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Visual dysfunction and its correlation with retinal changes in patients with Parkinson disease.

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SUBTITLE: Visual acuity, contrast sensitivity vision and color vision are affected in Parkinson disease. Visual dysfunction in these patients correlates with structural changes in the retina measured with Spectral domain OCT.

Abstract

Aim: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Parkinson disease (PD).

Methods: Patients with PD (n=37) and controls (n=37) underwent visual acuity (VA), color vision (using the Farnsworth and L´Anthony desaturated D15 color tests), and contrast sensitivity vision (CSV; using the Pelli Robson chart and CSV 1000E test) evaluation to measure visual dysfunction. Structural measurements of the retinal nerve fiber layer (RNFL), and macular and ganglion cell layer (GCL) thicknesses were obtained using spectral domain optical coherence tomography (SD-OCT). Comparison of obtained data and correlation analysis between functional and structural results were performed.

Results: VA (in all different contrast levels) and all CSV spatial frequencies were significantly worse in PD patients than in controls ($P < 0.05$). Color vision was significantly affected ($p<0.05$) based on the L´Anthony color test. Macular thinning was detected in the central, outer (inferior and temporal), and superior sectors ($p<0.05$), and the RNFL had significant thinning in the temporal quadrant ($p<0.05$). Significant GCL loss was observed in the superior and superonasal sectors and the GCL + minimum inner plexiform layer ($p<0.05$). CSV was the functional parameter most strongly correlated with structural measurements in PD. Color vision was associated with most GCL measurements. Macular thickness was strongly correlated with macular volume and functional parameters ($r >0.70$, $p<0.05$).

Conclusions: Patients with PD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. Macular and GCL measurements may be reliable indicators of visual impairment in PD patients.

Strengths and limitations of this study:

The present study provides further information on visual dysfunction in Parkinson disease (PD) patients and corroborates previously published results on this subject. In our study the parameters corresponding to visual acuity (VA), color vision (CV) and contrast sensitivity vision (CSV) were altered in PD patients, and CSV correlated with most of the structural data. We detected significant reductions in the macular, the retinal nerve fiber layer (RNFL), and the ganglion cell layer (GCL) thicknesses. The GCL correlated most with the visual function parameters.

We consider the sample size to be the most important limitation of this study. The small number of patients may have affected the significance of our results compared to previous studies; however, we detected significant reductions in the macular, RNFL, and GCL thicknesses (consisting with previous published results).

The strengths of this study should be resumed by the following points:

- We detected alteration in VA (at different contrast levels), CSV and CV in PD patients. CSV correlated with most of the structural data.
- We detected significant reductions in the macular, RNFL, and GCL thicknesses. The GCL correlated most with the visual function parameters.
- There are only 2 other published articles evaluating the association between visual dysfunction and morphologic parameters. Results provided by these previous studies differ from our results, possibly due to different measurement methods and sample size.
- CV in our study was assessed by L´Anthony´s and Farnsworth D15 color tests, which may provide more specific information about color deficiencies. These tests are not commonly used to evaluate color deficiencies in PD patients.

- Macular thickness and macular volume were strongly associated with functional parameters. This is the first study demonstrating strong correlation between structural and functional visual parameters in PD patients.

Introduction

Foveal vision alterations are associated with Parkinson disease (PD), and seem to be caused by dysfunction of the intraretinal dopaminergic circuitry and final retinal output to the brain.[1] Recent studies demonstrated retinal thinning in PD patients compared with healthy subjects.[2-5]

Several studies report a correlation between functional disability and axonal loss observed in the optic nerve in multiple sclerosis, another neurodegenerative process.[6,7] PD patients are also reported to have decreased contrast sensitivity and color vision, and altered visual evoked potentials.[1,8-13] To our knowledge, however, very few studies have assessed visual dysfunction in PD and its correlation with morphologic parameters.[14,15]

In the present study, we evaluated visual acuity (VA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, contrast sensitivity vision (CSV) using the CSV-1000E test and Pelli-Robson chart, and color vision using the Farnsworth and L´Anthony tests in PD patients and healthy controls to examine the association between visual dysfunction and morphologic parameters.

Material and methods

Thirty-seven eyes of 37 patients with definite PD and 37 eyes of 37 age- and sex-matched healthy individuals were recruited for the study. The study was performed at Miguel Servet University Hospital in Zaragoza, Spain. All procedures adhered to the tenets of the Declaration of Helsinki, and all participants provided informed consent to participate in the study.

The diagnosis of PD was based on standard clinical and neuroimaging criteria.[16] Information about disease severity was assessed using the Hoehn Yahr scale,[17] and disease duration and treatment were recorded. Exclusion criteria were the presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); intraocular pressure ≥21 mmHg; media opacifications; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. The healthy controls had no history and no evidence of ocular or neurologic disease of any nature; their best-corrected visual acuity (BCVA) was >20/30 based on the Snellen scale.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. Visual function was assessed by evaluating BCVA using an ETDRS chart, CSV using the CVS-1000E test and Pelli-Robson chart, and color vision using the Farnsworth desaturated D15 and L´Anthony desaturated D15 tests. Structural analysis of the retina was performed using Spectral domain (SD) optical coherence tomography (OCT) with the Cirrus High definition (HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), which included three different protocols: macular protocol (for macular thickness analysis), RNFL protocol, and ganglion cell protocol (for individual analysis of this layer).

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts - Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions with best correction.

Contrast sensitivity provides more complete information about visual function than visual acuity tests. CSV was evaluated in our patients using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under both monocular and binocular vision at a distance of 1 meter from the chart and under controlled fotopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 meters from the chart under monocular vision at 4 different spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprises four rows with 17 circular patches each. The patches present a grating that decreases in contrast moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardized values.

Color vision was assessed using the Color Vision Recorder (CVR) program. CVR software analyzes chromatic discrimination by classification of colors. The program includes the classic test of Farnsworth 100-hue (FM-100), Farnsworth - Munsell D15, and L´Anthony D15. All patients in the study were evaluated using the Farnsworth - Munsell D15 and L´Anthony D15 protocols and different output parameters such as the Confusion Index (C-index), the Color Confusion Index (CCI), the Confusion angle (Conf Ang), and the Scatter Index (S-index) were recorded.[18,19] The tests were performed under monocular vision.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis. The Cirrus OCT macular cube 512 x 128 protocol provides a macular volume measure and retinal thickness values for nine areas. These areas include a central 1-mm circle representing the fovea, and inner and outer rings measuring 3 mm and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each. The Cirrus OCT optic disc protocol generates images with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the mean, superior, inferior, temporal, and nasal thickness. Cirrus segmentation analysis for retinal layers also provides measurements of the GCL thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal sectors) and measurements of the mean and minimum GCL plus the inner plexiform layer (GCL + IPL) value of a set of 360 spokes, where each average represents the mean number of the pixels along that spoke that lies within the measurement annulus. The minimum is selected because the thinnest portion of the GCL + IPL in the perifoveal region is considered to indicate damage to the ganglion cells.

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). Due to the parametric distribution of the data, differences between evaluations of PD patients and healthy subjects were compared using Student´s t-test. The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of $p < 0.05$ were considered to indicate statistical significance. Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

Results

Thirty-seven patients with PD and 37 healthy controls were included in the study. The mean age of the patients with PD was 69 years (range: 58–74 years) and the mean age of the healthy controls was 68 years (range: 60–76 years). Age (p=0.361), sex (p=0.441), and intraocular pressure (p=0.720) did not differ significantly between healthy controls and patients with PD. Mean time from diagnosis of PD was 13.2 years and the median Hoehn Yahr stage was 2.7.

Functional parameters

PD patients had a lower BCVA at all three contrast levels of the ETDRS chart compared to the controls (0.18±0.26 in patients vs -0.065 ± 0.9 in controls at 100%, p=0.001; 0.59±0.21 vs 0.44±0.13 at 2.50%, p=0.01; and 0.61±0.23 vs 0.58±0.16 at 1.25%, p=0.009). CSV was affected in patients at all four spatial frequencies of the CSV 1000E chart (3, 6, 12, and 18 cpd) when analyzed based on the number of correct localized gratings (p=0.001, <0.001, <0.001, and 0.004 respectively). The Pelli Robson results also revealed a significant reduction in CSV in PD patients (1.71 in patients vs 1.89 in controls, p=0.02). Color vision (L´Anthony test indexes) was also affected in PD. The results are shown in Table 1.

	HEALTHY CONTROLS		PARKINSON DISEASE PATIENTS		SIGNIFICANCE (P)
	Mean	SD	Mean	SD	
VA ETDRS 100	-0.06	0.096	0.18	0.26	**0.001**
VA ETDRS 2.5	0.44	0.13	0.59	0.22	**0.010**
VA ETDRS 1.25	0.58	0.16	0.62	0.23	**0.009**
Pelli Robson	1.89	0.11	1.71	0.17	**0.002**
CSV 1000 3 cpd	1.72	0.16	1.49	0.35	**0.001**
CSV 1000 6 cpd	1.94	0.13	1.62	0.34	**0.000**
CSV 1000 12 cpd	1.62	0.17	1.26	0.41	**0.000**
CSV 1000 18 cpd	1.11	0.22	0.73	0.34	**0.004**
Farnsworth AC CCI	1.11	0.22	0.73	0.34	0.851
Farnsworth C- index	1.10	0.20	1.24	0.42	0.093
Farnsworth CCI	1.07	0.12	1.14	0.24	0.110
Farnsworth Conf Angle	63.90	11.15	65.84	7.49	0.392
Farnsworth S-index	1.56	0.22	1.64	0.39	0.278
Farnsworth time	78.67	28.96	82.91	33.10	0.616
L´Anthony AC CCI	1.05	0.19	1.02	0.18	0.489
L´ Anthony C-index	1.43	0.39	1.64	0.53	0.058
L´ Anthony CCI	1.30	0.23	1.44	0.37	0.066
L´ Anthony Conf Angle	62.31	14.74	71.91	9.25	**0.002**
L´ Anthony S-index	1.69	0.43	1.95	0.48	**0.020**
L´ Anthony time	77.14	25.99	84.09	39.31	0.431

Structural parameters

OCT measurements indicated significant differences in superior macular sectors, in the outer inferior, outer temporal, and central macular thickness (results are shown in Table 2, Figure 1). The segmentation analysis revealed a significantly reduced GCL in PD patients in the superior (81.64±7.08 µm in patients vs 84.55±4.32 µm in controls; p=0.032) and superonasal sectors (81.04±7.23 µm vs 85.28±4.78 µm; p=0.029). The minimum GCL+IPL value was also reduced (80.18±6.19 µm vs 82.45±3.60 µm; p=0.005). The RNFL was significantly reduced in the temporal quadrant in PD patients (Table 2, Figure 1).

	CONTROLS		**PARKINSON DISEASE**		
Structural parameters	Mean	SD	Mean	SD	P
Macular measurements					
Central macular thickness	254.75	17.903	248.96	17.765	**0.028**
Inner superior macular thickness	327.34	13.094	325.73	19.329	**0.019**
Inner nasal macular thickness	328.52	13.263	325.45	17.098	0.091
Inner inferior macular thickness	326.14	13.179	324.82	17.921	0.106
Inner temporal macular thickness	315.90	13.615	312.82	15.760	0.945
Outer superior macular thickness	284.76	9.418	279.44	17.981	**0.008**
Outer nasal macular thickness	302.41	12.167	299.18	17.064	0.074
Outer inferior macular thickness	277.79	10.755	273.76	16.798	**0.045**
Outer temporal macular thickness	271.52	10.992	266.23	18.987	**0.013**
Ganglion cell layer thickness					
Superior	84.55	4.323	81.61	7.087	**0.032**
Superonasal	85.28	4.780	81.04	7.234	**0.029**
Inferonasal	84.66	5.314	81.82	7.521	0.135
Inferior	84.34	5.052	81.91	6.252	0.389
Inferotemporal	85.79	4.003	83.73	4.860	0.233
Temporal	83.76	3.324	82.27	5.312	0.069
Average IPL+GCL	84.83	4.071	82.73	6.230	0.095
Min IPL+ GCL	82.45	3.601	80.18	6.194	**0.005**
RNFL thickness					
Average	96.17	6.714	94.88	11.505	0.105
Superior	117.90	10.965	118.68	16.861	0.115
Nasal	73.59	12.724	72.40	15.182	0.345

Inferior	128.14	14.060	123.20	22.907	0.075
Temporal	64.97	8.218	61.48	10.553	**0.027**

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in PD. The Pelli Robson CSV results correlated with GCL thickness in all sectors, although the association was not strong ($r < 0.5$). The superonasal (r=0.40, p=0.010), inferonasal (r=0.40, p=0.010), inferior (r=0.43, p=0.005), superotemporal sector (r=0.43, p=0.006), and mean GCL+IPL (r=0.45, p=0.004) values had the highest correlations. The Pelli Robson results also correlated with the thickness in different sectors of the RNFL (mean, superior, and inferior sectors). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCL measurements. The superonasal (r= 0.40, p= 0.013) and superotemporal (r= 0.44, p= 0.006) thickness, mean GCL +IPL thickness (r= 0.40, p= 0.012), and the minimum GCL + IPL (r= 0.40, p=0.011) at a spatial frequency of 6 cpd; and the superotemporal (r= 0.41, p= 0.01) thickness and the minimum GCL + IPL thickness (r= 0.43, p=0.006) at a spatial frequency of 18 cpd had the strongest correlations between CSV 1000E and GCL thickness. Spatial frequencies of 6 cpd and 18 cpd were strongly correlated with mean macular thickness (r= 0.79, p= 0.012; r= 0.77, p= 0.016, respectively) and macular volume (r= 0.78, p= 0.013; r= 0.78, p= 0.014, respectively).

Color vision assessed by the L´Anthony test was also associated with the structural parameters: both the L´Anthony CCI and C-index values were significantly correlated with most of the GCL measurements (see Table 3). A significant association between color vision and the RNFL parameters was only found in isolated sectors. All outer macular parameters were significantly correlated with the L´Anthony CCI and C-index results (see Table 3).

	L´Anthony´s color test					
	C-index	p	CCI	p	S-index	p
Macular thickness						
Central	-0.019	0.905	-0.059	0.716	-0.017	0.915
Inner superior	-0.146	0.369	-0.119	0.463	-0.167	0.302
Inner nasal	-0.055	0.735	-0.044	0.788	-0.040	0.807
Inner inferior	-0.073	0.654	-0.064	0.697	-0.074	0.649
Inner temporal	-0.049	0.764	-0.031	0.850	-0.126	0.439
Outer superior	**-0.377**	0.017	**-0.380**	0.015	-0.271	0.090
Outer nasal	**-0.341**	0.031	**-0.323**	0.042	-0.310	0.051
Outer inferior	**-0.360**	0.022	**-0.353**	0.025	**-0.375**	0.017

The strongest correlation was between the mean macular thickness and macular volume and the L´Anthony CCI, C-index, and S-index results. No significant correlations were found between the Farnsworth´s test parameters and structural measurements.

Outer temporal	**-0.360**	0.023	**-0.361**	0.022	**-0.350**	0.027
Macular average	***-0.691***	0.019	***-0.657***	0.028	***-0.709***	0.015
Macular volume	***-0.686***	0.020	***-0.647***	0.032	***-0.709***	0.015
Ganglion cell layer thickness						
Superior	**-0.380**	0.015	**-0.369**	0.019	-0.287	0.072
Superonasal	**-0.383**	0.015	**-0.337**	0.033	**-0.350**	0.027
Inferonasal	**-0.338**	0.033	**-0.313**	0.049	-0.268	0.094
Inferior	**-0.341**	0.031	-0.311	0.051	-0.282	0.078
Inferotemporal	-0.252	0.116	-0.263	0.101	-0.203	0.208
Temporal	**-0.403**	0.010	**-0.437**	0.005	**-0.314**	0.048
Average IPL+ GCL	**-0.381**	0.015	**-0.358**	0.023	**-0.319**	0.045
Minimun IPL+ GCL	**-0.338**	0.033	**-0.326**	0.040	-0.290	0.069

The VA ETDRS results correlated strongly with mean macular thickness and macular volume (see Table 4). There were significant but mild associations between the GCL parameters and VA at 100% (superonasal, inferonasal, and mean GCL + IPL thickness, r=-0.38, p=0.016; r=-0.35, p=0.016; and r=0.35, p=0.029, respectively) and 2.50% (superonasal sector, r=-0.36, p=0.023).

	Macular thickness	P value	Macular volumen	P value
VA ETDRS 100	-0.765	**0.006**	-0.761	**0.007**
VA ETDRS 1.25	-0.718	**0.013**	-0.715	**0.013**
VA ETDRS 2.50	-0.738	**0.010**	-0.729	**0.011**

Discussion

In the present study, we evaluated the visual function parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 37 patients with PD. Parameters corresponding to VA at different contrast levels, and all CSV tests results were altered in PD patients in comparison with healthy subjects. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Color vision was measured with two different tests, the Farnsworth and L´Anthony 15 D tests. These tests provide information for differentiating subjects with severe loss of color vision from those with milder color defects or normal color vision, and also can be used to evaluate acquired loss of color vision. In our study, only the L´Anthony test results were significantly altered in PD patients.

Previous studies have indicated that PD patients lose foveal contrast sensitivity to patterns to which normal observers are most sensitive (i.e., requiring the least contrast for detection).[8,9] Ganglion cells in the retina show adaptation to visual contrast and pool visual inputs over their receptive fields through an array of parallel bipolar cells with smaller receptive fields.[20] In the mammalian retina, color vision and contrast sensitivity are modulated through D1 and D2 receptors. These dopaminergic receptors are differentially located in the retinal layers and a complete lack of activation leads to signal dispersion and alterations in color vision and contrast sensitivity.[2]

Alteration of the retinal layers in PD was first demonstrated in 2004.[21] Since then, various studies have demonstrated different results.[3-5,21,22] A previous study carried out by our team suggested that macular thickness and the inner retinal layers were affected in PD patients.[23]

GCL thickness was inversely correlated with disease duration and PD severity, and predictive of axonal damage in these patients.[25] The present study included a smaller number of patients, which may have affected the significance of our results compared to previous studies. Despite the small number of subjects in the present study, however, we detected significant reductions in the macular, RNFL, and GCL thicknesses. The GCL correlated most with the visual function parameters: GCL thickness was directly associated with VA and CSV measured at all different spatial frequencies, and inversely correlated with the color vision indexes. Thus, GCL thinning is linked to color deficiencies, contrast sensitivity loss, and lower vision at different contrast levels in PD patients.

The degree of correlation is usually classified as low (<0.30), moderate (0.30–0.70), or strong (>0.70). Our results revealed a low and moderate degree of correlation between most parameters, consistent with findings in other neurodegenerative diseases.[24] Macular thickness and macular volume, however, were strongly associated with functional parameters (VA, CS, and L´Anthony CCI, C-index, and S-index). This strong association, to the best of our knowledge, has not been previously demonstrated in PD.

There are very few studies of the correlation between functional and structural parameters in PD patients. Adam et al [14] demonstrated a significant reduction in the inner retinal layer complex (RNFL + GCL + IPL) in PD patients, but no association with contrast sensitivity (measured with the Pelli Robson chart). A very recent study by Kaur et al [15] demonstrated a correlation between functional parameters and GCL thinning, consistent with our results. Kaur et al, however, found no significant alterations in VA or color vision in PD patients and the severity of the disease was not correlated with structural parameters, in contrast to other studies that demonstrate an association between macular and GCL thickness and disease duration and

severity.[23,25] Although the severity of the disease in our sample (based on the Hoehn Yahr scale) was similar to that in Kaur´s study, the duration of the disease in our study was higher than that in Kaur´s patients (13 years vs 5 years), which may account for some of the differences in the results between the two studies. These discrepancies (and similarities) support the need for more studies on this topic. Our results, together with previously published studies,[15,24] suggest that the GCL could be a reliable indicator of structural alterations in the retina of PD patients, demonstrating a significant correlation with functional tests in these patients. The results of the present study have important implications for clinical diagnosis and functional deficits in patients with PD, and highlight the importance of visual function tests in the evaluation of these patients.

In conclusion, visual dysfunction was significantly correlated with morphologic parameters in PD patients. PD patients present with a reduction in macular, RNFL, and GCL thickness, with changes in the GCL being most closely associated with visual dysfunction.

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No additional data available.

Contributorship:

V. Polo: Research project: organization. Statistical analysis and Manuscript: Review and critique.

M. Satue: Research project: Conception, design, organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

MJ Rodrigo: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

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E. Garcia-Martin: Research project: Conception, design, organization and execution. Statistical analysis: Design, execution, review and critique. Manuscript: Writing of the first draft, review and critique.

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Legends

Figure 1: Structural parameter means of macular, ganglion cell and retinal nerve fiber layer thickness obtained with Cirrus HD coherence tomography device, comparing healthy controls and patients with Parkinson's disease. HD, high definition; GCL, ganglion cell layer; IPL, inner plexiform layer; RNFL, retinal nerve fiber layer.

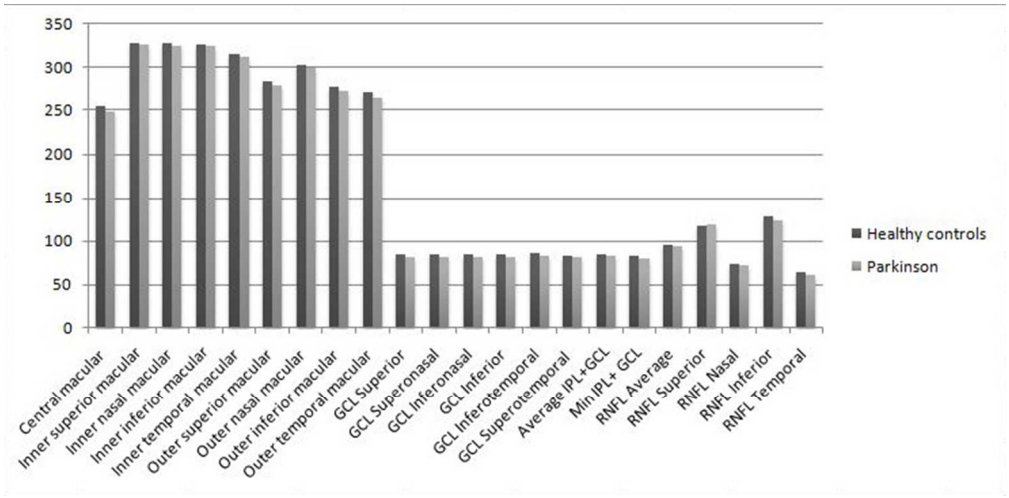
Table 1: Mean and standard deviation (SD) of visual functional parameters in healthy controls and subjects with Parkinson disease. Results in bold letters indicate statistical significance ($p<0.05$). Abbreviations: VA, visual acuity; ETDRS, early treatment diabetic retinopathy study; cpd, cicles per degree; AC CCI, age corrected color confusion index; CCI, color confusion index; C-index, confusion index; Conf Angle, confusion angle; S-index, scatter index; PD, Parkinson disease.

Table 2: Mean and standard deviation (SD) of structural parameters (retinal nerve fiber layer, ganglion cell layer and macular thicknesses) obtained with the Cirrus HD optical coherence tomography device in healthy controls and subjects with Parkinson disease. Bold letters indicate statistical significance ($p<0.05$). Abbreviations: IPL, inner plexiform layer; GCL, ganglion cell layer; RNFL, retinal nerve fiber layer; HD, high definition.

Table 3: Correlation between macular and ganglion cell layer structural measurements and color vision evaluated with L´Anthony´s color test in patients with Parkinson disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Abbreviations: GCL,

ganglion cell layer; IPL, inner plexiform layer; C-index, Confusion index; CCI, color confusion index; S-index, Scatter index.

Table 4: Correlation between visual acuity measured with ETDRS chart at different levels of contrast (in %) and macular structural measurements (thickness and volume) in patients with Parkinson disease. Correlation data in bold type are statistically significant (p value <0.05). Abbreviations: VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.



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Visual dysfunction and its correlation with retinal changes in patients with Parkinson disease.

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SUBTITLE: Visual acuity, contrast sensitivity vision and color vision are affected in Parkinson disease. Visual dysfunction in these patients correlates with structural changes in the retina measured with Spectral domain OCT.

Abstract

Objectives: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Parkinson disease (PD).

Methods: Patients with PD (n=37) and controls (n=37) were included in a observational cross-sectional study and underwent visual acuity (VA), color vision (using the Farnsworth and L´Anthony desaturated D15 color tests), and contrast sensitivity vision (CSV; using the Pelli Robson chart and CSV 1000E test) evaluation to measure visual dysfunction. Structural measurements of the retinal nerve fiber layer (RNFL), and macular and ganglion cell layer (GCL) thicknesses were obtained using spectral domain optical coherence tomography (SD-OCT). Comparison of obtained data and correlation analysis between functional and structural results were performed.

Results: VA (in all different contrast levels) and all CSV spatial frequencies were significantly worse in PD patients than in controls ($P < 0.05$). Color vision was significantly affected ($p<0.05$) based on the L´Anthony color test. Macular thinning was detected in the central, outer (inferior and temporal), and superior (inner and outer) sectors ($p<0.05$), and the RNFL had significant thinning in the temporal quadrant ($p<0.05$). Significant GCL loss was observed in the superior and superonasal sectors and the minimum GCL + inner plexiform layer ($p<0.05$). CSV was the functional parameter most strongly correlated with structural measurements in PD. Color vision was associated with most GCL measurements. Macular thickness was strongly correlated with macular volume and functional parameters ($r >0.70$, $p<0.05$).

Conclusions: Patients with PD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. Macular and GCL measurements may be reliable indicators of visual impairment in PD patients.

Strengths and limitations of this study:

The strengths of this study should be resumed by the following bullet points:

- We detected alteration in VA (at different contrast levels), CSV and CV in PD patients. CSV correlated with most of the structural data.
- We detected significant reductions in the macular, RNFL, and GCL thicknesses. The GCL correlated most with the visual function parameters.
- There are only 2 other published articles evaluating the association between visual dysfunction and morphologic parameters. Results provided by these previous studies differ from our results, possibly due to different measurement methods and sample size.
- CV in our study was assessed by L´Anthony and Farnsworth D15 color tests, which may provide more specific information about color deficiencies. These tests are not commonly used to evaluate color deficiencies in PD patients.
- Macular thickness and macular volume were strongly associated with functional parameters. This is the first time such a strong correlation is reported (r>0.70).

Introduction

Foveal vision alterations are associated with Parkinson disease (PD), and seem to be caused by dysfunction of the intraretinal dopaminergic circuitry and final retinal output to the brain.[1] Recent studies demonstrated retinal thinning in PD patients compared with healthy subjects.[2-5] Several studies report a correlation between functional disability and axonal loss observed in the optic nerve in multiple sclerosis, another neurodegenerative process.[6,7] PD patients are also reported to have decreased contrast sensitivity and color vision, and altered visual evoked potentials.[1,8-13] To our knowledge, however, very few studies have assessed visual dysfunction in PD and its correlation with morphologic parameters.[14,15]

In the present study, we evaluated visual acuity (VA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, contrast sensitivity vision (CSV) using the CSV-1000E test and Pelli-Robson chart, and color vision using the Farnsworth and L´Anthony tests in PD patients and healthy controls to examine the association between visual dysfunction and morphologic parameters.

Material and methods

Thirty-seven eyes of 37 patients with definite PD and 37 eyes of 37 age- and sex-matched healthy individuals were recruited for the study. The study was performed at Miguel Servet University Hospital in Zaragoza, Spain. All procedures adhered to the tenets of the Declaration of Helsinki, and all participants provided informed consent to participate in the study.

The diagnosis of PD was based on standard clinical and neuroimaging criteria.[16] Information about disease severity was assessed using the Hoehn Yahr scale [17] and the Unified Parkinson Disease Rating Scale III (UPDRS) [18]. Disease duration and treatment were recorded. Exclusion criteria were the presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); intraocular pressure ≥21 mmHg; media opacifications; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. The healthy controls had no history and no evidence of ocular or neurologic disease of any nature; their best-corrected visual acuity (BCVA) was >20/30 based on the Snellen scale.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. Visual function was assessed by evaluating BCVA using an ETDRS chart, CSV using the CVS-1000E test and Pelli-Robson chart, and color vision using the Farnsworth desaturated D15 and L´Anthony desaturated D15 tests. Structural analysis of the retina was performed using Spectral domain (SD) optical coherence tomography (OCT) with the Cirrus High definition (HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), which included three different protocols: macular protocol (for macular thickness analysis), RNFL protocol, and ganglion cell protocol (for individual analysis of this layer).

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts - Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions with best correction.

Contrast sensitivity provides more complete information about visual function than visual acuity tests. CSV was evaluated in our patients using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under both monocular and binocular vision at a distance of 1 meter from the chart and under controlled fotopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 meters from the chart under monocular vision at 4 different spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprises four rows with 17 circular patches each. The patches present a grating that decreases in contrast moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardized values.

Color vision was assessed using the Color Vision Recorder (CVR) program. CVR software analyzes chromatic discrimination by classification of colors. The program includes the classic test of Farnsworth 100-hue (FM-100), Farnsworth - Munsell D15, and L´Anthony D15. All patients in the study were evaluated using the Farnsworth - Munsell D15 and L´Anthony D15 protocols and different output parameters such as the Confusion Index (C-index), the Color Confusion Index (CCI), the Confusion angle (Conf Ang), and the Scatter Index (S-index) were recorded.[19,20] The tests were performed under monocular vision.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis. The Cirrus OCT macular cube 512 x 128 protocol provides a macular volume measure and retinal thickness values for nine areas. These areas include a central 1-mm circle representing the fovea, and inner and outer rings measuring 3 mm and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each. The Cirrus OCT optic disc protocol generates images with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal, and nasal thickness. Cirrus segmentation analysis for retinal layers also provides measurements of the GCL thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal sectors) and measurements of the average and minimum GCL plus the inner plexiform layer (GCL + IPL) value of a set of 360 spokes, where each average represents the mean number of the pixels along that spoke that lies within the measurement annulus. The minimum is selected because the thinnest portion of the GCL + IPL in the perifoveal region is considered to indicate damage to the ganglion cells.

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). Due to the parametric distribution of the data, differences between evaluations of PD patients and healthy subjects were compared using Student´s t-test. The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of $p < 0.05$ were considered to indicate statistical significance. Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

Results

Thirty-seven patients with PD and 37 healthy controls were included in the study. The mean age of the patients with PD was 69 years (range: 58–74 years) and the mean age of the healthy controls was 68 years (range: 60–76 years). Age (p=0.361), sex (p=0.441), and intraocular pressure (p=0.720) did not differ significantly between healthy controls and patients with PD. Mean time from diagnosis of PD was 13.2 years. The median Hoehn Yahr stage was 2.7, and the stage of PD based on the UPDRS was 25.06 (range: 7-39; Table 1).

Treatment was divided into three different categories: “Drugs that enhance dopamine levels” (carbidopa, levodopa and rasagiline), “dopaminergic drugs” (pramipexole, ropirinol, rotigotine), and “other”(amitriptiline, propranolol, clonazepam). “Drugs that enhance dopamine levels” was the most prescribed category (89% of patients) and combination therapy with levodopa and carbidopa was the most frequent treatment (44%). Sixty-four percent of treatments were categorized as “dopaminergic”, most of which were used in combination with drugs included in the previous category. A small percentage of patients (9%) were prescribed drugs with no dopaminergic effects.

PARAMETER	CONTROLS	PARKINSON DISEASE	p
Number of eyes (n)	37	37	-
Age, years, range	68 (60–76)	69 (58–74)	0.361
Men:Women (% of men)	24:13 (64.9)	23:14 (62.2)	0.441
Intraocular Pressure	15.58 (2.71)	15.12 (2.98)	0.720
Disease duration, years, mean (SD)	-	13.2 (5.77)	-
Hoehn Yahr, mean (SD)	-	2.7 (0.64)	-
UPDRS, mean (SD)	-	25.06 (8.24)	-

Functional parameters

PD patients had a lower BCVA at all three contrast levels of the ETDRS chart compared to the controls (0.18±0.26 in patients vs -0.065 ± 0.9 in controls at 100%, p=0.001; 0.59±0.21 vs 0.44±0.13 at 2.50%, p=0.01; and 0.61±0.23 vs 0.58±0.16 at 1.25%, p=0.009). CSV was affected in patients at all four spatial frequencies of the CSV 1000E chart (3, 6, 12, and 18 cpd) when analyzed based on the number of correct localized gratings (p=0.001, <0.001, <0.001, and 0.004 respectively). The Pelli Robson results also revealed a significant reduction in CSV in PD patients (1.71 in patients vs 1.89 in controls, p=0.02). Color vision (L´Anthony test indexes) was also affected in PD. The results are shown in Table 2.

	HEALTHY CONTROLS		PARKINSON DISEASE PATIENTS		SIGNIFICANCE (P)
	Mean	SD	Mean	SD	
VA ETDRS 100	-0.06	0.096	0.18	0.26	**0.001**
VA ETDRS 2.5	0.44	0.13	0.59	0.22	**0.010**
VA ETDRS 1.25	0.58	0.16	0.62	0.23	**0.009**
Pelli Robson	1.89	0.11	1.71	0.17	**0.002**
CSV 1000 3 cpd	1.72	0.16	1.49	0.35	**0.001***
CSV 1000 6 cpd	1.94	0.13	1.62	0.34	**0.000***
CSV 1000 12 cpd	1.62	0.17	1.26	0.41	**0.000***
CSV 1000 18 cpd	1.11	0.22	0.73	0.34	**0.004***
Farnsworth AC CCI	1.11	0.22	0.73	0.34	0.851
Farnsworth C- index	1.10	0.20	1.24	0.42	0.093
Farnsworth CCI	1.07	0.12	1.14	0.24	0.110
Farnsworth Conf Angle	63.90	11.15	65.84	7.49	0.392
Farnsworth S-index	1.56	0.22	1.64	0.39	0.278
Farnsworth time	78.67	28.96	82.91	33.10	0.616
L´Anthony AC CCI	1.05	0.19	1.02	0.18	0.489
L´ Anthony C-index	1.43	0.39	1.64	0.53	0.058
L´ Anthony CCI	1.30	0.23	1.44	0.37	0.066
L´ Anthony Conf Angle	62.31	14.74	71.91	9.25	**0.002***
L´ Anthony S-index	1.69	0.43	1.95	0.48	**0.020**
L´ Anthony time	77.14	25.99	84.09	39.31	0.431

Structural parameters

OCT measurements indicated significant differences in superior macular sectors, in the outer inferior, outer temporal, and central macular thickness (results are shown in Table 2, Figure 1). The segmentation analysis revealed a significantly reduced GCL in PD patients in the superior (81.64±7.08 µm in patients vs 84.55±4.32 µm in controls; p=0.032) and superonasal sectors (81.04±7.23 µm vs 85.28±4.78 µm; p=0.029). The minimum GCL+IPL value was also reduced

(80.18±6.19 µm vs 82.45±3.60 µm; p=0.005). The RNFL was significantly reduced in the temporal quadrant in PD patients (Table 3).

	CONTROLS		PARKINSON DISEASE		
Structural parameters	Mean	SD	Mean	SD	P
Macular measurements					
Central macular thickness	254.75	17.903	248.96	17.765	**0.028**
Inner superior macular thickness	327.34	13.094	325.73	19.329	**0.019**
Inner nasal macular thickness	328.52	13.263	325.45	17.098	0.091
Inner inferior macular thickness	326.14	13.179	324.82	17.921	0.106
Inner temporal macular thickness	315.90	13.615	312.82	15.760	0.945
Outer superior macular thickness	284.76	9.418	279.44	17.981	**0.008**
Outer nasal macular thickness	302.41	12.167	299.18	17.064	0.074
Outer inferior macular thickness	277.79	10.755	273.76	16.798	**0.045**
Outer temporal macular thickness	271.52	10.992	266.23	18.987	**0.013**
Ganglion cell layer thickness					
Superior	84.55	4.323	81.61	7.087	**0.032**
Superonasal	85.28	4.780	81.04	7.234	**0.029**
Inferonasal	84.66	5.314	81.82	7.521	0.135
Inferior	84.34	5.052	81.91	6.252	0.389
Inferotemporal	85.79	4.003	83.73	4.860	0.233
Temporal	83.76	3.324	82.27	5.312	0.069
Average IPL+GCL	84.83	4.071	82.73	6.230	0.095
Min IPL+ GCL	82.45	3.601	80.18	6.194	**0.005***
RNFL thickness					
Average	96.17	6.714	94.88	11.505	0.105
Superior	117.90	10.965	118.68	16.861	0.115
Nasal	73.59	12.724	72.40	15.182	0.345
Inferior	128.14	14.060	123.20	22.907	0.075
Temporal	64.97	8.218	61.48	10.553	**0.027**

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in PD. The Pelli Robson CSV results correlated with GCL thickness in all sectors, although the

association was not strong (r < 0.5). The superonasal (r=0.40, p=0.010), inferonasal (r=0.40, p=0.010), inferior (r=0.43, p=0.005), superotemporal sector (r=0.43, p=0.006), and average GCL+IPL (r=0.45, p=0.004) values had the highest correlations. The Pelli Robson results also correlated with the thickness in different sectors of the RNFL (average, superior, and inferior sectors). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCL measurements. The superonasal (r= 0.40, p= 0.013) and superotemporal (r= 0.44, p= 0.006) thickness, average GCL +IPL thickness (r= 0.40, p= 0.012), and the minimum GCL + IPL (r= 0.40, p=0.011) at a spatial frequency of 6 cpd; and the superotemporal (r= 0.41, p= 0.01) thickness and the minimum GCL + IPL thickness (r= 0.43, p=0.006) at a spatial frequency of 18 cpd had the strongest correlations between CSV 1000E and GCL thickness. Spatial frequencies of 6 cpd and 18 cpd were strongly correlated with average macular thickness (r= 0.79, p= 0.012; r= 0.77, p= 0.016, respectively) and macular volume (r= 0.78, p= 0.013; r= 0.78, p= 0.014, respectively).

Color vision assessed by the L´Anthony test was also associated with the structural parameters: The C-index and CCI results were significantly correlated with all outer macular parameters and most of the GCL measurements (see Table 4). A significant association between color vision and the RNFL parameters was only found in isolated sectors. (see Table 4).

	L´Anthony color test					
	C-index	p	CCI	p	S-index	p
Macular thickness						
Central	-0.019	0.905	-0.059	0.716	-0.017	0.9[illegible]
Inner superior	-0.146	0.369	-0.119	0.463	-0.167	0.3[illegible]
Inner nasal	-0.055	0.735	-0.044	0.788	-0.040	0.8[illegible]
Inner inferior	-0.073	0.654	-0.064	0.697	-0.074	0.6[illegible]
Inner temporal	-0.049	0.764	-0.031	0.850	-0.126	0.4[illegible]
Outer superior	**-0.377**	0.017	**-0.380**	0.015	-0.271	0.0[illegible]
Outer nasal	**-0.341**	0.031	**-0.323**	0.042	-0.310	0.0[illegible]
Outer inferior	**-0.360**	0.022	**-0.353**	0.025	**-0.375**	0.0[illegible]
Outer temporal	**-0.360**	0.023	**-0.361**	0.022	**-0.350**	0.0[illegible]
Macular average	***-0.691***	0.019	***-0.657***	0.028	***-0.709***	0.0[illegible]
Macular volume	***-0.686***	0.020	***-0.647***	0.032	***-0.709***	0.0[illegible]
Ganglion cell layer thickness						
Superior	**-0.380**	0.015	**-0.369**	0.019	-0.287	0.0[illegible]
Superonasal	**-0.383**	0.015	**-0.337**	0.033	**-0.350**	0.0[illegible]
Inferonasal	**-0.338**	0.033	**-0.313**	0.049	-0.268	0.0[illegible]
Inferior	**-0.341**	0.031	-0.311	0.051	-0.282	0.0[illegible]
Inferotemporal	-0.252	0.116	-0.263	0.101	-0.203	0.2[illegible]
Temporal	**-0.403**	0.010	**-0.437**	0.005	**-0.314**	0.0[illegible]
Average IPL+ GCL	**-0.381**	0.015	**-0.358**	0.023	**-0.319**	0.0[illegible]
Minimum IPL+ GCL	**-0.338**	0.033	**-0.326**	0.040	-0.290	0.0[illegible]

The strongest correlation was between the average macular thickness and macular volume and the L´Anthony CCI, C-index, and S-index results. No significant correlations were found between the Farnsworth´s test parameters and structural measurements.

The VA ETDRS results correlated strongly with average macular thickness and macular volume (see Table 5, Figures 1 and 2). There were significant but mild associations between the GCL parameters and VA at 100% (superonasal, inferonasal, and average GCL + IPL thickness, r=-

0.38, p=0.016; r=-0.35, p=0.016; and r=0.35, p=0.029, respectively) and 2.50% (superonasal sector, r=-0.36, p=0.023).

	Macular thickness	P value	Macular volume	P value
VA ETDRS 100	-0.765	**0.006**	-0.761	**0.007**
VA ETDRS 1.25	-0.718	**0.013**	-0.715	**0.013**
VA ETDRS 2.50	-0.738	**0.010**	-0.729	**0.011**

There was a significant correlation between Hoehn Yahr score and VA contrast level 2.50% (r=0.48, p=0.040), and CS measured with CSV 1000 at a space frequency of 12 cpd (r=-0.59, p=0.038). No correlations were detected between structural and disease severity parameters.

Discussion

In the present study, we evaluated the visual function parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 37 patients with PD. Parameters corresponding to VA at different contrast levels, and all CSV tests results were altered in PD patients in comparison with healthy subjects, prior to and after statistical correction for multiple tests. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Color vision was measured with two different tests, the Farnsworth and L´Anthony 15 D tests. These tests provide information for differentiating subjects with severe loss of color vision from those with milder color defects or normal color vision, and also can be used to evaluate acquired loss of color vision. In our study, only the L´Anthony test results were significantly altered in PD patients. L´Anthony test is less saturated than the Farnsworth color test, thus it is designed to detect more subtle color deficiencies. Our patients performed worse than controls in both tests (higher C-index and S-

index, reaching ranges similar to protanomalies) although only the differences in L´Anthony S-index were statistically significant, indicating that our patients had a (subtle) protanomaly (S-index of 1.95).

Previous studies have indicated that PD patients lose foveal contrast sensitivity to patterns to which normal observers are most sensitive (i.e., requiring the least contrast for detection).[8,9] Ganglion cells in the retina show adaptation to visual contrast and pool visual inputs over their receptive fields through an array of parallel bipolar cells with smaller receptive fields.[21] The parvo- and magnocellular ganglion cells are located in the RGC layer and take two different pathways for the identification of color and contrast at different frequencies.[22] RGC loss (as observed using SD-OCT) was recently identified as the cause of visual impairment in patients suffering from another neurodegenerative process, multiple sclerosis.[23] Thus, a similar process could be the cause of the contrast and color deficiencies in patients with PD. In addition, in the mammalian retina, color vision and contrast sensitivity are modulated through D1 and D2 receptors. These dopaminergic receptors are differentially located in the retinal layers and a complete lack of activation leads to signal dispersion and alterations in color vision and contrast sensitivity.[2]

Alteration of the retinal layers in PD was first demonstrated in 2004.[24] Since then, various studies have demonstrated different results.[3-5,24-27] Previous studies performed by our team confirmed that both macular thickness and the RNFL were affected in patients with PD, especially in the inferior and temporal quadrants.[4,5, 28] Moreover, Garcia-Martin et al demonstrated that the inner retinal layers were most affected in these patients, and that the GCL thickness was inversely correlated with disease duration and PD severity, and was predictive of axonal damage in these patients.[29] The present study included a smaller number of patients,

which may have affected the significance of our results compared to previous studies. Despite the small number of subjects in the present study, however, we detected significant reductions in the macular, RNFL, and GCL thicknesses. A significant reduction in the temporal sectors of the peripapillary RNFL thickness has been repeatedly observed by different groups [30, 31] and was confirmed in the present study. Two recent studies, however, detected no differences in the peripapillary RNFL thickness of PD patients compared to healthy controls using SD-OCT [26,27] and one study only found significant differences in the nasal quadrant.[32] More studies are required to clarify these contradictory observations.

In a previous study, we demonstrated that the retinal thickness corresponding to the papillomacular bundle (as measured with the Axonal Analytics software for Spectralis OCT) correlated ($r>0.70$) with some functional parameters (such as the mean defect and the pattern standard deviation of the automated perimetry) in patients with PD.[33] The GCL was not investigated at that time, however, and visual function parameters were reduced to perimetry and color vision measured with the Ishihara color test. The current study evaluated not only the RNFL but also the GCL thickness, and more visual function parameters were analyzed. The GCL correlated most with the visual function parameters: GCL thickness was directly associated with VA and CSV measured at all different spatial frequencies, and inversely correlated with the color vision indexes. Thus, GCL thinning is linked to color deficiencies, contrast sensitivity loss, and lower vision at different contrast levels in PD patients.

The degree of correlation is usually classified as low (<0.30), moderate (0.30–0.70), or strong (>0.70). Our results revealed a low and moderate degree of correlation between most parameters, consistent with findings in other neurodegenerative diseases.[34] Macular thickness and macular volume, however, were strongly associated with functional parameters (VA, CS, and L´Anthony

CCI, C-index, and S-index). This strong association, to the best of our knowledge, has not been previously demonstrated in PD.

There are very few studies of the correlation between functional and structural parameters in PD patients. Adam et al [14] demonstrated a significant reduction in the inner retinal layer complex (RNFL + GCL + IPL) in PD patients, but no association with contrast sensitivity (measured with the Pelli Robson chart). A very recent study by Kaur et al [15] demonstrated a correlation between functional parameters and GCL thinning, consistent with our results. Kaur et al, however, found no significant alterations in VA or color vision in PD patients and the severity of the disease was not correlated with structural parameters, in contrast to other studies that demonstrate an association between macular and GCL thickness and disease duration and severity.[28,29] Although the severity of the disease in our sample (based on the Hoehn Yahr scale) was similar to that in Kaur´s study, the duration of the disease in our study was higher than that in Kaur´s patients (13 years vs 5 years), which may account for some of the differences in the results between the two studies. These discrepancies (and similarities) support the need for more studies on this topic. Our results, together with previously published studies,[15, 29] suggest that the GCL could be a reliable indicator of structural alterations in the retina of PD patients, demonstrating a significant correlation with functional tests in these patients. The results of the present study have important implications for clinical diagnosis and functional deficits in patients with PD, and highlight the importance of visual function tests in the evaluation of these patients.

In conclusion, visual dysfunction was significantly correlated with morphologic parameters in PD patients. PD patients present with a reduction in macular, RNFL, and GCL thickness, with changes in the GCL being most closely associated with visual dysfunction.

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Competing interest:

The authors disclose no conflict of interest.

Data sharing:

No additional data available.

Contributorship:

V. Polo: Research project: organization. Statistical analysis and Manuscript: Review and critique.

M. Satue: Research project: Conception, design, organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

MJ Rodrigo: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

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MI. Fuertes: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

JM. Larrosa: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

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review and critique.

E. Garcia-Martin: Research project: Conception, design, organization and execution. Statistical analysis: Design, execution, review and critique. Manuscript: Writing of the first draft, review and critique.

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Legends

Figure 1 Correlation between the average macular thickness and visual acuity as measured with ETDRS optotipe at a contrast level of 100% in patients with Parkinson's disease.

Figure 2: Correlation between macular volume and visual acuity as measured with ETDRS optotipe at a contrast level of 100% in patients with Parkinson's disease.

Table 1: Epidemiologic and disease characteristics of patients with PD and healthy subjects, and statistical significance (P). Abbreviations: SD, standard deviation; UPDRS, Unified Parkinson Disease Rating Scale.

Table 2: Mean and standard deviation (SD) of visual functional parameters in healthy controls and subjects with Parkinson disease. Results in bold letters indicate statistical significance ($p<0.05$). The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0125$ for CSV 1000E measurements; $p<0.0083$ for Farnsworth and L´Anthony tests). Abbreviations: VA, visual acuity; ETDRS, early treatment diabetic retinopathy study; cpd, cycles per degree; AC CCI, age corrected color confusion index; CCI, color confusion index; C-index, confusion index; Conf Angle, confusion angle; S-index, scatter index; PD, Parkinson disease.

Table 3: Mean and standard deviation (SD) of structural parameters (retinal nerve fiber layer, ganglion cell layer and macular thicknesses) obtained with the Cirrus HD optical coherence

tomography device in healthy controls and subjects with Parkinson disease. Bold letters indicate statistical significance ($p<0.05$). The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0055$ for macular measurements; $p<0.0062$ for ganglion cell measurements and $p<0.01$ for RNFL measurements). Abbreviations: IPL, inner plexiform layer; GCL, ganglion cell layer; RNFL, retinal nerve fiber layer; HD, high definition.

Table 4: Correlation between macular and ganglion cell layer structural measurements and color vision evaluated with L'Anthony color test in patients with Parkinson disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; C-index, Confusion index; CCI, color confusion index; S-index, Scatter index.

Table 5: Correlation between visual acuity measured with ETDRS chart at different levels of contrast (in %) and macular structural measurements (thickness and volume) in patients with Parkinson disease. Correlation data in bold type are statistically significant (p value <0.05). Abbreviations: VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

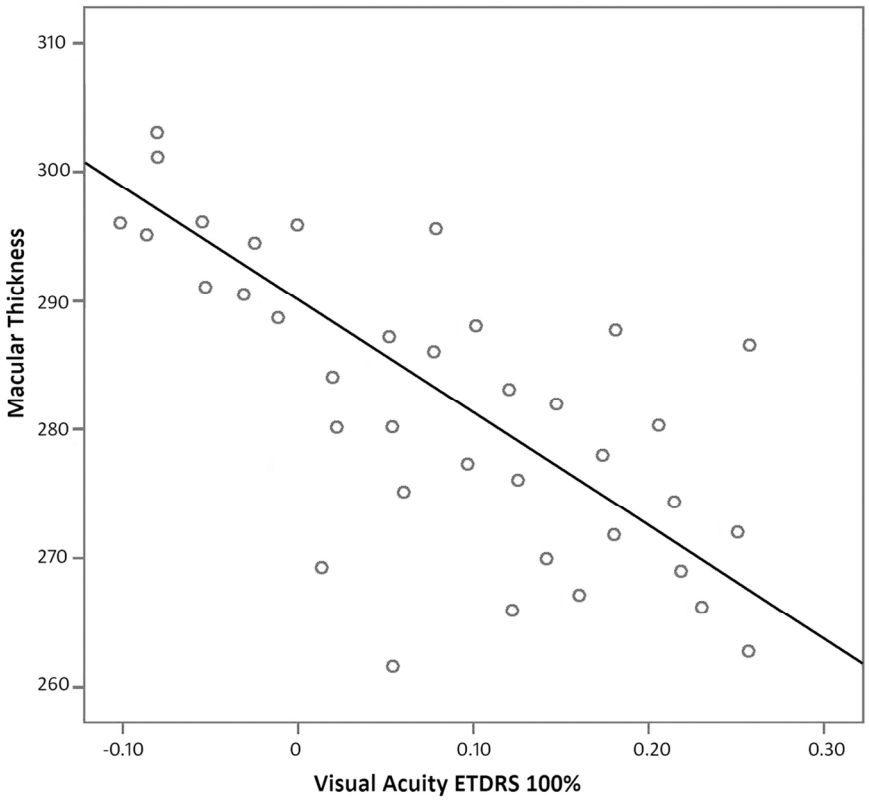

Figure 1: Correlation between the average macular thickness and visual acuity as measured with ETDRS optotipe at a contrast level of 100% in patients with Parkinson's disease.
118x100mm (300 x 300 DPI)

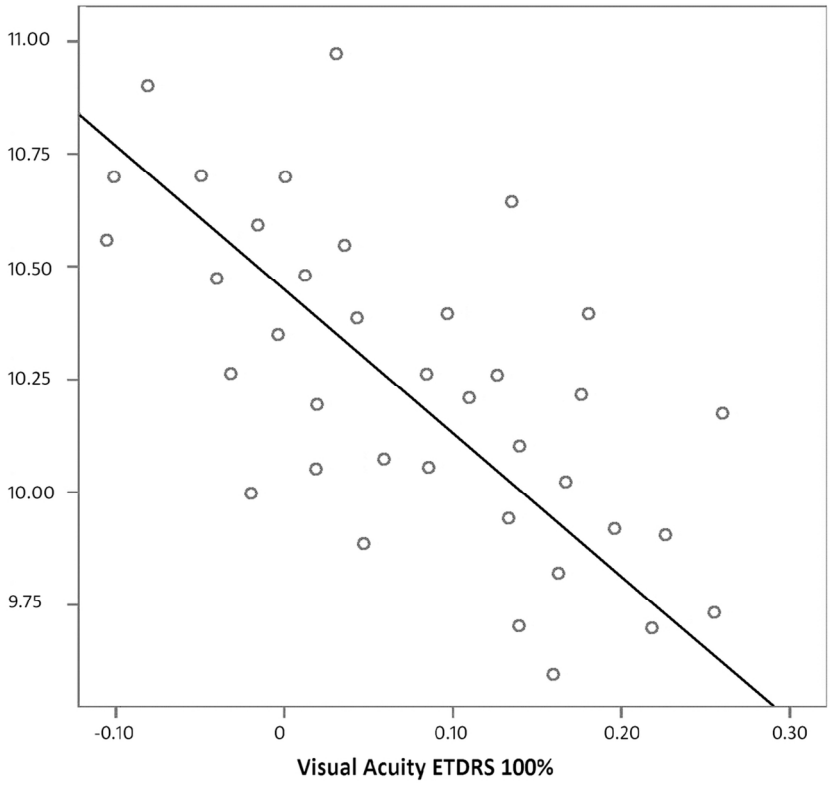

Figure 2: Correlation between macular volume and visual acuity as measured with ETDRS optotipe at a contrast level of 100% in patients with Parkinson's disease.
119x102mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	*(*a) Indicate the study's design with a commonly used term in the title or the abstract Check. This included in the abstract, methods, line 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Check. This is included in the abstract. Methods, from line 2 to results section line 9
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Check. This is included in the introduction. Paragraph 2.
Objectives	3	State specific objectives, including any prespecified hypotheses Check. This is included in the introduction. Paragraph 3.
Methods		
Study design	4	Present key elements of study design early in the paper Check. This is included in Methods, paragraph 1-3.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Check. This is included in Methods, paragraph 1-3.
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Check. This is included in Methods, paragraph 2
		(*b*) For matched studies, give matching criteria and the number of controls per case Check. This is included in Methods, paragraph 1
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Check. Outcomes and variables are explained in Methods, paragraphs 4-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Check. Details of measurements are included in paragraphs 4-8.
Bias	9	Describe any efforts to address potential sources of bias Check. This is included in paragraph 8, line 3-4.
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Check. Quantitative variables are explained in Methods, paragraphs 4-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding. Check. This is included in methods, paragraph 9
		(*b*) Describe any methods used to examine subgroups and interactions. Not applicable.
		(*c*) Explain how missing data were addressed Not applicable
		(*d*) If applicable, explain how matching of cases and controls was addressed Not applicable
		(*e*) Describe any sensitivity analyses Not applicable
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram This is a cross sectional study. Inclusion criteria were explained in methods. All elegible subjects were included in the study (37) as already explained, all completed the evaluation.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Check. This is included in Results, paragraph 1-2 and table 1.
		(b) Indicate number of participants with missing data for each variable of interest Not applicable.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure Check. Numbers and results of each variable are included in results.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Check. Main results include statistical results prior and post multiple comparisons adjustment.
		(*b*) Report category boundaries when continuous variables were categorized Check. Category of correlation is explained in discussion, paragraph 5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Not applicable
Discussion		
Key results	18	Summarise key results with reference to study objectives Check. This is included in results, paragraph 1.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Check, this is mentioned in paragraph 3 and 6.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Check. This is included and discussed along the discussion section.
Generalisability	21	Discuss the generalisability (external validity) of the study results Check. This is included and discussed along the discussion section
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Check. Not applicable.

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Visual dysfunction and its correlation with retinal changes in patients with Parkinson disease

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Visual dysfunction and its correlation with retinal changes in patients with Parkinson disease: an observational prospective study

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RUNNING TITLE: OCT and visual dysfunction in Parkinson disease

STUDY DESIGN AND SETTING: An observational prospective study, carried out at Miguel Servet University Hospital, in Zaragoza, Spain.

KEY WORDS: Parkinson disease, visual function, contrast sensitivity, ganglion cell layer.

WORD COUNT: 2500 words.

All subjects provided detailed consent to participate in this study, which was conducted in accordance with the guidelines established by the Ethics Committee of the Miguel Servet Hospital and based on the principles of the Declaration of Helsinki.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

The authors disclose no conflict of interest.

SUBTITLE: Visual acuity, contrast sensitivity vision and color vision are affected in Parkinson disease. Visual dysfunction in these patients correlates with structural changes in the retina measured with Spectral domain OCT.

Abstract

Objectives: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Parkinson disease (PD).

Methods: Patients with PD (n=37) and controls (n=37) were included in an observational cross-sectional study and underwent visual acuity (VA), color vision (using the Farnsworth and L´Anthony desaturated D15 color tests), and contrast sensitivity vision (CSV; using the Pelli Robson chart and CSV 1000E test) evaluation to measure visual dysfunction. Structural measurements of the retinal nerve fiber layer (RNFL), and macular and ganglion cell layer (GCL) thicknesses were obtained using spectral domain optical coherence tomography (SD-OCT). Comparison of obtained data and correlation analysis between functional and structural results were performed.

Results: VA (in all different contrast levels) and all CSV spatial frequencies were significantly worse in PD patients than in controls ($P < 0.05$). Color vision was significantly affected ($p<0.05$) based on the L´Anthony color test. Macular thinning was detected in the central, outer (inferior and temporal), and superior (inner and outer) sectors ($p<0.05$), and the RNFL had significant thinning in the temporal quadrant ($p<0.05$). Significant GCL loss was observed in the superior and superonasal sectors and the minimum GCL + inner plexiform layer ($p<0.05$). CSV was the functional parameter most strongly correlated with structural measurements in PD. Color vision was associated with most GCL measurements. Macular thickness was strongly correlated with macular volume and functional parameters ($r >0.70$, $p<0.05$).

Conclusions: Patients with PD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. Macular and GCL measurements may be reliable indicators of visual impairment in PD patients.

Strengths and limitations of this study:

The strengths of this study should be resumed by the following bullet points:

- We detected alteration in VA (at different contrast levels), CSV and CV in PD patients. CSV correlated with most of the structural data.
- We detected significant reductions in the macular, RNFL, and GCL thicknesses. The GCL correlated most with the visual function parameters.
- There are only 2 other published articles evaluating the association between visual dysfunction and morphologic parameters. Results provided by these previous studies differ from our results, possibly due to different measurement methods and sample size.
- CV in our study was assessed by L´Anthony and Farnsworth D15 color tests, which may provide more specific information about color deficiencies. These tests are not commonly used to evaluate color deficiencies in PD patients.
- Macular thickness and macular volume were strongly associated with functional parameters. This is the first time such a strong correlation is reported (r>0.70).

Introduction

Foveal vision alterations are associated with Parkinson disease (PD), and seem to be caused by dysfunction of the intraretinal dopaminergic circuitry and final retinal output to the brain.[1] Recent studies demonstrated retinal thinning in PD patients compared with healthy subjects.[2-5] Several studies report a correlation between functional disability and axonal loss observed in the optic nerve in multiple sclerosis, another neurodegenerative process.[6,7] PD patients are also reported to have decreased contrast sensitivity and color vision, and altered visual evoked potentials.[1,8-13] To our knowledge, however, very few studies have assessed visual dysfunction in PD and its correlation with morphologic parameters.[14,15]

In the present study, we evaluated visual acuity (VA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, contrast sensitivity vision (CSV) using the CSV-1000E test and Pelli-Robson chart, and color vision using the Farnsworth and L´Anthony tests in PD patients and healthy controls to examine the association between visual dysfunction and morphologic parameters.

Material and methods

Thirty-seven eyes of 37 patients with definite PD and 37 eyes of 37 age- and sex-matched healthy individuals were recruited for the study. The study was performed at Miguel Servet University Hospital in Zaragoza, Spain. All procedures adhered to the tenets of the Declaration of Helsinki, and all participants provided informed consent to participate in the study.

The diagnosis of PD was based on standard clinical and neuroimaging criteria.[16] Information about disease severity was assessed using the Hoehn Yahr scale [17] and the Unified Parkinson Disease Rating Scale part III score (UPDRS III) [18]. Disease duration and treatment were recorded. Exclusion criteria were the presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); intraocular pressure ≥21 mmHg; media opacifications; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. The healthy controls had no history and no evidence of ocular or neurologic disease of any nature; their best-corrected visual acuity (BCVA) was >20/30 based on the Snellen scale.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. Visual function was assessed by evaluating BCVA using an ETDRS chart, CSV using the CVS-1000E test and Pelli-Robson chart, and color vision using the Farnsworth desaturated D15 and L´Anthony desaturated D15 tests. Structural analysis of the retina was performed using Spectral domain (SD) optical coherence tomography (OCT) with the Cirrus High definition (HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), which included three different protocols: macular protocol (for macular thickness analysis), RNFL protocol, and ganglion cell protocol (for individual analysis of this layer).

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts - Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions with best correction.

Contrast sensitivity provides more complete information about visual function than visual acuity tests. CSV was evaluated in our patients using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under both monocular and binocular vision at a distance of 1 meter from the chart and under controlled fotopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 meters from the chart under monocular vision at 4 different spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprises four rows with 17 circular patches each. The patches present a grating that decreases in contrast moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardized values.

Color vision was assessed using the Color Vision Recorder (CVR) program. CVR software analyzes chromatic discrimination by classification of colors. The program includes the classic test of Farnsworth 100-hue (FM-100), Farnsworth - Munsell D15, and L´Anthony D15. All patients in the study were evaluated using the Farnsworth - Munsell D15 and L´Anthony D15 protocols and different output parameters such as the Confusion Index (C-index), the Color Confusion Index (CCI), the Confusion angle (Conf Ang), and the Scatter Index (S-index) were recorded.[19,20] The tests were performed under monocular vision.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis. The Cirrus OCT macular cube 512 x 128 protocol provides a macular volume measure and retinal thickness values for nine areas. These areas include a central 1-mm circle representing the fovea, and inner and outer rings measuring 3 mm and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each. The Cirrus OCT optic disc protocol generates images with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal, and nasal thickness. Cirrus segmentation analysis for retinal layers also provides measurements of the GCL thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal sectors) and measurements of the average and minimum GCL plus the inner plexiform layer (GCL + IPL) value of a set of 360 spokes, where each average represents the mean number of the pixels along that spoke that lies within the measurement annulus. The minimum is selected because the thinnest portion of the GCL + IPL in the perifoveal region is considered to indicate damage to the ganglion cells.

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). Due to the parametric distribution of the data, differences between evaluations of PD patients and healthy subjects were compared using Student´s t-test. To avoid a high false positive rate, the Bonferroni correction for multiple comparisons was calculated. The level of significance for each variable was established based on Bonferroni calculations.

The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of $p < 0.05$ were considered to indicate a significant correlation. Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

Results

Thirty-seven patients with PD and 37 healthy controls were included in the study. The mean age of the patients with PD was 69 years (range: 58–74 years) and the mean age of the healthy controls was 68 years (range: 60–76 years). Age (p=0.361), sex (p=0.441), and intraocular pressure (p=0.720) did not differ significantly between healthy controls and patients with PD. Mean time from diagnosis of PD was 13.2 years. The median Hoehn Yahr stage was 2.7, and the stage of PD based on the UPDRS III was 25.06 (range: 7-39; Table 1).

Treatment was divided into three different categories: "Drugs that enhance dopamine levels" (carbidopa, levodopa and rasagiline), "dopaminergic drugs" (pramipexole, ropirinol, rotigotine), and "other"(amitriptiline, propranolol, clonazepam). "Drugs that enhance dopamine levels" was the most prescribed category (89% of patients) and combination therapy with levodopa and carbidopa was the most frequent treatment (44%). Sixty-four percent of treatments were categorized as "dopaminergic", most of which were used in combination with drugs included in the previous category. A small percentage of patients (9%) were prescribed drugs with no dopaminergic effects.

PARAMETER	CONTROLS	PARKINSON DISEASE	p
Number of eyes (n)	37	37	-
Age, years, range	68 (60–76)	69 (58–74)	0.361
Men:Women (% of men)	24:13 (64.9)	23:14 (62.2)	0.441
Intraocular Pressure	15.58 (2.71)	15.12 (2.98)	0.720
Disease duration, years, mean (SD)	-	13.2 (5.77)	-
Hoehn Yahr, mean (SD)	-	2.7 (0.64)	-
UPDRS III, mean (SD)	-	25.06 (8.24)	-

Functional parameters

PD patients had a lower BCVA at all three contrast levels of the ETDRS chart compared to the controls (0.18±0.26 in patients vs -0.065 ± 0.9 in controls at 100%, p=0.001; 0.59±0.21 vs 0.44±0.13 at 2.50%, p=0.010; and 0.61±0.23 vs 0.58±0.16 at 1.25%, p=0.009). CSV was affected in patients at all four spatial frequencies of the CSV 1000E chart (3, 6, 12, and 18 cpd) when analyzed based on the number of correct localized gratings (p=0.001, <0.001, <0.001, and 0.004 respectively). The Pelli Robson results also revealed a significant reduction in CSV in PD patients (1.71 in patients vs 1.89 in controls, p=0.02). Color vision (Conf Angle in L´Anthony test) was also affected in PD. The results are shown in Table 2.

	HEALTHY CONTROLS		PARKINSON DISEASE PATIENTS		SIGNIFICANCE (P)
	Mean	SD	Mean	SD	
VA ETDRS 100	-0.06	0.096	0.18	0.26	0.001*
VA ETDRS 2.5	0.44	0.13	0.59	0.22	0.010*
VA ETDRS 1.25	0.58	0.16	0.62	0.23	0.009*
Pelli Robson	1.89	0.11	1.71	0.17	0.002*
CSV 1000 3 cpd	1.72	0.16	1.49	0.35	0.001*
CSV 1000 6 cpd	1.94	0.13	1.62	0.34	<0.001*
CSV 1000 12 cpd	1.62	0.17	1.26	0.41	<0.001*
CSV 1000 18 cpd	1.11	0.22	0.73	0.34	0.004*
Farnsworth AC CCI	1.11	0.22	0.73	0.34	0.851
Farnsworth C- index	1.10	0.20	1.24	0.42	0.093
Farnsworth CCI	1.07	0.12	1.14	0.24	0.110
Farnsworth Conf Angle	63.90	11.15	65.84	7.49	0.392
Farnsworth S-index	1.56	0.22	1.64	0.39	0.278
Farnsworth time	78.67	28.96	82.91	33.10	0.616
L´Anthony AC CCI	1.05	0.19	1.02	0.18	0.489
L´ Anthony C-index	1.43	0.39	1.64	0.53	0.058
L´ Anthony CCI	1.30	0.23	1.44	0.37	0.066
L´ Anthony Conf Angle	62.31	14.74	71.91	9.25	0.002*
L´ Anthony S-index	1.69	0.43	1.95	0.48	0.020
L´ Anthony time	77.14	25.99	84.09	39.31	0.431

Structural parameters

Based on Bonferroni corrections, OCT measurements indicated a significant difference in the minimum GCL+IPL value (80.18±6.19 µm vs 82.45±3.60 µm; p=0.005). However, we observed a clear tendency towards a reduction in superior macular sectors, in the outer inferior, outer temporal, and central macular thickness in PD patients compared to controls: the p value for these variables was <0.05 but did not meet Bonferroni significance (results are shown in Table 2). The segmentation analysis revealed a tendency towards reduced GCL in PD patients in the superior (81.64±7.08 µm in patients vs 84.55±4.32 µm in controls; p=0.032) and superonasal sectors (81.04±7.23 µm vs 85.28±4.78 µm; p=0.029); and the RNFL was reduced in the

temporal quadrant in PD patients (Table 3). These parameters however, did not meet the level of significance stablished by Bonferroni correction.

	CONTROLS		PARKINSON DISEASE		
Structural parameters	Mean	SD	Mean	SD	P
Macular measurements					
Central macular thickness	254.75	17.903	248.96	17.765	0.028
Inner superior macular thickness	327.34	13.094	325.73	19.329	0.019
Inner nasal macular thickness	328.52	13.263	325.45	17.098	0.091
Inner inferior macular thickness	326.14	13.179	324.82	17.921	0.106
Inner temporal macular thickness	315.90	13.615	312.82	15.760	0.945
Outer superior macular thickness	284.76	9.418	279.44	17.981	0.008
Outer nasal macular thickness	302.41	12.167	299.18	17.064	0.074
Outer inferior macular thickness	277.79	10.755	273.76	16.798	0.045
Outer temporal macular thickness	271.52	10.992	266.23	18.987	0.013
Ganglion cell layer thickness					
Superior	84.55	4.323	81.61	7.087	0.032
Superonasal	85.28	4.780	81.04	7.234	0.029
Inferonasal	84.66	5.314	81.82	7.521	0.135
Inferior	84.34	5.052	81.91	6.252	0.389
Inferotemporal	85.79	4.003	83.73	4.860	0.233
Temporal	83.76	3.324	82.27	5.312	0.069
Average IPL+GCL	84.83	4.071	82.73	6.230	0.095
Min IPL+ GCL	82.45	3.601	80.18	6.194	0.005*
RNFL thickness					
Average	96.17	6.714	94.88	11.505	0.105
Superior	117.90	10.965	118.68	16.861	0.115
Nasal	73.59	12.724	72.40	15.182	0.345
Inferior	128.14	14.060	123.20	22.907	0.075
Temporal	64.97	8.218	61.48	10.553	0.027

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in PD. The Pelli Robson CSV results correlated with GCL thickness in all sectors, although the

association was not strong (r < 0.5). The superonasal (r=0.40, p=0.010), inferonasal (r=0.40, p=0.010), inferior (r=0.43, p=0.005), superotemporal sector (r=0.43, p=0.006), and average GCL+IPL (r=0.45, p=0.004) values had the highest correlations. The Pelli Robson results also correlated with the thickness in different sectors of the RNFL (average, superior, and inferior sectors). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCL measurements. The superonasal (r= 0.40, p= 0.013) and superotemporal (r= 0.44, p= 0.006) thickness, average GCL +IPL thickness (r= 0.40, p= 0.012), and the minimum GCL + IPL (r= 0.40, p=0.011) at a spatial frequency of 6 cpd; and the superotemporal (r= 0.41, p= 0.01) thickness and the minimum GCL + IPL thickness (r= 0.43, p=0.006) at a spatial frequency of 18 cpd had the strongest correlations between CSV 1000E and GCL thickness. Spatial frequencies of 6 cpd and 18 cpd were strongly correlated with average macular thickness (r= 0.79, p= 0.012; r= 0.77, p= 0.016, respectively) and macular volume (r= 0.78, p= 0.013; r= 0.78, p= 0.014, respectively, Figure 1).

Color vision assessed by the L´Anthony test was also associated with the structural parameters: The C-index and CCI results were significantly correlated with all outer macular parameters and most of the GCL measurements (see Table 4). A significant association between color vision and the RNFL parameters was only found in isolated sectors. (see Table 4).

	L´Anthony color test					
	C-index	p	CCI	p	S-index	p
Macular thickness						
Central	-0.019	0.905	-0.059	0.716	-0.017	0.915
Inner superior	-0.146	0.369	-0.119	0.463	-0.167	0.302
Inner nasal	-0.055	0.735	-0.044	0.788	-0.040	0.807
Inner inferior	-0.073	0.654	-0.064	0.697	-0.074	0.649
Inner temporal	-0.049	0.764	-0.031	0.850	-0.126	0.439
Outer superior	**-0.377**	0.017	**-0.380**	0.015	-0.271	0.090
Outer nasal	**-0.341**	0.031	**-0.323**	0.042	-0.310	0.051
Outer inferior	**-0.360**	0.022	**-0.353**	0.025	**-0.375**	0.017
Outer temporal	**-0.360**	0.023	**-0.361**	0.022	**-0.350**	0.027
Macular average	***-0.691***	0.019	***-0.657***	0.028	***-0.709***	0.015
Macular volume	***-0.686***	0.020	***-0.647***	0.032	***-0.709***	0.015
Ganglion cell layer thickness						
Superior	**-0.380**	0.015	**-0.369**	0.019	-0.287	0.072
Superonasal	**-0.383**	0.015	**-0.337**	0.033	**-0.350**	0.027
Inferonasal	**-0.338**	0.033	**-0.313**	0.049	-0.268	0.094
Inferior	**-0.341**	0.031	-0.311	0.051	-0.282	0.078
Inferotemporal	-0.252	0.116	-0.263	0.101	-0.203	0.208
Temporal	**-0.403**	0.010	**-0.437**	0.005	**-0.314**	0.048
Average IPL+ GCL	**-0.381**	0.015	**-0.358**	0.023	**-0.319**	0.045
Minimum IPL+ GCL	**-0.338**	0.033	**-0.326**	0.040	-0.290	0.069

The strongest correlation was between the average macular thickness and macular volume and the L´Anthony CCI, C-index, and S-index results. No significant correlations were found between the Farnsworth´s test parameters and structural measurements.

The VA ETDRS results correlated strongly with average macular thickness and macular volume (see Table 5, Figures 2 and 3). There were significant but mild associations between the GCL parameters and VA at 100% (superonasal, inferonasal, and average GCL + IPL thickness, r=-

0.38, p=0.016; r=-0.35, p=0.016; and r=0.35, p=0.029, respectively) and 2.50% (superonasal sector, r=-0.36, p=0.023).

	Macular thickness	P value	Macular volume	P value
VA ETDRS 100	-0.765	**0.006**	-0.761	**0.007**
VA ETDRS 1.25	-0.718	**0.013**	-0.715	**0.013**
VA ETDRS 2.50	-0.738	**0.010**	-0.729	**0.011**

There was a significant correlation between Hoehn Yahr score and VA contrast level 2.50% (r=0.48, p=0.040), and CS measured with CSV 1000 at a space frequency of 12 cpd (r=-0.59, p=0.038). No correlations were detected between structural and disease severity parameters.

Discussion

In the present study, we evaluated the visual function parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 37 patients with PD. Parameters corresponding to VA at different contrast levels, and all CSV tests results were altered in PD patients in comparison with healthy subjects, prior to and after statistical correction for multiple tests. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Color vision was measured with two different tests, the Farnsworth and L´Anthony 15 D tests. These tests provide information for differentiating subjects with severe loss of color vision from those with milder color defects or normal color vision, and also can be used to evaluate acquired loss of color vision. In our study, only the L´Anthony Confusion Angle was significantly altered in PD patients. L´Anthony test is less saturated than the Farnsworth color test, thus it is designed to detect more subtle color deficiencies. Our patients performed worse than controls in both tests (higher C-index and S-

index, reaching ranges similar to protanomalies) although these differences did not reach statistical significance as established by Bonferroni correction. L´Anthony S-index p value was <0.05, indicating that our patients had a (subtle) tendency to protanomaly (S-index of 1.95).

In this study only one eye was tested per person. Some recent studies suggest asymmetrical involvement of the retina in PD and accept the incorporation of both eyes of each patient in the study.[21] Thus, the diagnostic yield in this study may have been lowered by including a potentially lesser affected eye. However, incorporating both eyes of a patient may sometimes be controversial: a minimum symmetric structural and functional alterations could have been masked and generated a % of dependence between measurements. The majority of authors consider the inclusion of only one eye of each patient adequate for statistical analysis because RNFL measurements correlate significantly between the two eyes; therefore, we included only one eye per patient.

Previous studies have indicated that PD patients lose foveal contrast sensitivity to patterns to which normal observers are most sensitive (i.e., requiring the least contrast for detection).[8,9] Ganglion cells in the retina show adaptation to visual contrast and pool visual inputs over their receptive fields through an array of parallel bipolar cells with smaller receptive fields.[22] The parvo- and magnocellular ganglion cells are located in the RGC layer and take two different pathways for the identification of color and contrast at different frequencies.[23] RGC loss (as observed using SD-OCT) was recently identified as the cause of visual impairment in patients suffering from another neurodegenerative process, multiple sclerosis.[24] Thus, a similar process could be the cause of the contrast and color deficiencies in patients with PD. In addition, in the mammalian retina, color vision and contrast sensitivity are modulated through D1 and D2 receptors. These dopaminergic receptors are differentially located in the retinal layers and a

complete lack of activation leads to signal dispersion and alterations in color vision and contrast sensitivity.[2]

Alteration of the retinal layers in PD was first demonstrated in 2004.[25] Since then, various studies have demonstrated different results.[3-5,25-28] Previous studies performed by our team confirmed that both macular thickness and the RNFL were affected in patients with PD, especially in the inferior and temporal quadrants.[4,5, 29] Moreover, Garcia-Martin et al demonstrated that the inner retinal layers were most affected in these patients, and that the GCL thickness was inversely correlated with disease duration and PD severity, and was predictive of axonal damage in these patients.[30] The present study included a smaller number of patients, which may have affected the significance of our results compared to previous studies. We could only detect a significant reduction in the minimum GCL+IPL thickness in PD patients compared to healthy subjects, after correction for multiple comparisons (using Bonferroni test). However, we detected a clear tendency towards a reduction in the macular, RNFL, and GCL thicknesses ($p<0.05$). A significant reduction in the temporal sectors of the peripapillary RNFL thickness has been repeatedly observed by different groups [31, 32] and was confirmed in the present study. Two recent studies, however, detected no differences in the peripapillary RNFL thickness of PD patients compared to healthy controls using SD-OCT [27,28] and one study only found significant differences in the nasal quadrant.[33] More studies are required to clarify these contradictory observations.

In a previous study, we demonstrated that the retinal thickness corresponding to the papillomacular bundle (as measured with the Axonal Analytics software for Spectralis OCT) correlated ($r>0.70$) with some functional parameters (such as the mean defect and the pattern standard deviation of the automated perimetry) in patients with PD.[34] The GCL was not

investigated at that time, however, and visual function parameters were reduced to perimetry and color vision measured with the Ishihara color test. The current study evaluated not only the RNFL but also the GCL thickness, and more visual function parameters were analyzed. The GCL correlated most with the visual function parameters: GCL thickness was directly associated with VA and CSV measured at all different spatial frequencies, and inversely correlated with the color vision indexes. Thus, GCL thinning is linked to color deficiencies, contrast sensitivity loss, and lower vision at different contrast levels in PD patients.

The degree of correlation is usually classified as low (<0.30), moderate (0.30–0.70), or strong (>0.70). Our results revealed a low and moderate degree of correlation between most parameters, consistent with findings in other neurodegenerative diseases.[35] Macular thickness and macular volume, however, were strongly associated with functional parameters (VA, CS, and L´Anthony CCI, C-index, and S-index). This strong association, to the best of our knowledge, has not been previously demonstrated in PD.

There are very few studies of the correlation between functional and structural parameters in PD patients. Adam et al [14] demonstrated a significant reduction in the inner retinal layer complex (RNFL + GCL + IPL) in PD patients, but no association with contrast sensitivity (measured with the Pelli Robson chart). A very recent study by Kaur et al [15] demonstrated a correlation between functional parameters and GCL thinning, consistent with our results. Kaur et al, however, found no significant alterations in VA or color vision in PD patients and the severity of the disease was not correlated with structural parameters, in contrast to other studies that demonstrate an association between macular and GCL thickness and disease duration and severity.[29,30] Although the severity of the disease in our sample (based on the Hoehn Yahr scale) was similar to that in Kaur´s study, the duration of the disease in our study was higher than

that in Kaur´s patients (13 years vs 5 years), which may account for some of the differences in the results between the two studies. These discrepancies (and similarities) support the need for more studies on this topic. Our results, together with previously published studies,[15, 30] suggest that the GCL could be a reliable indicator of structural alterations in the retina of PD patients, demonstrating a significant correlation with functional tests in these patients. The results of the present study have important implications for clinical diagnosis and functional deficits in patients with PD, and highlight the importance of visual function tests in the evaluation of these patients.

In conclusion, visual dysfunction was significantly correlated with morphologic parameters in PD patients. PD patients present with a reduction in macular, RNFL, and GCL thickness, with changes in the GCL being most closely associated with visual dysfunction.

Contributorship:

V. Polo: Research project: organization. Statistical analysis and Manuscript: Review and critique.

M. Satue: Research project: Conception, design, organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

MJ Rodrigo: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

S. Otin: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: review and critique.

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E. Garcia-Martin: Research project: Conception, design, organization and execution. Statistical analysis: Design, execution, review and critique. Manuscript: Writing of the first draft, review and critique.

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Data sharing

All relevant data are included in this manuscript. No additional data available.

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Legends

Figure 1: Correlation between the average macular thickness and contrast sensitivity vision as measured with the CSV 1000E test, at a spatial frequency of 6 cycles per degree, in patients with Parkinson's disease.

Figure 2: Correlation between the average macular thickness and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.

Figure 3: Correlation between macular volume and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.

Table 1: Epidemiologic and disease characteristics of patients with PD and healthy subjects, and statistical significance (P). Abbreviations: SD, standard deviation; UPDRS III, Unified Parkinson Disease Rating Scale part III.

Table 2: Mean and standard deviation (SD) of visual functional parameters in healthy controls and subjects with Parkinson disease. ~~Results in bold letters indicate statistical significance (p<0.05).~~ The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0125$ for VA ETDRS 100, 2.50 and 1.25; $p<0.0125$ for Pelli Robson and CSV 1000E measurements; $p<0.0083$ for Farnsworth and L´Anthony tests). Bold letters indicate parameters with p value <0.05. Abbreviations: VA, visual acuity; ETDRS, early treatment diabetic retinopathy study; cpd, cycles per degree; AC CCI, age corrected color

confusion index; CCI, color confusion index; C-index, confusion index; Conf Angle, confusion angle; S-index, scatter index; PD, Parkinson disease.

Table 3: Mean and standard deviation (SD) of structural parameters (retinal nerve fiber layer, ganglion cell layer and macular thicknesses) obtained with the Cirrus HD optical coherence tomography device in healthy controls and subjects with Parkinson disease. ~~Bold letters indicate statistical significance (p<0.05).~~ The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests (p<0.0055 for macular measurements; p<0.0062 for ganglion cell measurements and p<0.01 for RNFL measurements). Bold letters indicate parameters with p value <0.05. Abbreviations: IPL, inner plexiform layer; GCL, ganglion cell layer; RNFL, retinal nerve fiber layer; HD, high definition.

Table 4: Correlation between macular and ganglion cell layer structural measurements and color vision evaluated with L'Anthony color test in patients with Parkinson disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; C-index, Confusion index; CCI, color confusion index; S-index, Scatter index.

Table 5: Correlation between visual acuity measured with ETDRS chart at different levels of contrast (in %) and macular structural measurements (thickness and volume) in patients with Parkinson disease. Correlation data in bold type are statistically significant (p value <0.05). Abbreviations: VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

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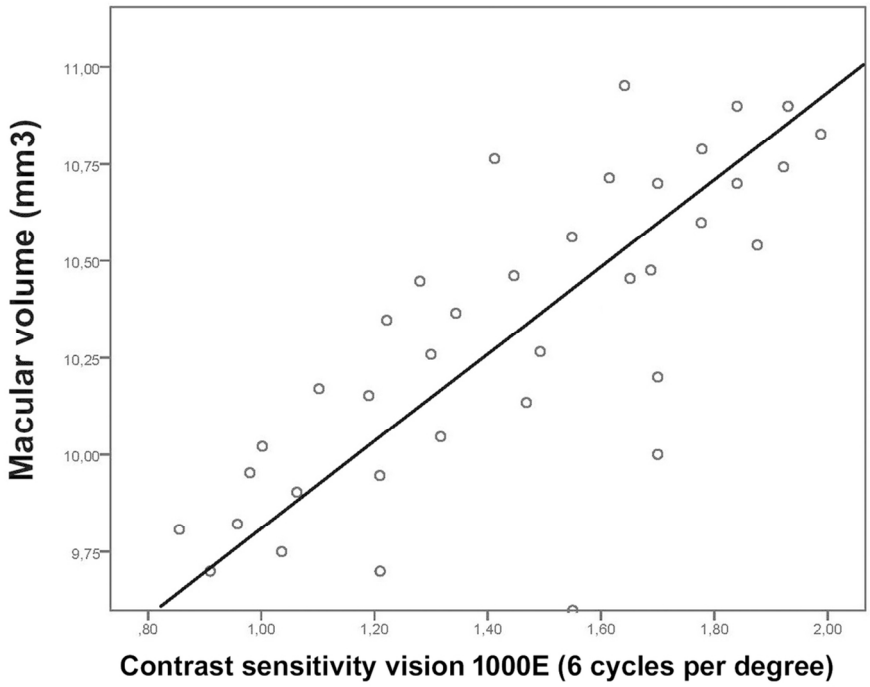

Figure 1: Correlation between the average macular thickness and contrast sensitivity vision as measured with the CSV 1000E test, at a spatial frequency of 6 cycles per degree, in patients with Parkinson's disease.
108x83mm (300 x 300 DPI)

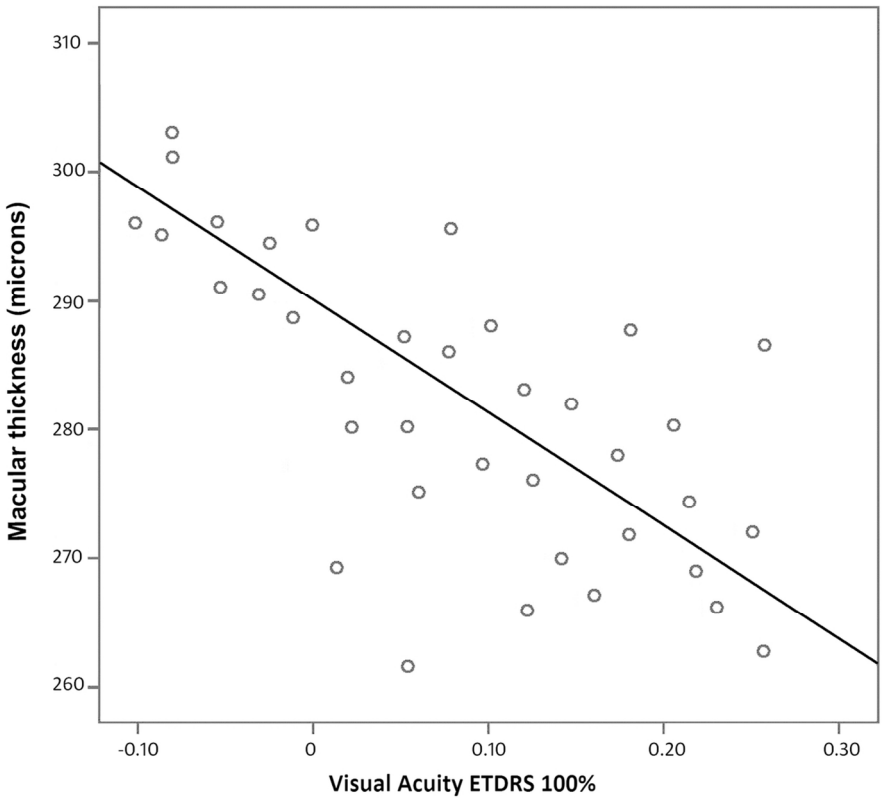

Figure 2: Correlation between the average macular thickness and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.
118x100mm (300 x 300 DPI)

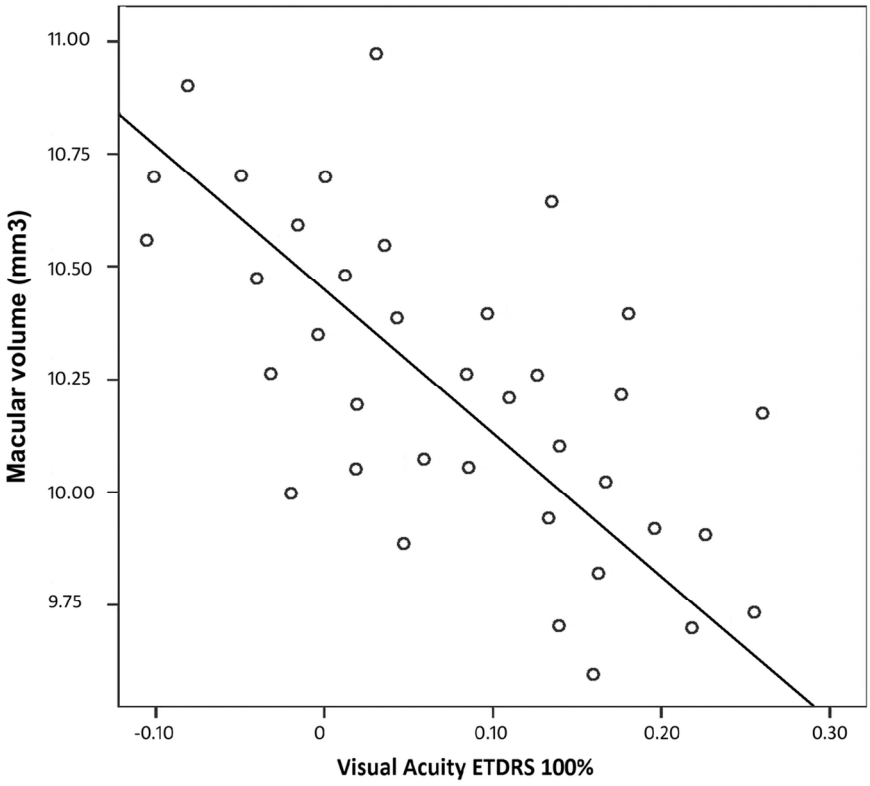

Figure 3: Correlation between macular volume and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.
119x102mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	*(*a) Indicate the study's design with a commonly used term in the title or the abstract Check. This included in the abstract, methods, line 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Check. This is included in the abstract. Methods, from line 2 to results section line 9
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Check. This is included in the introduction. Paragraph 2.
Objectives	3	State specific objectives, including any prespecified hypotheses Check. This is included in the introduction. Paragraph 3.
Methods		
Study design	4	Present key elements of study design early in the paper Check. This is included in Methods, paragraph 1-3.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Check. This is included in Methods, paragraph 1-3.
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Check. This is included in Methods, paragraph 2
		(*b*) For matched studies, give matching criteria and the number of controls per case Check. This is included in Methods, paragraph 1
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Check. Outcomes and variables are explained in Methods, paragraphs 4-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Check. Details of measurements are included in paragraphs 4-8.
Bias	9	Describe any efforts to address potential sources of bias Check. This is included in paragraph 8, line 3-4.
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Check. Quantitative variables are explained in Methods, paragraphs 4-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding. Check. This is included in methods, paragraph 9
		(*b*) Describe any methods used to examine subgroups and interactions. Not applicable.
		(*c*) Explain how missing data were addressed Not applicable
		(*d*) If applicable, explain how matching of cases and controls was addressed Not applicable
		(*e*) Describe any sensitivity analyses Not applicable
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram This is a cross sectional study. Inclusion criteria were explained in methods. All elegible subjects were included in the study (37) as already explained, all completed the evaluation.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Check. This is included in Results, paragraph 1-2 and table 1.
		(b) Indicate number of participants with missing data for each variable of interest Not applicable.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure Check. Numbers and results of each variable are included in results.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Check. Main results include statistical results prior and post multiple comparisons adjustment.
		(*b*) Report category boundaries when continuous variables were categorized Check. Category of correlation is explained in discussion, paragraph 5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Not applicable
Discussion		
Key results	18	Summarise key results with reference to study objectives Check. This is included in results, paragraph 1.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Check, this is mentioned in paragraph 3 and 6.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Check. This is included and discussed along the discussion section.
Generalisability	21	Discuss the generalisability (external validity) of the study results Check. This is included and discussed along the discussion section
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Check. Not applicable.

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study

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Visual dysfunction and its correlation with retinal changes in patients with Parkinson's disease: an observational cross-sectional study

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RUNNING TITLE: OCT and visual dysfunction in Parkinson disease.

STUDY DESIGN AND SETTING: An observational cross-sectional study, carried out at Miguel Servet University Hospital, in Zaragoza, Spain.

KEY WORDS: Parkinson disease, visual function, contrast sensitivity, ganglion cell layer.

WORD COUNT: 2500 words.

All subjects provided detailed consent to participate in this study, which was conducted in accordance with the guidelines established by the Ethics Committee of the Miguel Servet Hospital and based on the principles of the Declaration of Helsinki.

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

The authors disclose no conflict of interest.

SUBTITLE: Visual acuity, contrast sensitivity vision and color vision are affected in Parkinson disease. Visual dysfunction in these patients correlates with structural changes in the retina measured with Spectral domain OCT.

Abstract

Objectives: To evaluate visual dysfunction and its correlation with structural changes in the retina in patients with Parkinson disease (PD).

Methods: Patients with PD (n=37) and controls (n=37) were included in an observational cross-sectional study and underwent visual acuity (VA), color vision (using the Farnsworth and L´Anthony desaturated D15 color tests), and contrast sensitivity vision (CSV; using the Pelli Robson chart and CSV 1000E test) evaluation to measure visual dysfunction. Structural measurements of the retinal nerve fiber layer (RNFL), and macular and ganglion cell layer (GCL) thicknesses were obtained using spectral domain optical coherence tomography (SD-OCT). Comparison of obtained data and correlation analysis between functional and structural results were performed.

Results: VA (in all different contrast levels) and all CSV spatial frequencies were significantly worse in PD patients than in controls. Color vision was significantly affected based on the L´Anthony color test. Significant GCL loss was observed in the minimum GCL + inner plexiform layer. A clear tendency towards a reduction in several macular sectors (central, outer inferior, outer temporal and superior [inner and outer]) and in the temporal quadrant of the RNFL thickness was observed, although the difference was not significant. CSV was the functional parameter most strongly correlated with structural measurements in PD. Color vision was associated with most GCL measurements. Macular thickness was strongly correlated with macular volume and functional parameters ($r > 0.70$, $p < 0.05$).

Conclusions: Patients with PD had visual dysfunction that correlated with structural changes evaluated by SD-OCT. GCL measurements may be reliable indicators of visual impairment in PD patients.

Strengths and limitations of this study:

The strengths of this study should be resumed by the following bullet points:

- This study includes a complete assessment of visual function parameters and the evaluation of different retinal structures using Spectral domain Optical coherence tomography in patients with Parkinson disease.
- There are only 2 other published articles evaluating the association between visual dysfunction and morphologic parameters. Results provided by these previous studies differ from our results, possibly due to different measurement methods and sample size.
- Color vision in our study was assessed by L´Anthony and Farnsworth D15 color tests, which may provide more specific information about color deficiencies. These tests are not commonly used to evaluate color deficiencies in PD patients.
- As an important limitation to our study, we included one randomly selected eye per patient. The incorporation of both eyes of each patient in Parkinson disease studies is usually recommended due to asymmetrical involvement of the retina in this process.

Introduction

Foveal vision alterations are associated with Parkinson disease (PD), and seem to be caused by dysfunction of the intraretinal dopaminergic circuitry and final retinal output to the brain.[1] Recent studies demonstrated retinal thinning in PD patients compared with healthy subjects.[2-5] Several studies report a correlation between functional disability and axonal loss observed in the optic nerve in multiple sclerosis, another neurodegenerative process.[6,7] PD patients are also reported to have decreased contrast sensitivity and color vision, and altered visual evoked potentials.[1,8-13] To our knowledge, however, very few studies have assessed visual dysfunction in PD and its correlation with morphologic parameters.[14,15]

In the present study, we evaluated visual acuity (VA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart, contrast sensitivity vision (CSV) using the CSV-1000E test and Pelli-Robson chart, and color vision using the Farnsworth and L´Anthony tests in PD patients and healthy controls to examine the association between visual dysfunction and morphologic parameters.

Material and methods

Thirty-seven eyes of 37 patients with definite PD and 37 eyes of 37 age- and sex-matched healthy individuals were recruited for an observational cross-sectional study. The study was performed at Miguel Servet University Hospital in Zaragoza, Spain, and all evaluations were performed in one single visit. All procedures adhered to the tenets of the Declaration of Helsinki, and all participants provided informed consent to participate in the study.

The diagnosis of PD was based on standard clinical and neuroimaging criteria.[16] Information about disease severity was assessed using the Hoehn Yahr scale [17] and the Unified Parkinson Disease Rating Scale part III score (UPDRS III) [18]. Disease duration and treatment were recorded. Exclusion criteria were the presence of significant refractive errors (>5 diopters of spherical equivalent refraction or 3 diopters of astigmatism); intraocular pressure ≥21 mmHg; media opacifications; concomitant ocular diseases, including history of glaucoma or retinal pathology; and systemic conditions that could affect the visual system. The healthy controls had no history and no evidence of ocular or neurologic disease of any nature; their best-corrected visual acuity (BCVA) was >20/30 based on the Snellen scale.

All subjects underwent a complete neuro-ophthalmic evaluation that included pupillary, anterior segment, and funduscopic examination. Visual function was assessed by evaluating BCVA using an ETDRS chart, CSV using the CVS-1000E test and Pelli-Robson chart, and color vision using the Farnsworth desaturated D15 and L´Anthony desaturated D15 tests. Structural analysis of the retina was performed using Spectral domain (SD) optical coherence tomography (OCT) with the Cirrus High definition (HD) OCT (Carl Zeiss Meditec Inc, Dublin, CA), which included three different protocols: macular protocol (for macular thickness analysis), RNFL protocol, and ganglion cell protocol (for individual analysis of this layer).

LogMAR visual acuity (VA) was evaluated at three different contrast levels: 100% (HCVA, using ETDRS chart), 2.50%, and 1.25% (LCVA, using Low-Contrast Sloan Letter Charts - Precision Vision, LaSalle, IL-), The percentage indicating the level of contrast, i.e., 100% representing black letters over white background and 1.25% light grey letters over white background. All measurements were obtained under monocular vision and controlled lighting conditions with best correction.

Contrast sensitivity provides more complete information about visual function than visual acuity tests. CSV was evaluated in our patients using the Pelli-Robson chart and the CVS-1000E test. The Pelli-Robson chart comprises horizontal lines of capital letters organized into groups of three (triplets) with two triplets per line. Within each triplet, all letters have the same contrast. The contrast decreases from one triplet to the next, even within each line. All patients were evaluated under both monocular and binocular vision at a distance of 1 meter from the chart and under controlled fotopic conditions (85 cd/m^2). The score corresponding to the last triplet of letters seen by the patient was recorded.

The CSV-1000E instrument is used worldwide for standardized CSV and glare testing. All patients were evaluated at a distance of 2.5 meters from the chart under monocular vision at 4 different spatial frequencies (3, 6, 12, and 18 cycles per degree [cpd]). The chart comprises four rows with 17 circular patches each. The patches present a grating that decreases in contrast moving from left to right across the row. The patient indicates whether the grating appears in the top patch or the bottom patch for each column. Each contrast value for each spatial frequency was transformed into a logarithmic scale according to standardized values.

Color vision was assessed using the Color Vision Recorder (CVR) program. CVR software analyzes chromatic discrimination by classification of colors. The program includes the classic test of Farnsworth 100-hue (FM-100), Farnsworth - Munsell D15, and L´Anthony D15. All patients in the study were evaluated using the Farnsworth - Munsell D15 and L´Anthony D15 protocols and different output parameters such as the Confusion Index (C-index), the Color Confusion Index (CCI), the Confusion angle (Conf Ang), and the Scatter Index (S-index) were recorded.[19,20] The tests were performed under monocular vision.

Structural measurements of the retina were obtained using the Cirrus OCT device. The same experienced operator performed all scans and did not apply manual correction to the OCT output. We used an internal fixation target because it provides the highest reproducibility and rejected poor quality scans prior to data analysis. The Cirrus OCT macular cube 512 x 128 protocol provides a macular volume measure and retinal thickness values for nine areas. These areas include a central 1-mm circle representing the fovea, and inner and outer rings measuring 3 mm and 6 mm in diameter, respectively. The inner and outer rings are divided into four quadrants each. The Cirrus OCT optic disc protocol generates images with 200 linear scans enabling analysis of the RNFL of a 6-mm^3 area around the optic nerve. For each scan series of RNFL measurements, we assessed the average, superior, inferior, temporal, and nasal thickness. Cirrus segmentation analysis for retinal layers also provides measurements of the GCL thickness, evaluating six areas of the macular cube (superior, superonasal, inferonasal, inferior, inferotemporal, and superotemporal sectors) and measurements of the average and minimum GCL plus the inner plexiform layer (GCL + IPL) value of a set of 360 spokes, where each average represents the mean number of the pixels along that spoke that lies within the measurement annulus. The minimum is selected because the thinnest portion of the GCL + IPL in the perifoveal region is considered to indicate damage to the ganglion cells.

All data analyses were performed using SPSS software version 20.0 (SPSS Inc., Chicago, IL). Due to the parametric distribution of the data, differences between evaluations of PD patients and healthy subjects were compared using Student´s t-test. To avoid a high false positive rate, the Bonferroni correction for multiple comparisons was calculated. The level of significance for each variable was established based on Bonferroni calculations.

The linear correlation between structural and functional parameters was determined using Pearson's correlation coefficient. Values of $p < 0.05$ were considered to indicate a significant correlation. Each eye was considered separately, and one eye from each patient was randomly selected for analysis.

Results

Thirty-seven patients with PD and 37 healthy controls were included in the study. The mean age of the patients with PD was 69 years (range: 58–74 years) and the mean age of the healthy controls was 68 years (range: 60–76 years). Age (p=0.361), sex (p=0.441), and intraocular pressure (p=0.720) did not differ significantly between healthy controls and patients with PD. Mean time from diagnosis of PD was 13.2 years. The median Hoehn Yahr stage was 2.7, and the stage of PD based on the UPDRS III was 25.06 (range: 7-39; Table 1).

Treatment was divided into three different categories: "Drugs that enhance dopamine levels" (carbidopa, levodopa and rasagiline), "dopaminergic drugs" (pramipexole, ropirinol, rotigotine), and "other"(amitriptiline, propranolol, clonazepam). "Drugs that enhance dopamine levels" was the most prescribed category (89% of patients) and combination therapy with levodopa and carbidopa was the most frequent treatment (44%). Sixty-four percent of treatments were categorized as "dopaminergic", most of which were used in combination with drugs included in the previous category. A small percentage of patients (9%) were prescribed drugs with no dopaminergic effects.

PARAMETER	CONTROLS	PARKINSON DISEASE	p
Number of eyes (n)	37	37	-
Age, years, range	68 (60–76)	69 (58–74)	0.361
Men:Women (% of men)	24:13 (64.9)	23:14 (62.2)	0.441
Intraocular Pressure	15.58 (2.71)	15.12 (2.98)	0.720
Disease duration, years, mean (SD)	-	13.2 (5.77)	-
Hoehn Yahr, mean (SD)	-	2.7 (0.64)	-
UPDRS III, mean (SD)	-	25.06 (8.24)	-

Table 1.

Functional parameters

PD patients had a lower BCVA at all three contrast levels of the ETDRS chart compared to the controls (0.18±0.26 in patients vs -0.065 ± 0.9 in controls at 100%, p=0.001; 0.59±0.21 vs 0.44±0.13 at 2.50%, p=0.010; and 0.61±0.23 vs 0.58±0.16 at 1.25%, p=0.009). CSV was affected in patients at all four spatial frequencies of the CSV 1000E chart (3, 6, 12, and 18 cpd) when analyzed based on the number of correct localized gratings (p=0.001, <0.001, <0.001, and 0.004 respectively). The Pelli Robson results also revealed a significant reduction in CSV in PD patients (1.71 in patients vs 1.89 in controls, p=0.02). Color vision (Conf Angle in L´Anthony test) was also affected in PD. The results are shown in Table 2.

	HEALTHY CONTROLS		PARKINSON DISEASE PATIENTS		SIGNIFICANCE (P)
	Mean	SD	Mean	SD	
VA ETDRS 100	-0.06	0.096	0.18	0.26	0.001*
VA ETDRS 2.5	0.44	0.13	0.59	0.22	0.010*
VA ETDRS 1.25	0.58	0.16	0.62	0.23	0.009*
Pelli Robson	1.89	0.11	1.71	0.17	0.002*
CSV 1000 3 cpd	1.72	0.16	1.49	0.35	0.001*
CSV 1000 6 cpd	1.94	0.13	1.62	0.34	<0.001*
CSV 1000 12 cpd	1.62	0.17	1.26	0.41	<0.001*
CSV 1000 18 cpd	1.11	0.22	0.73	0.34	0.004*
Farnsworth AC CCI	1.11	0.22	0.73	0.34	0.851
Farnsworth C- index	1.10	0.20	1.24	0.42	0.093
Farnsworth CCI	1.07	0.12	1.14	0.24	0.110
Farnsworth Conf Angle	63.90	11.15	65.84	7.49	0.392
Farnsworth S-index	1.56	0.22	1.64	0.39	0.278
Farnsworth time	78.67	28.96	82.91	33.10	0.616
L´Anthony AC CCI	1.05	0.19	1.02	0.18	0.489
L´ Anthony C-index	1.43	0.39	1.64	0.53	0.058
L´ Anthony CCI	1.30	0.23	1.44	0.37	0.066
L´ Anthony Conf Angle	62.31	14.74	71.91	9.25	0.002*
L´ Anthony S-index	1.69	0.43	1.95	0.48	0.020
L´ Anthony time	77.14	25.99	84.09	39.31	0.431

Table 2

Structural parameters

Based on Bonferroni corrections, OCT measurements indicated a significant difference in the minimum GCL+IPL value (80.18±6.19 µm vs 82.45±3.60 µm; p=0.005). However, we observed a clear tendency towards a reduction in superior macular sectors, in the outer inferior, outer temporal, and central macular thickness in PD patients compared to controls: the p value for these variables was <0.05 but did not meet Bonferroni significance (results are shown in Table

2). The segmentation analysis revealed a tendency towards reduced GCL in PD patients in the superior (81.64±7.08 µm in patients vs 84.55±4.32 µm in controls; p=0.032) and superonasal sectors (81.04±7.23 µm vs 85.28±4.78 µm; p=0.029); and the RNFL was reduced in the temporal quadrant in PD patients (Table 3). These parameters however, did not meet the level of significance stablished by Bonferroni correction.

	CONTROLS		PARKINSON DISEASE		
Structural parameters	Mean	SD	Mean	SD	P
Macular measurements					
Central macular thickness	254.75	17.903	248.96	17.765	0.028
Inner superior macular thickness	327.34	13.094	325.73	19.329	0.019
Inner nasal macular thickness	328.52	13.263	325.45	17.098	0.091
Inner inferior macular thickness	326.14	13.179	324.82	17.921	0.106
Inner temporal macular thickness	315.90	13.615	312.82	15.760	0.945
Outer superior macular thickness	284.76	9.418	279.44	17.981	0.008
Outer nasal macular thickness	302.41	12.167	299.18	17.064	0.074
Outer inferior macular thickness	277.79	10.755	273.76	16.798	0.045
Outer temporal macular thickness	271.52	10.992	266.23	18.987	0.013
Ganglion cell layer thickness					
Superior	84.55	4.323	81.61	7.087	0.032
Superonasal	85.28	4.780	81.04	7.234	0.029
Inferonasal	84.66	5.314	81.82	7.521	0.135
Inferior	84.34	5.052	81.91	6.252	0.389
Inferotemporal	85.79	4.003	83.73	4.860	0.233
Temporal	83.76	3.324	82.27	5.312	0.069
Average IPL+GCL	84.83	4.071	82.73	6.230	0.095
Min IPL+ GCL	82.45	3.601	80.18	6.194	0.005*
RNFL thickness					
Average	96.17	6.714	94.88	11.505	0.105
Superior	117.90	10.965	118.68	16.861	0.115
Nasal	73.59	12.724	72.40	15.182	0.345
Inferior	128.14	14.060	123.20	22.907	0.075
Temporal	64.97	8.218	61.48	10.553	0.027

Table 3

Correlation between functional and structural parameters

CSV was the functional parameter most frequently associated with structural measurements in PD. The Pelli Robson CSV results correlated with GCL thickness in all sectors, although the association was not strong ($r < 0.5$). The superonasal ($r=0.40$, $p=0.010$), inferonasal ($r=0.40$, $p=0.010$), inferior ($r=0.43$, $p=0.005$), superotemporal sector ($r=0.43$, $p=0.006$), and average GCL+IPL ($r=0.45$, $p=0.004$) values had the highest correlations. The Pelli Robson results also correlated with the thickness in different sectors of the RNFL (average, superior, and inferior sectors). Measurements with the CSV 1000E at different spatial frequencies correlated significantly with most GCL measurements. The superonasal ($r= 0.40$, $p= 0.013$) and superotemporal ($r= 0.44$, $p= 0.006$) thickness, average GCL +IPL thickness ($r= 0.40$, $p= 0.012$), and the minimum GCL + IPL ($r= 0.40$, $p=0.011$) at a spatial frequency of 6 cpd; and the superotemporal ($r= 0.41$, $p= 0.01$) thickness and the minimum GCL + IPL thickness ($r= 0.43$, $p=0.006$) at a spatial frequency of 18 cpd had the strongest correlations between CSV 1000E and GCL thickness. Spatial frequencies of 6 cpd and 18 cpd were strongly correlated with average macular thickness ($r= 0.79$, $p= 0.012$; $r= 0.77$, $p= 0.016$, respectively) and macular volume ($r= 0.78$, $p= 0.013$; $r= 0.78$, $p= 0.014$, respectively, Figure 1).

Color vision assessed by the L´Anthony test was also associated with the structural parameters: The C-index and CCI results were significantly correlated with all outer macular parameters and most of the GCL measurements (see Table 4). A significant association between color vision and the RNFL parameters was only found in isolated sectors. (see Table 4).

	L´Anthony color test					
	C-index	p	CCI	p	S-index	p
Macular thickness						
Central	-0.019	0.905	-0.059	0.716	-0.017	0.9[illegible]
Inner superior	-0.146	0.369	-0.119	0.463	-0.167	0.3[illegible]
Inner nasal	-0.055	0.735	-0.044	0.788	-0.040	0.8[illegible]
Inner inferior	-0.073	0.654	-0.064	0.697	-0.074	0.6[illegible]
Inner temporal	-0.049	0.764	-0.031	0.850	-0.126	0.4[illegible]
Outer superior	**-0.377**	0.017	**-0.380**	0.015	-0.271	0.0[illegible]
Outer nasal	**-0.341**	0.031	**-0.323**	0.042	-0.310	0.0[illegible]
Outer inferior	**-0.360**	0.022	**-0.353**	0.025	**-0.375**	0.0[illegible]
Outer temporal	**-0.360**	0.023	**-0.361**	0.022	**-0.350**	0.0[illegible]
Macular average	***-0.691***	0.019	***-0.657***	0.028	***-0.709***	0.0[illegible]
Macular volume	***-0.686***	0.020	***-0.647***	0.032	***-0.709***	0.0[illegible]
Ganglion cell layer thickness						
Superior	**-0.380**	0.015	**-0.369**	0.019	-0.287	0.0[illegible]
Superonasal	**-0.383**	0.015	**-0.337**	0.033	**-0.350**	0.0[illegible]
Inferonasal	**-0.338**	0.033	**-0.313**	0.049	-0.268	0.0[illegible]
Inferior	**-0.341**	0.031	-0.311	0.051	-0.282	0.0[illegible]
Inferotemporal	-0.252	0.116	-0.263	0.101	-0.203	0.2[illegible]
Temporal	**-0.403**	0.010	**-0.437**	0.005	**-0.314**	0.0[illegible]
Average IPL+ GCL	**-0.381**	0.015	**-0.358**	0.023	**-0.319**	0.0[illegible]
Minimum IPL+ GCL	**-0.338**	0.033	**-0.326**	0.040	-0.290	0.0[illegible]

Table 4

The strongest correlation was between the average macular thickness and macular volume and the L´Anthony CCI, C-index, and S-index results. No significant correlations were found between the Farnsworth´s test parameters and structural measurements.

The VA ETDRS results correlated strongly with average macular thickness and macular volume (see Table 5, Figures 2 and 3). There were significant but mild associations between the GCL parameters and VA at 100% (superonasal, inferonasal, and average GCL + IPL thickness, r=-0.38, p=0.016; r=-0.35, p=0.016; and r=0.35, p=0.029, respectively) and 2.50% (superonasal sector, r=-0.36, p=0.023).

	Macular thickness	P value	Macular volume	P value
VA ETDRS 100	-0.765	**0.006**	-0.761	**0.007**
VA ETDRS 1.25	-0.718	**0.013**	-0.715	**0.013**
VA ETDRS 2.50	-0.738	**0.010**	-0.729	**0.011**

Table 5

There was a significant correlation between Hoehn Yahr score and VA contrast level 2.50% (r=0.48, p=0.040), and CS measured with CSV 1000 at a space frequency of 12 cpd (r=-0.59, p=0.038). No correlations were detected between structural and disease severity parameters.

Discussion

In the present study, we evaluated the visual function parameters and assessed the association between visual dysfunction and morphologic changes in the retina of 37 patients with PD. Parameters corresponding to VA at different contrast levels, and all CSV tests results were altered in PD patients in comparison with healthy subjects, prior to and after statistical correction for multiple tests. Moreover, contrast sensitivity was the most affected parameter in our study and correlated with most of the structural data. Color vision was measured with two different tests, the Farnsworth and L´Anthony 15 D tests. These tests provide information for

differentiating subjects with severe loss of color vision from those with milder color defects or normal color vision, and also can be used to evaluate acquired loss of color vision. In our study, only the L´Anthony Confusion Angle was significantly altered in PD patients. L´Anthony test is less saturated than the Farnsworth color test, thus it is designed to detect more subtle color deficiencies. Our patients performed worse than controls in both tests (higher C-index and S-index, reaching ranges similar to protanomalies) although these differences did not reach statistical significance as established by Bonferroni correction. L´Anthony S-index p value was <0.05, indicating that our patients had a (subtle) tendency to protanomaly (S-index of 1.95).

One important limitation of this study is that only one eye was tested per person. Some recent studies suggest asymmetrical involvement of the retina in PD and recommend the incorporation of both eyes of each patient in the study.[21] Thus, the diagnostic yield in this study may have been lowered by including a potentially lesser affected eye. In a similar way, including a randomly selected eye could be innapropiate for other neurological conditions, for example, a tumor compressing one optic nerve. However, incorporating both eyes of a patient may sometimes be controversial since a minimum symmetric structural and functional alterations could have been masked and generated a % of dependence between measurements.

Previous studies have indicated that PD patients lose foveal contrast sensitivity to patterns to which normal observers are most sensitive (i.e., requiring the least contrast for detection).[8,9] Ganglion cells in the retina show adaptation to visual contrast and pool visual inputs over their receptive fields through an array of parallel bipolar cells with smaller receptive fields.[22] The parvo- and magnocellular ganglion cells are located in the RGC layer and take two different pathways for the identification of color and contrast at different frequencies.[23] RGC loss (as observed using SD-OCT) was recently identified as the cause of visual impairment in patients

suffering from another neurodegenerative process, multiple sclerosis.[24] Thus, a similar process could be the cause of the contrast and color deficiencies in patients with PD. In addition, in the mammalian retina, color vision and contrast sensitivity are modulated through D1 and D2 receptors. These dopaminergic receptors are differentially located in the retinal layers and a complete lack of activation leads to signal dispersion and alterations in color vision and contrast sensitivity.[2]

Alteration of the retinal layers in PD was first demonstrated in 2004.[25] Since then, various studies have demonstrated different results.[3-5,25-28] Previous studies performed by our team confirmed that both macular thickness and the RNFL were affected in patients with PD, especially in the inferior and temporal quadrants.[4,5, 29] Moreover, Garcia-Martin et al demonstrated that the inner retinal layers were most affected in these patients, and that the GCL thickness was inversely correlated with disease duration and PD severity, and was predictive of axonal damage in these patients.[30] The present study included a smaller number of patients, which may have affected the significance of our results compared to previous studies. We could only detect a significant reduction in the minimum GCL+IPL thickness in PD patients compared to healthy subjects, after correction for multiple comparisons. However, we detected a clear tendency towards a reduction in the macular, RNFL, and GCL thicknesses. A significant reduction in the temporal sectors of the peripapillary RNFL thickness has been repeatedly observed by different groups [31, 32] and this reduction was also observed in the present study. Two recent studies, however, detected no differences in the peripapillary RNFL thickness of PD patients compared to healthy controls using SD-OCT [27,28] and one study only found significant differences in the nasal quadrant.[33] More studies are required to clarify these contradictory observations.

In a previous study, we demonstrated that the retinal thickness corresponding to the papillomacular bundle (as measured with the Axonal Analytics software for Spectralis OCT) correlated (r>0.70) with some functional parameters (such as the mean defect and the pattern standard deviation of the automated perimetry) in patients with PD.[34] The GCL was not investigated at that time, however, and visual function parameters were reduced to perimetry and color vision measured with the Ishihara color test. The current study evaluated not only the RNFL but also the GCL thickness, and more visual function parameters were analyzed. The GCL correlated most with the visual function parameters: GCL thickness was directly associated with VA and CSV measured at all different spatial frequencies, and inversely correlated with the color vision indexes. Thus, GCL thinning is linked to color deficiencies, contrast sensitivity loss, and lower vision at different contrast levels in PD patients.

The degree of correlation is usually classified as low (<0.30), moderate (0.30–0.70), or strong (>0.70). Our results revealed a low and moderate degree of correlation between most parameters, consistent with findings in other neurodegenerative diseases.[35] Macular thickness and macular volume, however, were strongly associated with functional parameters (VA, CS, and L´Anthony CCI, C-index, and S-index). This strong association, to the best of our knowledge, has not been previously demonstrated in PD.

There are very few studies of the correlation between functional and structural parameters in PD patients. Adam et al [14] demonstrated a significant reduction in the inner retinal layer complex (RNFL + GCL + IPL) in PD patients, but no association with contrast sensitivity (measured with the Pelli Robson chart). A very recent study by Kaur et al [15] demonstrated a correlation between functional parameters and GCL thinning, consistent with our results. Kaur et al, however, found no significant alterations in VA or color vision in PD patients and the severity of

the disease was not correlated with structural parameters, in contrast to other studies that demonstrate an association between macular and GCL thickness and disease duration and severity.[29,30] Although the severity of the disease in our sample (based on the Hoehn Yahr scale) was similar to that in Kaur´s study, the duration of the disease in our study was higher than that in Kaur´s patients (13 years vs 5 years), which may account for some of the differences in the results between the two studies. These discrepancies (and similarities) support the need for more studies on this topic. Our results, together with previously published studies,[15, 30] suggest that the GCL could be a reliable indicator of structural alterations in the retina of PD patients, demonstrating a significant correlation with functional tests in these patients. The results of the present study have important implications for clinical diagnosis and functional deficits in patients with PD, and highlight the importance of visual function tests in the evaluation of these patients.

In conclusion, visual dysfunction was significantly correlated with morphologic parameters in PD patients. PD patients present with a reduction in GCL thickness, which is closely associated with visual dysfunction.

Contributorship:

V. Polo: Research project: organization. Statistical analysis and Manuscript: Review and critique.

M. Satue: Research project: Conception, design, organization and execution. Statistical analysis: Review and critique. Manuscript: Writing of the first draft, review and critique.

MJ Rodrigo: Research project: organization and execution. Statistical analysis: Review and

critique. Manuscript: Writing of the first draft, review and critique.

S. Otin: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: review and critique.

R. Alarcia: Research project: organization and execution. Statistical analysis: Review and critique. Manuscript: review and critique.

MP. Bambo: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

MI. Fuertes: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

JM. Larrosa: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

LE. Pablo: Research project: execution. Statistical analysis: Review and critique. Manuscript: review and critique.

E. Garcia-Martin: Research project: Conception, design, organization and execution. Statistical analysis: Design, execution, review and critique. Manuscript: Writing of the first draft, review and critique.

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The authors disclose no conflict of interest.

Data sharing

All relevant data are included in this manuscript. No additional data available.

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Legends

Figure 1: Correlation between the average macular thickness and contrast sensitivity vision as measured with the CSV 1000E test, at a spatial frequency of 6 cycles per degree, in patients with Parkinson's disease.

Figure 2: Correlation between the average macular thickness and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.

Figure 3: Correlation between macular volume and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.

Table 1: Epidemiologic and disease characteristics of patients with PD and healthy subjects, and statistical significance (P). Abbreviations: SD, standard deviation; UPDRS III, Unified Parkinson Disease Rating Scale part III.

Table 2: Mean and standard deviation (SD) of visual functional parameters in healthy controls and subjects with Parkinson disease. The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0125$ for VA ETDRS 100, 2.50 and 1.25; $p<0.0125$ for Pelli Robson and CSV 1000E measurements; $p<0.0083$ for Farnsworth and L´Anthony tests). Abbreviations: VA, visual acuity; ETDRS, early treatment diabetic retinopathy study; cpd, cycles per degree; AC CCI, age corrected color confusion index; CCI, color confusion index; C-index, confusion index; Conf Angle, confusion angle; S-index, scatter

index; PD, Parkinson disease.

Table 3: Mean and standard deviation (SD) of structural parameters (retinal nerve fiber layer, ganglion cell layer and macular thicknesses) obtained with the Cirrus HD optical coherence tomography device in healthy controls and subjects with Parkinson disease. The asterisk indicates those values with statistical significance after Bonferroni correction for multiple tests ($p<0.0055$ for macular measurements; $p<0.0062$ for ganglion cell measurements and $p<0.01$ for RNFL measurements). Abbreviations: IPL, inner plexiform layer; GCL, ganglion cell layer; RNFL, retinal nerve fiber layer; HD, high definition.

Table 4: Correlation between macular and ganglion cell layer structural measurements and color vision evaluated with L'Anthony color test in patients with Parkinson disease. Data in bold type correspond to statistically significant correlations (p value <0.05). Abbreviations: GCL, ganglion cell layer; IPL, inner plexiform layer; C-index, Confusion index; CCI, color confusion index; S-index, Scatter index.

Table 5: Correlation between visual acuity measured with ETDRS chart at different levels of contrast (in %) and macular structural measurements (thickness and volume) in patients with Parkinson disease. Correlation data in bold type are statistically significant (p value <0.05). Abbreviations: VA, visual acuity; ETDRS, Early Treatment Diabetic Retinopathy Study.

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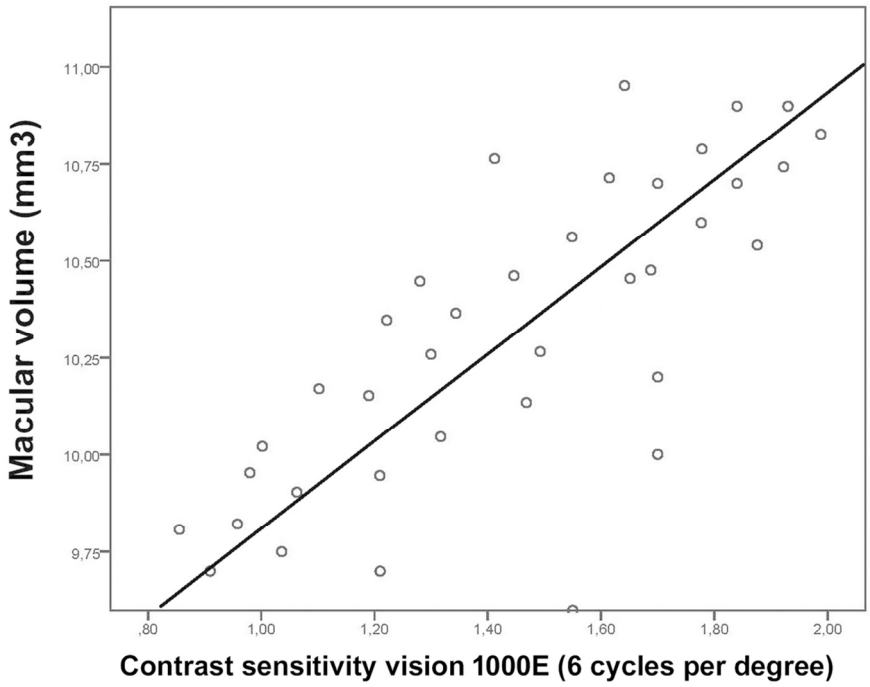

Figure 1: Correlation between the average macular thickness and contrast sensitivity vision as measured with the CSV 1000E test, at a spatial frequency of 6 cycles per degree, in patients with Parkinson's disease.
108x83mm (300 x 300 DPI)

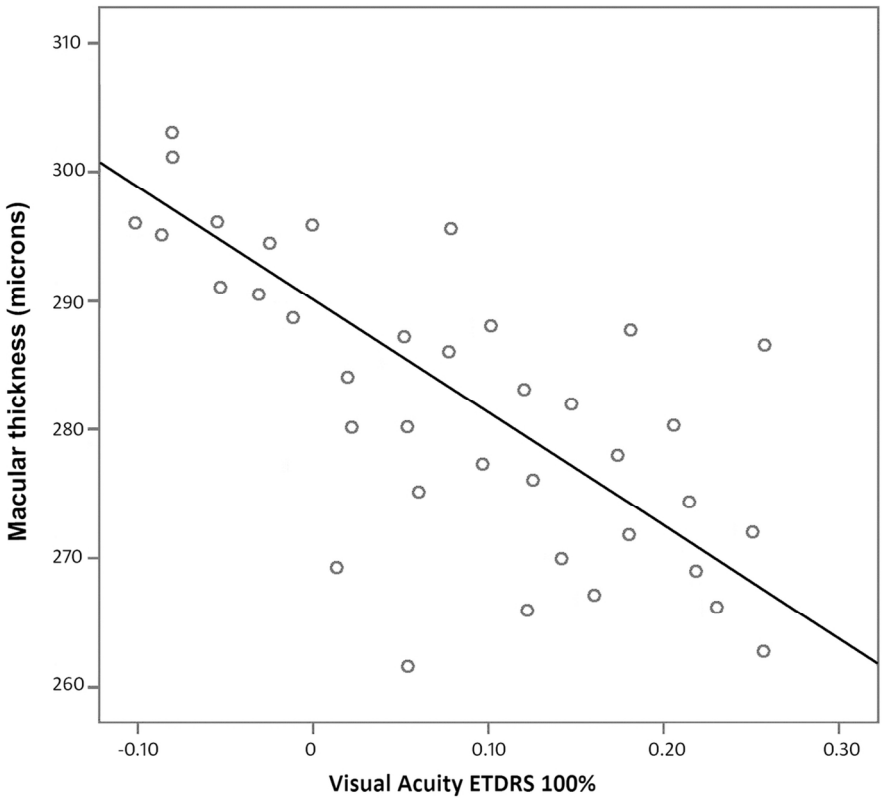

Figure 2: Correlation between the average macular thickness and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.
118x100mm (300 x 300 DPI)

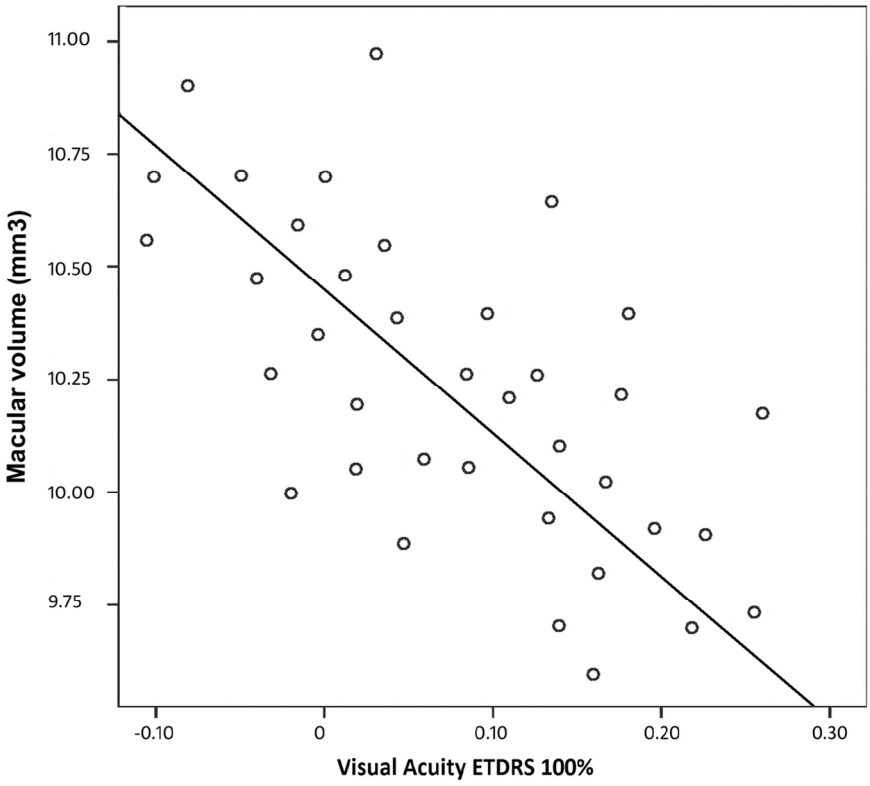

Figure 3: Correlation between macular volume and visual acuity as measured with ETDRS optotype at a contrast level of 100% in patients with Parkinson's disease.
119x102mm (300 x 300 DPI)

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	*(*a) Indicate the study's design with a commonly used term in the title or the abstract Check. This included in the abstract, methods, line 1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Check. This is included in the abstract. Methods, from line 2 to results section line 9
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Check. This is included in the introduction. Paragraph 2.
Objectives	3	State specific objectives, including any prespecified hypotheses Check. This is included in the introduction. Paragraph 3.
Methods		
Study design	4	Present key elements of study design early in the paper Check. This is included in Methods, paragraph 1-3.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Check. This is included in Methods, paragraph 1-3.
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Check. This is included in Methods, paragraph 2
		(*b*) For matched studies, give matching criteria and the number of controls per case Check. This is included in Methods, paragraph 1
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Check. Outcomes and variables are explained in Methods, paragraphs 4-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Check. Details of measurements are included in paragraphs 4-8.
Bias	9	Describe any efforts to address potential sources of bias Check. This is included in paragraph 8, line 3-4.
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Check. Quantitative variables are explained in Methods, paragraphs 4-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding. Check. This is included in methods, paragraph 9
		(*b*) Describe any methods used to examine subgroups and interactions. Not applicable.
		(*c*) Explain how missing data were addressed Not applicable
		(*d*) If applicable, explain how matching of cases and controls was addressed Not applicable
		(*e*) Describe any sensitivity analyses Not applicable
Results		

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram This is a cross sectional study. Inclusion criteria were explained in methods. All elegible subjects were included in the study (37) as already explained, all completed the evaluation.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Check. This is included in Results, paragraph 1-2 and table 1.
		(b) Indicate number of participants with missing data for each variable of interest Not applicable.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure Check. Numbers and results of each variable are included in results.
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Check. Main results include statistical results prior and post multiple comparisons adjustment.
		(*b*) Report category boundaries when continuous variables were categorized Check. Category of correlation is explained in discussion, paragraph 5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Not applicable

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Not applicable
Discussion		
Key results	18	Summarise key results with reference to study objectives Check. This is included in results, paragraph 1.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Check, this is mentioned in paragraph 3 and 6.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Check. This is included and discussed along the discussion section.
Generalisability	21	Discuss the generalisability (external validity) of the study results Check. This is included and discussed along the discussion section
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Check. Not applicable.

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.