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Analysis of Ganglion Cell Complex and Retinal Nerve Fiber Layer Thicknesses in Turkish Population

Türk Halkında Ganglion Hücre Kompleksinin ve Retina Sinir Lifi Kalınlığının Değerlendirilmesi

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Öz

Amaç: Sağlıklı bireylerde yaş ve cinsiyetin ganglion hücre kompleksi ve retina sinir lifi kalınlığı üzerine etkisinin değerlendirilmesi amaçlandı.
Hastalar ve Yöntem: Bu kesitsel çalışmada Kasım 2012 - Mayıs 2013 tarihleri arasında yaşları 10-84 arasında değişen 393 sağlıklı katılımcıyı değerlendirdik. Yaşa bağlı değişikler lineer regresyon analizi ve Pearson korelasyon analizi ile incelendi. Retina sinir lifi kalınlığı ve ganglion hücre kompleksi arasındaki ilişki pearson korelasyon testi ile değerlendirildi.
Bulgular: Tüm populasyonun ortalama retina sinir lifi kalınlığı 108.94±9.77 µm idi ve yıllık 0.101 µm azalmaktaydı. En önemli azalma üst temporal bölümde görüldü. Üst kadran hariç retina sinir lifi kalınlığında cinsiyete göre fark bulunamadı. Ortalama ganglion hücre kompleksi kalınlığı 97.45±6.42 µm olarak bulundu ve her yıl 0.043 µm azalmaktaydı. Cinsiyetle ganglion hücre kalınlığı arasında bir ilişki bulunmazken retina sinir lifi ile ganglion hücre kompleksi arasında anlamlı korelasyon tespit edilmiştir.
Sonuç: Retina sinir lifi kalınlığı ve ganglion hücre kompleksi kalınlığı yaşla birlikte önemli ölçüde azalmaktadır ancak ganglion hücre kalınlığı daha az etkilenmektedir.

Anahtar Kelimeler: Optik kohorens tomografi, retina sinir lifi kalınlığı, ganglion hücre kompleksi, Türk halkı

Abstract

Aim: To determine the effects of age and sex on retinal ganglion cell complex thickness and retinal nerve fiber layer thickness in the eyes of healthy individuals.
Patients and Methods: We evaluated 393 healthy subjects aged 10-84 years in a cross-sectional study between November 2012 and May 2013. Linear regression analysis and Pearson's correlation were performed to analyze the difference in the age-related changes. We evaluated the relationship between the retinal nerve fiber layer thickness and ganglion cell complex thickness using Pearson's correlation.
Results: The whole population mean retinal nerve fiber layer thickness was 108.94±9.77 µm, and decreased by 0.101 µm/year. The most significant decrease was in the supero-temporal sector. There was no difference in the retinal nerve fiber layer thickness between sexes, except for the superior quadrant. The mean ganglion cell complex thickness was 97.45±6.42 µm, and decreased by 0.043 µm/year. There was no relationship between sex and ganglion cell complex thickness. The mean retinal nerve fiber layer and ganglion cell complex thicknesses exhibited a significant correlation.
Conclusion: The ganglion cell complex thickness and retinal nerve fiber layer thickness decreased significantly with age but ganglion cell complex thickness was less affected.

Key words: Optical coherence tomography, retinal nerve fiber layer, ganglion cell complex, Turkish population

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INTRODUCTION

Glaucoma is an optic neuropathy characterized by the progressive loss of retinal ganglion cells (RGCs) and their respective axons (1). The human retina contains >1 million RGCs, and ~50% are located within 4.5 mm of the center of the fovea (2). Because the macular region contains a significant portion of the RGCs, an evaluation of ganglion cell changes may assist in the diagnosis of glaucoma (3-6). In recent years, the evaluation of the ganglion cell complex thickness (GCCT) and retinal nerve fiber layer thickness (RNFLT) has become important in the diagnosis and follow up of glaucoma patients (4,7-10). The ganglion cell complex (GCC) comprises three tissue layers: the retinal nerve fiber layer (RNFL), RGCs, and the inner-plexiform layer. RNFL and GCC thicknesses may vary according to factors such as race, age, axial length, and refraction (11-14).

There is no commercially available optical coherence tomography (OCT) device with a database for the Turkish population. To our knowledge, although studies of the RNFL in healthy Turkish populations using various OCT devices have been performed, no such data on the GCC are available in healthy adult Turkish population (15-19). Therefore, we aimed to evaluate RNFL and GCC thicknesses in Turkish subjects according to age and sex.

PATIENTS AND METHODS

This study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from the healthy participants after they were provided information about the nature and possible consequences of the study. The study protocol was approved by Bozok University Faculty of Medicine, Non-interventional Clinical Trial (Decision No:2013,6/10). The current study was performed at the Ophthalmology Clinic of Bozok University Medical Faculty between November 2012 and May 2013. A total of 393 (212 female and 181 male) healthy individuals were enrolled. The subjects were healthy individuals employed in the hospital or were associates of the patients. The best-corrected visual acuity was determined using the Snellen chart. After slit-lamp and fundus examination using a 90-D lens, the same physician determined the intraocular pressure (IOP) by Goldmann applanation tonometry. Then, the peripapillary RNFL and GCC thicknesses were determined using the RTVue-100 SD-OCT device (Optovue Inc., Fremont, CA, USA). Measurements were taken in triplicate for each eye and B-scan image segmentations were inspected on the screen. The best provided signal strength higher than 60 was used for analysis. Subjects who had undergone intraocular surgery, an IOP >21 mmHg, diabetic retinopathy, a refractive error >±2D, a corrected visual acuity worse than 20/20, a history of glaucoma, ocular trauma, or a history of uveitis, were excluded. Moreover, patients who had any sign of peripapillary choroidal atrophy and an abnormal ophthalmoscopic examination of the optic nerve head, macula, and retinal vasculature were excluded.

Participants were classified according to decade between the ages of 10 and 69 years. Subjects >70 years were evaluated as a single group. The measurements were performed without pupil dilation by the same researcher.

OCT Measurements

Measurements were performed using the RTVue-100 SD OCT instrument with software version 6.1. The working principle of the device is described in detail elsewhere (20). The following parameters were evaluated: the mean RNFLT around the optic nerve head; the four-quadrants RNFLT (inferior, nasal, superior, and temporal); all eight RNFLT sectors (TU: temporal-upper; ST: supero-temporal; IT: infero-temporal; TL: temporal lower; SN: supero-nasal; NU: nasal-upper; NL: nasal lower; and IN: infero-nasal); and the mean GCC, IGCC, and SGCC thicknesses.

Statistical Analysis

We used the Statistical Package for the Social Sciences (SPSS) software (Worldwide Headquarters SPSS Inc. 18.0 Windows package program) for statistical analysis. Descriptive findings are given as the means ± standard deviation. The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Comparisons between groups were performed using Student's t-test; one-way analysis of variance was used for multiple comparisons between groups. Pearson's correlation test was performed for correlation analysis.Linear regression analysis was performed to determine the mean decrease in the RNFLT and GCC thickness according to age. Annual rates of change in the RNFLT and GCC thickness are shown as percentages/year. A value of $p<0.05$ was considered to indicate statistical significance.

RESULTS

The right eyes of 393 subjects were analyzed according to sex and age. There were 212 (53.95%) females and 181 (46.05%) males. The mean age was 39.90±17.57 (range, 10-84) years. The distributions of the subjects according to age group

Table 1. Distribution of subjects according to age groups and gender

Age groups	10-19	20-29	30-39	40-49	50-59	60-69	≥70
Female	40	28	34	48	34	19	9
Male	26	26	33	34	37	16	9
Total	66	54	67	82	71	35	18

Table 2. Retinal nerve fiber layer thickness measurements (mean±SD in micrometers) according to gender

	Average	**Superior**	**Temporal**	**Inferior**	**Nasal**
Female (n=212)	109.79±9.72	134.69±15.16	83.97±10.98	142.29±18.32	78.00±11.49
Male (n=181)	107.95±9.78	130,65±15.17	85.11±11.16	139.52±16.57	76.41±11.42
p	0.06	0.01	0.31	0.12	0.17

and gender are given in Table 1. The mean RNFLT was 108.94±9.77 (range, 77-140) μm. Although the mean RNFLT was greater in females than in males, the difference was not significant (109.80±9.71 and 107.94±9.72 μm, respectively, p=0.06). The RNFLTs of the superior, temporal, inferior, and nasal quadrants were 132.8±15.8, 84.5±11.1, 140.7±18.1, and 77.5±11.7 μm, respectively, independent of sex. The superior RNFLT was significantly greater in females than in males (134.69±15.16 and 130.65±15.17 μm, respectively, p=0.01). Table 2 shows the mean and four-quadrants RNFLTs of both sexes. The mean RNFLT exhibited a significant inverse correlation with age (p<0.001, r= -0.17), while the greatest correlation was for the superior RNFLT (p<0.001, r= -0.18). The decrease in the mean RNFLT was 0.101 μm/year (95% confidence interval (CI), -0.151, -0.051; linear regression analysis, p=0.001), with the most significant decrease being in the ST sector (0.197 μm/year, 95% CI, -0.293,-0.101; linear regression analysis, p<0.001). Detailed data on the mean, four quadrants, eight sectors and linear regression of the RNFLT according to age group are presented in Table 3.

The mean GCC, SGCC, and IGCC thicknesses of all population were 97.45±6.42, 97.3±6.7, and 97.9±6.7 μm, respectively. The male and female mean GCC thicknesses were 97.70±6.53 and 97.24±6.34 μm, respectively (p=0.48). The decrease in the mean GCC was 0.043 μm/year (95% CI, -0.079, -0.007; linear regression analysis, p=0.019), while the most significant decrease was in the SGCC thickness (0.053 μm/year, 95% CI, -0.092,-0.015; linear regression analysis, p=0.007). Detailed information according to age group is presented in Table 4. The mean GCC thickness exhibited a significant inverse correlation

Table 3. Retinal nerve fiber layer thickness measurements (mean±SD in micrometers) and linear regression analysis according to age groups.

	Overall (n=393)	**10-19 y (n=67)**	**20-29 y (n=54)**	**30-39 y (n=67)**	**40-49 y (n=82)**	**50-59 y (n=71)**	**60-69 y (n=35)**	**70 and over (n=18)**	**p1**	**Slope μm/y**	**%95 CI**	**p2**
Mean RNFLT(μm)	108,9±9,8	111,3±8,7^{a}	109,3±9,0^{ab}	109,1±10,1^{ab}	109,4±9,0^{ab}	110,1±9,7^{ab}	104,6±10,0^{bc}	100,3±12,5^{c}	<0.001	-0.101	-0,151, -0,051	0,001
Quadrants (μm)												
Superior	132,8±15,8	137,4±14,0^{a}	134,0±15,1^{a}	132,4±15,8^{a}	132,2±16,1^{a}	133,6±15,8^{a}	129,9±16,2^{ab}	117,4±13,5b^{c}	<0.001	-0,153	-0,298, -0,067	<0,001
Temporal	84,5±11,1	84,4±9,6^{a}	85,7±9,3^{a}	86,9±13,6^{a}	85,2±10,6^{a}	84,3±10,6^{a}	80,8±10,1^{a}	78,8±14,4^{a}	0.061	-0.080	-0,142, -0,018	0,012
Inferior	140,7±18,1	144,4±16,7^{a}	140,8±18,7^{a}	139,4±18,1^{a}	140,8±18,7^{a}	143,1±17,3^{a}	133,8±16,5^{a}	136,0±23,2^{a}	0.076	-0,078	-0,177, 0.021	0.122
Nasal	77,5±11,7	79,5±11,7a	77,6±11,2^{ab}	76,5±11,7^{ab}	78,4±12,9^{ab}	79,1±10,9^{a}	73,3±10,7^{ab}	69,28±10,7^{b}	0.007	-0,072	-0,137, -0,008	0.028
RNFLT sectors(μm)												
ST	145.7±17.3	150.5±15.8^{a}	148.9±19,3^{ab}	145.3±16.2^{a}	145.9±18.2^{ab}	145.9±18.2^{ab}	137.9±16.4^{bc}	131.3±13.1^{c}	0.001	-0.197	-0,293, -0.101	<0.001
SN	120.0±18.5	124.1±18.0^{a}	121.7±17.6^{a}	117.1±16.4^{ab}	120.4±18.4^{a}	121.6±18.6^{a}	120.0±21.8^{a}	102.8±16.0^{b}	0.002	-0,127	-0.231, -0.022	0.018
NU	82.3±13,0	84,8±13.6^{a}	83,2±12.1^{ab}	83.9±17.6^{ab}	82.2±13,1^{ab}	84.2±11.9^{ab}	77.1±11.7^{ab}	73.7±13.3^{b}	0.014	-0.112	-0.189, -0.034	0.005
NL	72,3±11.3	74.4±11.8^{a}	72.1±11.3^{a}	71.2±10.8^{a}	72.6±10.9^{a}	74.1±11,4^{a}	69.0±11.7^{a}	67.1±9.5	0.075	-0.040	-0.104, 0.024	0.220
IN	128.1±23.7	131,5±23.7^{a}	126.9±25.8^{a}	126.4±23.5^{a}	129.6±23.1^{ab}	129.4±24.3^{a}	122.2±22.1^{a}	124.5±22.4^{a}	0.559	-0.044	-0.179, 0.091	0.518
IT	154,6±18.8	156.6±16.9^{a}	155.5±17.2^{ab}	155.7±20.9^{a}	155.2±17,9^{ab}	156.7±18.9^{a}	144.1±17.8^{b}	148.5±22,5^{ab}	0.020	-0.121	-0.227, -0,015	0.026
TL	81,3±12,1	81.2±10.0^{a}	83.7±11.4^{a}	83.0±14,0^{a}	80.9±11.9^{a}	80.0±11.1^{a}	79.0±13.1^{a}	77.7±17.7^{a}	0.303	-0.077	-0.145, -0.008	0.028
TU	87,7±13.2	87.0±12.4^{a}	87.4±11.3^{a}	89.7±15.7^{a}	88.8±12.3^{a}	89.2±14.0^{a}	82.8±14.9^{a}	81.7±14.9^{a}	0.068	-0.052	-0.127, -0.022	0.169

* Abbreviations: RNFLT; Retinal nerve fiber layer thickness, ST; supero-temporal, SN; supero-nasal, NU; nasal-upper, NL; nasal lower, IN; infero-nasal, IT; infero-temporal, TL; temporal lower TU; Temporal-upper,
Different subscripts in a row indicate statistically significance difference.
p1 shows that intergroup difference significance with ANOVA
p2 shows that significance of linear regression analysis

Table 4. Ganglion cell complex thickness measurements (mean±SD in micrometers) and linear regression analysis according to age groups.

	Total (n=393)	10-19 age (n=67)	20-29 age (n=54)	30-39 age (n=67)	40-49 age (n=82)	50-59 age (n=71)	60-69 age (n=35)	70 and over (n=18)		P1	Slope µm/y	%95 CI	P2
GCC	97.5±6.4	97.9±5.3[a]	97.1±5.5[ab]	98.4±6.5[a]	98.3±6.4[a]	98.4±6.6[a]	94.0±7.3[b]	92.6±6.4[b]	<0.001	-0.043	-0.079, -0.007		0.019
IGCC	97.9±6.6	98.2±5.4[a]	97.5±5.3[ab]	98.7±6.1[a]	98.8±6.6[a]	98.7±7.1[a]	93.8±8.1[b]	93.2±6.8[b]	<0.001	-0.046	-0.083, -0.008		0.017
SGCC	97.3±6.7[a]	98.7±6.1[a]	99.4±6.3[a]	97.6±7.6[a]	96.9±6.9[a]	96.3±6.6[a]	96.7±7.7[a]	96.1±7.0[a]	0.135	-0.053	-0.092, -0.015		0.007

* Abbreviations: GCC Ganglion cell complex, IGCC Inferior ganglion cell complex ,SGCC Superior ganglion cell complex
Different subscripts in a row indicate statistically significance difference
p1 shows that intergroup difference significance with ANOVA
p2 shows that significance of linear regression analysis

with age (p=0.02, r=-0.12), with the greatest correlation for the SGCC thickness (p=0.01, r=-0.14). The mean GCC thickness and RNFLT exhibited a significant correlation (p<0.001, r=0.630). Although the IGCC thickness was significantly correlated with the inferior RNFLT, no correlation was found between the SGCC thickness and the superior RNFLT (p<0.001, r=0.494 and p=0.106, r=0.082, respectively).

DISCUSSION

We investigated the variation in GCC thickness and RNFLT measurements obtained by RTVue OCT in healthy subjects according to sex and defined age group, and the correlation between the GCC thickness and RNFLT. Although several studies have investigated the relationship between age and the RNFLT using other OCT devices, we found no data on the relationship between the GCC and age in the healthy Turkish population (15-17). In this study, we demonstrated that the RNFLT and GCC thickness are inversely correlated with age. A strong correlation was also found between the RNFLT and GCC thickness in healthy individuals.

Currently, two in vivo methods of determine the RNFLT are available scanning laser polarimetry and OCT. OCT technology has changed considerably in recent years with the incorporation of spectral-domain (SD) imaging, which offers important advantages over traditional time-domain (TD)-OCT techniques (21). The TD-OCT and SD-OCT determined RNFLT measurements are dissimilar, and RNFLT values differ between individual SD-OCT devices, and are thus not interchangeable (22). Therefore, studies of the variation in the RNFLT in a normal population using SD-OCT are required. To our knowledge, this is the first study to evaluate RNFLT and GCC thickness in healthy Turkish individuals using RTVue. We also investigated, for the first time, the correlation between these two parameters in healthy Turkish subjects.

Relationships among the RNFLT, Age and Sex

The overall mean RNFLT was 108.94±9.77 µm. Although this RNFLT is slightly greater than in previous studies of the Stratus, Spectralis, and Cirrus devices, it is compatible with an earlier study using an RTvue OCT device (13, 15, 16, 23, 24). In a study that compared three OCT devices for healthy and glaucomatous eyes, and found that the mean and four quadrant values obtained using the RTvue were greater than those using Stratus, Spectralis, and Cirrus OCT in both groups (23). In agreement with previous reports, we found that the mean inferior quadrant had the greatest thickness, followed by the superior, temporal and nasal quadrant. Previous studies reported that 56-79% of normal eyes comply with the thickest inferiorly and thinnest temporally (ISNT) rule (25-26). There are also articles that do not obey this rule and support our finding. The Singapore Chinese Eye Study (27) reported a descending RNFLT in the order inferior, superior, temporal, and nasal sectors. Because normal eyes likely exhibit some variation in the distribution of the RGC axons, this may play a role in normal eyes that do not comply with the ISNT rule.

The normal variability in the RNFLT may differ according to race, age, sex, refractive error, and axial length (11). Many researchers have examined the impact of ethnic factors on the RNFLT. Caucasians have a lower RNFLT compared to Hispanics, African–Americans, and Asians (12, 13). In two studies performed using SD-OCT found that the mean RNFLT was 102 µm in Japanese whereas that it was 97.2 µm in German (28, 29). In a study that compared three OCT devices, the mean RNFLT was 11.5 µm greater with the RTvue than with the Cirrus OCT and 4.5 µm greater than with the Stratus OCT in the same population (23). Therefore, a normative database for each device and ethnicity is required to evaluate disorders properly.

We demonstrated that the mean RNFLT and four-quadrants RNFLT, with the exception of the inferior quadrant, decrease with age. Although some studies

did not report a decrease in the RNFLT with age, most did show such a decrease (13, 15-17, 30-34). These results are consistent with previous histological studies that reported a decrease in the density of photoreceptors and ganglion cells with age (35, 36). An age-related decrease in the RNFLT was observed, together with a reduction of 4000 axons/year, in a histological study (37). The reported rates of RNFLT change ranged from -1.5 to -3.3 μm/decade. The mean RNFLT changed at a rate of -0.16 μm/year and by quadrant the mean superior RNFLT showed the greatest tendency to decrease with age, whereas the change was minimal in the inferior quadrant (-0.23 and -0.08 μm/year, respectively) (38). Similarly reported that the mean RNFLT slope was -0.24 μm/year, while for the superior and inferior quadrants it was -0.43 and -0.15 μm/year, respectively (33). Alasil et al (13) showed that the greatest decline was in the superior quadrant (0.21 μm/year), followed by the mean RNFLT (0.15 μm/year). We found a decrease in the mean RNFLT of 0.101 μm/year. This is similar to but somewhat lower than reported in the literature. One possible explanation for this is related to the inclusion of only individuals with 20/20 visual acuity. Although these findings support our result, contradictory data have been reported. In the literature also showed that the decrease in the superior and inferior quadrants was at a greater rate than in the temporal and nasal quadrants and the another study reported that the greatest absolute slopes were for the inferior and superior quadrants, and the clock hour 1 (32, 39). The cause of these differences is unknown, but it may be related at least in part to ethnic differences.

Although we found that the mean RNFLT was slightly greater in females, there was no significant difference between the sexes, with the exception of the superior quadrant. Kılıç et al (15) reported that the mean RNFLTs in males in females were 99.8 and 102.1 μm, respectively, but without any significant difference. They also reported that the temporal quadrant thickness was greater in females. Although these findings suggest that gender has a slight effect on the RNFLT, it appears to have no significant impact in the literature (12, 13, 28, 29, 33).

Relationships among the GCC, Age and Sex

We demonstrated that the GCC thickness decreased in older subjects and that there was no difference according to gender. We found that the GCC thickness was less affected by age than the RNFLT. The decrease in the mean GCC thickness was 0.043 μm /year. Kita et al (40) found no significant relationship between the GCC thickness and age, but reported a partial regression coefficient for the GCC of -0.15 for Hungarians and -0.11 for Japanese. These studies included a small simple size, 52 Hungarian and 50 Japanese. Similarly demonstrated that the GCC thickness did not change according to to age in young myopic subjects (41). In contrast, some research demonstrated that the GCC thickness decreased by 0.159 μm/year, although they found no relationship with sex (42). Girkin et al (43) reported that the GCC thickness decreased by 0.1 μm/year. We speculate that GCC change isn't uniform due to the GCC comprises three tissue layers. These tissues may have different response to aging process. Significant loss of retinal ganglion cells occurred in 12-week old mice although inner plexiform layer stratification remained unchanged at this stage (44). Other possible situation may be residual tissue. Complete loss of retinal ganglion cells in humans leaves a residual RNFL thickness due to residual blood vessels and glial cells (45).

CONCLUSION

The ganglion cell complex thickness and retinal nerve fiber layer thickness decreased significantly with age but ganglion cell complex thickness was less affected. Racial and ethnic changes may lead to differences which may result in delays in the diagnosis of disorders. Therefore, ethnicity should be taken into account to evaluate disorders properly.

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