence Tomography (OCT) evaluation, have reported a lower retinal thickness among PD patients as compared to healthy subject [6]. In agreement with these observations we have recently described a thinning of inner retinal layers in PD patients at early stage of disease [7]. Moreover, we have also reported a positive correlation between inner retinal thickness and microvascular density in the foveal region, known to be involved in visual acuity and colour vision [7].

Visual impairment has been reported in 40–60% of iRBD subjects, especially colour vision dysfunction [8,9] and the risk of conversion to parkinsonian clinical forms seems to be higher among iRBD with abnormal colour discrimination [2,9]. On this ground retinal impairment could be part of the neurodegenerative process and it could be present also during the prodromal phase of PD, before the motor onset. To the best of our knowledge, only one study recently evaluated macular retinal thickness using retinal segmentation analysis, reporting a thinning of ganglion cell complex (GCC) in iRBD as compared to healthy controls (HCs) [10]. Unlike PD, up to date no studies investigated the possible presence of microvascular impairment already in the prodromal phase of PD, such as in iRBD.

Thus, in this study, we evaluated the thickness of retinal layers and the microvascular retinal pattern in a group of iRBD patients compared to PD and healthy subjects using, respectively, OCT and Spectral-Domain OCT angiography (OCT-A).

2. Materials and methods

2.1. Study population

Three groups of subjects were enrolled: PD patients, iRBD patients and HCs.

We analysed PD patients enrolled in our previous study [7]. In detail, early PD patients attending the "Parkinson's Disease and Movement Disorders Centre" of the University of Catania and fulfilling the MDS-PD diagnostic criteria for clinically established or clinically probable PD were enrolled [7,11].

iRBD were enrolled among both subjects previously identified in a population-based study investigating iRBD prevalence in the community of Catania [12] and patients attending to the Clinic of Neurology of the University of Catania. Diagnosis of definite iRBD (dRBD) was sought on the base of a video-polysomnographic recording (VPSG), showing the lack of the physiological atonia during REM sleep phase associated to abnormal motor behaviors or vocalizations, scored according to the American Academy of Sleep Medicine (2014). When VPSG was not available, confirmation of the presence of RBD was performed by a board certified sleep expert (LG) using a semi-structured interview based on the RBD screening questionnaire (RBDSQ) [13]. These patients were diagnosed as probable iRBD (pRBD) [14].

A group of HCs was selected from caregivers of PD and iRBD patients attending our centre. All controls underwent a neurological examination by trained neurologists, to exclude any neurological disease.

Neurological examination was performed by neurologists expert in movement disorders. Motor impairment was evaluated with the Unified Parkinson's Disease Rating Scale part-III (UPDRS-III) and the Hoehn and Yahr (HY) scale. For PD patients clinical and pharmacological data were collected from patient's medical records.

2.2. Ophtalmologic evaluation

All subjects underwent a complete ophthalmologic examination, performed at "Ophthalmology Clinic" of University of Catania. It included a standardized clinical examination with the assessment of visual acuity, intraocular pressure (IOP), fundus examination, slit-lamp biomicroscopy, OCT and OCT-A. We excluded all subjects with a history of ocular trauma, ocular surgery, ocular diseases involving retina, optic nerve, cornea or macula, IOP>21 mmHg, cataract, systemic condition that could impair visual system, such as diabetes mellitus, uncontrolled hypertension or hypotension, cardiovascular diseases and any other neurological disease.

2.3. High-definition Optical Coherence Tomography (HD-OCT) and Spectral-Domain optical coherence tomograph-angiography (SD-OCT-A) imaging

Macular retinal thickness and peripapillary retinal nerve fiber layer (RNFL) thickness were assessed using the Cirrus HD-OCT model 5000 (Carl Zeiss Meditec, Inc). SD-OCT-A of the macula and peripapillary plexus were performed using AngioVue XR Avanti (Optovue Inc, Fremont, California, USA). OCT and OCT-A protocols have been extensively reported elsewhere [7]. In brief, following layers were analysed: RNFL, Ganglionic Cell Layer (GCL), Inner Plexiform Layer (IPL), Inner Nuclear Layer (INL), Outer Plexiform Layer (OPL), Outer Nuclear Layer (ONL), Retinal Pigment Epithelium (RPE). AngioVue automatically segments the area into four layers, including superficial capillary plexus layer (SCP), deep capillary plexus layer (DCP), that are, in turn, subdivided into foveal, parafoveal, superior and inferior area.

2.4. Data collection

Retinal images of both left and right eye were acquired for each subject. The quality of each ocular image was evaluated by expert ophthalmologists (MR,AL,AR,NC) and eyes whose ocular measurements were not of good quality were excluded from the analysis. All collected data have been entered in ad hoc created database using Excel software and each subject was identified using a unique identification code to protect anonymity. Before analysis, a consistency check has been performed for all the variables in the database.

2.5. Statistical analysis

Data were analysed using STATA 12.1 software (StataCorp, College Station, TX, United States). Quantitative variables were described using mean and standard deviation. The difference between means was evaluated by the *t*-test and ANOVA-test while the difference between proportions by the Chi-squared test. In case of a not normal distribution, appropriate non-parametric tests were performed. To evaluate the possible association between iRBD and the thickness of each retinal layer an unconditional logistic regression analysis was performed. Multivariate analysis was performed considering age and sex as *a priori* confounders. Moreover, considering the influence of blood pressure values on microvascular pattern, all results were adjusted also by presence of hypertension on medical history, using multivariate model. The odds ratios (OR) with 95% confidence intervals (CI) and p value (two-tailed test, $\alpha = 0.05$) were calculated. Pearson correlation analysis was performed to evaluate the presence of correlation between retinal layers thickness and microvascular pattern. These data were also adjusted for age, sex and hypertension. The significance level was set at 0.05 and the ninety-five confidence intervals (95% CI) were calculated.

2.6. Standard protocol approvals, registrations and patient consents

This study was carried out in accordance with Declaration of Helsinki and approval from the local ethical committee (Ethical Committee Catania 1) was obtained. All the participants have been asked to sign an informed consent prior to be included in the study.

3. Results

3.1. Descriptive analysis

Twenty-one PD patients [12 men, 57.1%; age (means ± SD) 61.5 ± 6.5], 19 iRBD subjects [11 men, 57.9%; age 58.8 ± 13.3 years] and 17

HCs [9 men, 52.9%; age 65.1 ± 10.7] were enrolled in the study. Age and sex were not significantly different across the three groups (Table 1).

PD patients were at an early stage of disease, with a mean duration from the clinical onset to the neurological evaluation of 27.4 ± 14.3 months (disease duration). History of hypertension was significantly more frequent among PD patients as compared to both iRBD and controls (Table 1).

Among the enrolled iRBD subjects, 9 (47.4%) underwent a VPSG and they were all diagnosed as dRBD (4 from the population-based study and 5 from patients attending to our Clinic of Neurology), while the others were considered as pRBD.

There were not significant differences in age, sex and UPDRS-III score between probable and definite RBD (Supplementary table 1).

3.2. OCT analysis–comparison of macular retinal layers thickness among PD, iRBD and HCs

A total of 41 eyes from 21 patients with PD, 37 eyes from 19 subjects with iRBD and 33 eyes from 17 HCs were analysed using OCT. One eye from PD patients, 1 eye from iRBD subjects and 1 eye from HCs were excluded because of the poor quality of OCT.

The thickness of each retinal layer was not significantly different between right and left eye in PD, HCs, and iRBD groups. Thus, data from both eyes were considered to perform statistical analysis. Due to the lack of statistically significant differences in the thickness of each retinal layer between pRBD and dRBD, iRBD subjects were analysed considering a single group (Supplementary table 2).

The thickness of macular RNFL, GCL, IPL, INL, OPL, ONL was statistically different across the three study groups (Table 2). In particular, all retinal layers, except for RPE, have been found to be significantly thinner in iRBD patients compared to HCs and, even more, in PD patients (Table 2). Comparing PD patients and iRBD subjects, RNFL and GCL resulted to be significantly thinner in PD, while significant differences were not found in the other layers (Table 2).

These findings have been confirmed by multivariate logistic regression analysis, adjusting by age, sex and hypertension (Supplementary table 3).

Table 1
Demographic and clinical characteristics.

	PD n. 21 (n. 41 eyes)	iRBD n. 19 (n. 37 eyes)	HCs n. 17 (n. 33 eyes)	p-value[a] iRBD vs PD	p-value[a] iRBD vs HCs
Men, *n (%)*	12 (57.1%)	11 (57.9%)	9 (52.9%)	0.96	0.76
Age at OCT *(years)*	61.5 ± 6.5	58.8 ± 13.3	65.1 ± 10.7	0.41	0.14
Age at onset *(years)*	59.3 ± 7.0	/	/	/	/
Disease duration *(months)*	27.4 ± 14.3	/	/	/	/
HY stage	1.9 ± 0.4	/	/	/	/
UPDRS-ME score	25.0 ± 6.9	5.6 ± 4.3	3.2 ± 2.7	**<0.001**	0.07
Education *(years)*	10.5 ± 3.3	9.6 ± 2.9	11.0 ± 3.7	0.37	0.22
LD, *n (%)*	11 (52.4%)	/	/	/	/
LED *(mg)*	127.4 ± 142.7			/	/
Hypertension, *n (%)*	12 (57.1%)	4 (21.1%)	4 (23.5%)	**0.02**	0.86

Legend: PD (Parkinson's disease); iRBD (Idiopathic Rapid eye movement sleep Behavior Disorder); HCs (healthy controls); HY (Hoehn and Yahr scale); UPDRS-ME (Unified Parkinson's Disease Rating Scale part III); LD (Levodopa); LED (Levodopa Equivalent Dose).

[a] Using *t*-test.

Table 2
Thickness of retinal layers in PD, iRBD and HCs group, assessed by OCT segmentation analysis.

	PD n. 21 (41 eyes)	iRBD n. 19 (37 eyes)	HCs n. 17 (33 eyes)	p-value[a] (PD vs iRBD)	p-value[a] (iRBD vs HCs)	ANOVA p-value[b]
RNFL	13.4 ± 1.9	15.4 ± 1.8	17.8 ± 2.2	<0.001	<0.001	<0.001
GCL	16.1 ± 3.2	19.2 ± 2.7	21.4 ± 2.2	<0.001	<0.001	<0.001
IPL	21.4 ± 2.9	22.5 ± 2.8	24.2 ± 2.1	0.10	0.005	<0.001
INL	20.7 ± 5.5	23.2 ± 3.9	28.2 ± 4.5	0.02	<0.001	<0.001
OPL	28.5 ± 6.3	27.1 ± 3.4	31.2 ± 4.8	0.21	<0.001	0.003
ONL	86.1 ± 12.6	84.9 ± 9.0	97.7 ± 7.7	0.63	<0.001	<0.001
RPE	15.6 ± 1.6	16.1 ± 1.1	16.3 ± 1.7	0.10	0.63	0.12
G ON	92.8 ± 9.4	96.9 ± 9.2	101.1 ± 8.0	0.06	0.05	<0.001
SUP ON	112.8 ± 16.5	114.8 ± 10.0	120.6 ± 9.6	0.52	0.02	0.03
TEMP ON	70.5 ± 9.8	72.9 ± 8.6	78.3 ± 7.8	0.25	0.01	0.001
INF ON	120.2 ± 15.0	119.2 ± 9.2	119.4 ± 9.2	0.73	0.94	0.92
NAS ON	73.0 ± 10.2	79.5 ± 8.5	80.9 ± 8.2	0.003	0.48	<0.001

Legend: PD (Parkinson's disease), iRBD (Idiopathic Rapid eye movement sleep Behavior Disorder), HCs (healthy controls), RNFL: Retinal Nerve Fiber Layer, GCL: Ganglionic Cell Layer, IPL: Inner Plexiform Layer, INR: Inner Nuclear Layer, OPL: Outer Plexiform Layer, ONL: Outer Nuclear Layer, RPE: Retinal Pigment Epithelium, G ON: Global Optic Nerve, SUP ON: Superior sector - Optic Nerve, TEMP ON: Temporal sector - Optic Nerve, INF ON: Inferior sector - Optic Nerve, NAS ON: Nasal sector - Optic Nerve.

[a] Using *t*-test, to compare two groups.

[b] Using ANOVA-test, to compare three groups.

3.3. OCT analysis–comparison of peripapillary RNFL among PD, iRBD and HCs

The overall optic disc and its superior and temporal sectors were thinner in iRBD and PD as compared to HCs (Table 2). These findings have been confirmed by multivariate logistic regression analysis, adjusting by age, sex and hypertension (Supplementary table 3). Moreover, comparing PD and iRBD subjects, the overall optic disc and its nasal sector resulted to be significantly thinner in PD group, also after adjusting by age, sex and hypertension (Table 2 and supplementary table 3).

3.4. OCT-A analysis–comparison of microvascular density among PD, iRBD and HCs and association between retinal thickness and microvascular pathway

All PD patients and HCs underwent OCT-A evaluation while microvascular density was assessed in 14 out of 19 iRBD subjects (7 men, 50%; mean age 58.6 ± 13.4 years) One eye from PD patients, 1 eye from iRBD subjects and 1 eye from HCs were excluded because of the poor quality of OCT-A.

Comparing iRBD subjects with PD patients and HCs, a higher microvascular retinal density was found in iRBD group along the whole DCP, including its superior, inferior and parafoveal areas (Table 3). In iRBD, DCP resulted to be markedly increased with respect to superficial

Table 3
Microvascular density pathway among PD, iRBD and HCs, assessed by OCT-A.

	PD n.21 (n. 41 eyes)	iRBD n.14 (n. 27 eyes)	HCs n.17 (n. 33 eyes)	p-value[a] (PD vs RBD)	p-value[a] (RBD vs HCs)	ANOVA p-value[b]
Foveal thickness	254.3 ± 19.2	250.6 ± 28.8	259.9 ± 16.8	0.52	0.12	0.21
SCP whole	44.6 ± 4.4	43.0 ± 4.6	43.9 ± 3.8	0.15	0.43	0.30
SCP superior	44.3 ± 4.6	43.0 ± 4.6	43.5 ± 4.6	0.24	0.66	0.44
SCP inferior	44.9 ± 4.3	43.0 ± 4.6	43.7 ± 3.8	0.09	0.52	0.18
SCP fovea	19.3 ± 5.7	15.6 ± 4.8	18.4 ± 5.9	0.006	0.05	0.02
SCP parafovea	46.9 ± 4.5	45.7 ± 5.1	46.1 ± 4.3	0.31	0.77	0.52
DCP whole	47.8 ± 4.3	50.5 ± 3.1	47.8 ± 3.7	0.005	0.003	0.006
DCP superior	48.0 ± 4.8	50.2 ± 3.0	48.1 ± 3.9	0.03	0.02	0.06
DCP inferior	47.6 ± 4.3	50.9 ± 3.2	47.5 ± 3.8	0.001	0.001	0.001
DCP fovea	33.8 ± 6.6	31.6 ± 5.8	32.9 ± 7.9	0.16	0.47	0.40
DCP parafovea	49.8 ± 4.5	52.5 ± 3.4	49.9 ± 3.6	0.009	0.01	0.01

Legend: SCP: Superficial capillary plexus, DCP: deep capillary plexus.
[a] Using *t*-test, to compare two groups.

[b] Using ANOVA-test, to compare three groups.

capillary density, as revealed by the ratio between superficial and deep capillary vascularization (Supplementary table 4). Indeed, considering each retinal sector, the ratio between superficial and deep capillary density resulted to be significantly lower in iRBD subjects as compared to both PD and HCs. Comparing iRBD and PD, this finding has been confirmed also by multivariate logistic regression analysis, adjusting by age, sex and hypertension (Supplementary table 4).

When conducting the correlation analysis, we did not find any significant correlations between retinal layers thickness and retinal microvascular pathway among iRBD subjects and HC subjects.

4. Discussion

Our study demonstrated a thinning of different retinal layers (RNFL, GCL, IPL, INL, OPL and ONL) in both PD and iRBD patients with respect to healthy subjects. Interestingly, iRBD presented a retinal thickness that was intermediate between HCs and PD patients. This observation supports the hypothesis that retinal impairment is an early sign of neurodegeneration, occurring in the prodromal phase of PD, when only premotor symptoms are present, such as RBD.

Indeed, iRBD is considered one the most important marker of prodromal PD [4] and the presence of olfactory dysfunction, visual impairment, subtle motor signs, autonomic symptoms and abnormal dopaminergic imaging are supposed to be potential neurodegenerative biomarkers in iRBD, increasing the risk of conversion to PD [2,8,9,15].

Visual disturbances described in neurodegenerative diseases have been suggested to be related to dysfunction both in the visual cortex and in the retina [16]. OCT is a non-invasive and cheap technique used to investigated retina and optic disc. In several OCT studies, peripapillary RNFL resulted to be thinner in PD patients as compared to healthy subjects [6,17]. Moreover, many studies performing retinal segmentation analysis showed a thinning of RNFL, GCL, IPL, INL and OPL in PD patients [6] and it has been hypothesized that retinal thickness can be related to the visual impairment frequently reported by PD patients, also at early stage of disease [5]. Consistent with these data, we found a thinning of peripapillary RNFL in PD patients as compared to healthy controls. Moreover, the thickness of each retinal layer resulted to be lower in PD patients as compared to HCs, except for RPE.

To the best of our knowledge, only two studies evaluated retinal thickness in iRBD. In particular, Yang and coll. reported that peripapillary RNFL is thinner in iRBD subjects with respect to controls [18]. Moreover, a thinning of peripapillary RNFL was found also in PD with RBD as compared to PD without RBD, suggesting RBD as a worsening factor [18]. However, the authors assessed the RNFL thickness in the peripapillary area of iRBD subjects but not the thickness of each retinal layer in the macular area. Conversely, Lee and coll. observed a thinning of ganglion cell complex (GCC) in iRBD as compared to HCs [10], with a value that laid between PD and controls.

In our study we evaluated the retinal thickness of iRBD patients using a retinal segmentation analysis by OCT. In agreement with data reported by Lee and coll. [10], we found a thinning of retinal layers in iRBD and in PD, as compared to healthy subjects, with values in iRBD group that are intermediate between HCs and PD patients. Thus, it could be hypothesized that retinal impairment occurs already in the prodromal phase of PD, representing an early sign of neurodegeneration. Then, retinal thinning could worsen with the progression of the neurodegeneration, reflecting a continuum of neuronal damage that begins already in iRBD patients and that continues up to the onset of PD, among which we found a macular RNFL and GCL even thinner than in iRBD patients. The latter finding could be explained by the evidences that dopamine, physiologically released in the human retina by dopaminergic neurons [19], has a trophic role on retinal cells, including ganglionic cells. However, loss of dopaminergic cells and lower dopamine level have been described in PD patients' retina [20]. Moreover, dopaminergic neurons form synapses with ganglionic cells, providing not only a trophic support but also modulating visual pathway, whose output is represented by RNFL, axonal fibers of ganglionic cells [6].

Furthermore, phosphor-αSYN inclusions, histopathological hallmark of PD, have been found also outside of basal ganglia [21]. Phosphor-αSYN inclusions have been described also in the retina of PD patients and animal models, especially in RNFL, GCL and IPL [22]. Moreover, the phosphor-αSYN density in the retina has been described to significantly correlate with phosphor-αSYN density in the post-mortem brain of PD [23]. Thus, both dopamine depletion and abnormal alfa-synuclein $(\alpha-\mathrm{SYN})$ deposition in the retina could, at least partly, explain retinal impairment in PD. αSyn aggregates have been detected in submandibular gland nerve of iRBD patients [24] and, more interestingly, post-mortem studies revealed the presence of α- SYN deposition also in the brain of iRBD patients [25], indicating a neurodegenerative process at least in some iRBD subjects.

Nevertheless, the main novelty of our study was the assessment of microvascular retinal pattern in iRBD. Indeed, vascular degeneration has been well described in PD patients but not in iRBD subjects. In particular, vascular impairment has been reported in PD subjects not only in brain regions associated to dopaminergic neuron degeneration (substantia nigra and brain stem nuclei), but also in regions not associated to dopaminergic degeneration, such as the middle frontal gyrus [26]. Moreover, in PD subjects a higher rate of string vessels has been reported as compared to healthy controls [27]. Conversely, to the best of our knowledge, this is the first study that evaluated retinal vascularization in a group of iRBD patients. Using OCT-A, we found that deep capillary density was remarkably higher than superficial capillary density in iRBD. Indeed, considering each retinal sector, the ratio between superficial and deep capillary density resulted to be significantly lower

in iRBD subjects as compared to both PD and HCs.

We have not a clear explanation for such findings. Among possible factors, vascular remodeling due to an aSYN-induced inflammation might be supposed [22,28,29]. Indeed, it is known that abnormal protein deposition could activate microglial cells [30], leading to inflammation [31] and subsequent vascular changes, such as vasodilatation and neoangiogenesis [28]. Increased microglial activation has been reported in the retina of genetic rodent models of PD [22,30] and higher levels of pro-inflammatory factors have been described in post-mortem brains and cerebrospinal fluid of PD patients [32,33]. Interestingly, post-mortem studies revealed the presence of $\alpha-$ SYN deposition also in the brain of iRBD patients [24,25] and, recently, microglial activation has been observed also in the *substantia nigra* of iRBD [34]. No data are available on the presence of $\alpha-$ SYN aggregates in the human retina of iRBD. Nevertheless, since retina is an extension of the brain, it could not be entirely excluded that inflammatory response occurring in the brain could also involve the retina [30].

As we previously said, we have not a clear explanation for our findings. Considering that RBD is the strongest prodromal feature of PD [4] and that inflammation leads to neurodegeneration [29,30], it could be speculated that RBD is a clinical-physiopathological "intermediate" condition between HC and PD, in which inflammation is more prominent than neurodegeneration, leading to a more prominent vasodilatation in DCP with respect to PD, where, conversely, neurodegeneration becomes more marked than inflammation. Nevertheless, this is just a speculation and further studies are needed to validate our hypothesis.

Several limits of our study should be taken into consideration in interpreting results. In particular, one important limit is related to the small size of our sample, because of which we cannot exclude that such findings might be due, at least partly, to chance. Moreover, not all iRBD subjects underwent an OCT-A evaluation. Thus, further studies with larger population are needed to verify our findings.

The lack of polysomnographic confirmation for some iRBD patents should be also taken into consideration. Diagnosis of RBD was, in fact, confirmed by VPSG recording only in nine subjects.

Nevertheless, considering VPSG as "gold standard", the RBD1Q has been reported to have high values of sensitivity and specificity, respectively 93.8% and 87.2%, with a positive predictive value (PPV) of 87.9% and a negative predictive value (NPV) of 93.4% [35]. In addition, patients whose RBD1Q was positive were extensively evaluated by a neurologist expert on sleep disorders in order to exclude other causes of secondary RBD before that pRBD diagnosis was made. Moreover, after comparing the thickness of each retinal layer between dRBD and pRBD, no significant differences were found. Nevertheless, the lack of polysomnographic confirmation for some iRBD patents should be taken into account for the interpretation of data.

In conclusion, retina resulted to be thinner in iRBD as compared to HCs, with a microvascular pattern different from both PD and HCs. All these findings point out the possible role of retina as a biomarker of neurodegeneration in iRBD and the opportunity to use non-invasive tools to select and monitor people at risk to evolve into neurodegenerative diseases. OCT and OCT-A might be some of these tools, certainly never alone and always along with more specific and largely accepted instruments. Extreme attention has been focused on iRBD, because it represents a "window of opportunity" in which experimental neuroprotective drugs could be tested, in order to act in the prodromal phases, before the occurrence of symptomatic and irreversible damage [9].

Author statements

Nicoletti Alessandra: Conception and design of the study; analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; final approval of the version to be submitted, Rascunà Cristina: Conception and design of the study; analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; final approval of the version to be submitted, Calogero Edoardo Cicero: Conception and design of the study; analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; final approval of the version to be submitted, Clara Grazia Chisari: Acquisition of data; revising the article critically for important intellectual content; final approval of the version to be submitted, Andrea Russo: Conception of the study; acquisition of data; revising the article critically for important intellectual content; final approval of the version to be submitted, Loretta Giuliano: Conception of the study; revising the article critically for important intellectual content; final approval of the version to be submitted, Niccolò Castellino: Acquisition of data; revising the article critically for important intellectual content; final approval of the version to be submitted, Claudio Terravecchia: Conception of the study; interpretation of data; drafting the article and revising it critically for important intellectual content; final approval of the version to be submitted, Marco Grillo: Acquisition of data; revising the article critically for important intellectual content; final approval of the version to be submitted, Antonio Longo: Conception of the study; acquisition of data; revising the article critically for important intellectual content; final approval of the version to be submitted, Teresio Avitabile: Conception of the study; revising the article critically for important intellectual content; final approval of the version to be submitted, Mario Zappia: Conception of the study; revising the article critically for important intellectual content; final approval of the version to be submitted, Michele Reibaldi: Conception and design of the study; analysis and interpretation of data; drafting the article and revising it critically for important intellectual content; final approval of the version to be submitted.

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Declaration of competing interest

All authors have approved the final article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.05.031.

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