

Original Article

# Retinal nerve fiber layer thickness in children with $\beta$ -thalassemia major



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## Abstract

**Purpose:** To measure the retinal nerve fiber layer thickness (RNFLT) in children with  $\beta$ -thalassemia major and to compare with healthy controls.

**Methods:** A total of 47 patients with  $\beta$ -thalassemia major and 51 healthy controls were included. Each subject underwent a standard ophthalmological examination. RNFLT measurements were performed using optical coherence tomography.

**Results:** Mean age of the patient group and healthy controls were  $13.7 \pm 2.1$  and  $14.3 \pm 2.2$  years, respectively. Mean peripapillary RNFL thickness was  $94 \mu\text{m}$  in the patient group, and  $100 \mu\text{m}$  in the control group ( $p < 0.01$ ). In patients with  $\beta$ -thalassemia major, RNFL was thinner in all quadrants than control subjects. Within the  $\beta$ -thalassemia major group neither average RNFLT nor each four quadrant RNFLT were correlated with the age, serum ferritin or serum hemoglobin levels ( $p > 0.05$ ).

**Conclusion:** In this study, we observed RNFL was thinner in patients with  $\beta$ -thalassemia major. Thinning of RNFL did not correlate with hemoglobin or ferritin levels.

**Keywords:**  $\beta$ -thalassemia major, Retinal nerve fiber layer thickness, Optical coherence tomography

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## Introduction

$\beta$ -thalassemia major, a disease of defective globin  $\beta$ -chain synthesis, is characterized by anemia caused by a combination of hemolysis and ineffective erythropoiesis, and iron overload.<sup>1</sup> The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world.<sup>2</sup> Anemia, growth retardation, pathological fracture of long bones, leg ulcers, hepatosplenomegaly, and congestive heart failure are main clinical characteristics of these patients. Ocular manifestations included cataract, optic neuropathy, retinal pig-

ment epithelium (RPE) degeneration, RPE mottling, retinal venous tortuosity, vitreoretinal hemorrhages and obliteration of iris pattern. These ocular changes may occur as a result of the disease process or as a side effect of iron chelators.<sup>3–5</sup>

The patients with  $\beta$ -thalassemia major suffer from iron overload as a consequence of recurrent transfusions and ineffective erythropoiesis.<sup>6</sup> Although iron is crucial for the synthesis of neurotransmitters, optic nerve myelination and visual phototransduction cascade, excessive iron may be toxic.<sup>7</sup> Iron has a catalytic role to produce reactive oxygen species and free radicals which may lead to oxidative damage.<sup>6,8</sup>

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Oxidative damage caused by free radicals include DNA strand breakage, lipid peroxidation and biomolecule degradation. Free radicals and iron accumulation have been implicated in the pathogenesis of some other neurological disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.<sup>9–11</sup> Although the impact of excessive iron on optic nerve is not clear, it has been known that the retina and RPE are particularly prone to oxidative damage caused by iron overload.<sup>12</sup>

Retinal nerve fiber layer thickness (RNFLT) measurement provides important clinical data for understanding the disorders of optic nerve and with advances in technology even to obtain high quality sections of retina and retinal nerve fiber layer has been possible.

In the present study, we aimed to investigate the RNFLT in  $\beta$ -thalassemia major patients with normal ocular findings.

## Subjects and methods

Forty-seven patients with  $\beta$ -thalassemia major and fifty-one healthy controls were included in the study. All subjects and parents were informed about the goals of the study and informed consent was obtained. This study followed the tenets of the Declaration of Helsinki and the protocol was approved by the local Ethics Committee.

$\beta$ -thalassemia major diagnosis was confirmed with hematological and electrophoretic studies. Hemoglobin (gr/dl) and ferritin (ng/ml) levels and the data regarding the type and duration of chelating therapy were obtained. These children were receiving regular blood transfusions approximately once a month, and deferasirox and deferepiron were used as chelating agents.

Each subject underwent a standard ophthalmological examination including best-corrected visual acuity (BCVA), slit lamp examination, Goldmann applanation tonometer, and funduscopy.

Exclusion criteria included any corneal or retinal abnormalities, optic disc disorders and cup/disc ratio abnormalities, any history of ocular surgery or ocular trauma, intraocular pressure higher than 21 mmHg in either eye, any neurological disease, history of prematurity, myopia or hyperopia >6.0 diopters.

The peripapillary RNFLT measurements were performed with a Cirrus HD spectral domain OCT (Carl Zeiss Meditec, Dublin, CA) by the same masked technician. The peripapillary RNFLT of the temporal, nasal, inferior and superior quadrants and the average thickness of the RNFLT were obtained with the optic disc 200 × 200 cube scan protocol along a circle with a diameter of 3.45 mm around the center of the disc. Signal strengths >7 were included in the study.

## Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Kolmogorov Smirnov test was used to determine whether the distributions of metric discrete and continuous variables were normal. Metric discrete and continuous variables were shown as mean  $\pm$  standard deviation (SD) or median (IQR), where applicable.

While the mean difference between groups was compared by using Student's *t* test, otherwise, Mann-Whitney *U* test

was applied for comparisons of the median values. Nominal data were analyzed by Pearson's Chi-Square test. The relationship between clinical measurements was evaluated by Spearman's correlation test.

A *p* value less than 0.05 was considered statistically significant.

## Results

A total of 47 children were eligible to participate in the study. In the  $\beta$ -thalassemia major group, 26 were female and 21 were male, and the mean age was  $13.7 \pm 2.1$  years (range 12–17) years. In the control group 31 (60.8%) of 51 patients were female and 20 (39.2%) were male. Mean age was  $14.3 \pm 2.2$  (range 13–16). Mean (range) intraocular pressure (IOP) of the control group and  $\beta$ -thalassemia major group were 12.8 (10–18) and 13.2 (9–19) mmHg, respectively. There was no significant difference between the two groups in terms of age, gender, IOP and refractive status (*p* > 0.05).

Serum ferritin level [median (IQR)] of the patients with  $\beta$ -thalassemia major were 1800 (1450) (ng/ml) and mean  $\pm$  SD hemoglobin levels were  $8.7 \pm 0.45$  (gr/dl). All patients with  $\beta$ -thalassemia major were under deferasirox or deferepiron therapy as a chelating agent.

In patients with  $\beta$ -thalassemia major right and left average, right nasal, right inferior, right and left temporal RNFLT were significantly thinner in comparison to control subjects (*p* < 0.05) (Table 1). Right and left superior, left nasal and left inferior quadrants RNFLT were statistically similar between the groups (*p* > 0.05).

Within the  $\beta$ -thalassemia major group neither average RNFLT nor each individual four quadrant RNFLT were correlated with the age, serum ferritin or serum hemoglobin levels (*p* > 0.05) (Table 2).

## Discussion

In the current study, patients with  $\beta$ -thalassemia major showed thinner average RNFLT and RNFL in nasal, inferior and temporal quadrants were also significantly thinner in the patient group than control subjects. Patients with thalassaemia may present with various ocular manifestations

**Table 1.** The comparison of the RNFLT between thalassaemia major group and control group.

|  | Thalassaemia Group (n:47) | Control Group (n:51) | <i>P</i> value   |
|--|---------------------------|----------------------|------------------|
| <i>Average RNFLT (<math>\mu</math>m)</i> |                           |                      |                  |
| Right                                    | 94 (10)                   | 100 (17)             | <b>0.003</b>     |
| Left                                     | 91 (11)                   | 98 (14)              | <b>0.005</b>     |
| <i>Superior</i>                          |                           |                      |                  |
| Right                                    | 120 (16)                  | 124 (26)             | 0.063            |
| Left                                     | 122 (14)                  | 127 (19)             | 0.492            |
| <i>Nasal</i>                             |                           |                      |                  |
| Right                                    | 66 (15)                   | 79 (24)              | <b>&lt;0.001</b> |
| Left                                     | 63 (18)                   | 73 (20)              | 0.098            |
| <i>Inferior</i>                          |                           |                      |                  |
| Right                                    | 119 (26)                  | 129 (25)             | <b>&lt;0.001</b> |
| Left                                     | 121 (19)                  | 128 (19)             | 0.199            |
| <i>Temporal</i>                          |                           |                      |                  |
| Right                                    | 73 (9)                    | 79 (13)              | <b>&lt;0.001</b> |
| Left                                     | 70 (9)                    | 76 (10)              | <b>0.018</b>     |

\* Values represent median (IQR), bold values mean statistically significant.

**Table 2.** The correlations of the RNFLT (right and left eyes) with age, hemoglobin and ferritin levels in the  $\beta$ -thalassemia major group.

|                      | Age     |         | Serum Hemoglobin |         | Serum Ferritin |         |
|----------------------|---------|---------|------------------|---------|----------------|---------|
|                      | r value | p value | r value          | p value | r value        | p value |
| <i>Average RNFLT</i> |         |         |                  |         |                |         |
| Right                | 0.055   | 0.701   | -0.103           | 0.470   | 0.016          | 0.911   |
| Left                 | 0.036   | 0.803   | -0.135           | 0.344   | -0.056         | 0.699   |
| <i>Superior</i>      |         |         |                  |         |                |         |
| Right                | -0.072  | 0.617   | 0.028            | 0.845   | -0.027         | 0.851   |
| Left                 | 0.127   | 0.376   | 0.059            | 0.679   | -0.146         | 0.306   |
| <i>Nasal</i>         |         |         |                  |         |                |         |
| Right                | 0.187   | 0.190   | -0.073           | 0.611   | 0.030          | 0.832   |
| Left                 | 0.105   | 0.464   | -0.067           | 0.642   | 0.081          | 0.574   |
| <i>Inferior</i>      |         |         |                  |         |                |         |
| Right                | 0.163   | 0.253   | -0.048           | 0.738   | -0.117         | 0.415   |
| Left                 | 0.143   | 0.315   | -0.171           | 0.231   | -0.125         | 0.384   |
| <i>Temporal</i>      |         |         |                  |         |                |         |
| Right                | -0.100  | 0.487   | -0.238           | 0.092   | 0.100          | 0.485   |
| Left                 | -0.184  | 0.195   | -0.202           | 0.156   | 0.004          | 0.978   |

including color vision anomalies, night blindness, cataract, visual field defects, decreased visual acuity and optic neuropathy.<sup>3</sup> Although the impact of thalassemia major on the optic nerve is not clear, the possible combined effect of iron overload, iron-mediated oxidative stress and the use of chronic chelator therapy may contribute to the pathogenesis of ocular involvement.

Iron is essential for many cellular reactions in neural tissue however excess iron can be extremely damaging due to the release of reactive oxygen species.<sup>12</sup> An experimental study indicated that neurodegeneration appears in transgenic mice because of the break down of blood-brain barrier due to iron-mediated free radical generation which accelerates iron deposition into tissues.<sup>13</sup> Iron-mediated oxidative stress and cytosolic iron levels play a major role in neurodegeneration and neuron survival in CNS.<sup>14</sup> In several neurodegenerative diseases such as MS, oxidative stress plays a major role<sup>15</sup> and iron toxicity has been suggested to participate in the neurodegenerative process.<sup>16</sup>

The role of iron in the pathogenesis of the eye diseases has been widely studied.<sup>17</sup> Iron exists in very low levels in both aqueous humor and vitreous<sup>18</sup> unless inflammation breaks down the barriers inducing iron deposition in the tissues.<sup>19</sup> There is strong evidence relating to oxidative damage mediated by iron-related proteins that contribute to the pathogenesis of some optic nerve diseases such as optic neuritis, ischemic optic neuropathy and glaucoma.<sup>20-22</sup> Although the mechanism is not fully understood, it has been suggested that reactive oxygen species play a critical role in the signaling pathway of ganglion cell death through apoptosis. Although no direct link between optic neuropathies and excessive iron has been shown, the ability for iron to form highly toxic reactive oxygen species may play a role in optic neuropathy.<sup>23</sup> Thalassemia is a disease of iron overload in several tissues and iron accumulation has been shown to cause hepatic, endocrine or cardiac complications.<sup>24</sup> The impact of iron overload on the optic nerve ultrastructure and function is not clear. In patients with  $\beta$ -thalassemia major, we found the average and three quadrants (nasal, inferior and temporal) RNFLT thinner when compared to normal subjects. We may speculate that thinning of the RNFLT may be related with oxidative stress and cellular death through apoptosis due to iron accumulation in the optic nerve. Likewise in a

recent study, researchers found RNFLT thinner in children with thalassemia major than controls in all quadrants.<sup>25</sup> In the same study, patients with iron deficiency anemia presented RNFLT thinning in inferior quadrant. Türkyılmaz and associates reported average, superior and inferior quadrant RNFLT thinning in patients with iron deficiency anemia.<sup>26</sup> Recently Acer et al. demonstrated that there was no statistically significant difference in RNFLT thickness in children with thalassemia minor compared with healthy controls.<sup>27</sup> Arifoğlu et al. reported that mean values of subfoveal, nasal, temporal choroidal, and macular thickness for the four quadrants were significantly lower in patients with  $\beta$ -thalassemia minor than in healthy controls. Even though, no statistically significant differences for retinal nerve fiber layer thickness were noted between the two groups.<sup>28</sup> We hypothesized that iron is crucial for normal functioning of the neuronal cells but iron accumulation may accelerate ganglion cell death. Future experimental or clinical studies are needed to understand the impact of iron overload on the ultrastructure of the optic nerve.

Chelating agents are widely used in managing patients with chronic iron overload and all our patients were under treatment with chelators. Although iron chelators were shown as anti-neurodegenerative<sup>29</sup> and found neuroprotective in animal models of brain injury,<sup>30</sup> some ocular side effects such as cataracts, retrobulbar optic neuritis, pigmentary retinopathy and vitelliform maculopathy has been reported previously.<sup>31</sup> Although the role of the chelator agents on the thickness and the function of the optic nerve is not known, this issue needs to be further investigated.

The application of OCT in the field of ophthalmology provide non-invasive and rapid information about many ocular diseases. OCT is widely used for the diagnosis and follow-up of optic nerve diseases.<sup>32,33</sup> Although OCT devices have an integrated normative database for adults, no such data are available for children under the age of 18. Elia et al.<sup>33</sup> presented normative values for Cirrus OCT among caucasian healthy children, and found the temporal quadrant the thinnest ( $69.35 \pm 11.28$  mm), followed by the nasal ( $71.30 \pm 13.45$  mm), superior ( $123.65 \pm 19.49$  mm) and inferior ( $130.18 \pm 18.13$  mm) quadrants. In our study, the nasal quadrant of the healthy controls showed the thinnest RNFLT, followed by the temporal, inferior and superior quadrants. This difference

may be a result of variances in the age, race or gender distribution in between study groups.

As previously reported, RNFL measurements might change significantly with ethnicity differences,<sup>34</sup> axial length and refractive error in children.<sup>35</sup> Since both our control and patient groups were from the same geographical region, ethnic difference was not applicable to our results. There was no difference in the refractive status in between our patient group and healthy subjects. Indeed some studies showed that these measurements had minimal impact on the RNFL measurements<sup>36</sup> and this influence is lower for Caucasian children compared to other races.<sup>33,37</sup>

In transfusion dependent diseases like thalassemia major, iron accumulates in the body and possibly may lead to toxicity in ocular tissues. Aksoy and associates showed a negative correlation between RNFLT and serum ferritin levels.<sup>25</sup> Similarly, increased number of ocular involvement reported with increasing levels of serum ferritin and serum iron.<sup>3</sup> We found RNFLT was not correlated with age, serum hemoglobin and serum ferritin levels in the patients with thalassemia major. Similar to our study, Acer et al. showed no correlation between retinal nerve fiber layer thickness and hemoglobin values in patients with thalassemia minor.<sup>27</sup>

However this study has several limitations. Number of the patients underwent OCT measurements were relatively low due to poor cooperation in younger children. Additionally, prospective studies with a longer follow up time may give more informative data for this patient group.

## Conclusions

Thalassemia major may affect optic nerve ultrastructure and functional capability in terms of direct iron overload, iron-related oxidative stress and the adverse reactions of the chelator agents. We observed the RNFL thinner in patients with thalassemia major than in controls and this apparent difference may be the sum of the mentioned consequences. The impact of the iron overload and chelator therapy on the RNFLT should be investigated in future studies.

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## Conflict of interest statement

None of the authors has conflict of interest with this submission.

## References

- Olivieri O, De Franceschi L, Capellini MD, et al. Oxidative damage and erythrocyte membrane transport abnormalities in thalassemias. *Blood* 1994;**84**(1):315–20.
- Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Diseases* 2010;**5**:11.
- Taneja R, Malik P, Sharma M, et al. Multiple transfused thalassemia major: ocular manifestations in a hospital-based population. *Indian J Ophthalmol* 2010;**58**(2):125–30.
- Arden GB, Wonke B, Kennedy C, et al. Ocular changes in patients undergoing long-term desferrioxamine treatment. *The British J Ophthalmol* 1984;**68**(12):873–7.
- De Doncker R, Casteels I, Leys A, et al. Desferrioxamine and ocular toxicity, causal or concomitant relationship. *Bulletin de la Societe belge d'ophtalmologie* 1986;**220**:61–7.
- Ghone RA, Kumbar KM, Suryakar AN, et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. *Indian J Clin Biochem: IJCB* 2008;**23**(4):337–40.
- Song D, Dunaief JL. Retinal iron homeostasis in health and disease. *Front Aging Neurosci* 2013;**5**:24.
- Bazvand F, Shams S, Borji Esfahani M, et al. Total antioxidant status in patients with major beta-thalassemia. *Iran J Pediat* 2011;**21**(2):159–65.
- Honda K, Casadesus G, Petersen RB, et al. Oxidative stress and redox-active iron in Alzheimer's disease. *Ann New York Acad Sci* 2004;**1012**:179–82.
- Gotz ME, Double K, Gerlach M, et al. The relevance of iron in the pathogenesis of Parkinson's disease. *Ann New York Acad Sci* 2004;**1012**:193–208.
- Craelius W, Migdal MW, Luessenhop CP, et al. Iron deposits surrounding multiple sclerosis plaques. *Arch Pathol Lab Med* 1982;**106**(8):397–9.
- He X, Hahn P, Iacovelli J, et al. Iron homeostasis and toxicity in retinal degeneration. *Progr Retinal Eye Res* 2007;**26**(6):649–73.
- Castelnuovo PA, Garrett RS, Palinski W, et al. Abnormal iron deposition associated with lipid peroxidation in transgenic mice expressing interleukin-6 in the brain. *J Neuropathol Exp Neurol* 1998;**57**(3):268–82.
- Macco R, Pelizzoni I, Consonni A, et al. Astrocytes acquire resistance to iron-dependent oxidative stress upon proinflammatory activation. *J Neuroinflamm* 2013;**10**:130.
- Haider L, Fischer MT, Fischer JM, et al. Oxidative damage in multiple sclerosis lesions. *Brain: J Neurol* 2011;**134**(Pt 7):1914–24.
- Nunez MT, Urrutia P, Mena N, et al. Iron toxicity in neurodegeneration. *Biomaterials: Int J Role Metal Ions Biol, Biochem Med* 2012;**25**(4):761–76.
- Goralska M, Ferrell J, Harned J, et al. Iron metabolism in the eye: a review. *Exp Eye Res* 2009;**88**(2):204–15.
- McGahan MC, Fleisher LN. A micromethod for the determination of iron and total iron-binding capacity in intraocular fluids and plasma using electrothermal atomic absorption spectroscopy. *Anal Biochem* 1986;**156**(2):397–402.
- McGahan MC, Fleisher LN. Inflammation-induced changes in the iron concentration and total iron-binding capacity of the intraocular fluids of rabbits. *Graefes Arch Clin Exp Ophthalmol = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie* 1988;**226**(1):27–30.
- Qi X, Lewin AS, Sun L, et al. Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. *Invest Ophthalmol Visual Sci* 2007;**48**(2):681–91.
- Abu-Amero KK, Bosley TM. Increased relative mitochondrial DNA content in leucocytes of patients with NAION. *British J Ophthalmol* 2006;**90**(7):823–5.
- Farkas RH, Chowdhury I, Hackam AS, et al. Increased expression of iron-regulating genes in monkey and human glaucoma. *Invest Ophthalmol Visual Sci* 2004;**45**(5):1410–7.
- Loh A, Hadziahmetovic M, Dunaief JL. Iron homeostasis and eye disease. *Biochim Biophys Acta* 2009;**1790**(7):637–49.
- Borgna-Pignatti C, Gamberini MR. Complications of thalassemia major and their treatment. *Expert Rev Hematol* 2011;**4**(3):353–66.
- Aksoy A, Aslan L, Aslankurt M, et al. Retinal fiber layer thickness in children with thalassemia major and iron deficiency anemia. *Seminars Ophthalmol* 2014;**29**(1):22–6.
- Turkylmaz K, Oner V, Ozkasap S, et al. Peripapillary retinal nerve fiber layer thickness in children with iron deficiency anemia. *Eur J Ophthalmol* 2013;**23**(2):217–22.
- Acer S, Balci YI, Pekel G, et al. Retinal nerve fiber layer thickness and retinal vessel calibers in children with thalassemia minor. *SAGE Open Med* 2016;**4**, 2050312116661683.
- Arifoglu HB, Kucuk B, Duru N, et al. Assessing posterior ocular structures in beta-thalassemia minor. *Int Ophthalmol* 2017.
- Gumienna-Kontecka E, Pyrkosz-Bulska M, Szebesczyk A, et al. Iron chelating strategies in systemic metal overload, neurodegeneration and cancer. *Curr Med Chem* 2014;**21**(33):3741–67.
- van der Kooij MA, Groenendaal F, Kavelaars A, et al. Combination of deferoxamine and erythropoietin: therapy for hypoxia-ischemia-induced brain injury in the neonatal rat? *Neurosci Lett* 2009;**451**(2):109–13.
- Simon S, Athanasiov PA, Jain R, et al. Desferrioxamine-related ocular toxicity: a case report. *Indian J Ophthalmol* 2012;**60**(4):315–7.
- Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science* 1991;**254**(5035):1178–81.

33. Elia N, Pueyo V, Altemir I, et al. Normal reference ranges of optical coherence tomography parameters in childhood. *British J Ophthalmol* 2012;**96**(5):665–70.
34. Samarawickrama C, Wang JJ, Huynh SC, et al. Ethnic differences in optic nerve head and retinal nerve fibre layer thickness parameters in children. *British J Ophthalmol* 2010;**94**(7):871–6.
35. Oner V, Ozgur G, Turkyilmaz K, et al. Effect of axial length on retinal nerve fiber layer thickness in children. *Eur J Ophthalmol* 2014;**24**(2):265–72.
36. El-Dairi MA, Asrani SG, Enyedi LB, et al. Optical coherence tomography in the eyes of normal children. *Arch Ophthalmol* 2009;**127**(1):50–8.
37. Tariq YM, Samarawickrama C, Pai A, et al. Impact of ethnicity on the correlation of retinal parameters with axial length. *Invest Ophthalmol Visual Sci* 2010;**51**(10):4977–82.