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ORIGINAL ARTICLE

Ganglion cell complex analysis in thalassemia major patients measured by optical coherence tomography

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Abstract

To analyze the changes in ganglion cell complex (GCC), peripapillary retinal nerve fiber layer (RNFL) thickness and central macular thickness (CMT) on spectral domain optical coherence tomography (OCT) in patients with thalassemia major. Forty one eyes of 41 patients with thalassemia major and 41 eyes of 41 healthy subjects were included in this prospective and comparative study. Peripapillary RNFL thickness, CMT and macular GCC thickness were evaluated with OCT (Cirrus HD-OCT 5000 Carl Zeiss Meditec, Inc, Dublin, CA, USA) in all patients and healthy controls. Additionally, disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight were analyzed in thalassemia major group. RNFL thickness values were lower in the thalassemia patients but the difference was not statistically significant (except superior quadrant) and there was no significant differences in the mean CMT measurements. GCC thickness was thinner in all areas (average, superior, inferior, superior-temporal, inferior-temporal, superior-nasal, inferior-nasal) but only the thinning in the inferior-temporal was statistically significant. GCC and RNFL thickness changes occur earlier than CMT changes in β-thalassemia major patients. GCC thickness measurements can be used for follow-up in combination with other diagnostic methods.

Keywords: *Iron, thalassemia major, optical coherence tomography, ganglion cell complex, retinal nerve fiber layer*

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Introduction

Beta thalassemia (β- thalassemia) is one of the most common hereditary blood disorder characterized by genetic mutation and resulting in defective β-globin chain synthesis. It leads to hypochromic microctic anemia with erythrocyte displasia and destruction [1,2]. β- thalassemia major patients have severely diminished β- globin synthesis and requires regular red blood cell transfusions lifelong to survive. Regular transfusion regimen results with iron accumulation which leads to multiple organ failure. Iron chelator drugs are applied to diminish iron accumulation and prevent it's toxic effects [3,4]. Deferoxamine is the oldest iron chelator drug administered through subcutaneous injection. In recent years two forms of oral chelators (Deferasirox and Deferiprone) were introduced [5,6].

Thalassemia and it's management drugs can lead to various ocular problems such as retinal pigment epithelium degeneration, angioid streaks, venous tortuosity, visual field defects, deterioration of color vision, decreased visual acuity and optic neuropathy [7,8]. Iron deposition in ocular tissues and iron chelator treatments may play role in the pathogenesis of ocular involvement. Patients with β-thalassemia are mainly exposed to oxidative stress due to iron overload and antioxidants play an essential role in protection of the cells from oxidative damage [9].

Optical coherence tomography (OCT) can perform non-invasive in vivo evaluations have come into clinical use to evaluate the thickness of the peripapillary retinal nerve fiber layer (RNFL), ganglion cell complex (GCC), and choroid layer in various disorders [10]. High-resolution spectral domain-OCT techniques and computerized algorithms for image analysis have further improved the segmentation and measurement of specific retinal layers such as the GCC which is defined as the three innermost retinal layers: the nerve fiber layer, the ganglion cell layer, and the inner plexiform layer.

This study was conducted to assess the changes of macular GCC, peripapillary RNFL thickness and central macular thickness (CMT) in multi-transfused β-thalassemia major patients and compare with healty controls. Additionally, their relationship with disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight were aimed to be studied.

Materials and Methods

Study population and design

This cross-sectional, comparative study was conducted between June 2020 and September 2020. The study was organized in accordance with the ethical standards and informed consent was obtained patients and their guardians.

We recruited a sample of 41 patients with β-thalassemia major and 41 randomly selected age-sex matched healty controls, aged between 13 and 48. Right eye of each patient was used for analysis. We excluded from the study patients with previous ocular trauma and ocular surgery, refractive error more than ±3 diopters, uveitis, glaucoma, retina and optic nerve diseases, any systemic diseases other than thalassemia and it's complications. Diabetes mellitus cases secondary to thalassemia were excluded from the study.

The diagnosis of β-thalassemia major was based on complete blood count, peripheral blood evaluation, hemoglobin electrophoresis and genetic mutation analysis of the patients. Serum hemoglobin concentration, ferritin level, the dosage and duration of chelation therapy, number of transfusion per year and patient's weight were recorded in β-thalassemia major patients.

All participants underwent a complete ophthalmologic examination including best corrected visual acuity (BCVA) using the decimal system, intraocular pressure (IOP) by air-puff tonometry, slit lamp biomicroscopy and dilated fundus examination, OCT measurements (peripapillary RNFL thickness, CMT and macular GCC thickness). Additionally disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight were recorded in β-thalassemia major patients.

We obtained images after dilatation of pupil with Cirrus HD-OCT 5000 (Carl Zeiss Meditec, Inc, Dublin, CA, USA) by the same examiner. The Optic Disc Cube 200 × 200 protocol was used and average RNFL thickness and RNFL value by quadrants (superior, inferior, temporal and nasal) on a measurement circle 3.46 mm in diameter were calculated. The Macular Cube 512 × 128 protocol was used for the CMT and GCC thickness measurements. The Cirrus HD-OCT ganglion cell analysis algorithm was used to process the data. Images with movement artifacts, signal strength below 7/10, segmentation errors or poor centralization were rejected to ensure accurate results.

Statistical Analysis

Statistical analysis performed with IBM SPSS Statistics software ver. 25 (IBM Corp., Armonk, NY). Chi-square test was used to compare the categorical variables. Normality assumption of numerical variables was assessed with Shapiro Wilk test. Mann Whitney U test was used to compare non-normal distributed variables for two groups. Independent samples t test was used to compare normally distributed variables for two groups. Spearman Correlation Analaysis was used for relations between numerical variables. A p-value <0.05 was considered statistically significant.

Results

We evaluated a total of 41 patients with β-thalassemia major and 41 sex and age matched healty controls during the study period. Of the 41 patients, 22 (%53.7) were female and 19 (%46.3) were male in both groups. The mean age for the β-thalassemia major and control group were 24.34±8.58 years [range 13-48].

The values and comparisons of RNFL thickness in average and all four quadrants (superior, inferior temporal, nasal), CMT and GCC thickness (average, superior, inferior, superior-temporal, inferio-temporal, superior-nasal and inferio-nasal quadrants) are summarized in Table 1.

Although the average and all four quadrants RNFL thickness values were lower in the thalassemia group, the difference was not statistically significant, except superior quadrant (p=0.024 in superior quadrant). There was no significant differences in the mean CMT measurements. The GCC thickness was thinner in patients with β-thalassemia major in all areas (average, superior, inferior, superior-temporal, inferior-temporal, superior-nasal, inferior-nasal) but only the thinning in the inferior-temporal was statistically significant (p=0.025).

In β-thalassemia major group, the mean disease duration was 23.5± 8.6 years. The mean serum ferritin level and hemoglobin concentration were 1522.3±1777,8 ng/ml and 9.3±0.7 g/dL, respectively. Deferasirox was used in all thalassemia patients as chelation therapy. The mean dose and duration of chelation therapy were 1519,2± 529.9 mg and 21.3±9 years, respectively. The annual number of transfusions was 34.3±6 per year. Mean weight of thalassemia patients was 52.8± 11.6 kg.

The correlation between disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight and OCT parameters are shown in Table 2 and 3. In correlation analyses, there was no statistically significant influence of disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight for any RNFL thickness and CMT measurement in the β-thalassemia major group.

Although disease duration was negatively correlated with GCC thickness in all areas, it was statistically significant only in the average GCC thickness (p=0.045). There was a positive correlation between GCC thickness and the serum ferritin level (p=0.037 for inferior, p=0.025 for superior-nasal) and a negative correlation between GCC thickness and hemoglobin level (p=0.047 for superior, p=0.041 for inferior,

Table 1. The value and statistical comparison of OCT measurements.

	Thalassemia group	Control group	p
RNFL thickness (µm)			
Average	99.7 ± 7.8	100.9 ± 18.7	0.134
Superior	120.1 ± 13.9	128.2 ± 18.3	0.024
Inferior	133.8 ± 16.6	136.7 ± 18.9	0.463
Temporal	66.7 ± 7.7	69.1 ± 9.5	0.223
Nasal	77.3 ± 11.6	80.3 ± 17.2	0.357
CMT (µm)	244.2 ±18.2	244.6 ± 19	0.963
GCC thickness (µm)			
Average	83.1 ± 5.7	85.3 ± 4.6	0.055
Superior	84.1 ± 6.1	85.9 ± 5.5	0.170
Inferior	82.2 ± 6.4	83.9 ± 5.3	0.192
Superior-temporal	81.5 ± 5.8	83.1 ± 4.9	0.163
Inferior-temporal	82.2 ± 6.3	85.2 ± 5.6	0.025
Superior-nasal	84.7 ± 6	86.7 ± 5.3	0.061
Inferior-nasal	83.9 ± 5.9	85.8 ± 4.8	0.103

CMT: central macular thickness, GCC: ganglion cell complex, RNFL: retinal nerve fiber layer

Table 2. Correlation between disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight and OCT parameters.

		Disease duration	Ferritin	Hemoglobin	Chelation duration	Chelation dose	Transfusion count/ year	Patient's weight
RNFL thickness								
Average	r	-0.086	-0.074	-0.167	-0.188	-0.144	0.034	0.070
	p	0.591	0.646	0.297	0.238	0.383	0.831	0.664
Superior	r	-0.046	0.041	-0.145	-0.138	-0.056	0.011	0.111
	p	0.774	0.799	0.367	0.390	0.737	0.946	0.491
Inferior	r	-0.085	0.105	-0.258	-0.223	-0.053	0.129	0.205
	p	0.599	0.513	0.104	0.161	0.747	0.421	0.198
Temporal	r	-0.101	0.237	0.075	-0.112	0.116	-0.121	0.058
	p	0.532	0.136	0.642	0.488	0.484	0.450	0.719
Nasal	r	-0.149	-0.170	-0.223	-0.164	-0.284	0.019	-0.035
	p	0.352	0.287	0.161	0.304	0.080	0.906	0.827
CMT	r	-0.112	0.036	0.022	-0.146	-0.026	-0.241	-0.016
	p	0.487	0.823	0.889	0.364	0.874	0.128	0.919

CMT: central macular thickness, RNFL: retinal nerve fiber layer

Table 3. Correlation between disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight and GCC thickness.

		Disease duration	Ferritin	Hemoglobin	Chelation duration	Chelation dose	Transfusion count/ year	Patient's weight
Average	r	-0.314	0.293	-0.291	-0.287	0.052	0.071	-0.077
	p	0.045	0.063	0.065	0.069	0.752	0.658	0.634
Superior	r	-0.284	0.304	-0.312	-0.256	0.058	0.094	-0.034
	p	0.072	0.053	0.047	0.107	0.724	0.560	0.835
Inferior	r	-0.278	0.328	-0.321	-0.265	0.102	0.037	-0.060
	p	0.078	0.037	0.041	0.094	0.535	0.819	0.708
Superior-temporal	r	-0.223	0.144	-0.374	-0.167	0.076	0.074	-0.060
	p	0.161	0.371	0.016	0.298	0.647	0.644	0.708
Inferior-temporal	r	-0.233	0.232	-0.306	-0.184	0.040	0.044	-0.005
	p	0.143	0.144	0.052	0.249	0.808	0.785	0.974
Superior-nasal	r	-0.253	0.349	-0.233	-0.225	0.101	0.157	-0.046
	p	0.110	0.025	0.143	0.157	0.540	0.327	0.775
Inferior-nasal	r	-0.316	0.287	-0.263	-0.328	0.013	0.101	-0.126
	p	0.044	0.068	0.097	0.036	0.939	0.531	0.431

p=0.016 for superior-temporal). No significant correlation was found between GCC thickness and chelation therapy duration, except inferior-nasal area (p=0.036). Chelation therapy dose, transfusion count and patient's weight were not correlated with GCC thickness.

Discussion

Although the mechanism is not fully understood, β-thalassemia major patients represent various ocular manifestations. In previous studies, pathological findings including retinal pigment epithelium degeneration, angioid streaks, venous tortuosity, visual field defects, deterioration of color vision, decreased visual acuity, optic neuropathy, thicker lens and cataract have been reported in β-thalassemia major patients [4,7,8,11].

Iron is essential for many metabolic processes but excess iron can be toxic to tissues. In retina, iron is particularly critical for the visual phototransduction cascade for isomerohydrolase activity and catalyzing the conversion of hydrogen peroxide to hydroxyl radical which is the most damaging of the reactive oxygen species. Excessive generation of free

radicals can cause oxidative damage to biological macromolecules such as DNA, lipids, carbohydrates and proteins. Increased intraocular iron has been shown to cause oxidative injury to the retina [12-14]. Manafikhi et al. reported that thalassemic patients had lower total antioxidant capacity compared to healthy subjects and there was no relationship to disease severity [14].

Iron overload is unavoidable in thalassemia patients due to life-long transfusions. Iron- chelating drugs are used to prevent transfusion related complications. Deferoxamine has well-documented ocular side effects including retinal changes ranges from peripheral pigmentary changes to bulls-eye maculopathy [15,16]. Deferasirox is a new iron chelator drug that have been introduced as an orally effective alternative for deferoxamine. There are studies showing that it may be toxic to the eye although it's side effects are less compared to Deferoxamine. In a recent study, ERG and mfERG responses were reduced in thalassemic patients regardless of the type of chelation therapy they received [17]. On the contrary Sakamoto et al. suggested that iron-chelating agents was protected retinal neurons against excitoneurotoxicity via reduction of iron content and oxidative stress in the rats [18]. The toxic effects of iron chelation therapy on the retina and its mechanism were not fully understood.

We speculate that iron accumulation in retinal tissues, increased oxidative stress and decreased antioxidant capacity may lead damage to retinal structures and accelerate ganglion cell injury in β-thalassemia major patients.

In the current study, we found that RNFL thickness values were lower in the thalassemia patients but the difference was not statistically significant (except superior quadrant) and there was no significant differences in the mean CMT measurements. As in our study, Acer et al. found retinal nerve fiber layer thickness and subfoveal choroidal thickness was not statistically significant different in children with thalassemia minör [19]. Aksoy et al. reported that peripapillary RNFL was thinner in tha-major in all quadrants and thinning of the RNLF was correlated with hemoglobin value and ferritin level, but not with number of transfusions and visual acuity [20]. In β -thalassemia major patients Uzun et al. also observed that RNFL was thinner in all quadrants than control subjects and thinning was not correlate with hemoglobin or ferritin levels [21]. According to our study neither average and four quadrants RNFL thickness nor CMT were correlated with the disease duration, serum ferritin level, hemoglobin concentration, the dosage and duration of chelation therapy, count of transfusion, patient's weight.

Although the RNFL thickness and CMT has been investigated widely, GCC thickness changes in β-thalassemia major patients have been evaluated less. In our study we found the GCC thickness was thinner in patients with β-thalassemia major in all areas (average, superior, inferior, superior-temporal, inferior-temporal, superior-nasal, inferior-nasal) but only the thinning in the inferior-temporal was statistically significant. In correlation analyses, disease duration was negatively correlated with GCC thickness in all areas, it was statistically significant only in the average GCC thickness. Also hemoglobin level was negatively correlated with GCC thickness in superior, inferior, and superior-temporal area. There was a positive correlation between GCC thickness and the serum ferritin level in inferior and superior-nasal areas. No significant correlation was found between GCC thickness and chelation therapy duration (except inferior-nasal area), Chelation therapy dose, transfusion count and patient's weight. In β -thalassemia major patients Ulusoy et al. observed that peripapillary RNFL thickness in all quadrants, CMT and macular average GCC were thinner but there were no statistically difference. They also reported that RNFL, CMT, and GCC thicknesses were not correlated with hemoglobin, hematocrite, ferritin, and other demographic characteristics [22]. Our study showed that GCC thickness changes occur earlier than RNFL and CMT changes in β-thalassemia major patients. GCC thickness can be used for earlier diagnosis and follow-up in combination with other diagnostic methods. The limitations of this study was the relatively small number of cases.

Conclusion

Since life expectancy in thalassemia major patients has improved over time, the risk of ocular abnormalities due to diease and drugs used in treatment increases. Therefore, regular long-term follow-up of these patients has become more important to prevent further sight-threatening ocular complications. GCC thickness can be used for earlier diagnosis and follow-up along with other imaging methods.

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Conflict of interest
The authors have no conflicts of interest declared.

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