· Original article ·

Investigation of relationship of iris color with retinal nerve fiber layer, macula and choroid thickness in healthy individuals

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虹膜颜色与视网膜神经纤维层和黄斑及脉络膜厚度关系的研究

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摘要

目的:明确眼虹膜颜色与眼轴长度、眼压、视网膜神经纤维层厚度(RNFL)、黄斑厚度、脉络膜厚度是否相关。

方法:前瞻性横断面研究。92 例(92 眼)正常个体根据虹膜颜色分为深色组(DCE)和浅色组(LCE)。用标准光学相干断层扫描(OCT)分析 RNFL 和黄斑厚度,EDI(Electronic data interchange)分析脉络膜厚度。脉络膜厚度在中央凹及水平位置距离中央凹 1500μm 的鼻侧、颞侧进行测量。

结果:92 眼中,深色组 62 眼(67.4%)平均年龄 29.22±5.86y,浅色组 30 眼(32.6%)平均年龄 28.86±6.50y。各组间平均年龄、眼轴长度、黄斑厚度、脉络膜厚度及眼压无显著性差异($P>0.05$)。视网膜神经纤维层厚度的变化取决于测量象限,而 LCE 个体在全眼球,鼻侧和颞侧象限较低($P\leqslant0.022$)。

结论:深色组和浅色组个体间眼内压(IOP),黄斑厚度和脉络膜厚度无显著差异,且视网膜神经纤维层厚度较低。

关键词:视网膜神经纤维层厚度;脉络膜厚度;眼睛颜色;虹膜颜色

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Abstract

• AIM: To determine whether there was a significant relationship between eye iris color with axial length, intraocular pressure, retinal nerve fiber layer (RNFL) thickness, macular thickness and choroidal thickness.

• METHODS: A prospective cross-sectional study involving 92 eyes of 92 healthy volunteers. These were divided into dark colored-eye (DCE) and light-colored eye (LCE) groups according to iris color. The RNFL and macular thicknesses were analysed with standard optical coherence tomography (OCT) protocol while choroidal thickness was analysed with electronic data interchange (EDI) protocol in all subjects. Choroidal thickness was measured at the fovea, 1500 μm nasal and 1500 μm temporal to the fovea in a horizontal section.

• RESULTS: Of the 92 eyes included, 62 (67.4%) were dark-colored while 30 (32.6%) were light-colored. The mean age was 29.22±5.86y in the subjects with DCE and 28.86±6.50y in those with LCE. No significant difference was detected in mean age, axial length, macular thickness, choroidal thickness and intraocular pressure (IOP) between the groups ($P>0.05$). However, RNFL thicknesses varied depending on the quadrant measured, and were lower in both global and the nasal and temporal quadrants for individuals with LCE ($P\leqslant0.022$).

• CONCLUSION: No significant differences were found in IOP, macular thickness and choroid thickness between individuals with DCE and LCE. Meanwhile, the RNFL thickness is lower.

• KEYWORDS: retinal nerve fiber layer thickness; choroid thickness; eye color; iris color
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INTRODUCTION

Iris color, which determines eye color, is one of the factors defining the physical characteristics of the human. Genetic characteristics and ultraviolet light (UV) from the sun affects eye color in humans. Darker skin and iris color in individuals living in warm climates can be explained by the protective mechanism of melanin against the effect of

UV. This can be explained by melanin intensity[1]. Changes in iris color can also be related to systemic diseases such as Down syndrome, neurofibromatosis, albinism or ocular disorders such as herpetic infection, trauma and pigment dispersion syndrome. Many classifications have been used to categorize iris color[2-5]. However, in many genetic studies, eyes or eye photographs have only used the limited classification of blue, hazel/green or brown[6-7]. Previously, the relationship between iris color and several ocular disorders have been investigated, including cataract, glaucoma, age-related macular degeneration (AMD), uveal melanoma[8-9]. Moreover, there are studies advocating that uveal melanoma is more common in individuals with light colored eyes (LCE), proposing LCE as a prognostic factor[10-11].

Primarily, cataract development has been investigated, which is one of the most important causes of blindness. In some studies investigating the relationship between iris color and cataract, it was reported that some types of cataract were more common in dark brown eyes when compared to individuals with LCE[8,12-15]. There are many studies proposing that cataract formation is more frequent in brown eyes since they have greater melanin content compared to blue eyes[16]. Melanin intensity was discussed as an underlying mechanism. It was concluded that heat absorption will be as high as melanin intensity and the facilitating effect of heat will be greater on cataract development in the lens.

In the present study, we aimed to investigate the relationship of iris color with axial length, intraocular pressure (IOP), retinal nerve fiber layer (RNFL) thickness, macular thickness and choroidal thickness in healthy volunteers presented to our clinic.

SUBJECTS AND METHODS

Patient Selection This prospective, cross-sectional study included 92 eyes of 92 healthy volunteers presented to the Ophthalmology Clinic of Kayseri Training and Research Hospital. The study was approved by the Institutional Ethics Committee of Kayseri Training and Research Hospital. The study was conducted in accordance to the Helsinki Declaration. Written informed consent was obtained from all participants before entering the study.

Examination Protocol and Measurements In all cases, a detailed ophthalmological examination was performed by a single clinician, including best corrected visual acuity (BCVA), adjusted intraocular pressure measurement by applanation tonometry, biomicroscopy evaluation of anterior and posterior segments, and gonioscopy. All participants were white and had similar ethnic characteristics. Healthy volunteers were divided into two groups according to eye color: those with dark-colored eyes (brown and dark brown colored eyes) and those with light-colored (hazel/green and blue colored eyes). Other eye colors and heterochromic eyes were excluded. If both eyes met all the inclusion criteria, only one eye per patient was randomly selected. Detailed history was obtained regarding systemic disorders. Macular, choroidal and RNFL thickness, axial length and mean adjusted intraocular pressure were compared between the both groups.

Exclusion Criteria The subjects with any retinal, macular disorder or optic nerve disease, those with glaucoma, uveitis, marked cataract, those had undergone laser or ocular surgeries, those with systemic diseases such as hypertension or diabetes mellitus that may interference with choroidal thickness, those on systemic drug therapy, those with ocular pathologies such as higher degrees of myopia (more than -5.00 D) or hyperopia (more than +3.00 D) that may cause interference with axial thickness, smokers, and those with pseudoexfoliation syndrome were excluded.

The intraocular lens (IOL) Master (Carl Zeiss Meditec, Dublin, CA) was used for ocular biometry in order to measure axial length, repeating until five valid values were obtained. Axial length was measured from the corneal vertex to the retinal pigment epithelium (RPE). The results of axial length measurements were interpreted on the basis of the signal-to-noise ratio being above 2.0 and the appearance of graphs.

Optical Coherence Tomography (OCT) Imaging RNFL, macular and choroidal thicknesses were performed by using OCT (software version 5.6.4.0; Spectralis OCT Heidelberg Engineering, Dossenheim, Germany). Scans for all participants were performed with pupillary dilatation under the same intensity of dim room lighting by the same experienced technician. An internal fixation target was also used in all scans with a real-time eye tracking system to adjust for eye motion. The macular thickness (μm) was determined automatically and was analyzed by OCT software. The fast macular thickness map included a 25 line raster volume scan, 20×20 degrees centered on the fovea. Scans were obtained in high speed mode with the automated real time (ART) feature enabled and set at nine frames for raster scans was utilized for the macular measurements. The infrared scanning laser ophthalmoscope (IRSLO) scan angle was set at 30 degrees for all scans acquired. We selected the macular map analysis protocol on the Spectralis instrument to display numeric averages of the measurements for each of nine subfields as defined by the early treatment diabetic retinopathy study (ETDRS) circle grid. The diameters of the concentric circles were 1 mm, 3 mm, and 6 mm for macular scan.

The peripapillary RNFL thickness parameters were automatically calculated by using the fast RNFL mode. Scans were obtained in high speed mode with the ART feature enabled, set at 16 frames, and divided into sectors as temporal, temporal superior, temporal inferior, nasal, nasal inferior, nasal superior. This software provided a thickness profile across the temporal-superior-nasal-inferior temporal areas of the standard 12-degree circular scan. The software also calculated the average thickness values (μm) for the global and each of 6 sectors centered on the optic disc. The Spectralis instrument uses a signal-to-noise (SNR in dB) estimate for quality score (QS). After all exposures, the non-

Table 1 Comparison of axial length and IOP values between groups mean±SD

	DCE group (n=62)	LCE group (n=30)	P
Axial length (mm)	23.65±0.78	23.50±0.88	0.419
IOP (mmHg Appl.)	15.74±2.47	15.00±1.81	0.148

DCE: Dark colored eye; LCE: Light colored eye; IOP: Intraocular pressure.

Table 2 Comparison of macular thickness between groups mean±SD

Macular thickness (μm)	DCE group(n=62)	LCE group(n=30)	P
Central	262.12±18.12	267.76±14.53	0.144
Superior inner	351.50±15.43	349.63±10.63	0.552
Temporal inner	334.50±16.54	335.36±12.98	0.802
Inferior	347.24±16.47	345.73±11.46	0.653
Nasal inner	348.51±18.37	348.60±12.45	0.982
Superior outer	304.88±12.47	304.33±7.40	0.823
Temporal outer	290.96±13.10	291.23±9.51	0.921
Inferior outer	296.70±13.23	295.06±8.93	0.540
Nasal outer	326.04±17.38	321.16±11.87	0.169

DCE: Dark colored eye; LCE: Light colored eye.

Table 3 Comparison of RNFL thickness measurements between groups mean±SD

RNFL thickness (μm)	DCE group (n=62)	LCE group (n=30)	P
Global	102.90±9.03	98.33±7.58	0.019
Temporal	78.35±11.96	68.30±13.55	0.000
Temporal superior	115.50±18.93	116.93±22.56	0.734
Temporal inferior	115.04±20.62	115.53±22.16	0.918
Nasal	75.17±12.13	69.33±9.18	0.022
Nasal superior	139.58±18.69	133.30±16.31	0.119
Nasal inferior	145.90±19.38	143.70±10.00	0.560

RNFL: Retinal nerve fiber layer; DCE: Dark colored eye; LCE: Light colored eye.

centered scans and scans with signal strength <20 dB were excluded from the study.

Choroidal thickness was measured by enhanced depth imaging (EDI) using the spectral-domain OCT. Each section, consisting of 30 average scans, was obtained in a 15×30-degree rectangle centered at the macula. Choroidal thickness was determined as the distance from the outer surface of the hyper-reflective line, referred to as the "RPE" layer, to the hyper-reflective line of the inner sclera border. The scan was measured at the fovea, 1500 μm nasal and 1500 μm temporal to the fovea in a horizontal section. The axial resolution is 3.9 mm digital, which is the same for routine OCT images obtained by Spectralis OCT. Measurements were evaluated by 2 independent ophthalmologists (Gülhan A, Yüce Y), and the mean value was used for analysis. The measurements were performed at the same time of day to avoid diurnal fluctuations.

Statistical Analysis Statistical analysis was performed by using the SPSS software package version 20.0 (IBM Corporation, Armonk, NY). Quantitative data were expressed as mean and standard deviation. Independent-samples t-test was used to compare the groups for mean axial length, intraocular pressure, choroidal, macular and RNFL thicknesses. In all analyses, P values <0.05 was considered statistically significant.

RESULTS

The study included 92 eyes of 92 healthy volunteers. Of these, 32 (34.8%) were men while 60 (64.2%) were women. Of the 92 eyes included, 62 (67.4%) were dark-colored while 30 (32.6%) were light-colored. The mean age was 29.22±5.86y in the subjects with DCE and 28.86±6.50 years in those with LCE. No significant difference was detected in mean age between the groups (P>0.05). No significant difference was detected in axial length and IOP between groups (P=0.419 and P=0.148, respectively) (Table 1).

No significant difference was detected in macular thickness when measurements obtained in nine segments were compared between groups (P>0.05) (Table 2).

RNFL thickness was measured in global and 6 segments. RNFL thicknesses in global, temporal and nasal quadrants were found to be significantly higher in the DCE group when compared to the LCE group (P=0.019, P=0.000 and P=0.022, respectively). No significant difference was detected in RNFL thickness measured in the temporal superior, temporal inferior, nasal superior and nasal inferior quadrants between groups (P=0.734, P=0.918, P=0.119 and P=0.560, respectively) (Table 3).

No significant differences were detected in central, nasal and temporal choroidal thicknesses between groups (P=0.749, P=0.621 and P=0.245, respectively) (Table 4).

Table 4 Comparison of choroidal thickness between groups mean±SD

Choroidal thickness (μm)	DCE group (n=62)	LCE group (n=30)	P
Central	322.12±86.35	328.33±88.30	0.749
Nasal	386.62±86.73	396.13±84.48	0.621
Temporal	340.93±81.58	361.66±75.24	0.245

DCE: Dark colored eye; LCE: Light colored eye.

DISCUSSION

Skin and iris color can express variations based on characteristics of ethnic origin. Individuals with dark-colored skin and hair also have darker iris color. In the literature, there are many studies investigating effects of melanin on the eye by considering iris color as a parameter. The studies focusing on the effects of iris color on age relateal macular degeneraion (AMD) development evaluated the hypothesis proposing that ocular melanin absorbs light and prevents progression to AMD by scavenging free radicals from retinal cells. Some authors advocate that iris color has no effect on AMD development[17-25] while others advocate that AMD development is more common among individuals with light-colored eyes[20,25-28]. In a study by Tomany *et al*[29], it was found that the RPE depigmentation rate was significantly lower in cases younger than 65y, whereas there was no significant difference in those older than 65y. This result was interpreted as the protective effect of melanin decreasing with advancing age.

Menon *et al*[16] measured melanin intensity in brown and blue eyes and found that there was no significant difference in melanin intensity of iris between them, but melanin intensity was greater in ciliary body, retinal pigment epithelium(RPE) and choroid in brown eyes than in those with blue eyes. Together with studies reporting lower AMD frequency in individuals with DCE than in those with LCE, this result supports the hypothesis that melanin intensity in individuals with DCE may have a protective effect against AMD development by exerting a protective effect on retinal cells.

To best of our knowledge, there is no study evaluating relationship between macular thickness and iris color in the literature. In our study, no significant relationship was detected between iris color and macular thickness obtained from in all quadrants. Given the younger mean age in our study population, this result suggests that environmental factors may play an important role in the development of AMD in individuals with light-colored eyes with time.

In the literature, there are a limited number of studies focusing on iris color and glaucoma. In a study on 3,654 cases aged 49-97y, Mitchell *et al*[30] found a relationship between iris color and IOP, indicating higher IOP in DCE with greater melanin content.

In a study comparing iris color with central corneal thickness (CCT) and IOP, Semes *et al*[31] found lower CCT and higher mean CCT and adjusted IOP in individuals with DCE when compared to those with LCE.

In a study on 1,973 eyes of 1,012 patients by Jonas *et al*[32], no significant differences were found in optic nerve diameter, neuroretinal rim alpha and beta zone peripapillary atrophy diameters between individuals with DCE and LCE. Similar results were found in patients with ocular hypertension, or open-angle and closed-angle glaucoma. Authors found the highest neuroretinal rim thickness in the brown-eye group in those with normal-tension glaucoma. As a result, the authors found no significant difference regarding glaucoma development and progression.

In our study, no significant difference was detected in mean IOP between groups. However, the finding that there is lower RNFL thickness in the temporal and nasal quadrants in individuals with light-colored eyes suggests that LCE may be more vulnerable to glaucomatous damage because of lower RNFL thickness compared to DCE. There is a need for long-term studies with greater study sample numbers, including patients with glaucoma, for further understanding of this topic.

In the literature, there are studies investigating the relationship between axial length and age, retinal vein occlusion or refractive defects such as a higher degree of myopia[33-35]. We also compared groups regarding axial length with the idea that the differences of melanin intensity between individuals with DCE and LCE can interfere with axial length. No significant relationship was detected between iris color and axial length. The choroid consists of vascular and connective tissues and it is supplied by branches of the ophthalmic artery. It supplies the outer layer of the retinal. Moreover, the choroid is involved in maintaining heat balance and removal of debris in the ocular tissues. To the best of our knowledge, there has been no study investigating choroid thickness and iris color and consequently we believe that our study may be unique in this topic. We investigated the vascular difference that may result from any difference in intensity in melanin granules within the RPE between DCE and LCE. We failed to find significant difference between the both groups.

In conclusion, no significant differences were found in intraocular pressure, macular thickness and choroid thickness between individuals with DCE and LCE. Interestingly, we found that the RNFL thickness is lower in the nasal and temporal quadrants for LCE, and the differences in mean RNFL thickness between people with LCE and those with DCE that is difficult to interpret. The clinical significance of this finding is unclear.

Larger, more diverse studies with longer follow-up times are needed to gain further understanding in terms of glaucomatous risk.

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