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Utility of Retinal Thickness Analyzer in Early Diagnosis of Glaucomatous Damage

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Abstract. *Aim: The utility of retinal thickness analysis (RTA) in the primary open-angle glaucoma (POAG) early diagnosis and in the follow-up was evaluated. Materials and Methods: The nerve fibre layer thickness was analysed with the RTA by repeated examinations in POAG, ocular hypertension (OH) and healthy subjects (H). Results: In the POAG group, a statistically significant reduction of mean retinal thickness (MRT) compared to the H group was evidenced both in the total area examined and in each of the four quadrants. The visual field evaluation classified the POAG group's visual fields in stage 1 and the OH group's visual fields in stage 0. Conclusion: RTA reveals a variation of mean retinal thickness, even before a functional and/or morphological optic nerve alteration appears. RTA is a complementary tool with the exams usually applied in the diagnosis of glaucoma, even though the information obtained is not specific for glaucoma.*

One of main ophthalmological problems not yet solved today is early open-angle glaucoma diagnosis (1-3). Currently, different semiological criteria and instrumental tools are available to underline the glaucomatous optic nerve and retinal nervous fiber layer damage: tonometric and tonographic criteria, provocative tests, morphological study of the ocular structures, visual evocated potentials, visual function analysis (conventional and unconventional computerized perimetry), blood flow study in orbital and ocular district (echo-colour-Doppler) (4, 5). However, these methods do not always allow for an early damage diagnosis (6-8). Actually, the availability of instruments able to measure retinal thickness [scanning laser ophthalmoscopy (SLO) under Argon-blue; optic nerve head analyzer (ONHA) imagenet; scanning laser polarimetry (GDx); retinal thickness analyzer (RTA); optical coherence tomography (OCT)] focus the researchers' interest on the study of retinal nerve fibre layer (RNFL) anatomical damage at the posterior pole (8-11).

By now it is well-known that the anatomical damage on ganglion cells and nerve fibres begins before perimetric examination is able to reveal glaucomatous functional alterations (1, 2, 5, 8). This problem is not satisfactorily solved by new perimetric techniques either (5, 12). For that reason, alternative methods were developed to directly quantify the anatomical damage on ganglion cells and RNFL (8-10, 12).

It is known, actually, that anatomical alternations in glaucoma would begin in ganglion extra-papillary retinal fibres (6, 7). The loss of these ganglion fibres, only when considerable, causes a progressive reduction of the neural rim, a progressive extension of the physiological cupping and consequent visual field alterations (1, 2, 6, 7). Studies on experimentally-induced glaucoma in monkeys showed a substantial loss of ganglion retinal cells into the macular area (6). The ganglion cell layer is a substantial fraction (30-34%) of the retinal thickness at the posterior pole (9, 10). The retinal thickness analyzer (RTA), used in this study, is a method that measures thickness at the posterior pole and usually applied in the macular disease (8, 9, 12).

Based on this knowledge, the present research has been undertaken with the aim to investigate the role of some variables of the RTA in the open-angle glaucoma early diagnosis and in the follow-up of glaucomatous patients. In particularly, the nerve fibre layer thickness was analyzed by repeat examinations.

Patients and Methods

Groups of patients enrolled in this study. The study protocol was approved by the Ethical Committee of Sapienza University of Rome and, in compliance with the Helsinki Declaration, informed written consent was obtained from all subjects before enrolment.

- First group (OH): 30 patients (30 eyes) with ocular hypertension (OH), without pharmacological therapy and a follow-up of one year

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Key Words: Fiber layer thickness, ocular hypertension, open-angle glaucoma, retinal thickness analyzer, visual field.

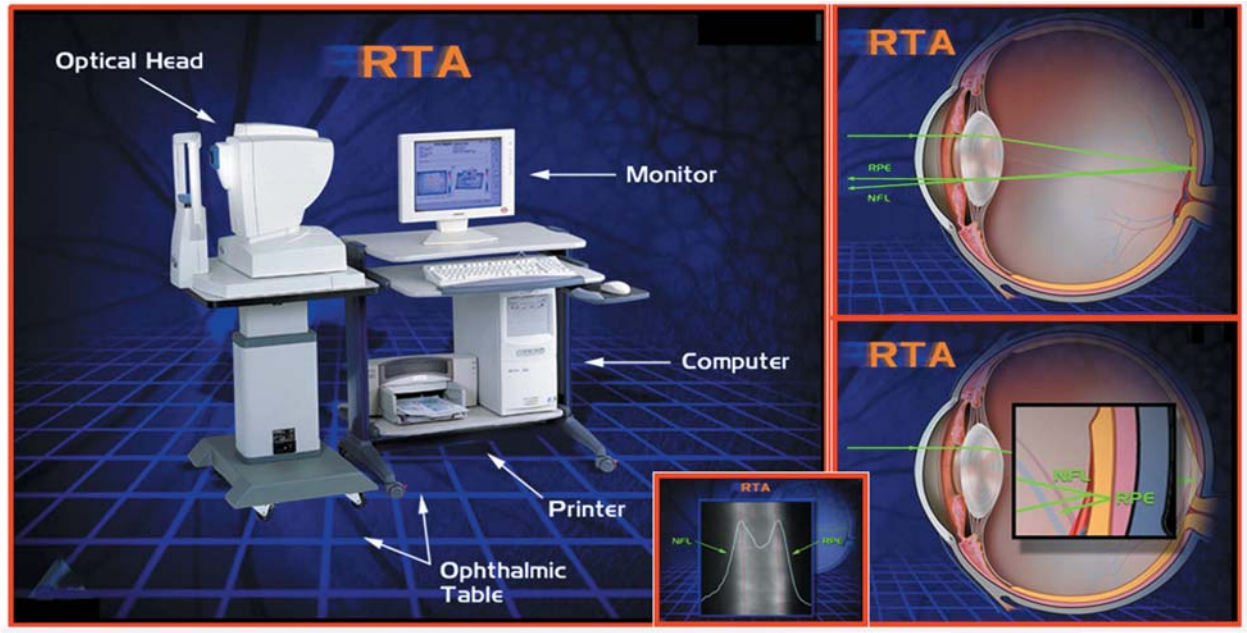

Figure 1. *Retinal thickness analyzer (RTA).*

at least. Patients did not show any visual field defect. The intraocular pressure (I0P) was between 21 and 23 mmHg and the horizontal cup/disc ratio was between 0.5 and 0.1.

- Second group (POAG): 30 patients (30 eyes) with a diagnosis of primary open-angle glaucoma (POAG) cup/disc ratio >0.5. They have been in therapy with 0.5% timolol maleate since 3 years and showed visual field defects.
- Third group (H): 40 healthy subjects (H) (40 eyes) without ocular disease and refraction defects. All patients presented to gonioscopy (Goldmann three mirrors lens) a fourth grade angle according to Shaffer classification and were carefully investigated.

i) Computerized perimetric examinations repeated three times by means of Humphrey program 30-2 full threshold test at six months distance. The Glaucoma Staging System (GSS) was used to classify the visual field defects and indentify the severity of glaucomatous functional damage (13). The mean deviation (MD) of the retinal sensitivity in decibel (dB) was calculated in the four quadrants;

ii) Morphological examination of the optic nerve head by slit lamp biomicroscopy and cup/disc ratio evaluation with Volk + 90D lens;

3) Measurements of the retinal thickness at the posterior pole using the RTA (Talia Technology Ltd., Mevaseret Zion, Israel). The measurements were repeated after six months.

Exclusion criteria. (i) Patients with refractive defects >±2 dioptres and alterations of the corneal thickness; (ii) Patients with subterminal visual fields; (iii) Patients with corneal and lens opacity and not dilatable pupils; (iv) Patients undergone ocular discomfort, cataract, glaucoma and retinal detachment surgery; (v) Patients affected with diabetes mellitus, systemic arterial hypertension and other systemic diseases.

Fundus RTA image registration. The RTA is a combination of a digital video camera and a green helium-neon laser biomicroscope (wave length 543 nm) that projects on *fundus* a monochromatic parallel beam (Figure 1) (9, 14, 15).

A green laser slit is projected on the *fundus* at an angle and its intersection with the retina is imaged. The distance between the intersection with the vitreo-retinal interface and that with the retinal-retinal pigment epithelium interface is directly proportional to the retinal thickness. Scanning RTA is a further development of the method, which aims to provide analysis of multiple optical cross-sections yielding a detailed map of the retinal thickness (9, 12, 15). More specifically, the laser slit is projected on the retina and scans, in 200 or 400 ms, the *fundus* across a 2×2 mm area, yielding 10 optical cross-sections that are digitally recorded. On *fundus*, the beam has the dimension in a slit of 2 mm in length and 14 μm in amplitude. Nine such scans are performed by providing the subject with nine fixation targets (Figure 2).

The composite covers the central 20˚ of the *fundus*. The appropriate fixation can be verified by examining a *fundus* image that is acquired simultaneously with the scan. The optical cross sections are analysed by an operator-free algorithm. The return signals reflected by the retina are recorded by a video camera; the data obtained, by transversal sections through the computer's processing, supply a false colour retinal map that can be bi- or tri-dimensional. Retinal thickness increases progressively from blue to red.

The scanning performance and image quality is compromised by relevant alterations of the lens and corneal thickness alterations. RTA makes up for refractive defect up to three diopters, otherwise the patient has to correct his refractive error wearing contact lens. Two validity indexes (Type 1 and Type 2) are given at each test. Tests with validity Type 1 index <50% and Type 2 index <80% were excluded from analysis of results.

Statistical methods. The mean retinal thickness was calculated in each of four quadrants about central 20˚ by fovea. Comparisons

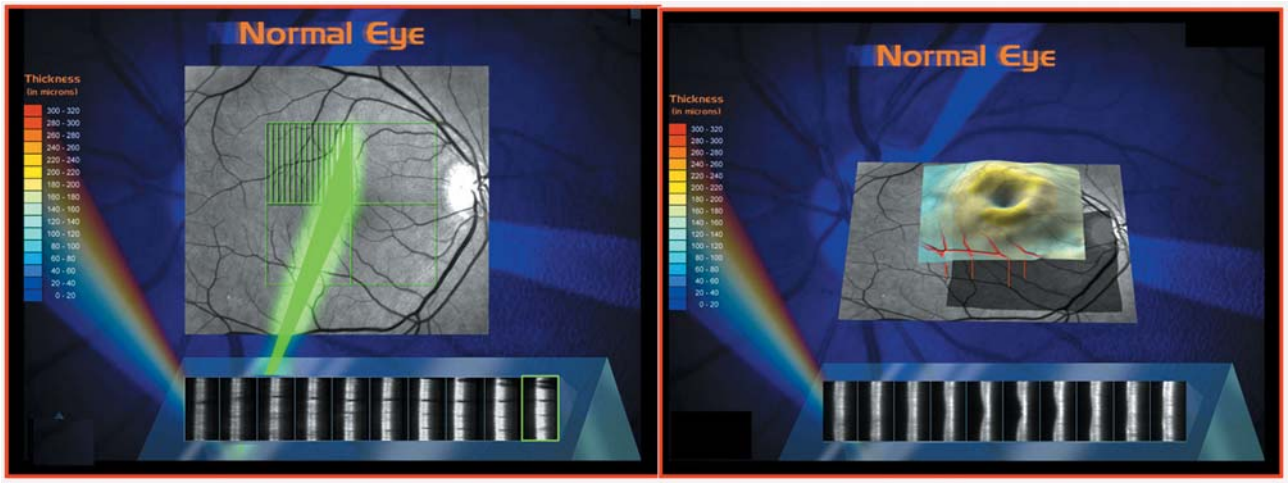

Figure 2. *Nerve fibre layer thickness analyzed with the retinal thickness analyzer (RTA) in normal eyes.*

marked between three groups (POAG, OH, H) were performed by the Mann- Whitney test with Bonferroni's correction. Comparisons for paired data at different times were performed by the Wilcoxon signed-rank test.

Results

Mean age was of 39.2±3.2 years in the OH group, 50±5 years in the POAG group and 32±4 years in the H group. Patients of the POAG group presented a mean IOP of 16±2 mmHg.

The healthy subjects were submitted to two RTA consecutive tests in the same day by which a mean variation of retinal thickness of ±11.5 μm was underlined in the total area examined; the group's mean total retinal thickness was 215.10±25.63 μm. Thus, the exam's reproducibility was tested and it resulted in accordance with clinical trials data (Figure 3) (15).

In all performed examinations, mean retinal thicknesses for each quadrant was calculated: Superior-Temporal (ST), Superior-Nasal (SN), Inferior-Temporal (IT) and Inferior-Nasal (IN). The mean retinal thicknesses obtained in the first tests were compared among the three groups. On the base of exclusion criteria, the groups remained numerically the same. In the POAG group, a statistically significant reduction of mean retinal thickness (MRT) -compared to H group- was noted, both in total examined area (Δ_{tot} 45.5 μm) and in each quadrant (Table I, Figure 4A). The patients' percentages into the POAG group with MRT were: ST quadrant 93% Δm 62.14±40.15 μm; SN quadrant 83% Δm 35.98±30.11 μm; IT quadrant 90% Δm 51.38±45.2 μm; IN quadrant 93% Δm 32.35±26.2 μm.

In the OH group -compared to H group- a statistically significant reduction of MRT ($p<0.05$, Δ_{tot} 15.5 μm) was observed only in the ST quadrant (Table I, Figure 4A).

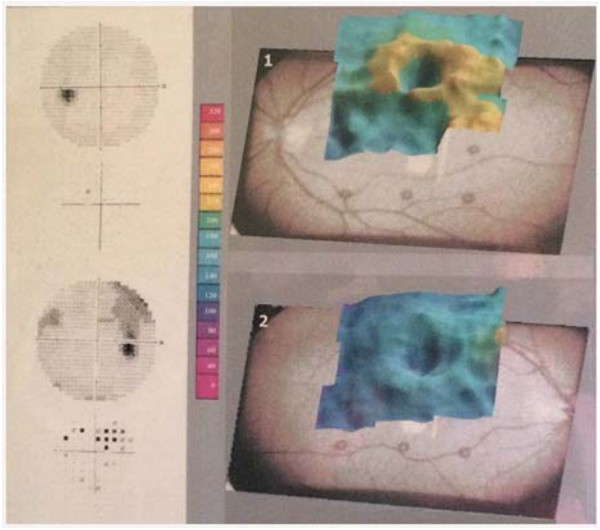

Figure 3. *Examples of the 3-dimensional maps in 1) an ocular hypertension (OH) patient and in 2) a primary open-angle glaucoma (POAG) patient with their relative automated visual fields.*

The patients' percentage with MRT reduction was: ST quadrant 90% Δm 33.29±20.8 μm. In the POAG group compared to OH group, the MRT resulted statistically significant ($p<0.05$) thinned in the previous group: both in the total examined area (Δ_{tot} 30 μm) and in three of the four quadrants (except for the quadrant ST) (Table I, Figure 4A). The RTA examination was repeated after six months in the POAG group and did not show a further statistically significant reduction of the MRT, both in the total examined area and in each quadrant (Table II and Figure 4B).

Table I. *Mean retinal thickness (M)±standard deviation (SD) in each of the three groups for each quadrant (Q) and in the total area. Level of significance of the three groups' retinal thickness (in μm) comparisons.*

Q	1 POAG M±SD	2 OH M±SD	3 H M±SD	1 *vs.* 2	1 *vs.* 3	2 *vs.* 3
ST	166.35±25.25	195.20±41.65	228.49±40.96	NS	<0.001	0.05
SN	173.29±17.02	197.63±25.14	209.27±19.98	<0.05	<0.001	NS
IT	168.78±33.94	208.14±48.95	220.16±41.7	<0.05	<0.001	NS
IN	170.15±20	197.67±27.61	202.5±20.66	< 0.05	<0.001	NS
Total	169.65±20.26	199.66±32.63	215.10±25.63	<0.05	<0.001	NS

Patients with primary open-angle glaucoma (POAG, n=30), patients with ocular hypertension (OH, n=30) and healthy subjects (H, n=40). Superior-Temporal (ST), Superior-Nasal (SN), Inferior-Temporal (IT) and Inferior-Nasal (IN).

This could, if confirmed by long-term data, hypothesize a stability of glaucomatous disease by a pharmacological and tonometric reward.

In OH patients, after six months, a further statistically significant reduction ($p<0.0033$) of the MRT only in the SN quadrant was found (Table II and Figure 4C).

The visual field evaluation, based on mean deviation (MD) and corrected pattern standard deviation (CPSD) values (according to GSS), classified the POAG group's visual fields in stage 1 (wide defect: 19 eyes, localized defect: 11 eyes) and the OH group's visual fields in stage 0 (13). Re-evaluation between the previous visual fields with those performed six months later did not show any statistically significant variations of retinal sensibility in the total neither in the four quadrants. This functional stability of the visual fields was found in both groups.

Table II. *Comparisons of primary open-angle glaucoma (POAG) and ocular hypertension (OH) groups for each quadrant, between mean retinal thickness (in μm) to time 0 (TO) and time 1 (T1).*

POAG	T(0) mean±SD in μm	T(1) mean±SD in μm	*p*-Value
ST	166.35±25.25	163.94±29.66	NS
SN	173.29±17.02	174.57±13.90	NS
IT	168.78±33.94	166.43±22.72	NS
IN	170.15±20	172.04±16.93	NS
Total	169.65±20.26	169.24±16.34	NS
OH	**T(0) mean±SD in μm**	**T(1) mean±SD in μm**	***p*-Value**
ST	195.20±41.65	192.90±43.23	NS
SN	197.63±25.14	190.37±21.44	0.0033
IT	208.14±48.95	192.39±35.02	NS
IN	197.67±27.61	190.89±20.96	NS
Total	199.66±32.63	191.64±24.82	NS

Superior-Temporal (ST), Superior-Nasal (SN), Inferior-Temporal (IT) and Inferior-Nasal (IN).

Discussion

This study has been undertaken with the aim to investigate the role of certain variables of the RTA in the open-angle glaucoma early diagnosis and in the follow-up of glaucomatous patients by repeated examinations. The involvement of retinal cells in glaucoma is supported by a number of psychophysical, electrophysiological and morphological studies (2, 5, 6-10, 16). In particular, they showed a reduction and delay of electroretinogram A, B and N waves in glaucomatous eyes, which were also comparable to those observed in early cone-rod dystrophy (5, 17, 18). The present research suggests that the inner and outer retina layers can be functionally abnormal in subjects affected by glaucoma.

Moreover, an increased photoreceptor thickness in glaucoma has been reported by Ishikawa *et al.* (10), whereas in the study by Fan *et al.* (19) the foveal was measured because this location has the highest density of cones (central one degree of the macula). The authors compared the photoreceptor layer thickness between normal and glaucomatous eyes using spectral domain optical coherence tomography (SD-OCT). They reported that there were significant differences in foveal photoreceptor thicknesses and not in the parafoveal area between the normal and mild glaucoma groups (19), whereas no difference was found comparing normal and moderate to advanced glaucomatous eyes. This concurs with the observation that cone (but not rod) swelling is associated with glaucoma (19). The authors concluded that the increased foveal thickness observed in mild glaucoma could be explained by cone swelling, infiltration of glial cells, inflammatory cells and increased extracellular matrix deposition (19). However, this is in contrast with what reported by other authors who, in recent case series, showed losses in cone density and thinning of the photoreceptor outer segments in patients with glaucoma (20, 21).

Therefore, *in vivo* measurement of photoreceptors on the fovea provides an approach to study the association between photoreceptors and glaucoma.

On the other hand, in our study, the analysis of data obtained through previous RTA control, in particular in the POAG group, would confirm the clinical diagnosis and would emphasize anatomic damage central in the 20° of the

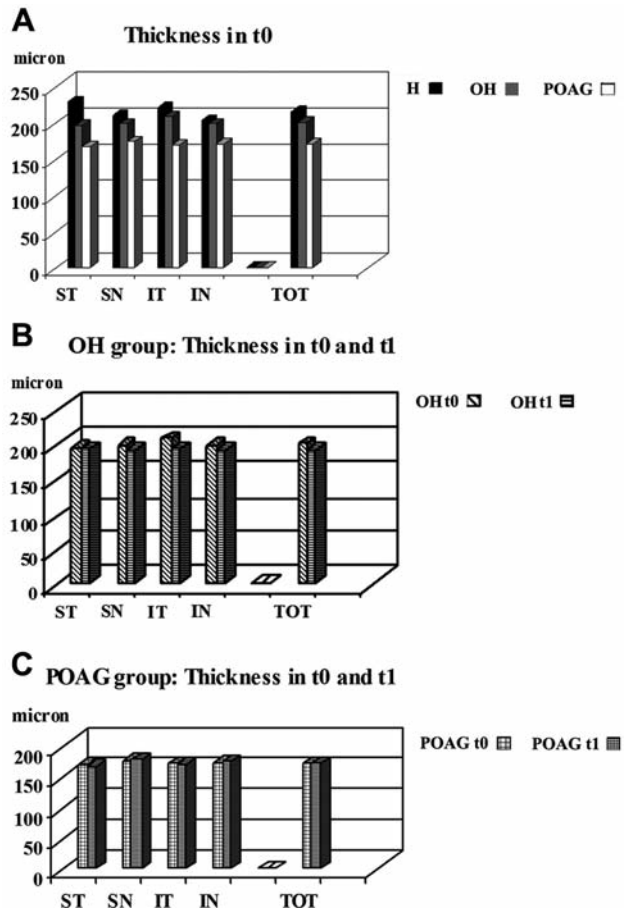

Figure 4. *Mean retinal thicknesses compared for the three groups. Panel A): Mean retinal thickness values in time 0 (t0) of three groups tested. In the x-axis the considered quadrants are displayed, whereas in the y-axis thicknesses in μm are displayed. Healthy subjects = H; Panel B: Comparison in the patients with ocular hypertension (OH) between mean retinal thickness in time 0 (t0) and time 1 (t1). In the x-axis the considered quadrants are displayed, whereas in the y-axis thicknesses in μm are displayed; Panel C: Comparison in the patients with primary open-angle glaucoma (POAG) between mean retinal thickness in time 0 (t0) and time 1 (t1). In the x-axis the considered quadrants are displayed, whereas in the y-axis thicknesses in μm are displayed.*

fundus. The thickness loss, only in one quadrant in the OH group, could make a distinction between ocular hypertensive patients and those in whom the hypertension induced a structural damage of retinal fibers, not yet recorded by computerized perimetry; stage 0 according to GSS (13).

RTA repeated after six months in the POAG group, did not demonstrate any further significant reduction of retinal thickness, both in the total examined area and in the four quadrants. This could, if confirmed by long-term data, make one think about a stability of glaucoma disease obtained by a good pressure control with a potential pharmacological reward.

We concur with several studies that the posterior pole retinal thickness decreases in early- and moderate-stage glaucoma. In addition, the reduction of perifoveal retinal thickness is correlated with visual field loss. *In vivo* measurements of posterior pole retinal thickness may help distinguish between normal and glaucomatous eyes. In fact, some studies showed that in many glaucomatous eyes (up to 81.8%) there was a relationship between retinal thickness loss and visual field defects (4, 9, 22, 23).

Conclusion

Based on the fact that alterations of nerve fiber layers are not exclusive for glaucoma but can be present even in neurological disease and sometimes in normal eyes, our RTA data provide supplementary and interesting information when, especially, considering that myopic aspects on the posterior pole makes it difficult to understand results obtained by this instrument as the difficulty to identify the early glaucoma anatomic alterations limits the diagnostic specificity of RTA. Thus, RTA could be a valid tool complementary to perimetric examination and to the morphometric evaluation of the optic nerve head.

Conflicts of Interest

The Authors report no conflicts of interest and have no proprietary interest in any of the materials mentioned in this article.

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