Alzheimer Disease: OCT Retinal and Choroidal Thickness

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Abstract: The aim of this study is to compare macular retinal layers and choroidal thicknesses of patients with Alzheimer's disease (AD) with those of patients without other known ophthalmological pathology, using spectral domain optical coherence tomography. Multiple linear regression analysis was applied to assess the effects of age, gender, spherical equivalent, visual acuity, intraocular pressure, axial length and mean arterial pressure on macular retinal layers thickness. Fifty eyes of 50 patients (mean age 73.10; SD=5.36 years) with a diagnosis of mild AD and 152 eyes of 152 patients without AD (mean age 71.03; SD=4.62 years) were included. There was a thinning in the peripheral ring of the ganglion cell layer (GCL) in the AD group (S6 $p < 0.001$; T6 and N6 $p = 0.001$). In the superior sectors of the inner plexiform layer (IPL), differences between the two groups also remained statistically significant after Bonferroni correction (S3 $p = 0.001$ and S6 $p < 0.001$). In the outer layers we did not observe differences statistically significant for AD group. These layers' thicknesses were associated with statistical significance with gender (in inner and outer nuclear layers), age and choroidal thickness (CT) (in photoreceptor layer). In the AD patients group, CT was significantly thinner than in the first group of patients without AD, in all 13 locations ($p<0.001$), and age was relevant factor. Patients with AD showed a significant reduction in retinal layers and choroidal thickness. The thinnest macular measurements were found mostly in the inner layers, GCL and IPL, at superior sectors (pericentral and peripheral rings). This thinning may reflect a retinal characteristic of AD, related with both primary retinal lesion and transsynaptic retrograde degeneration and the choroidal thinning probably reflects the importance of vascular factors in the pathogenesis of this disease.

1 INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia and is a long-term progressive neurodegenerative disorder with great social impact. (Association Alzheimer's Dementia1, 2012). The earliest AD pathological change in the central nervous system (CNS) is the accumulation of amyloid β (Aβ), derived from abnormal processing of amyloid precursor protein (APP). (Perl DP, 2010). This process can begin a decade before the onset of the clinical syndrome of dementia. Visual symptoms occur frequently among the earliest complaints in AD patients, contributing to further impairment in the quality of life. (Burns A et al, 2009; Querfurth HW, Laferla FM, 2010) However, the results of visual tests are dependent on the patient's understanding, memorization and compliance to the rules and the test instructions, which depends on the cognitive status of each patient.

Visual defects in AD patients were initially thought to be solely due to parietal and primary visual cortex pathology. However, increasing evidence has demonstrated that anterior visual pathway degeneration also plays a role. Hinton et al. first provided histopathological evidence of optic neuropathy and degeneration of retinal ganglion cells (RGC) in patients with AD, with reduced number of RGCs and reduced retinal nerve fiber layer (RNFL) thickness (Hinton DR et al., 1986). Later post-mortem studies showed that degeneration of the ganglion cell layer (GCL) occurs preferentially in superior and inferior sectors, as well as in the central retina, in particular the temporal foveal region (Blancs JC, Torigoe Y et al., 1996; (Blancs JC, Schmidt SY et al., 1996).

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Initial in vivo non-invasive studies of optic neuropathy in patients with AD using fundus photographs showed RNFL abnormalities, as well as macular changes and optic nerve head abnormalities (increased cup-to-disc ratio and decreased neurorretinal rim). (Tsai CS et al., 1991; Hedges TR et al., 1996; Kromer R et al., 2013).

In addition, in vivo studies have shown reduced macular thickness and volume in patients with AD (Iseri PK et al., 2006) reporting macular changes in the ganglion cell complex (GCC), comprising the GCL and the inner plexiform layer (IPL) (Mochos MM et al., 2012 Marziani E et al., 2013; Ascaso FJ at el., 2014; Garcia-Martin ES et al., 2014; Cheung CYL et al., 2015).

The aim of this study is to identify the retinal and choroidal macular regions more affected and the layers where the retinal thinning is more pronounced, considering potential confounding variables such as age, gender, spherical equivalent, best corrected visual acuity (BCVA), axial length, intraocular pressure (IOP) and mean arterial pressure (MAP).

2 MATERIALS AND METHODS

2.1 Subjects Groups

This cross-sectional study was conducted at the Ophthalmology and Neurology Departments of the Central Hospital Lisbon Center (CHLC), between 2014 and 2016. Consecutive AD patients sent by the Neurology Department for ophthalmological screening were observed for inclusion/exclusion criteria. The inclusion criteria were AD patients with age between 65 and 78 years old with normotensive eyes and ability to understand the study.

Exclusion criteria were: refractive error > 5 diopters (D) or/and axial length > 25 mm in the studied eye; known diagnosis of diabetes; retinal diseases; glaucoma or ocular hypertension; uveitis; neurodegenerative diseases; significant media opacities that precluded fundus imaging. Other relevant known neurologic pathology, such as neurodegenerative diseases, other types of dementia, previous stroke or uncertain or indeterminate diagnosis were also excluded.

Patient's informed consent was obtained before participation in this study. The principles of the Declaration of Helsinki were respected and the study was approved by our institutional Ethics Committee.

Fifty patients with AD (AD group) and 152 patients without AD (control group) were recruited from the Neurology department of CHLC.

2.2 Study Procedures

After a pre-screening visit where demographic, background history, full ophthalmological examination with visual acuity, anterior segment examination, tonometry, indirect ophthalmoscopy and ultrasonic biometry were recorded, patients were assigned to a specific study visit where the following methodology was taken: Goldmann applanation tonometry and spectral domain optical coherence tomography. One eye of each subject was randomly selected.

2.2.1 Visual Acuity

BCVA for each eye was measured using Snellen charts and converted to the logarithm of the minimum angle of resolution (logMAR).

2.2.2 Intraocular Pressure

IOP was measured before pupillary dilation with Goldmann applanation tonometry and a mean of 3 measurements was taken.

2.2.3 Spectral Domain Optical Coherence Tomography Imaging

All eyes were examined with SD-OCT (Spectralis Heidelberg Engineering, Germany, software version 6.0) at the same time of the day from 2 PM to 4 PM. For macular measurements, subjects were studied using the “fast macular volume” preset, consisting of a 25-line horizontal raster scan covering 20° × 20°, centered on the fovea (consisting of 25 high-resolution scans). In the same session, enhanced depth imaging scans (EDI) were also performed to improve the quality of choroidal imaging according to the previously reported method (Spaide et al., 2008).

The new Spectralis automatic segmentation software was used to obtain individual retinal layer thickness measurements including: RNFL, GCL, IPL, inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), retinal pigment epithelium (RPE) and photoreceptor layer (PR).

The thickness values were calculated for the nine Early Treatment Diabetic Retinopathy Study (ETDRS) sectors/regions (Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie

House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group.' (1991). This ETDRS plots consist in three concentric rings of 1, 3 and 6 mm diameter centered at the fovea and two outer rings subdivided in 4 sectors. Each sector was designated the fovea or central sector (C), the pericentral ring (ETDRS sectors: S3, T3, I3 and N3) and the peripheral ring (ETDRS sectors: S6, T6, I6 and N6).

The OCT images were obtained by one ophthalmologist (A.S.) and were assessed by other two ophthalmologists (J.P.C. and R.P.), masked to the patients' diagnosis, who verified the automatic segmentation and the automatic position of the ETDRS grid, correcting when necessary. Choroidal thickness (CT) was measured manually from the hyperreflective line, corresponding to the retinal pigment epithelium (RPE, to the hyporreflective line, corresponding to the sclerochoroidal interface, as previously described. (Tavares Ferreira J et al., 2016).

2.2.4 Mean Arterial Pressure

Blood pressure was measured in the seated position by an automatic sphygmomanometer and systolic and diastolic blood pressure (SBP and DBP) were recorded. Mean arterial pressure (MAP) was calculated using the following formula:

$$MAP = DBP + 1/3\ (SBP - DBP).$$

2.2.5 Statistical Analysis

Demographics and clinical characteristics of patients were described with frequencies (percentages) and with mean (SD: standard deviation) or with median and interquartile range (IQR: 25th percentile-75th percentile), as appropriate. Nonparametric Chi-Square test and Mann-Whitney tests were applied.

Linear regression models were used to identify the variables which may explain the variability of macular retinal layers thicknesses. The variables group, gender, age, IOP, axial length, spherical equivalent, MAP, and BCVA were considered in this analysis. Variables with a p-value <0.25 in the univariable analysis were selected as candidates for the multivariable models. Multivariable regression models regarding PR layer in sectors C, S3, I3, N3, and T3, also considered the variable CT subfoveal, 1000 superior, inferior, nasal, and temporal of the fovea, respectively. Normality assumption of the residuals was verified using Kolmogorov–Smirnov goodness-of-fit test with Lilliefors correction. A level of significance α=0.05 was considered. Data were analysed using the Statistical Package for the Social Sciences for Windows (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.)

3 RESULTS

3.1 Patient Demographics and Clinical Characteristics

A total of 50 AD patients (16 males) were included in the AD group and 152 patients without AD (55 males) were included in the control group. Concerning gender, no significant differences were found between AD and control groups (32.0% vs 36.2%; p=0.591). The mean age was 73.1 (SD=5.36) years in AD group and 71.0 (SD=4.62) years in control group (p=0.011).

The demographic, clinical and ophthalmologic characteristics of the 2 groups, including BCVA, IOP, spherical equivalent, axial length, MAP, therapy with diuretics and antihypertensive medication are summarized and compared in Table 1.

Table 1: Demographic and clinical characteristics of the patients by group.

	Alzheimer Group (n=50)	Control Group 1 (n=152)	p
Age (years)	73.1 (5.36)	71.0 (4.62)	<0.001
Male gender n (%)	16 (32)	55 (36)	
BCVA (logMAR)	0.121 (0.153)	0.040 (0.073)	<0.001
IOP – Goldmann (mmHg)	15.52 (2.62)	14.72 (2.51)	0.66
Spherical Equivalent (D)	0.995 (1.43)	0.700 (1.64)	0.344
Axial length (mm)	22.44 (0.91)	22.49 (0.99)	0.668
Mean Arterial Pressure (mmHg)	98.91 (94.67-103.33)	97.87 (93.75-101.25)	0.287
Therapy			0.058

Results are expressed as mean (SD) or median (IQR), as appropriate; Best Corrected Visual Acuity (BCVA); Intra-Ocular Pressure (IOP).

3.2 OCT Measurements - Macular Retinal Thickness

In multivariable linear regression models considering factors such as age, gender, visual acuity, IOP, spherical equivalent, axial length, MAP, therapy and antihypertensive medication we have observed a thinning of the GCL for AD group in three of the four pericentral sectors (S3 p= 0.016; T3 p= 0.050 and N3 p= 0.043) and in three of the four peripheral sectors (S6 p < 0.001; T6 p= 0.001 and N6 p= 0.001). In fact, the mean values of GCL decreased between 2.29 and 3.28 µm in AD group when compared with control group, and for each ten years increase of life, the mean values of the layers also decreased between 1.28 and 2.71 µm.

In the IPL, all sectors excepting the central and N6, had thinner thicknesses with statistical significance for AD group (S3 p= 0.001; T3 p= 0.003; I3 p= 0.005; N3 p= 0.018; S6 p < 0.001; T6 p= 0.015 and N6 p= 0.010). Results showed that the mean values of IPL decreased between 1.23 and 2.61 µm in AD group, and for each ten years increase of life, the mean values of the layers also decreased between 1.02 and 1.48 µm.

After Bonferroni correction, the sectors still statistically thinner in the inner layers for AD group were localized in the GCL peripheral sectors S6, T6 and N6, and in the superior sectors S3 and S6 of the IPL.

For the other layers other than GCL and IPL for multivariable regression models results regarding RNFL, INL, ONL, RPE and PR layers), results of the multivariable regression models after Bonferroni correction, showed a mean thickening of 1.49 µm at T3 sector in the RNFL for AD group; a mean thickening of 5.22 µm at C sector in the INL for male gender; a mean thickening of 5.82 µm at I3 sector in the ONL for male gender and a mean thinning of 3.11 µm at C sector in the PR for each 10 years increase of life. Additionally, the pericentral sectors of PR layer had a positive association with choroidal thickness after Bonferroni correction

3.3 EDI-OCT Measurements – Choroidal Thickness

In all 13 locations the differences were statistically significant (p<0.001).

In the multivariable regression models, after adjusting for age, spherical equivalent, BCVA, IOP, axial length and MAP we identified significant differences in CT between the groups in all 13 locations. In all locations, except at 500 and 1000 µm superior and 1000 µm inferior of the fovea, independently from the group, age was negatively associated with CT with a mean decrease between 15.5 and 28.4, for each 10 additional years. Spherical equivalent was also associated with CT in four locations, namely 1000 µm nasal, 1500 µm nasal, 1500 µm superior and 1500 µm inferior of the fovea. For each increase of 1 D in spherical equivalent value, the CT was thicker in ADG with increases between 5.3 and 7.2 µm.

4 DISCUSSION

Diagnosis of AD can be made with high accuracy by using clinical, neuropsychological, and imaging assessments. Fortunately, in ophthalmology, we have the possibility to measure neuronal layers in a non-invasive way with OCT technology.

Since 2001, peripapillary RNFL thinning has been demonstrated with time domain TD-OCT and spectral-domain SD-OCT studies (Parisi). However, differences have been reported regarding which retinal quadrants are most affected. More recent OCT studies have reported also macular changes in ganglion cell complex (GCC), comprising the ganglion cell layer (GCL) and inner plexiform layer (IPL) (Mochos MM et al., 2012 Marziani E et al., 2013; Ascaso FJ at el., 2014; Garcia-Martin ES et al., 2014; Cheung CYL et al., 2015).

In addition, one study using SD-OCT showed a diffuse reduction of the RNFL and GCL combined in AD (Marziani E et al., 2013), although the authors were not able to determinate which layer was most affected by AD. Other studies have demonstrated IPL thinning in AD patients (Ascaso FJ et al., 2014; Ong YL et al., 2014; Cheung CY et al., 2014). This reduction of ganglion cell complex thickness (GC-IPL and RNFL layers) in AD occurs to a larger extent than that accounted for age-related GC-IPL loss alone (about 0.3 µm/year) (Cheung CY et al., 2014). Macular GC-IPL thinning may be a more sensitive marker of earlier neurodegeneration in Mild Cognition impairment (MCI) and AD than evaluation of the RT. ()

Whether or not an association exists between retinal changes and severity of dementia also remains a controversial issue. While most studies concluded that OCT can be used to detect early abnormalities in AD, the majority reported no statistically significant differences between MCI and AD patient groups [Oktem EO et al., 2014; Paquet C et al., 2007; Kesler A et al., 2011). Only one TD-

OCT study reported correlation between MMSE scores and macular volume (Iseri PK et al., 2006).

Two meta-analyses by K.L. Thomson et al. and G. Coppola et al. also tried to determine the utility of OCT as a tool for evaluating disease progression, and prognostic significance of GC-IPL and RNFL thickness, but their conclusions failed to determine an association between RNFL and the clinical severity of dementia (Thomson KL et al., 2015; Coppola G et al., 2015). A recent SD-OCT study found that reduced grey matter volumes of occipital and temporal lobes were associated with thinning of the GC-IPL and peripapillary RNFL in individuals without dementia (Ong YT et al., 2015). Since those cortical regions are an early site of deposition of senile plaques and NFTs, the findings by Y.-T. Ong and co-workers raises the possibility that GC-IPL thinning may reflect neurodegenerative changes in the brain, even before the clinical onset of dementia.

In our study, we used SD-OCT to compare retinal thickness in mild AD patients with a large control group. In the multivariable analysis, after adjustment for age, gender, BCVA, IOP, axial length, spherical equivalent and MAP, the GCL and the IPL were thinner especially in superior sectors. This thinning of the superior macular sectors in AD patients would explain the predominantly inferior visual field defects previously described in AD (Kesler A et al., 2011; Lu Y et al., 2010; Berisha F et al., 2007; Kirbas S et al., 2013; Bambo MP et al., 2015) and probably associated to the increased risk of falls in these patients.

The sensitivity of pericentral sectors between 1-3 mm from the fovea, in SD-OCT, was already reported by our group of research as the macular sector is more affected in neuro-ophthalmological diseases (Costa L et al., 2015) and can provide a clue for the probable neuro-ophthalmological etiology of some OCT findings.

When we analysed the different areas of the macula of both groups, we observed a normal distribution of each layer thickness, with a thicker nasal quadrant than temporal and a thicker superior than inferior quadrant. The multivariable analysis considering retinal thickness as the dependent variable support the importance of AD in the thinning of superior sectors of GCL and IPL, nearly eliminating the classically described superior-inferior asymmetry. The higher statistical significance in the upper sectors allows, in some way, to differentiate the diagnosis of glaucomatous optic neuropathy from another possible AD related neuropathy. Also, Armstrong (Armstrong RA, 1996) found a greater density of senile plaques and neurofibrillary tangles in the cuneal gyrus than in the lingual gyrus, suggesting the explanation for the predominantly inferior field defects reported by Trick et al. in AD (Trick GL et al., 1995). Like in others studies, the increasing age is responsible for changes in retinal layer thickness, as studied by Ooto et al. (Ooto S et al., 2011). Demirkaya et al postulated a lose of 2.06 μm of pericentral GCL and of 0.92 μm of peripheral IPL over a period of 20 years, probably due to a diffuse loss of neural tissue (Demirkaya et al., 2013).

The principle role of the choroid is to supply oxygen to the outer retina up to the level of the inner nuclear membrane and, therefore, the neurosensory retina in the foveal avascular zone derives blood from the choroid. Vascular choroidal changes can therefore occur in patients with vascular risk factors as AD.

In vivo studies have demonstrated that CT varies topographically within the posterior pole and is inversely correlated with age. It decreases approximately 16 μm for each decade of life. A study reviewed 54 eyes and demonstrated that CT was thinnest nasally and thickest subfoveally. Additionally, choroidal thickness was found to be highly correlated with age, axial length, and refraction, emphasizing the importance of controlling for these variables when studying any patient population. More interestingly, choroidal thickness varied on a diurnal basis by as much as 33 μm (ranging from 8 to 65) in one study, suggesting that it can be a highly variable measure of choroidal vasculature and further emphasizing the need to develop novel approaches to reliably assess choroidal vascular health in vivo.

Patients with AD have an altered microvascular network in the retina (narrower retinal venules and a sparser and more tortuous retinal vessels) compared with matched controls. The accumulation of Aß and development of neurofibrillary tangles (NFTs) cause neurotoxicity, neuronal and synaptic loss, and vascular angiopathy. The role of choroidal vasculature in the pathogenesis of AD is unknown, but the results in this study of choroidal thinning in patients with AD when compared with controls support previous results of others studies. When we analysed the pattern of CT in the control and AD groups, both had a normal distribution of CT, with a thicker superior quadrant than the inferior and a thicker temporal than nasal quadrant.

The models of multifactorial linear regression for the dependent variable choroidal thickness support the importance of Alzheimer disease as a risk factor for choroidal atrophy besides aging.

Our study had some limitations. The first one concerned to the different age distribution of the two groups. However, to overcome this drawback, all the regression models were adjusted by age which has been proved to be very important in some inner and outer layers. CT measurements were done manually, however, the measurements were done by 3 independent persons and this manual technique already been proved to have a high intra-observer and inter-observer reproducibility. Secondly, the hydration status, that may affect the CT, was not taken into account. To this extent, we try to decrease any circadian variability by performing the measurements at the same time of the day and in the same location and environment. Also, the automatic segmentation and centration of ETDRS grid could have resulted in imprecise measurements, although it was confirmed by 2 independent persons.

5 CONCLUSIONS

Patients with AD showed a significant thinning in pericentral and peripheral sectors of the inner layers. The thinnest macular measurements were found mostly in the inner layers and superior sectors. After Bonferroni correction, the most affected regions were localized in the GCL S6, T6 and N6, and in the IPL S3 and S6. These OCT findings in AD support the direct retinal involvement but also suggest the contribution of transynaptic retinal degeneration in the physiopathology of retinal and visual dysfunction in AD. Patients with AD showed a choroidal thinning that was statistically significant in the 13 locations studied at 1.5 mm centered on the fovea. This thinning may reflect the importance of choroidal vascular factor in the pathogenesis of this disease and may aid in the diagnoses of "Alzheimer's choroidopathy" not related with age.

However, further studies are needed to clarify some questions that remain to be answered before considering OCT a useful clinical tool for early detection of dementia and assessment of disease progression in AD.

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