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Complete article title:

Clinical and Structural Parameters in Autosomal Dominant Optic Atrophy Patients: A Cross-Sectional Study Using Optical Coherence Tomography.

Running title:

Structural Parameters in Autosomal Dominant Optic Atrophy

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Key words

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Optical coherence tomography

Ganglion cell layer

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Abstract

Background

Autosomal Dominant Optic Atrophy (ADOA) is a hereditary optic neuropathy characterized by retinal ganglion cell degeneration and optic nerve fiber loss. This study aimed to investigate the correlation between clinical and structural parameters in ADOA patients using optical coherence tomography (OCT) and explore potential clinical biomarkers.

Methods

A cross-sectional, case-control observational study included 27 ADOA patients and 27 age and sex-matched healthy controls. Clinical evaluations, OCT imaging, and OCT angiography (OCTA) were performed to assess visual function and retinal changes. Statistical analyses were conducted to establish correlations between clinical and OCT parameters.

Results

ADOA patients exhibited gradual bilateral vision loss, central scotomas, and optic disc pallor. Structural OCT analysis revealed significant reductions in central macular thickness, macular volume, ganglion cell complex (GCC), and peripapillary retinal nerve fiber layer compared to controls. Correlation analysis demonstrated associations between worsening clinical parameters (best corrected visual acuity, Sloan Letters Low Contrast Chart 25%, Pseudoisochromatic Test) and increased OCT damage (structural and OCTA). GCC emerged, at least at exploratory terms, as

the most important clinical biomarker in ADOA patients given its multiple positive functional associations, while OCTA parameters correlated with visual field defects.

Conclusion

Our study reveals significant correlations between clinical and structural parameters in ADOA patients, highlighting the importance of OCT in assessing disease severity. GCC measurement shows promise as a clinical biomarker, aiding in disease monitoring. OCTA parameters offer potential early biomarkers for vascular changes. These findings contribute to understanding ADOA pathophysiology and may improve patient diagnosis and management. Further research is warranted to validate these findings and explore potential therapeutic interventions.

Autosomal Dominant Optic Atrophy (ADOA), also known as Optic Atrophy 1 (OMIM#165500) or Kjer disease, is the most common form of hereditary optic neuropathy(1). ADOA belongs to the group of inherited optic neuropathies, which are genetic conditions consisting of the degeneration of optic nerve fibers, primarily affecting retinal ganglion cells (RGC). These cells' axons collectively form the optic nerve.

The estimated prevalence of ADOA ranges from 1 in 35 000 to 1 in 50 000 individuals worldwide(2,3) although it can be as high as 1 in 1 000 in specific populations. The penetrance of AODA is approximately 70%, but it can vary from 100%(4) to 43%(5) based on families, specific mutations, and study criteria(2,6). The clinical diagnosis of ADOA presents challenges to physicians due to its heterogeneity, making it difficult to ascertain solely based on clinical presentation(7).

The most common genetic association with ADOA is a mutation in OPA1 gene, located on chromosome 3q28q29. OPA1 encodes a dynamin-related GTPase, which is expressed in the inner mitochondrial membrane. This GTPase plays a role in maintaining mitochondrial morphology and regulating apoptosis(8,9). Mutations in the OPA1 gene can lead to loss of GTPase function, disrupting the integrity of the respiratory chain and compromising the energy supply to RGC, ultimately leading to their apoptosis(10). To date, more than 700 OPA1 mutations have been reported, (https://databases.lovd.nl/shared/genes/OPA1) primarily clustered in the catalytic GTPase domain (exons 8-15) and the dynamin central domain (exons 16-23)(11).

ADOA is typically diagnosed in the first decade of life and is characterized by a progressive bilateral visual loss, centrocecal scotoma, and blue-yellow dyschromatopsia(8). A key ophthalmoscopic finding is symmetric optic atrophy with temporal pallor of the optic disc(10), sometimes accompanied by a temporal grey crescent(8).

Optical coherence tomography (OCT) is a non-invasive technique that has proven effective in diagnosing and monitoring several optic neuropathies, including glaucoma, Leber Hereditary Optic Neuropathy (LHON), and Non-Arteritic Ischemic Optic Neuropathy(12,13). Post-mortem histopathology studies in ADOA patients have reported selective degeneration of RGC, particularly in the papillo-macular bundle, with secondary ascending optic atrophy and related changes in the optic pathways. This suggests that RGC degenerate first, with optic atrophy developing subsequently(1). Previous studies utilizing time-domain and spectral-domain OCT (SD-OCT) have shown a reduction of temporal and inferotemporal quadrants' thickness of the peripapillary retinal nerve fiber layer (pRNFL)(14). Additionally, ADOA patients have been found to exhibit thinner retinal ganglion cell–inner plexiform layer (RGC-IPL) compared to healthy controls(9,15). Studies utilizing SD-OCT have further confirmed the selective degeneration of inner retinal layers in ADOA patients, including the pRNFL and RGC(3,16–18). More recent publications have described changes in OCT angiography (OCTA) in these patients including a decrease in retinal vessel density associated with RGC-IPL thinning(19).

The main objective of this study is to investigate the relationship between OCT findings and clinical tests, with the aim of improving the understanding of the

correlation between structural lesions and the functional status of the RGC-IPL in patients with ADOA.

Methods

Study population:

This cross-sectional, case-control observational study included 27 patients (54 eyes) affected by molecularly confirmed ADOA with OPA1 mutation from 16 unrelated pedigrees and 27 age and sex-matched healthy controls (54 eyes). Due to the rarity of AODA, both eyes of each patient were enrolled and compared with an equal number of eyes from the controls. Participants were recruited from the Neuroophthalmology Department outpatient clinic at Hospital Clínic of Barcelona. Prior to participation, written informed consent was obtained from all adult participants and from the legal guardian for those under the age of 18. The study was approved by the Ethics and Investigation Committee of Hospital Clínic of Barcelona (HCB/2018/0631) and adhered to the principles of the Declaration of Helsinki.

Inclusion Criteria:

Consecutive patients with ADOA confirmed by genetic OPA1 mutations were included in the study. Inclusion criteria for the ADOA group were individuals aged 8 years or older. The control group consisted of age and sex-matched heathy subjects recruited during routine ophthalmologic examinations. The inclusion criteria for the control group was as follows: best-corrected visual acuity (BCVA) of at least 0.0 LogMAR, normal intraocular pressure (<21mmHg), normal appearance of the optic disc, absence of significant ocular diseases, and no family history of glaucoma or systemic diseases with potential ocular involvement, such as diabetes mellitus.

Exclusion Criteria:

Both groups were subjected to the same exclusion criteria, which included refractive errors higher or equal to 7 diopters of spherical equivalent; any retinal o optic nerve pathologies other than ADOA, any systemic disorder known to affect retinal vessel density and any media opacities likely to compromise image acquisition.

Clinical evaluation:

All participants underwent an extensive ophthalmic examination including BCVA using ETDRS charts, Sloan Letters Low Contrast Chart 25% (SL25) (Cat. No. L220, Lighthouse Enterprises, NY, USA), Hardy Rand Rittler (HRR) Pseudoisochromatic Plates Test, slit-lamp biomicroscopy, fundoscopy, intraocular pressure determination with Goldmann applanation tonometer, manifest refraction, automated perimetry (*Humphrey*®, Carl Zeiss Meditec; Jena, Germany; SITA Standard) resulting in mean defect (MD), colour fundus photography, OCT and OCTA (Cirrus SD-OCT, software v6.0, Carl ZeissMeditec).

Instrumentation:

The study utilized structural SD-OCT to acquire retinal images for analysis. The SD-OCT scans were obtained using the following protocols: /1/ Optic Disc Cube 200×200: This protocol was used for imaging the optic disc, and it consisted of 200 horizontal scan lines, each with 200 A-scans, for measuring pRNFL thickness. /2/ Macular Cube 512×128: This protocol was employed to obtain macular thickness parameters, including central macular thickness (CMT), macular volume (MV) and ganglion cell complex (GCC); which were calculated using the built-in software. It consisted of a 6×6mm macular cube with a series of 128 horizontal scan lines, each composed of 512 A-scans, and a central horizontal B-HD scan. /3/ OCTA Cubes:

OCTA imaging was performed using a 6×6mm (245×245) scanning protocol with a three-dimensional macular cube centered on the fovea and the optic nerve, respectively. OCTA parameters included vessel density (VD, mm/mm^2), vascular perfusion (VP), and foveal avascular zone (FAZ) characteristics, such as FAZ area, perimeter, and circularity, which were calculated using the built-in software.

Scans were conducted without pupil dilation, and all structural SD-OCT scans were performed by the same operator to ensure consistency. Internal fixation was used for image acquisition, with the exception of patients with poor central vision who were instructed to look in the lateral direction to achieve an optimal view of the optic disc on the operator's screen. External fixation was employed when permitted by the fellow eye.

Exclusion criteria for image quality included a low-quality index (<6), presence of blinking artifacts, motion artifacts due to poor fixation, and dioptric media opacities obscuring the view of retinal vasculature. No participants, whether patients or controls, were excluded based on these image quality criteria.

Statistical analysis:

Categorical variables were described using absolute frequencies and percentages. Quantitative variables were presented as mean and standard deviation (M±SD) with their respective ranges. The normality of the distributions was assessed using the Shapiro-Wilk test. T-test, Wilcoxon rank-sum test and chi-squared test were used accordingly. Associations between clinical variables and disease characteristics were examined using either Pearson or Spearman correlation coefficient determination, with Bonferroni correction applied to account for multiple comparisons. Backward stepwise linear regression models were then developed, incorporating variables with

a significance level less than 0.1 in the univariate analysis as independent factors. A bilateral type I error of 5% was established. A sample size of 27 cases in the study group (ADOA) was calculated according to anticipated means(20) of GCC as primary objective outcome, with a targeted 80% power and 0.05 alpha. The statistical software package STATA v.15.1 (StataCorp, College Station, Texas, USA) was utilized for all data analysis.

Results

The baseline demographic characteristics of the study population are summarized in Table 1. The study included 54 eyes of 27 patients with ADOA, compared with 54 eyes of 27 healthy controls. Among the participants, there were 9 men and 18 women, with an average age of 36.0 ± 16.8 years. The distribution of age and sex was similar between ADOA patients and controls. Clinical characteristics, such as BCVA, SL25, and HRR colour test, were significantly worse in ADOA patients compared to controls. However, visual field MD did not show a significant mean difference between groups given the high variability of campimetric features in ADOA patients. Regarding structural OCT parameters, CMT, MV, GCC, and pRNFL were all statistically significantly reduced in ADOA patients compared to controls (Table 1). Moreover, vascular characterization by OCTA showed optic nerve head (ONH) VD to be thinner in ADOA cases. By contrast, no differences were found in macular VD, neither inner macular ring, outer macular ring nor complete area.

Correlation analysis between clinical and anatomical parameters is provided in Table 2. Univariate correlation coefficients between BCVA and OCT parameters resulted in a relationship between lower BCVA and lower GCC. Low contrast test SL25 directly correlated with GCC, while the HRR colour test showed direct associations with GCC and pRNFL. In addition, visual field MD correlated with macular VD values, inner sectors, outer sectors and total measurement.

Furthermore, multivariate regression analysis on clinical variables by pRNFL, GCC and OCTA (ONH and macula VD) parameters controlled by age was undertaken

(Supplementary material, Table 3). BCVA was reported to be independently associated with GCC (ß coefficient -0.02; p=0.017). By contrast, SL25 was found to be independently related to pRNFL (ß coefficient 0.34; p<0.001). HRR colour test was reported to be independently associated with also age (ß coefficient -0.11, p=0.016), GCC (ß coefficient 0.37; p=0.018) and ONH VD (ß coefficient 0.57; p=0.019). Additionally, MD was reported to be independently associated with macula VD (ß coefficient -0.58; p=0.025).

Discussion

The aim of our study was to investigate the correlation between clinical and structural parameters in patients with ADOA compared to healthy controls. Our findings demonstrate significant differences between ADOA patients and controls not only in clinical parameters but also in OCT and OCTA measurements.

In line with previous reports, ADOA patients exhibited clinical features such as low visual acuity, central, centrocecal, or paracentral scotomas on visual field evaluation, and symmetric temporal or diffuse optic disc pallor. Clinical features were confirmed with the presence of OPA1 mutations in the ADOA group, sustaining the genetic basis of the disease(4).

The structural OCT analysis revealed significant reductions in CMT, MV, GCC, pRNFL and macular VD in ADOA patients compared to healthy controls. These findings are consistent with previous studies that have demonstrated inner retinal layer degeneration in ADOA patients using OCT(3,7,17,18). The observed thinning of retinal layers indicates progressive RGC loss and GCC thinning on OCT is the cause of vison impairment observed in ADOA patients.

Therefore, these OCT-based structural findings could be considered biomarkers in ADOA disease. Even though such patients present with varying degrees of visual function impairment, the ability to provide objective correlations between OCT findings and visual function could be of great interest.

Correlation analysis further strengthened the link between clinical and structural parameters in ADOA. We found that worsening clinical parameters, such as BCVA, SL25, HRR colour test and even visual field results, were associated with greater OCT damage in terms of OCT and OCTA parameters. This suggests that as the clinical impairment worsens, structural changes in the retina and optic nerve become more evident, highlighting the close relationship between functional deficits and retinal tissue damage.

Interestingly, we could consider GCC, at least at exploratory terms, as the most important clinical biomarker in ADOA patients given its multiple positive functional associations. GCC thickness, which represents the combined thickness of the ganglion cell layer and inner plexiform, showed significant and independent correlations with clinical parameters (Figure 1). This suggests that GCC measurement may serve as a valuable clinical indicator for disease severity and progression in ADOA, aiding in disease monitoring and treatment decisions.

Furthermore, our study revealed correlations between macular OCTA parameters and visual field defects as measured by MD. However, such results should be carefully considered given the high dispersion of MD results in the study group. This association could relate retinal vascular perfusion and visual fields deficits in ADOA patients. These OCTA parameters may potentially be also proposed as early biomarkers for disease detection and provide insights into vascular changes in ADOA. In conclusion, our study highlights the correlation between clinical and structural parameters in patients with ADOA and thus their potential biomarker use.

However, it is essential to acknowledge some limitations of the present investigation. The cross-sectional design restricts our ability to establish causality between clinical and structural changes. Longitudinal studies would be valuable in understanding disease progression. Additionally, a larger sample size and longer follow-up period would enhance the generalizability and robustness of our findings. In addition, even though ADOA is typically diagnosed in the first decade of life, our sample mean age is somehow older, thus reflecting the natural history of patients who were diagnosed in childhood and are now being followed into adulthood. In summary, future research should focus on further exploring the potential of GCC as a clinical biomarker and investigating the utility of OCTA parameters as early disease indicators in ADOA.

Overall, our study provides valuable insights into the relationship between clinical and structural parameters in ADOA, especially GCC and OCTA measurements, paving the way for improved disease monitoring and potentially leading to earlier interventions and better patient outcomes. Given that OCT outcomes correlate with function, OCT can be used as an objective surrogate endpoint in treatment decision-making and even clinical trials, especially useful in pediatric populations where consistent functional assessments are challenging.

In conclusion, our study demonstrates significant correlations between clinical and structural parameters in patients with ADOA. The observed differences in clinical and OCT measurements underscore the impact of RGC loss and optic nerve fiber thinning in ADOA pathogenesis. GCC and OCTA measurements emerge as promising clinical biomarkers. These findings have implications for disease monitoring and management, contributing to better diagnosis and care for ADOA

patients. Further research is needed to validate these findings and explore potential therapeutic interventions.

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Figure Legends

Figure 1: Correlations between clinical and structural parameters. A. Lower best-corrected visual acuity (BCVA) correlates with lower ganglion cell complex (GCC). B Lower HRR colour test correlates with lower GCC.

Supplemental Digital Content

Table 3. Multivariate linear regression analysis between clinical variables and structural features.

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