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Analysis of optic disk color changes in Alzheimer's disease: A potential new biomarker

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ABSTRACT

Purpose: In the present study, we evaluated changes in the retinal nerve fiber layer (RNFL) and optic disk color (i.e., the level of paleness as an indirect sign of axonal loss) in patients with Alzheimer's disease (AD) compared with healthy controls. The usefulness of this method as a new biomarker for AD was also evaluated.

Methods: Fifty-six patients with mild or moderate AD and 56 sex-and age-matched healthy subjects were included in this cross-sectional study. All subjects underwent a complete neuro-ophthalmologic examination, including analysis of the RNFL thickness with Cirrus optical coherence tomography (OCT). One photograph of the optic disk was obtained using a Canon CF 60 DSi retinograph, and new colorimetric analysis software (Laguna ONhE) was used to detect color changes in the photographs based on hemoglobin (Hb) values as reference pigment.

Results: Mean Hb percentage and Hb content in the outer ring, which corresponds with the neuroretinal rim, calculated by the Laguna ONhE program were significantly lower in AD patients than in healthy controls ($P < 0.005$). OCT measurements revealed that the mean RNFL thickness was significantly decreased in AD patients compared with healthy controls ($P < 0.003$).

Conclusion: Analysis of the optic disk color assessed by Laguna ONhE software revealed papillary paleness due to axonal loss and perfusion alterations, even in the early stages of AD. Application of this simple method in routine clinical practice may provide a good biomarker of AD.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, and its prevalence is increasing due to the aging of the general population. Scientific interest in this form of dementia continues to increase, and intense efforts have focused on the discovery of new biomarkers because of frequent delays in the diagnosis using currently available diagnostic methods. Visual symptoms are often among the earliest complaints of patients with AD and have been reported for more than 40 years, but they were initially considered to be of strictly cortical origin. In the last 20 years, some studies have described pathologic changes in the retina and optic nerve [1,2] that could appear during the preclinical stages of AD.

Optical coherence tomography (OCT) reveals thinning of the retinal nerve fiber layer (RNFL) [3,4] and central retina [5] in AD patients compared with healthy subjects. The RNFL comprises axons originating in retinal ganglion cells that eventually form the optic nerve, namely, the first neurons of the visual pathway. In animal models of AD and age-related macular degeneration, extracellular deposits of amyloid beta are a common pathologic feature [6]. The ability to observe microcirculation using a noninvasive technique such us fundus examination [7] makes the retina and optic nerve head a focus for potential biomarkers of AD.

Axonal loss in the optic nerve is also observed as a progressive pallor by fundus examination with an ophthalmoscope. The human eye, however, cannot quantify axonal loss or detect early axonal loss unless more than 50% of nerve fibers are lost. The Laguna ONhE (optic nerve head hemoglobin) program is a new software program designed by a group of ophthalmologists and engineers that allows for the measurement of hemoglobin (Hb) levels at the optic nerve head using conventional fundus color photographs that compensate for different variables, such as illumination or lens

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absorption and diffusion. Use of Laguna ONhE for early diagnosis of glaucomatous optic neuropathy has demonstrated high precision and reproducibility compared with the classic functional and structural tests used in glaucoma [8]. This new technology is easy to implement in routine clinical practice because it requires only a good photograph of the optic nerve head, and it has also proved useful for analyzing axonal loss in neuro-ophthalmologic patients, such as those with multiple sclerosis (MS) [9] or Parkinson's disease [10].

The aim of this study was to investigate changes in the RNFL as assessed by a high definition OCT in patients with AD, and to perform a colorimetric analysis of the optic disk in patients with AD by utilizing the Laguna ONhE program.

2. Materials and methods

The study design followed the Declaration of Helsinki Principles and the study protocol was approved by the Clinical Research Ethics Committee of Aragon (Zaragoza, Spain). Informed written consent was obtained from all participants.

2.1. Subjects and measurement protocol

Required inclusion criteria were: best-corrected visual acuity (BCVA) of 20/40 or better, refractive error within ±5.00 diopters equivalent sphere and ±2.00 diopters astigmatism, and transparent ocular media (nuclear color/opalescence, cortical or posterior subcapsular lens opacity <1), according to the Lens Opacities Classification System III system [11]. Exclusion criteria included previous intraocular surgery, diabetes, or other diseases affecting the visual field or neurologic system, and current use of medications that could affect visual function. We also included as exclusion criteria glaucoma signs (applanation intraocular pressure over 20 mm Hg, previous intraocular surgery, cup-to-disk ratio of 0.5 or higher, or arcuate nerve fiber bundle visual field defects). Some studies have found a significantly high rate of occurrence of glaucoma among patients with AD, so it is important to avoid this possible bias [12,13].

Fifty-six patients with mild or moderate AD and 56 sex-and age-matched healthy subjects were included in the study from September 2012 through December 2013. Diagnosis of AD was determined by neurologists according to the National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association [14] and the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) criteria [15]. Twelve patients with severe dementia were not included due to their inability to complete an ophthalmologic examination and to provide reliable test results. Each subject underwent the Mini Mental State Examination [16], a practical method for grading cognitive state. The MMSE is a 10-min bedside measure of impaired thinking in underdeveloped, uneducated, diseased, or very old populations. The summed score of the individual items indicates the severity of cognitive impairment. Deterioration in cognition is indicated by decreasing scores of repeated tests over time. The items on the MMSE include tests of orientation, registration, recall, calculation, attention, naming, repetition, comprehension, reading, writing, and drawing. A score of 30 indicates 100% correct. The mean score for a community-dwelling population over 65 years old is about 27. Patients with AD lose 3–4 points per year of illness after the onset of memory disturbance, although there is wide variability [17].

All subjects underwent a complete neuro-ophthalmologic examination, including assessment of BCVA; color vision evaluation (using Ishihara's isochromatic charts); eye movement; pupillary, anterior segment, and fundoscopic examinations; Goldmann applanation tonometry; and OCT examinations using the Cirrus HD OCT (Carl Zeiss Meditec, Dublin). One photograph of the optic disk was obtained using a Canon CF 60 DSi retinograph (Canon Incorporation, Tokyo, Japan) connected to a Canon EOS 1DS Mark III body camera. Each eye was considered separately and only one eye of each subject was randomly included in the analyses.

2.2. OCT evaluation

OCT scans were performed to obtain measurements of the peripapillary RNFL. The same experienced operator performed all scans. No manual correction was applied to the OCT output. Scan quality was assessed before the analysis, and poor-quality scans were rejected.

The Cirrus OCT optic disk protocol generates 200 × 200 voxel images from 200 linear scans that are performed by 200A-scans. This option analyzes a 6-mm cube around the optic nerve. In each series of scans, mean RNFL thickness, quadrant RNFL thickness (superior, inferior, temporal, and nasal), and thickness at the 12 clock hours of 30° RNFL were analyzed. The hour sectors were assigned a number from position H1 to H12 in the clockwise direction for the right eye, and in the counter-clockwise direction for the left eye.

2.3. Optic disk photograph evaluation

The Laguna ONhE program (Insoft SL., Tenerife, Spain) analyzed three spectral components of optic nerve head photographs: blue, green, and red. Red light is mainly reflected by optic nerve head areas with high Hb levels and in contrast, areas with a low Hb component reflect a lower proportion of the red component compared to the green and blue components [8]. The Laguna ONhE software uses mathematical algorithms for automatic component segmentation to perform a semiautomatic delimitation of the optic nerve head border and to identify the central retinal vessels. The program uses the amount of Hb in the central retinal vessels (i.e., the red color of these vessels) as a reference, and establishes different Hb concentrations in each of the 24 sectors in which the software divides the papilla image (Fig. 1A). The software grades the level of paleness of the different sectors in the optic disk and consequently the decreased in the microvascularization and the extent of ganglion cell axon loss that has occurred.

There are several factors that hinder the automatic analysis of the optic nerve head images: lighting issues, type of camera used, saturation level of the images, patient cooperation, etc. For this reason, the program has a pre-processing system of the images that prepares and improves retinal images for the subsequent segmentation stage. This pre-processing system prevents continued analysis of photographs that meet certain luminance and saturation criteria, and in turn, allows for the use of images from different cameras. Finally, the influence of the lens status was compensated for by analyzing the differences between the green and blue components before calculating the Hb content.

2.4. Statistical analysis

This was an observational, prospective cross-sectional study. All data analyses were performed using SPSS software version 20.0 (SPSS Incorporation, Chicago, IL) statistical software. The Kolmogorov–Smirnov test was used to assess sample distribution. Given the parametric distribution of the data, the OCT and Hb parameters between the healthy and AD groups were compared using Student's *t*-test with Bonferroni's correction for multiple comparisons. Correlations were examined by Pearson's test.