scientific reports

Check for updates

OPEN

Structural and vascular assessment of the optic nerve head and macula in chronic Chagas disease

Cristiana L. M. Agra[1,2]✉, Camila V. Ventura[1,2,3], Letícia D. da Fonte[1], Marcela R. V. Oliveira[1], Marília Leal[1], Maria Elisa L. S. M. Assunção[4], Maria da Glória A. M. Cavalcanti[4], Silvia M. M. Alves[4], Ana Karine Soares[3], Liana O. Ventura[1,3] & Tiago S. Prata[5]

Chagas disease (CD), a parasitic infection, may have ocular repercussions in its cardiologic form, since a history of heart disease of other etiologies already has been established as a risk factor for neuropathies and maculopathies. The aim of the present study was to investigate preclinical structural and vascular optic nerve head (ONH) and macular parameters in patients with chronic CD. Nineteen patients with CD and 19 healthy subjects were evaluated with optical coherence tomography, optical coherence tomography angiography, and Laguna ONhE® software. The main outcome measures were the glaucoma discriminant factor, average peripapillary retinal nerve fiber layer thickness, macular ganglion cell complex thickness, peripapillary vascular density (VD), foveal and parafoveal thickness, foveal avascular zone area, and total foveal and parafoveal VD from the superficial and deep capillary plexus that were compared between the two groups. No significant differences were observed among the studied variables. Although our findings suggested that the cardiovascular dysfunction resulting from chronic CD does not seem to cause significant structural or vascular preclinical changes to the ONH and the macula, the results herein benefit this patient population and may provide important preliminary information about the ocular impairment caused by the condition and its possible systemic complications.

Keywords Chagas disease, Optic nerve, Macula, Optical coherence tomography angiography, Laguna ONhE®, Hemoglobin

Chagas Disease (CD), a parasitic infection caused by *Trypanosoma cruzi*[1], affects millions of people, mainly in Latin America, and migration to developed countries has spread the disease globally[2–4]. The condition has serious medical, social, and economic consequences, is one of the main causes of death from parasitic diseases in Brazil, and is considered by the World Health Organization to be a neglected disease[1,5,6].

CD can be classified into acute and chronic phases based on its laboratory and clinical evolution[3]. The main clinical forms of the chronic phase can be indeterminate, digestive, or cardiac (with or without ventricular dysfunction). The latter is of great medical importance because of its high morbidity and mortality[3].

Although still underexplored in CD, ophthalmologic findings deserve attention because they can provide important information about the disease progression and its systemic complications[7]. Ocular involvement, possibly beyond what is seen in routine ophthalmologic examinations, may be found in patients with CD; therefore, these patients should undergo careful assessment including more specific tests to assess in detail the ocular structures not observed in routine tests.

A history of heart disease of other etiologies already has been established as a risk factor for glaucoma[8]. Studies have linked reduced optic nerve head (ONH) perfusion to glaucoma development[9]. Changes in the ONH and retinal nerve fiber layer (RNFL) have been observed in patients with chronic CD with cardiac involvement with and without ventricular dysfunction, suggesting a potential association with cardiac problems[7]. In addition, retinal involvement in patients with chronic CD heart disease has been previously investigated, with published reports of anomalous electroretinography findings and retinal pigment abnormalities observed in this population[10].

[1]Department of Ophthalmology, Altino Ventura Foundation (FAV), Avenida Maurício de Nassau, 2075, Recife, PE 52171-011, Brazil. [2]Department of Ophthalmology, HOPE Eye Hospital, Recife, PE, Brazil. [3]Department of Research, Altino Ventura Foundation, Recife, PE, Brazil. [4]Department of Cardiology, Casa de Chagas - PROCAPE, Recife, PE, Brazil. [5]Department of Ophthalmology, Federal University of São Paulo, São Paulo, SP, Brazil. ✉email: clmagra@gmail.com

Variables	CD group	Control group	*p* value
Age (years) (mean ± SD)	60.2 ± 10.8 (n = 19)	58.5 ± 9.3 (n = 19)	0.609*
Sex n (%)			
Female	14 (73.7%)	16 (84.2%)	0.693†
Male	5 (26.3%) (n = 19)	3 (15.8%) (n = 19)	
Systemic arterial hypertension n (%)			
Yes	8 (42.1%)	7 (36.8%)	0.740‡
No	11 (57.9%) (n = 19)	12 (63.2%) (n = 19)	
BCVA (logMAR) (median, min – max)	0 (0–0.4) (n = 19)	0.1 (0–0.5) (n = 19)	0.132§
Intraocular pressure (mean ± SD)	13.6 ± 3.8 (n = 19)	10.3 ± 1.3 (n = 19)	0.487§
Disease duration (years) (mean ± SD)	9.2 ± 3.2 (n = 18)	Does not apply	–

Table 1. Study participants and control group sociodemographic and clinical characteristics. CD, Chagas Disease; SD, standard deviation; BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution. * Student's T, † Fisher exact test, ‡ Chi-square test, § Mann–Whitney test.

	CD group (n = 19)	Control group (n = 19)	*p* value
Peripapillary RNFL thickness (µm) (mean ± SD)	108.0 ± 12.6	112.0 ± 13.5	0.301*
Peripapillary VD (%) (mean ± SD)	53.0 ± 3.8	52.4 ± 2.6	0.669*

Table 2. Optic nerve head SD-OCT and angioanalytics results: comparison between patients with CD and healthy subjects. SD-OCT, spectral domain optical coherence tomography; CD, Chagas Disease; RNFL, retinal nerve fiber layer; µm, micra; SD, standard deviation; VD, vascular density. *Student's T.

Retinal imaging examinations, such as optical coherence tomography angiography (OCTA), are important to assess the ophthalmologic findings and determine the need for treatment in other systemic diseases[11–13], OCTA is a non-invasive imaging modality that assesses retinal and ONH vasculature that differentiates glaucomatous from normal eyes and detects changes correlated with visual field (VF) findings and OCT structural changes, such as peripapillary RNFL and macular alterations[14]. Interestingly, reduced vessel density in the retinal layers has been reported using OCTA in patients with heart disease before clinical fundus signs[15]. Another relatively new method of structural evaluation of the ONH involves the estimation of the ONH hemoglobin (ONHHb) concentration from color fundus photographs by detecting color differences based on the principle that Hb is its only pigment[16,17]. This analysis, made using the Laguna ONhE® software (Laguna Health, New York, NY) previously showed a significant association with both functional and structural damage in optic nerve diseases such as glaucoma[17].

Considering this, one could hypothesize that cardiovascular dysfunction resulting from chronic CD could lead to significant structural or vascular changes in the ONH and macula. Therefore, the aim of this study was to investigate the preclinical morphologic changes in the optic nerve and macula, including structural and vascular ONH and macular parameters obtained from OCT, OCTA, and Laguna ONhE software in patients with chronic CD and normal funduscopy findings.

Results

Thirty-eight eyes of 38 patients were included in this study: 19 from patients with CD (mean age ± standard deviation [SD], 60.2 ± 10.8 years) and 19 eyes of healthy control subjects (mean age ± SD, 58.5 ± 9.3 years). Most participants in both groups were women. Table 1 shows the clinical and sociodemographic characteristics of the participants. Ten (52.6%) patients had the chronic cardiac form (CCF) without ventricular dysfunction, six (31.6%) had CCF with ventricular dysfunction, and three (15.8%) had an indeterminate form. The groups did not differ significantly in the presence of systemic arterial hypertension, best-corrected visual acuity (BCVA), and intraocular pressure values. Twelve participants reported CD diagnosis more than 10 years previously. It was not possible to obtain data on the disease duration for one patient, who was unable to report it.

In subjects with CD, the mean axial lengh (AL) was 22.8 ± 0.7 mm and the mean pachymetry value was 531.4 ± 39.2 µm, similar to those of the general population. All subjects with CD who could perform the VF test obtained satisfactory results. The mean values for mean deviation (MD) and pattern standard deviation (PSD) were, respectively, − 1.7 ± 1.6 decibels (dB) and 1.1 ± 1.0 dB. Five VF tests were excluded due to unreliable results.

Tables 2 and 3 show the spectral-domain OCT (SD-OCT) and Angioanalytics software findings obtained from both groups and a comparative evaluation of the ONH and macula, respectively. Considering the ONH, no structural or vascular parameters evaluated suggested a greater probability of glaucoma in the population with

Parameter (mean ± SD)	CD group (n = 19)	Control group (n = 19)	*p* value
Foveal thickness (µm)	245.0 ± 22.3	252.0 ± 18.6	0.301*
Parafoveal thickness (µm)	314.0 ± 17.5	317.0 ± 11.4	0.517*
GCC thickness (µm)	94.9 ± 6.9	97.3 ± 6.8	0.296*
FAZ area (mm^2)	0.32 ± 0.1	0.30 ± 0.1	0.540*
Total SCP VD (%)	43.8 ± 4.3	45.2 ± 3.5	0.261*
Foveal SCP VD (%)	15.9 ± 8.1	18.2 ± 7.1	0.194§
Parafoveal SCP VD (%)	46.8 ± 4.6	47.9 ± 3.8	0.414§
Total DCP VD (%)	50.1 ± 3.9	50.5 ± 3.2	0.680*
Foveal DCP VD (%)	30.2 ± 9.3	32.4 ± 7.9	0.493§
Parafoveal DCP (%)	53.0 ± 4.1	52.8 ± 3.2	0.883*

Table 3. Macular angioanalytics results: comparison between patients with CD and healthy subjects. CD, Chagas Disease; SD, standard deviation; GCC, ganglion cell complex; FAZ, foveal avascular zone; VD, vascular density; SCP, superficial capillary plexus; DCP, deep capillary plexus; * Student's T, § Mann t-test.

CD compared with the healthy individuals in the evaluated sample. Furthermore, the macular assessments also were similar between the groups.

Regarding the Laguna ONhE software findings, the ONHHb concentration did not differ significantly between healthy individuals and patients with CD. The mean glaucoma discriminant function (GDF) index values for the CD and control groups were 16.5 ± 18.0 and 23.9 ± 19.0, respectively ($p = 0.290$). Four patients were excluded automatically from the analysis by the software because of lower quality fundus photographs secondary to light saturation.

In a subanalysis, comparing the ONH and macular variables in the eyes of patients with ventricular dysfunction and the eyes of the control group, there was no significant difference ($p \geq 0.279$).

Figure 1 shows the ONH SD-OCT, OCTA, and Laguna ONhE assessments of a patient with CD. The macular vascular and structural assessments of one patient with CD are shown in Fig. 2.

Discussion

This study explored the association between chronic CD and ophthalmologic alterations. The aim was to identify if the preclinical structural and vascular parameters of the ONH and retina obtained by OCT, OCTA, and Laguna ONhE in patients with CD could indicate an increased risk of developing neuropathy or present retinal repercussions related to the systemic condition in otherwise healthy eyes. A comparison between the disease and control groups did not reveal significant differences in the evaluated ocular parameters. To the best of our knowledge, this is the first study to apply OCTA and Laguna ONhE software technologies to analyze the eyes of patients with chronic CD.

The literature contains few data regarding CD and ophthalmologic fndings. Villas-Bôas et al. (2019) analyzed a group of patients with CD and reported a higher frequency of ONH pallor, peripapillary hemorrhage, notch, and RNFL defects on retinography. SD-OCT also found decreased peripapillary RNFL thickness, a greater cup-to-disc ratio, and a lower neural rim area. These findings were more significant in patients with CCF and heart failure than in those without left ventricular dysfunction and those with the indeterminate form. Their findings suggested that cardiac dysfunction in CD might have potentiated these alterations[7]. However, it should be noted that subjects with established glaucoma and glaucoma suspects were included in their sample, which introduces a bias and possibly contributed to those findings. Retinal involvement in chronic CD also has been explored previously[10]. Electroretinography performed in these patients detected selective rod dysfunction. An autoimmune response to *T. cruzi* has been suggested. Fluorescein angiography was performed, but no vascular aspects were explored in the study[10]. Since the probability of a higher frequency of glaucoma and retinopathy in the CD population was raised, we sought to detect early vascular signs before any clinical alterations. In the current study, which investigated possible preclinical structural and vascular parameters of the ONH and retina, we found no differences in the preclinical structural and vascular parameters of the ONH and retina between the patients with CD and controls. Our results suggested that for our population with chronic CD who had no other clinical ophthalmologic alterations, annual follow-up with funduscopy might be sufficient; however, this can only be concluded from longitudinal research. The reduced sample size and the small proportion of cardiac patients with ventricular dysfunction are other study limitations.

We believe it is important to provide a deeper discussion about the imaging examinations we performed in our study. Clinically measuring the ocular blood flow is challenging[9] and OCTA has emerged as a dye-free option for evaluating the ONH and retinal microvasculature[14]. The literature indicates a strong correlation between OCTA measurements and VF and OCT results[14,18]. Initial assessments in eyes with primary open-glaucoma (POAG) using OCTA revealed a reduced flow index and VD in the ONH and peripapillary regions compared with controls[19,20]. Subsequent studies have reported reduced VD in the superficial macular regions of glaucomatous eyes[18,21,22]. Moreover, an evaluation found reduced VD in a sector of the perimetrically intact hemifield despite normal RNFL thickness, suggesting that OCTA changes may precede the structural findings[23]. This supports the notion of identifying vascular alterations before clinical and structural manifestations in patients with chronic CD. Regarding the Laguna ONhE software, it indirectly measures ONH perfusion[24]. Its good reproducibility and accuracy in the early diagnosis of glaucomatous neuropathy has been demonstrated[17].

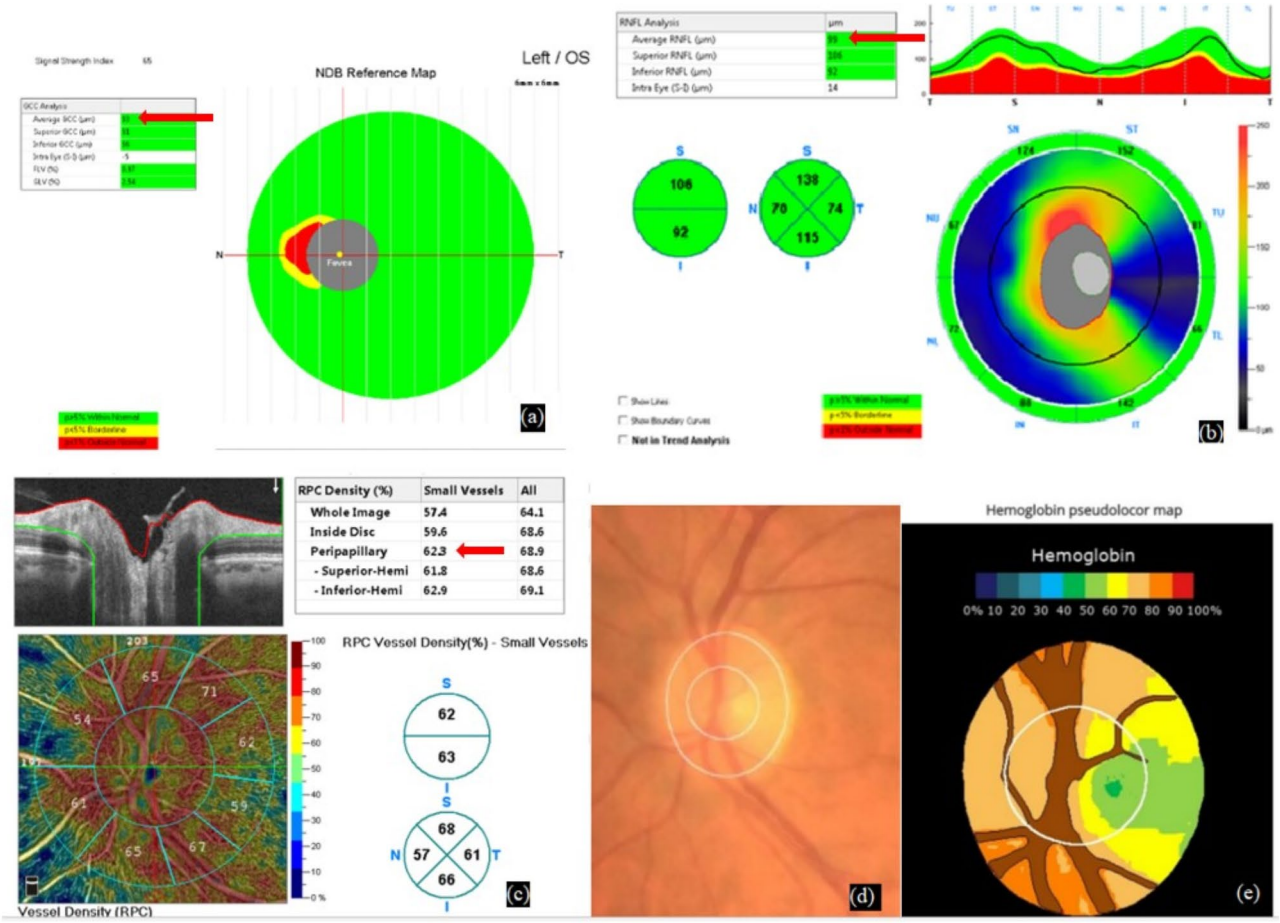

Fig. 1. (**A**) Ganglion cell complex (GCC), (**B**) optic nerve head (ONH) and (**C**) angiodisc maps. The red arrows indicate the values obtained for analysis. (**D**) Retinography with ONH segmentation and (**E**) colorimetric analysis of the ONH provided by the Laguna ONhE software. The colorimetric scale in the Laguna image (**E**) indicates the amount of haemoglobin content present in the disc.

Alterations in ONHHb levels may occur before visual impairment in early glaucoma stages[17]. The GDF index shows a high correlation with the RNFL measured by OCT and functional damage observed on automated perimetry[17,24,25]. The OCTA parameters have been compared to Laguna ONhE, with similar diagnostic validity in POAG[16]. Laguna ONhE software was included in this study alongside OCTA since it could represent a more accessible and equally efficient ophthalmologic evaluation. It is less costly, which is advantageous for the population evaluated, considering that CD is essentially a disease of rural areas and urban emigrants with poorer socioeconomic conditions[1,16].

Some studies have investigated the possible clinical implications of CD-related blood flow and vascular changes in both the optic nerve and retina functions. The vascular theory for the development of glaucoma considers the insufficient blood supply resulting from the reduced ocular blood flow responsible for its pathogenesis[9]. The instability in the blood flow to the ONH leads to ischemia, oxidative stress, and apoptosis[26–28]. Several studies have reported an association between cardiovascular conditions and glaucoma since they promote reduced cardiac output[9,26]. This context could indicate that Chagas syndrome is a risk factor for optic neuropathies. Regarding the possibility of maculopathy, CCF may involve coronary heart disease, and the coronary artery is correlated potentially with the retinal vessels via the ophthalmic artery. The retinal microvasculature has been proposed as an indirect method for evaluating the coronary circulation. A previous report cited decreased VD in the superficial capillary plexus (SCP) and deep capillary plexus (DCP) in OCTA before patients exhibited clinical fundus signs, suggesting OCTA as a measurement method for detecting early-stage coronary heart disease[15]. In addition, microvascular changes observed in CD include microcirculatory spasms, endothelial dysfunction, and higher platelet activity, which also can contribute to macular ischemia[3].

Our study had some limitations that should be considered while interpreting its results. The main limitation of the present study was the small number of participants. Ocular involvement supposedly is connected to the cardiac and general vascular dysfunction present in the chronic phases of the condition, and therefore associated with the duration of the disease. Although most participants were diagnosed with CD more than 10 years previously and a subanalysis also was performed that attempted to explore possible differences between the

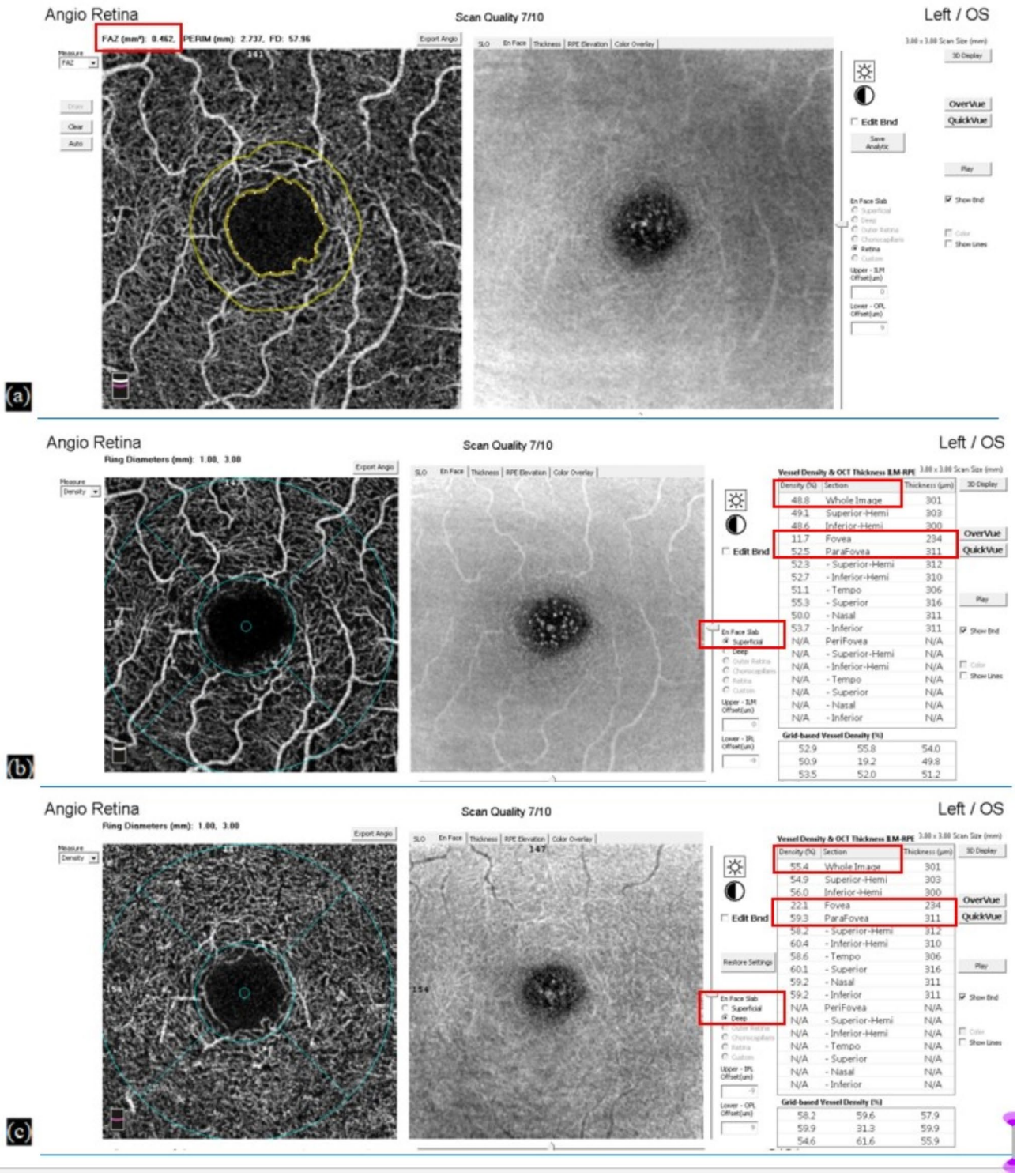

Fig. 2. Macular angioanalytic images provided by the Angiovue software: (**A**) foveal avascular zone area automatically calculated by the program; (**B**) superficial and (**C**) deep capillary plexus. Red boxes indicate the evaluated plexus, the density and thickness values obtained for analysis.

group with ventricular dysfunction and the control group, it should be considered that this is a small sample and therefore lacks adequate statistical power to reach further conclusions.

However, it is justified by the thorough selection of patients to exclude potential biases such as the presence of diabetes, suspected glaucoma, and common vascular retinopathies in CD, including hypertensive retinopathy. Additionally, CD is a less prevalent disease, and patients with previous etiologic treatment were excluded.

In addition, as a cross-sectional study, our findings do not allow the investigation of possible cause-effect relationships but only associations.

In conclusion, our results showed no differences between healthy eyes and those with CD, suggesting that the cardiovascular dysfunction resulting from chronic CD does not seem to lead to significant structural or vascular preclinical changes to the ONH and macula. However, longitudinal research with larger samples should be considered to confirm these findings, since the disease continues to expand to developed countries and still comprises a considerable portion of the world's population.

Material and methods

Ethical criteria

This was an observational and cross-sectional study involving patients with chronic CD attending *Casa de Chagas* – PROCAPE in Recife, Pernambuco, Brazil, and healthy volunteers followed by the Ophthalmology Department of the Altino Ventura Foundation, Recife, PE, Brazil. The Institutional Review Board of the Ethical Committee of the Federal University of São Paulo (IRB protocol number: 4.286.085), the Altino Ventura Foundation (IRB protocol number: 5.866.610), and the *Casa de Chagas*—PROCAPE (IRB protocol number: 4.321.880) approved the study, which was conducted according to the tenets of the Declaration of Helsinki. All participants were informed about the study and provided informed consent before the beginning of the examination. Both groups underwent ophthalmologic assessments at the Altino Ventura Foundation between February 2020 and December 2023.

Participants

The inclusion criteria were laboratory-confirmed CD, clinical chronic CD with heart disease, or the indeterminate form classified by a cardiologist that had not received etiologic treatment for at least 5 years before the beginning of the study. Individuals younger than 18 years of age with cardiologic conditions other than CD, neurologic diseases, and diabetes were excluded. The ophthalmologic exclusion criteria included the inability to perform the examinations; spherical refractive errors between -1.5 diopters (D) and + 4.0 D; astigmatism greater than – 2.5 D; a history of posterior segment intraocular surgery, ocular trauma, photocoagulation, presence of significant media opacities, and other chorioretinal disorders, such as epiretinal membranes, vascular diseases, or vitreomacular traction. Patients with pathological funduscopy findings, glaucoma, or suspected glaucoma also were excluded, with the aim of detecting alterations in ophthalmologic examinations before any clinical fundus signs of disease. A control group of healthy subjects with the same exclusion criteria was included and matched for age and the presence of systemic arterial hypertension.

Study protocol

Data collected included age, sex, presence of comorbidities (systemic arterial hypertension and diabetes mellitus), disease duration (years), history of previous etiologic treatment, and clinical CD classification. Heart disease was classified according to the Latin American guidelines for the diagnosis and treatment of chagasic cardiomyopathy[3]. The indeterminate chronic form (A) included individuals who do not present with clinical symptoms or electrocardiographic changes. The CCF included patients without ventricular dysfunction (B1), in which individuals have electrocardiographic changes and discrete echocardiographic changes but do not have ventricular dysfunction, and patients with ventricular dysfunction (B2) characterized by global ventricular dysfunction, but without previous or current signs and symptoms of heart failure.

Ophthalmologic assessment

All participants underwent an ophthalmologic assessment that included measurement of the BCVA expressed as the logarithm of the minimum angle of resolution units, slit-lamp biomicroscopy, intraocular pressure measurement with Goldmann applanation tonometry, and a dilated fundus examination. In the presence of normal funduscopy, retinography (CR2, Canon, Inc., New York, NY, USA) was performed, followed by SD-OCT and OCTA (RTVue XR; Avanti, Optovue, Fremont, CA, USA) of the ONH and macula. Retinography images then were analyzed using the Laguna ONhE software. In addition, pachymetry (OD1, Axis Tecnologia Médica, São Paulo, Brazil), AL (IOL Master 500, Carl Zeiss Meditec, Dublin, CA, USA), and VF testing (Humphrey SITA—Standard 10-2, Carl Zeiss Meditec) were performed in patients with chronic CD.

Considering the VF tests, the PSD and MD were determined in the CD group using the 10-2, White III, and SITA Fast strategies. Tests with greater than 15% false positives, 33% loss of fixation, or false negatives were excluded. In addition, examinations with artifacts such as edge defects, loss of fixation, fatigue effects, and other pathological alterations also were excluded[17].

SD-OCT and OCTA analysis

The same experienced examiner performed all SD-OCT and OCTA measurements to minimize variability. For the ONH and macular OCTA analyses, the same examiner obtained three sets of images from each participant from both groups at one visit, and considered the possibility of variations in the results between different examinations. For each analyzed variable, the mean of the three values was obtained. One eye was included in the research, and its selection was based on the best-quality images. When the image quality was equal, one eye was selected randomly. A signal strength index below 5/10, poor quality images secondary to media opacities, poor coloration or fixation, and the presence of artifacts were disregarded. Artifacts were defined as more than one-third of the images without delineation between capillaries[11].

The AngioVue system (version 2015.100.0.35, RTVue-XR SD-OCT, Optovue Inc.) and its algorithm, the split spectrum amplitude decorrelation angiography (AngioVue) method of acquiring images has been described previously[13,29]. The flow index (mm^2) and VD (%) are the two parameters used by OCTA to quantify the

ocular circulation. In the assessed area, the mean decorrelation values corresponded to the flow index, and the percentage area occupied by vessels corresponded to the VD[29].

The ONH OCTA scan was performed covering an area of 4.5 × 4.5 mm centered around the disc. The slab used in glaucoma analysis was a radial peripapillary capillary (RPC) slab that delineates the vessels in the RNFL. The RPC slab is delimited from the internal limiting membrane (ILM) to the posterior boundary of the RNFL[14]. The peripapillary VD of the small vessels (%) was obtained from this slab.

The macular OCTA scan covered an *en-face* foveal centralized area of 3 × 3 mm. The SCP extends from 3 µm below the ILM to 15 µm below the inner margin of the internal plexiform. The DCP included an area 15 µm below the internal margin of the internal plexiform layer and 70 µm below the outer margin of the outer plexiform layer. A circular grid centered on the fovea was provided for both plexuses with inner and outer rings of 1.0 and 3.0 mm in diameter, respectively, delimiting the fovea and parafovea[11,13,30]. In the current study, the data collected from the macular assessment were the foveal and parafoveal thicknesses (µm); the total (9 mm^2), parafoveal, and foveal VD (%) from the SCP and DCP, along with the foveal avascular zone (FAZ) area (mm^2) of the SCP. The FAZ is automatically provided by the device.

For the structural assessment, all participants underwent SD-OCT imaging. An ONH protocol map with a 3.45-mm diameter centered on the optic disc was obtained and used for the measurement of the average peripapillary RNFL thickness (µm). The ganglion cell complex (GCC) map protocol was used for the macular assessment to obtain the average GCC thickness (µm). The map is comprised of 7-mm-long vertical and horizontal scans centered 1 mm temporal to the fovea and measures the inner retinal thickness, which includes the nerve fiber layer, ganglion cell layer, and inner plexiform layer, which together make up the GCC[31]. Scans with a signal strength index greater than 40 and without segmentation failures were considered.

Laguna ONhE® analysis

Conventional color fundus retinographies of all subjects were analyzed using the Laguna ONhE software to measure the amount of ONHHb, represented by the GDF index. The method for acquiring the GDF values was described previously[24]. Basically, the central retinal vessels and ONH tissue are defined by automatic segmentation based on three spectral components of the OHN: red (R), green (G), and blue (B). The software understands that the red component reflects areas with high Hb content compared with green and blue. The formula (R-G)/R then was applied to the pixels of the vessels and tissue, and the result was almost linearly proportional to the amount of Hb present. Finally, the ONHHb levels and GDF were determined by colorimetric analysis[24]. The GDF is expressed as an absolute number from + 75 to − 90, which represents the probability that the ONHHb is within the normal range (positive values), borderline, or outside the normal range (negative values)[32]. The software automatically excluded from the analysis lower quality fundus photographs secondary to light saturation.

Statistical analysis

Statistical analysis was performed using the Jamovi software (version 2.3.28, the Jamovi project, Sydney, Austrália). The Shapiro–Wilk test was used to assess data normality. For the quantitative variables, descriptive analysis was carried out using measures of position, such as the mean and median, and measures of dispersion, including SD and maximal and minimal values. Absolute and relative frequency distributions were created for the qualitative variables. When investigating the relationships between the variables and the CD and control groups, a comparative analysis was executed that considered its qualitative or quantitative nature. For qualitative variables, absolute and relative cross-distributions were drawn, and the chi-square test was used to check the significance of the relationship. Fisher's exact test was used to compare sex variables between groups. For quantitative variables, the Student's t-test was used. As an alternative to the t-test, the Mann–Whitney test was used for data outside the normal range. The level of significance was set at 0.05 with a 95% confidence interval. To calculate the sample power, the size of the effect (minimal difference between the groups), the sample size observed and the significance level were defined. Considering the size of the effect equal to a unit, the sample size observed equal to 19, and a significance level of 0.05, the power achieved was 85.1%. In general, the desired power is greater than or equal to 80%.

Data availability

Data are available upon request by contacting Cristiana Agra, MD (clmagra@gmail.com).

Received: 22 October 2024; Accepted: 13 January 2025
Published online: 17 January 2025

References

1. Dias, J. C. P. et al. II Brazilian consensus on Chagas disease. *Epidemiol. Serv. Saúde* **25**, 7–86 (2016).
2. World Health Organization. Chagas disease (American trypanosomiasis). Preprint at https://www.who.int/health-topics/chagas-disease (2015).
3. Andrade, J. P. et al. I Diretriz latino-americana para o diagnóstico e tratamento da cardiopatia chagásica. *Arq. Bras. Cardiol.* **97**(2), 1–48 (2011).
4. Schmunis, G. A. The globalization of Chagas disease. *ISBT Sci. Ser.* **2**, 6–11 (2007).
5. Brasil. Ministério da Saúde, Secretaria de Vigilância em Saúde. Guide to health surveillance. *Ministério da Saúde*. 01–727 (2019).
6. World Health Organization. Sustaining the drive to overcome the global impact of neglected tropical diseases: second WHO report on neglected diseases. Preprint at https://www.who.int/publications/i/item/9789241564540 (2013).
7. Villas-Bôas, F. S. et al. Association between Chagas disease and changes in the optic nerve and retinal nerve fiber layer. *Arq. Bras. Oftalmol.* **82**(3), 183–188 (2019).
8. Gordon, M. O. The ocular hypertension treatment study. *Arch. Ophthalmol.* **120**, 714–720 (2002).

9. Flammer, J. et al. The impact of ocular blood flow in glaucoma. *Prog. Retin. Eye Res.* **21**, 359–393 (2002).
10. Matsumoto, S. C. et al. Retinal dysfunction in patients with chronic Chagas' disease is associated to anti-*Trypanosoma cruzi* antibodies that cross-react with rhodopsin. *FASEB J.* **20**(3), 550–552 (2006).
11. Agra, C. L. M., Lira, R. P. C., Pinheiro, F. G., Sá, L. H. S. E. & Filho, V. T. F. B. Optical coherence tomography angiography: Microvascular alterations in diabetic eyes without diabetic retinopathy. *Arq. Bras. Oftalmol.* **84**(2), 149–157 (2021).
12. Alnawaiseh, M., Lahme, L., Treder, M., Rosentreter, A. & Eter, N. Short-term effects of exercise on optic nerve and macular perfusion measured by optical coherence tomography angiography. *Retina* **37**(9), 1642–1646 (2017).
13. Agemy, S. A. et al. Retinal vascular perfusion density mapping using optical coherence tomography angiography in normals and diabetic retinopathy patients. *Retina* **35**, 2353–2363 (2015).
14. Rao, H. L. et al. Optical coherence tomography angiography in glaucoma. *J. Glaucoma* **29**(4), 312–321 (2020).
15. Wang, J. et al. Retinal and choroidal vascular changes in coronary heart disease: An optical coherence tomography angiography study. *Biomed. Opt. Express.* **10**(4), 1532–1544 (2019).
16. Mendez-Hernandez, C. et al. Diagnostic validity of optic nerve head colorimetric assessment and optical coherence tomography angiography in patients with glaucoma. *Br. J. Ophthalmol.* **105**, 957–963 (2021).
17. Rocha, J. A. G. et al. Optic nerve head hemoglobin levels in glaucoma: A structural and functional correlation study. *J. Ophthalmol.* **2021**, 1–8 (2021).
18. Yarmohammadi, A. et al. Optical coherence tomography angiography vessel density in healthy, glaucoma suspect and glaucoma eyes. *Invest. Ophthalmol. Vis. Sci.* **57**(9), 451–459 (2016).
19. Jia, Y. et al. Optical coherence tomography angiography of optic disc perfusion in glaucoma. *Ophthalmology* **121**(7), 1322–1332 (2014).
20. Liu, L. et al. Optical coherence tomography angiography of the peripapillary retina in glaucoma. *JAMA Ophthalmol.* **133**(9), 1045–1052 (2015).
21. Rao, H. L. et al. Regional comparisons of optical coherence tomography angiography vessel density in primary open-angle glaucoma. *Am. J. Ophthalmol.* **171**, 75–83 (2016).
22. Rao, H. L. et al. A comparison of the diagnostic ability of vessel density and structural measurements of optical coherence tomography in primary open angle glaucoma. *PLoS ONE* **77**, 1–13 (2017).
23. Pradhan, A. Z. S., Dixit, S., Sreenivasaiah, S., Rao, H. L. & Jayasree, P. A sectoral analysis of vessel density measurements in perimetrically intact regions of glaucomatous eyes: An optical coerence tomography angiography study. *J. Glaucoma* **27**(6), 525–531 (2018).
24. Gonzalez de la Rosa, M. et al. Measuring hemoglobin levels in the optic nerve head: Comparisons with other structural and functional parameters of glaucoma. *Invest. Ophthalmol. Vis. Sci.* **54**(1), 482–489 (2013).
25. Gonzalez-Hernandez, M., Saavedra, J. S. & Gonzalez-de-la, R. M. Relationship between retinal nerve fiber layer thickness and hemoglobin present in the optic nerve head in glaucoma. *J. Ophthalmol.* **2017**, 1–10 (2017).
26. Meira-Freitas, D., Melo, L. A. S., Almeida-Freitas, D. B. & Paranhos, A. Glaucomatous optic nerve head alterations in patients with chronic heart failure. *Clin. Ophthalmol.* **6**, 623–629 (2012).
27. Choi, B. R. et al. Factors associated with decreased cerebral blood flow in congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am. J. Cardiol.* **97**(9), 1365–1369 (2006).
28. Harris, A. et al. The role of optic nerve blood flow in the pathogenesis of glaucoma. *Ophthalmol. Clin. North Am.* **18**(3), 345–353 (2005).
29. Jia, Y. et al. Quantitative OCT angiography of optic nerve head blood flow. *Biomed. Opt. Express.* **3**(12), 3127–3137 (2012).
30. Coscas, F. et al. Normative data for vascular density in superficial and deep capillary plexuses of healthy adults assessed by optical coherence tomography angiography. *Invest. Ophthalmol. Vis. Sci.* **57**(9), 211–223 (2016).
31. Rao, H. L. et al. Comparison of different spectral domain optical coherence tomography scanning areas for glaucoma diagnosis. *Ophthalmology* **117**(9), 1692–1699 (2010).
32. Mendez-Hernandez, C., Rodriguez-Uña, I., Gonzalez-de-la, R. M., Arribas-Pardo, P. & Garcia-Feijoo, J. Glaucoma diagnostic capacity of optic nerve head haemoglobin measures compared with spectral domain OCT and HRT III confocal tomography. *Acta Ophthalmol.* **94**(7), 697–704 (2016).

Acknowledgements

We thank the patients with Chagas disease who participated in the study. We also thank the informations and support of the Casa de Chagas – PROCAPE, in Recife – PE, Brazil.

Author contributions

C.L.M.A. was responsible for the study conception and design, ophthalmological assessments, literature review, drafting of the report, and reviewing the final manuscript. C.V.V. and T.S.P. conceived of the study design and critically revised the manuscript. A.K.S. and L.V.V. provided critical inputs and reviewed the manuscript. L.O.F., M.R.V.O., and M.L. contributed to literature review and ophthalmological assessments. M.E.L.S.M.A., M.G.A.M.C., and S.M.M.A. contributed to cardiological assessments. All authors revised and approved the final version of the manuscript.

Funding

This study was partially supported by the Altino Ventura Foundation of Recife, PE, Brazil.

Declarations

Competing interests

The authors declare no competing interests.

Patient consent for publication

Obtained.

Additional information

Correspondence and requests for materials should be addressed to C.L.M.A.