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Assessment of visual function and the neuroretina in subjects diagnosed with congenital anomaly of color vision

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Abstract: This cross-sectional and observational study includes 50 eyes of subjects with color blindness and 50 eyes of control subjects. Visual function (visual acuity, contrast sensitivity, and color vision) and neuroretinal structure were assessed in all subjects using optical coherence tomography (OCT). Significant thinning of the retinal nerve fiber layer, ganglion cell layer, and retina were observed in the color blindness group. Significant thinning was also recorded in layers that involve photoreceptor nuclei (between the outer limiting layer and the Bruch membrane and between the outer plexiform layer and the outer limiting membrane). OCT evaluation based on retinal segmentation is a rapid (5–10 minutes) non-invasive technique and seems to be a good biomarker of color blindness.

1. Introduction

Neural image processing begins in the neuroretina, in the neural cells. The retinal photoreceptors (cones and rods) capture the light signal from the environment and transform it into a nerve impulse through a series of enzymatic and biochemical reactions. The nerve impulse reaches the ganglion cells, whose axons join to form the optic nerve which conducts the electrical signal to the optic chiasm, the optic tract, the lateral geniculate body and the primary visual area in the occipital lobe of the cerebral cortex.

There are approximately 120 million rods and 6 million cones in the retina. The rods are mainly located in the peripheral retinal area, while the cones decrease in number towards this area and are more abundant in the central area, in the fovea [1]. The rods are responsible for response at low light intensity (i.e., in scotopic vision, with very low illumination below 0.01 candelas per square meter [cd/m^2]), while the cones are responsible for response at high light intensity (i.e., in photopic vision, with good illumination corresponding to ≥ 3 cd/m^2). In this way, the two together enable vision at different light intensities, as each type is responsible for the corresponding vision. At medium light intensity (mesopic illumination, from 3 cd/m^2 down to 0.01 cd/m^2) they work simultaneously.

In addition, the interior of each photoreceptor contains a visual pigment: a molecule made up of a protein (opsin) and a derivative of vitamin A (11-cis-retinal). This molecule changes its formation when light strikes it, triggering the enzymatic and biochemical reactions that begin visual processing.

The rods contain a pigment called rhodopsin. Cones can contain any of three types of opsin, each of which is sensitive to a characteristic wavelength: erythropsin (in long-wavelength

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cones [L-cones], corresponding to the color red), chloropsin (in medium-wavelength cones [M-cones], corresponding to the color green), and cyanopsin (in short-wavelength cones [S-cones], corresponding to the color blue). [1,2].

Color blindness is an anomaly of color vision characterized by a reduced ability to distinguish colors. The degree of color blindness varies widely, ranging from difficulty in distinguishing different shades of color to total loss of color vision. Color blindness is classified into 3 groups (Table 1).

Table 1. Summary of the three main groups of color blindness, with their subgroups.

TYPE OF COLOR BLINDNESS	SUBTYPE	PIGMENT AFFECTED	CAUSE	DEFICIENCY
Anomalous trichromatism	Deuteranomaly	Chlorolabe	Absence of affected pigment	Red-Green
	Protanomaly	Erythrolabe		
	Tritanomaly	Cyanolabe		Yellow-Blue
Dichromatism	Deuteranonopia	Chlorolabe	Absence of affected pigment	Red-Green
	Protanopia	Erythrolabe		Red-Green
	Tritanopia	Cyanolabe		Yellow-Blue
Monochromatism & achromatopsia	Monochromatism	All	Presence of a single pigment	Monochromatic vision
	Achromatopsia	All	Total absence of pigments	Achromatic vision

Another way to classify color deficiencies is by dividing them into three categories: red-green color blindness (the most common), blue-yellow color blindness, and complete color blindness (monochromacy, much less prevalent).

In red-green color blindness, the types of deficiencies are deuteranomaly (the most common type of red-green color blindness, caused by an anomaly of the M-cones and in which greens look more red), protanomaly (the opposite of deuteranomaly, this deficiency makes red look more green and less bright and is caused by an anomaly of the L-cones), and protanopia and deuteranopia (the inability to detect any difference between red and green altogether).

In blue-yellow color blindness, there is reduced sensitivity in the blue-sensitive S-cone cells. The deficiencies are tritanomaly (which makes blue and green hard to differentiate along with red and yellow) and tritanopia (which makes it difficult to tell the difference between certain combinations of colors like blue/green, purple/red, and yellow/pink; surrounding colors will look less bright).

There are many tests to detect color vision deficiencies, such as pseudoisochromatic tests (composed of colored circles of variable size and intensity that contain a number or line that is indistinguishable to the pathological eye, e.g., the Ishihara test), tests for sorting colors according to their hue or saturation (the Farnsworth-Munsell and Lanthony tests) and anomaloscopes (colorimeters used for color matching that perform comprehensive analysis, such as the Nagel anomaloscope) [3].

The Farnsworth D-15, Lanthony D-15 and Farnsworth-Munsell 100 are sorting tests that comprise sheets of different stimuli that must be sorted according to tone or hue. The subject's color vision evaluation is based on the number of errors they make during sorting, classifying their vision as either normal trichromatic or one of the different types and degrees of color blindness. Subjects with normal color vision are capable of sorting colors by hue with minimal error, while those with impaired color vision are incapable of doing so and produce a high percentage of errors.

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There is currently no treatment for color vision deficiencies. However, there are aids that allow color-blind subjects to improve their color perception, enabling clearer discrimination of the different shades of a color [4]. This is the case of contact lenses and glasses with selective filters, which act on the wavelengths affected in each type of deficiency (red-green or yellow-blue), improving visualization of the different absorption spectra affected [4]. There are also aids (applications) that facilitate visualization of electronic device screens by modifying their contrast and tone.

Given that color blindness is due to the lack or malfunction of at least one type of cone, it seems logical to posit that these subjects will present a reduction in retinal thickness, in particular in the photoreceptor layer, and that this thinning may be quantified by optical coherence tomography (OCT). [4]

This study assesses whether the existence of color vision deficiency or color blindness involves impairment of visual acuity, contrast sensitivity and color vision and results in variations in retinal nerve fiber layer (RNFL) thickness, macular area, retinal ganglion cell complex and retinal layers that contain photoreceptors (bastons and cones) versus subjects with normal color vision within the same age and gender distribution.

2. Material and methods

A cross-sectional, observational, non-interventional study was conducted. Data collection was carried out at Miguel Servet University Hospital. A sample of subjects was studied and classified according to the presence or absence of color vision impairment. The tests performed in both groups assessed visual function: visual acuity (VA) with the Early Treatment Diabetic Retinopathy Study (ETDRS) optotype at 3 contrast levels (100%, 2.5%, 1.25%), contrast sensitivity using the Pelli-Robson test, color vision using the Color Vision Recorder (CVR) (Farnsworth Panel D-15, Lanthony Desaturated D-15, Farnsworth-Munsell 100), and neuroretinal structure using OCT focusing on measurement of the RNFL, the ganglion cell complex (GCC) (axonal and macular) and the photoreceptor layer (layer between the outer limiting layer and the Bruch membrane and layer between the outer plexiform layer and the outer limiting membrane), both overall and by quadrant and sector. Fifty eyes of 50 subjects diagnosed with color blindness and 50 eyes of 50 control subjects were evaluated.

Inclusion criteria were transparency in the optical media and visual acuity > 0.5 on the Snellen scale when measured monocularly (to avoid including amblyopic eyes that could alter the results) [5].

Exclusion criteria were high refractive defects (spherical equivalent > 5.00 D or astigmatism > 3.00 D), anisometropia > 2 D, pharmacological treatment potentially toxic to the retina and/or optic nerve, ocular diseases capable of altering retinal anatomy, intraocular pressure > 21 mmHg or suspected glaucomatous pathology, previous retinal and/or glaucoma surgery and other causal alterations of acquired decrease in color vision or optic neuropathy.

Visual acuity was measured using the ETDRS optotype, consisting of three films of different contrasts: 100%, 2.5% and 1.25%. This back-illuminated test uses the logMAR scale to measure visual acuity at 3 contrast levels, keeping the number of letters present in each row constant and maintaining a progression of 0.1 logarithmic units between them [6]. The test ends when the subject is unable to identify every letter in a line. Each subject was seated 4 meters from the test and vision was corrected for distance if necessary. Visual acuity was measured for all 3 contrasts, monocularly and under high mesopic illumination.

Contrast sensitivity was assessed using the Pelli-Robson test, which consists of an eye chart made up of 8 lines with 6 letters on each, the difference being that the contrast of these lines decreases for each trio [7]. The test ends when the subject is unable to identify the letters of a trio or can only distinguish one of them. Each subject was placed 1 meter from the test and

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distance or intermediate vision was corrected if necessary. Contrast sensitivity was measured monocularly and under photopic illumination.

To identify and classify the different color vision deficiencies, the subjects were assessed using the Color Vision Recorder (CVR) software, in which the Farnsworth Panel D-15, Lanthony Desaturated D-15 and Farnsworth-Munsell 100 sorting tests were performed. These tests allow diagnosis of type of color vision, as well as quantification of its severity [8,9]. Each subject was placed 40 cm from the computer and near vision was corrected if necessary. Color vision was assessed monocularly and under photopic illumination.

OCT was performed using the Spectralis spectral domain OCT (SD-OCT) device [10] and three protocols were used to record the thickness of the peripapillary neuroretinal structures and all the retinal layers: **RNFL** (glaucoma), to measure the thickness of the RNFL, starting from the temporal quadrant of the optic nerve and continuing in the TSNIT (Temporal-Superior-Nasal-Inferior-Temporal) direction; **RNFL-N** (axonal), to measure the same but starting and ending in the nasal quadrant of the optic nerve with the aim of improving the reproducibility and reliability of measurements taken in the temporal sector; and **Fast Macular**, to measure macular thickness and segment the different retinal layers. The following retinal layers were recorded: RNFL, ganglion cell layer (GCL), the layer between the outer limiting layer and the Bruch membrane, and the layer between the outer plexiform layer and the outer limiting membrane.

The fast macular protocol provides information on the thickness of the retina in the macular region, dividing it into several sectors (nasal inner and outer, superior inner and outer, temporal inner and outer, and inferior inner and outer) (Fig. 1). The Spectralis OCT software uses the images provided by this protocol to automatically delimit the retinal layer (Fig. 2) and to provide the thicknesses of each layer. This segmentation protocol makes it possible to determine the specific thickness of each layer and identify thinning or alterations in each retinal cell type.

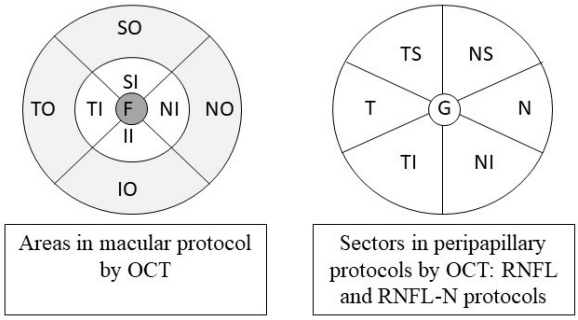

Fig. 1. (a) Representation of areas in macular protocol by optical coherence tomography (OCT) in the right eye. Abbreviations: Fovea (F), Nasal inner (NI), Nasal outer (NO), Superior inner (SI), Superior outer (SO), Temporal inner (TI), Temporal outer (TO), Inferior inner (II) and Inferior outer (IO). (b) Representation of six sectors in two peripapillary protocols by OCT (RNFL and RNFL-N protocols) around the optic disc in the right eye. Abbreviations: Temporal (T), Temporal Superior (TS), Temporal Inferior (TI), Nasal (N), Nasal Superior (NS) and Nasal Inferior (NI), the average value of RNFL thickness globally (G).

The thicknesses of the RNFL and RNFL-N protocols were provided in the 6 sectors of the optic disc: Temporal (T), Temporal Superior (TS), Temporal Inferior (TI), Nasal (N), Nasal Superior (NS) and Nasal Inferior (NI). They also represent the average value of RNFL thickness globally (G) (Fig. 1).

Each subject was positioned with their forehead and chin resting on the apparatus. Their vision was uncorrected and directed straight ahead. The necessary protocol was selected for each measurement and the values were taken monocularly and with scotopic illumination in the room.

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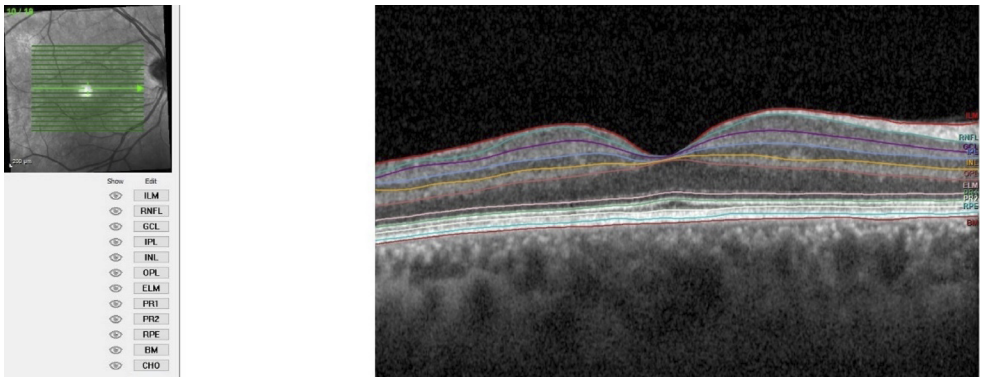

Fig. 2. Representation of the layers of the retina delimited and measured by the Spectralis OCT software's automatic segmentation protocol. Abbreviations: ILM, inner limiting membrane; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ELM, external limiting membrane; PR1, photoreceptor inner segment; PR2, photoreceptor outer segment; RPE, retinal pigment epithelium; BM, Bruch membrane; CHO, choroid.

Statistical analysis: The study variables were collected in a database using Microsoft Excel and imported into the Statistical Package for the Social Sciences (SPSS version 20.0, SPSS Inc. Chicago, IL, USA) for statistical analysis. The results obtained for the quantitative variables were expressed as mean and standard deviation, while the results obtained for the qualitative variables were expressed as frequencies and percentages. The Kolmogorov-Smirnov test was used to check that the quantitative variables conformed to normality. Although most of them did, it was decided to use non-parametric tests to analyze the study variables because some parameters did not have a normal distribution. Therefore, variables were compared between the two groups using the Mann–Whitney U test. For all analyses in this study, the significance level used was $p \leq 0.05$. In tables with several variables we have added the Bonferroni test to counteract the multiple comparison problem. Lastly, we have performed comparative analysis of neuroretinal thicknesses between the different types of color blindness (deuteranomaly, protanomaly, and undetermined) [11].

3. Results

Before starting the analysis, we verified that the two study groups were comparable by checking that there were no significant differences with respect to the age variable, the mean age being 23.71 ± 11.14 years old in the group of control subjects and 25.54 ± 11.25 years old in the group of color-blind subjects ($p = 0.501$); no statistically significant differences were found.

The results obtained in the VA and color vision tests are shown in Table 2.

Although the difference in VA between the two groups was not statistically significant, the results in the group of control subjects were slightly higher than those in the group of color-blind subjects (higher VA was observed in all contrasts in the control group).

The Pelli-Robson test showed no significant difference in contrast sensitivity between the control group (1.91 ± 0.13) and the color-blind group (1.93 ± 0.09) ($p = 0.321$).

The results obtained in the assessment of color vision using the CVR program's three sorting tests showed significantly better results in the group of control subjects (Table 2, Fig. 3), except for the time-taken-to-complete-the-test variable.

The measurements of the neuroretinal structure obtained using the Spectralis SD-OCT device to measure RNFL thicknesses are shown in Table 3 both for the glaucoma protocol (RNFL fast)

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Table 2. Mean and standard deviation of VA using the ETDRS optotype, and of Lanthony D-15, and Farnsworth-Munsell 100 color vision tests for the control group and the color-blind group, and statistical significance (p-value) of the comparison between the two groups obtained by the non-parametric Mann–Whitney U test.[a]

		CONTROL SUBJECTS	COLOR-BLIND SUBJECTS	SIGNIFICANCE (P)
VA	100%	1.10 ± 0.19	1.07 ± 0.26	0.302
	2.5%	0.39 ± 0.12	0.38 ± 0.11	0.512
	1.25%	0.26 ± 0.10	0.22 ± 0.08	0.132
FARNS-WORTH	Time (seconds)	64.33 ± 25.89	70.11 ± 32.12	0.555
	CCI	1.00 ± 0.02	2.79 ± 0.63	**< 0.001**
	AC CCI	1.00 ± 0.03	2.78 ± 0.64	**< 0.001**
	C-index	1.00 ± 0.02	3.40 ± 0.77	**< 0.001**
	S-index	1.47 ± 0.02	4.31 ± 1.50	**< 0.001**
	Confusion angle (°)	62.16 ± 2.54	4.87 ± 15.92	**< 0.001**
LANTHONY D-15	Time (seconds)	85.43 ± 48.02	74.01 ± 37.70	0.417
	CCI	1.11 ± 0.05	2.58 ± 0.37	**< 0.001**
	AC CCI	0.98 ± 0.08	2.35 ± 0.37	**< 0.001**
	C-index	1.05 ± 0.09	3.08 ± 0.42	**< 0.001**
	S-index	1.45 ± 0.12	3.39 ± 1.34	**< 0.001**
	Confusion angle (°)	59.44 ± 5.12	3.03 ± 16.09	**< 0.001**
FARNSWORTH-MUNSELL100	Time (seconds)	403.17 ± 134.78	396.88 ± 158.00	0.325
	TES	39.75 ± 25.62	244.91 ± 69.36	**< 0.001**
	Square root of TES	6.27 ± 1.98	15.04 ± 2.38	**< 0.001**
	TPES-RG	21.07 ± 14.26	156.90 ± 45.37	**< 0.001**
	TPES-BY	21.99 ± 14.91	89.15 ± 48.71	**< 0.001**
	C-index	1.17 ± 0.19	2.93 ± 0.47	**< 0.001**
	S-index	1.35 ± 0.10	1.76 ± 0.31	**< 0.001**
	Confusion angle (°)	52.35 ± 9.87	5.54 ± 19.54	**< 0.001**

[a]Abbreviations: CCI (Color Confusion Index), AC CCI (Age-Corrected Color Confusion Index), C-index (Confusion Index), S-index (Selectivity Index), TES (Total Error Score), TPES-RG (Total Primary Energy Supply Red-Green), TPES-BY (Total Primary Energy Supply Blue-Yellow).

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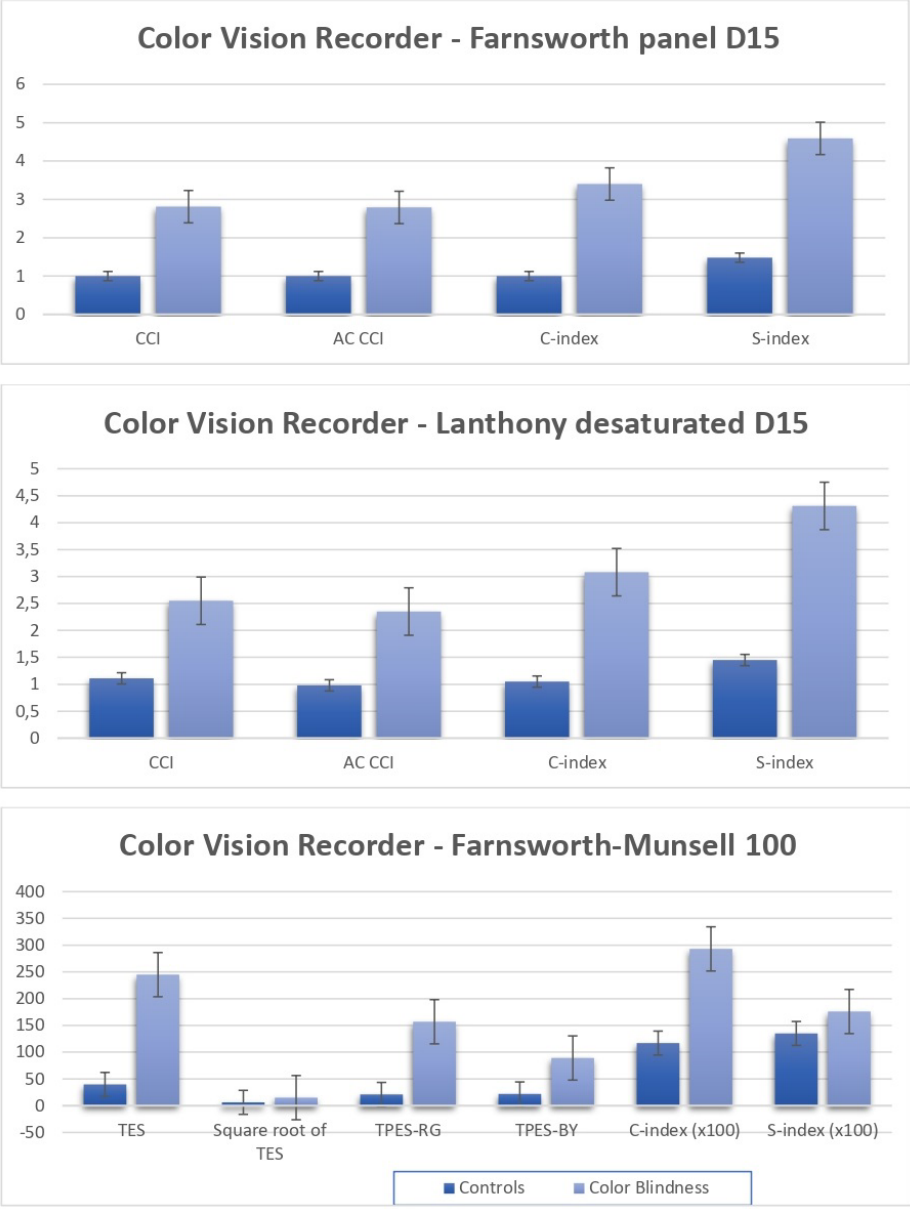

Fig. 3. Comparison representation of the mean and standard deviation of the parameters obtained using the Farnsworth Panel D-test, the Lanthony Desaturated D-15, and the Farnsworth-Munsell 100 tests, for the control group and the color-blind group.

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and the axonal protocol (RNFL-N). Lesser RNFL thickness could be observed in all sectors in the color-blind group versus the control group, except in the upper nasal quadrant and the Nasal/Temporal Ratio of the axonal protocol, where the values were slightly higher in the color-blind group. These differences were significant as regards the average thickness parameter and the lower temporal and nasal sectors of the glaucoma protocol, and in the lower temporal sector of the axonal protocol (Table 3, Fig. 4).

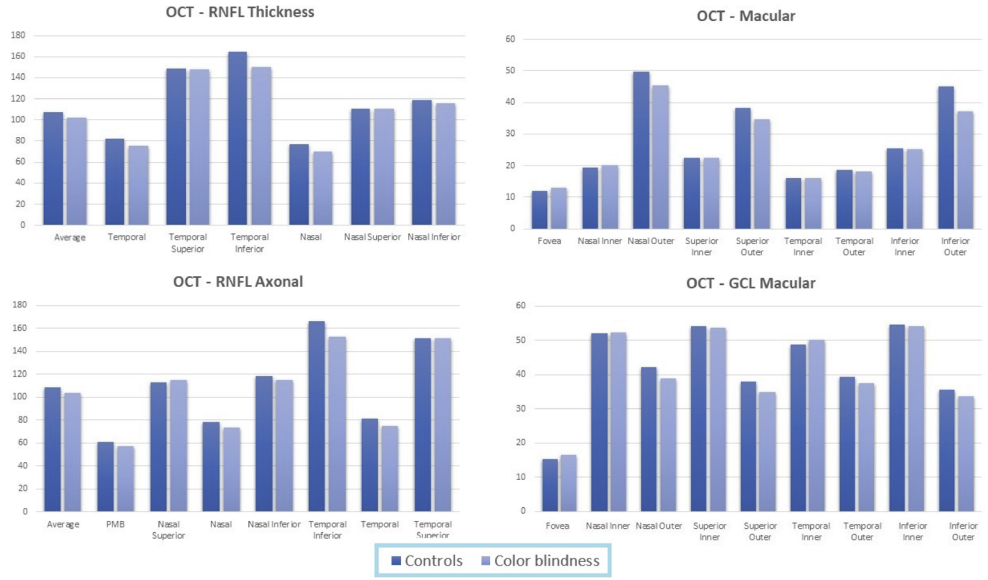

Fig. 4. Comparison representation of the mean of the parameters obtained by OCT of the retinal ganglion cell complex, for the control group and the color-blind group.

The retinal and ganglion cell layer thicknesses in the macular region can be seen in Table 4 and Fig. 4. Lesser retinal thickness was observed in the macular area in all retinal sectors in the group of color-blind subjects versus the control group, the difference being significant in the total volume, the maximum central point and in the outer nasal, outer superior and outer inferior quadrants. In the GCL, significantly greater thickness was observed in the outer nasal and upper outer quadrants in the control group versus the color-blind group.

The retinal thicknesses between the outer limiting layer and the Bruch membrane and between the outer plexiform layer and the outer limiting membrane are listed in Table 5. Significant thinning was recorded in almost all sectors in the group of subjects with color vision deficiencies (Fig. 5).

Classification by color-vision deficiency type in our 50-subject sample identified deuteranomaly in 27 subjects (54%), protanomaly in 13 subjects (26%), and undetermined deficiency type in 10 subjects (20%). Comparative analysis of neuroretinal thicknesses between the different types of color blindness (deuteranomaly, protanomaly, and undetermined) was performed using the ANOVA test. No statistical differences were found in terms of retinal layer thickness between groups by quadrant or sector.

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Table 3. Mean and standard deviation of the parameters obtained using OCT of the retinal nerve fiber layer for the control group and the color-blind group, and statistical significance (p-value) of the comparison between the two groups with a non-parametric Mann–Whitney U test.[a]

		CONTROL SUBJECTS	COLOR-BLIND SUBJECTS	SIGNIFICANCE (P)
RNFL GLAUCOMA	Global (μm)	107.55 ± 10.52	102.53 ± 6.42	0.037
	Temporal (μm)	82.32 ± 16.41	75.34 ± 12.31	0.120
	Temporal Superior (μm)	148.89 ± 22.59	148.26 ± 12.43	0.760
	Temporal Inferior (μm)	164.35 ± 16.56	150.34 ± 15.32	**0.008**
	Nasal (μm)	76.83 ± 16.34	70.27 ± 11.13	**0.037**
	Nasal Superior (μm)	109.92 ± 18.78	110.45 ± 19.83	0.879
	Nasal Inferior (μm)	118.92 ± 24.76	115.12 ± 13.31	0.447
RNFL AXONAL	Global (μm)	108.71 ± 10.19	103.98 ± 6.88	0.071
	PMB (μm)	61.01 ± 8.67	57.72 ± 8.09	0.206
	N/T Ratio	1.01 ± 0.29	1.02 ± 0.30	0.901
	Nasal Superior (μm)	113.01 ± 18.72	113.97 ± 19.55	0.776
	Nasal (μm)	78.81 ± 18.99	73.38 ± 12.67	0.179
	Nasal Inferior (μm)	118.47 ± 23.14	114.90 ± 16.79	0.402
	Temporal Inferior (μm)	165.76 ± 17.12	152.65 ± 13.71	**0.011**
	Temporal (μm)	81.50 ± 16.87	74.76 ± 12.67	0.113
	Temporal Superior (μm)	151.65 ± 19.33	151.37 ± 10.52	0.876

[a]The sectors are delimitated by OCT around the optic disc for peripapillary area. Abbreviations: RNFL, retinal nerve fiber layer.

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Table 4. Mean and standard deviation of retinal thickness in the macular area and ganglion cell layer for the control group and the color-blind group, and statistical significance (p-value) of the comparison between the two groups with a non-parametric Mann–Whitney U test.[a]

		CONTROL SUBJECTS	COLOR-BLIND SUBJECTS	SIGNIFICANCE (P)
RETINAL THICKNESS	Total Volume (mm^3)	0.94 ± 0.12	0.86 ± 0.09	**0.008**
	Fovea (μm)	11.93 ± 1.71	12.99 ± 1.64	**0.046**
	Nasal inner (μm)	19.41 ± 1.67	20.31 ± 1.88	0.181
	Nasal outer (μm)	49.63 ± 7.77	45.21 ± 6.00	**0.034**
	Superior inner (μm)	22.46 ± 2.73	22.49 ± 2.11	0.800
	Superior outer (μm)	38.37 ± 5.34	34.79 ± 4.44	**0.016**
	Temporal inner (μm)	16.20 ± 1.15	16.16 ± 1.11	0.701
	Temporal outer (μm)	18.59 ± 1.75	17.99 ± 1.06	0.244
	Inferior inner (μm)	25.43 ± 2.75	25.21 ± 2.48	0.901
	Inferior outer (μm)	45.04 ± 7.74	37.08 ± 4.80	**< 0.001**
	Centre (μm)	0.93 ± 2.01	1.38 ± 2.10	0.097
	Central maximum (μm)	25.26 ± 3.01	28.79 ± 4.00	**< 0.001**
GCL THICKNESS	Fovea (μm)	15.32 ± 3.01	16.50 ± 3.14	0.244
	Nasal inner (μm)	52.10 ± 5.97	52.32 ± 4.79	0.564
	Nasal outer (μm)	42.20 ± 3.11	38.86 ± 3.47	**0.005**
	Superior inner (μm)	54.21 ± 4.31	53.59 ± 3.78	0.711
	Superior outer (μm)	37.84 ± 3.44	34.93 ± 2.99	**< 0.001**
	Temporal inner (μm)	48.63 ± 4.87	50.16 ± 4.41	0.200
	Temporal outer (μm)	39.23 ± 5.00	37.41 ± 3.97	0.197
	Inferior inner (μm)	54.63 ± 5.06	54.20 ± 3.69	0.754
	Inferior outer (μm)	35.59 ± 3.99	33.66 ± 3.69	0.134
	Centre (μm)	3.13 ± 3.10	3.85 ± 3.01	0.387
	Central maximum (μm)	38.70 ± 8.33	42.80 ± 8.14	0.099

[a]The sectors are delimitated by OCT in the macular area the optic disc for peripapillary area.
Abbreviations: GCL (Ganglion Cell Layer).

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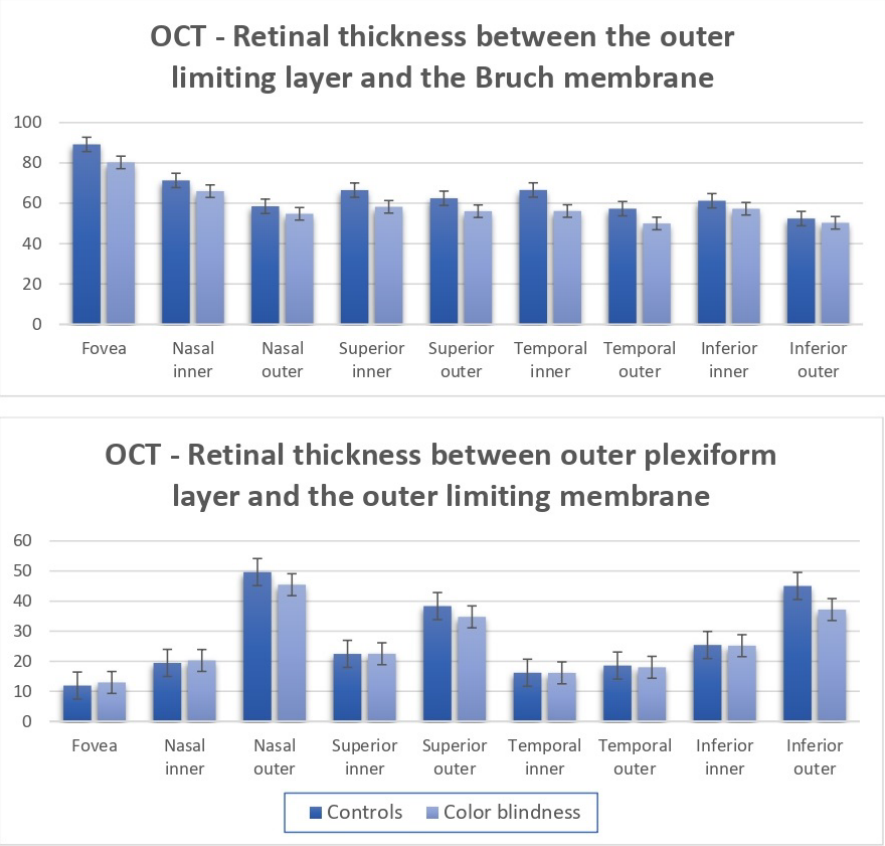

Fig. 5. Comparison representation of the mean and standard deviation of the parameters obtained by OCT of the photoreceptor layers (the layer between the outer limiting layer and the Bruch membrane and the layer between the outer plexiform layer and the outer limiting membrane), for the control group and the color-blind group.

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Table 5. Mean and standard deviation of the parameters obtained by OCT of the retinal thicknesses between the outer limiting layer and the Bruch membrane and between the outer plexiform layer and the outer limiting membrane for the control group and the color-blind group, and statistical significance (p-value) of the comparison between the two groups with a non-parametric Mann–Whitney U test.[a]

		CONTROL SUBJECTS	COLOR-BLIND SUBJECTS	SIGNIFICANCE (P)
THICKNESS BETWEEN OUTER LIMITING LAYER AND BRUCH MEMBRANE	Total Volume (mm^3)	2.22 ± 0.08	2.18 ± 0.11	0.101
	Fovea (μm)	87.76 ± 4.86	82.72 ± 5.41	**0.003***
	Nasal inner (μm)	81.98 ± 2.90	79.40 ± 4.53	0.056
	Nasal outer (μm)	78.54 ± 3.69	77.61 ± 4.44	0.100
	Superior inner (μm)	79.80 ± 3.18	77.32 ± 4.09	**0.033**
	Superior outer (μm)	78.59 ± 2.21	75.14 ± 4.18	**0.002***
	Temporal inner (μm)	80.32 ± 3.01	77.66 ± 3.87	**0.023**
	Temporal outer (μm)	76.84 ± 2.77	74.53 ± 2.98	**0.004**
	Inferior inner (μm)	78.90 ± 3.00	75.45 ± 3.45	**< 0.001***
	Inferior outer (μm)	75.94 ± 2.31	74.95 ± 3.23	0.098
	Centre (μm)	95.69 ± 7.21	88.60 ± 4.99	**< 0.001**
	Central maximum (μm)	98.43 ± 7.75	91.87 ± 5.31	**0.003**
THICKNESS BETWEEN OUTER PLEXIFORM LAYER AND OUTER LIMITING MEMBRANE	Total Volume (mm^3)	1.72 ± 0.19	1.61 ± 0.21	0.187
	Fovea (μm)	89.20 ± 11.71	80.22 ± 7.77	**0.004**
	Nasal inner (μm)	71.34 ± 10.32	66.02 ± 7.02	0.091
	Nasal outer (μm)	58.51 ± 5.02	54.80 ± 5.14	0.077
	Superior inner (μm)	66.49 ± 12.44	58.24 ± 10.22	**0.018**
	Superior outer (μm)	62.46 ± 7.80	56.11 ± 5.01	**0.006**
	Temporal inner (μm)	66.61 ± 19.15	56.17 ± 7.31	**< 0.001***
	Temporal outer (μm)	57.32 ± 7.59	50.02 ± 5.97	**< 0.001***
	Inferior inner (μm)	61.28 ± 9.25	57.30 ± 7.01	0.111
	Inferior outer (μm)	52.43 ± 5.88	50.35 ± 6.00	0.198
	Centre (μm)	104.53 ± 12.67	93.94 ± 13.78	**0.010**
	Central maximum (μm)	115.94 ± 9.67	107.24 ± 10.44	**0.005**

[a]Bold lettering indicates significant differences ($p \leq 0.05$) and an * indicates significant differences that overcome Bonferroni correction for multiple comparisons.

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4. Discussion

In this study we assessed both visual function (VA, contrast sensitivity and color vision) and neuroretinal structure using OCT in subjects affected by color blindness versus control subjects. Our hypothesis was that subjects with color blindness would show thinning in some neuroretinal structures, especially in the retinal photoreceptor layers. Until a few years ago, neuroretinal structures could only be measured by histological study. However, recent advances in both OCT and segmentation techniques with which to linearly analyze the thickness of each retinal layer now allow us to evaluate layer thickness, including photoreceptors, using a non-invasive *in vivo* technique based on Fourier domain OCT.

On the one hand, quantification of VA using the ETDRS optotype test in the two study groups showed no statistically significant differences, although VA was slightly lower in the color-blind group. On the other, the data corresponding to contrast sensitivity, obtained using the Pelli-Robson test, showed a slight and statistically non-significant increase in this visual capacity in the group of color-blind subjects. This improvement in contrast sensitivity in subjects with color blindness was observed in the study by Ilhan *et al.* [12], who argued that it was due to a decrease in blur and defocus of the retinal image due to a decrease in chromatic noise (color fluctuation) caused by chromatic aberrations of the visual system and photoreceptors, at all spatial frequencies, in subjects with abnormal color vision and specifically in subjects with congenital red-green deficiency. However, this could also cause an increase in VA in these subjects, which was not observed in our study.

Statistically significant differences were recorded between the two groups when conducting the Farnsworth D-15, Lanthony D-15 and Farnsworth-Munsell 100 color vision tests.

Finally, in our study we used Spectralis spectral domain OCT to obtain measurements of the thickness of the RNFL and CCL, both in the macular and peripapillary areas, as well as of the thicknesses of the layers that make up the photoreceptors. In general, no statistically significant differences were observed between the two study groups, with the group of color-blind subjects having lower thicknesses in both layers versus the group of control subjects. Similar results were observed in the study by Gupta *et al.* [13], who also found no significant differences between the retinal morphology of control subjects and that of subjects with red-green color deficiency. However, Ozsoy *et al.* have not found differences in the the thickness of the central macula, RNFL, or ganglion cell layer between subjects with congenital red-green visual deficit and controls subjects. This may be because they included fewer sample size in the study and only a single subtype of colour vision deficit [14].

We have found more thinning in macular volume in outer sectors, although the density of cones is higher in inner sectors. This suggests a potential concentrical affectation of cones in this deficiency.

On the other hand, isolated areas showing a significant increase in the thickness of both layers were observed in control subjects. This was especially the case in the macular area, the retinal area where the number of photoreceptors is greatest. This, therefore, could be diminished in subjects with dichromatism, in which one of the three cones is absent. Theoretically, in trichromats the distribution and density of the cones would not be altered since the deficiency is due to the incorrect functioning of a cone type rather its absence, so the thickness would not be reduced.

Conversely, the greater the degree of color blindness, the greater the involvement of the retinal structure. Although this study did not include any subjects with a high degree of such involvement, this fact has been previously demonstrated by other authors who studied the morphological characteristics of the retina in subjects with cone dysfunction. These include Barthelmes *et al.* [15], who observed reduced thicknesses in the fovea of subjects with blue monochromatism, but did not record any retinal involvement in subjects with achromatopsia.

Trichromat, dichromat, and monochromat are terms used in the vision science community to refer to different possible configurations of the human visual system with three, two, or one

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channel of color information, respectively. However, these terms are simplified to a great extent because the true capability of a color vision system also depends on the degree of overlap between the channels, on perceptual noise within the channels, and on the cognitive processing capability to decipher these signals in the visual cortex of the brain. Most cases of color blindness are considered anomalous trichromacy, which means the subject is effectively functioning somewhere between trichromacy (normal color vision with 3 channels) and dichromacy (2 channels).

In this study using Spectralis OCT, significant reductions in neuroretinal thickness were recorded in subjects diagnosed with color blindness versus the group of control subjects, both in the thickness of the RNFL (average and lower temporal and nasal quadrant thicknesses), of the retina in the macular region (total volume and outer sectors), and of the GCC in the macular area (outer nasal and outer superior sectors). This reduction in number could be due to the characteristic absence of the different cone types in dichromatism. In addition, a slight increase in VA was observed in control subjects, but the opposite was true for contrast sensitivity, which showed better results in color-blind subjects. In neither case, however, were the differences statistically significant.

Specific analysis of retinal layers that involve photoreceptor nuclei shows significant reduction in individuals with color blindness in almost all sectors. Our study therefore demonstrates a clear thinning of this layer caused by reduction in photoreceptor number. As our findings show, color blindness is associated with thinning in the retinal layer between the outer limiting layer and the Bruch membrane and in the layer between the outer plexiform layer and the outer limiting membrane. OCT study with retinal segmentation therefore seems to be an extra marker of color blindness with potential utility in the case of doubt regarding diagnosis.

The main limitation of this study is the small sample size in both groups, meaning further research should consider increasing the number of subjects and thus increasing the reliability and validity of the study.

Another limitation is that when using OCT data, neuroretinal thinning in color deficiencies is unspecified. However, big data or learning machine studies exploiting greater processing power and bioengineering techniques may optimize OCT's ability to detect the specific layers affected in these conditions and provide a biomarker with which to facilitate diagnosis.

5. Conclusions

We have found significantly reduced thicknesses in the RNFL and several retinal layers (ganglion cell and photoreceptor layers), demonstrating that color blindness is associated with thinning in retinal and RNFL thickness, and in the retinal layers that involve photoreceptor nuclei. OCT study with retinal segmentation therefore seems to be a marker of color blindness of utility in clinical practice in the case of doubt regarding diagnosis. This analysis could be useful in evaluating the effectiveness of potential therapies such as gene treatment.

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Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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