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Retinal nerve fiber layer in beta-thalassemia major: a comparative study

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Beta-thalassemia is a hereditary blood disorder which occurs due to defective beta-globin gene synthesis. It is characterized by blood deficiency caused by ineffective erythropoiesis, hemolysis, and iron overload due to repeated transfusions. The ***purpose*** *of the is study to find out the changes in retinal nerve fiber layer (RNFL) thickness as a result of Beta-thalassemia major and to compare the values with healthy individuals.* ***Material and methods.*** *This cross-sectional study was performed on a total of 56 beta-thalassemia major cases and 64 healthy controls from December 2023 to June 2024. The mean age of the cases (18.42 ± 4.05 years) and controls (17.45 ± 4.02 years) was significantly similar (p = 0.190). All the subjects undergone standard ophthalmological examination followed by RNFL thickness measurement using Heidelberg Spectralis OCT (Optical Coherence Tomography).* ***Results.*** *The RNFL thickness showed significant difference between the case and control groups. The p-value for global, inferior, nasal, and temporal RNFL thickness was < 0.001, while for superior RNFL p-value was 0.006. A significant correlation of global, inferior, and nasal RNFL measurements was found with the duration of thalassemia (p = 0.01, p = 0.01, p < 0.001), however, the global and nasal RNFL were found to be thinner in the cases receiving iron chelation monotherapy (p = 0.048, p = 0.006).* ***Conclusion.*** *The study concludes that the RNFL was significantly thinner in beta-thalassemia major cases in comparison to healthy controls. Moreover, only global, inferior, and nasal RNFL revealed to have a significant correlation with the thalassemia duration and iron chelation therapy.*

Keywords: beta-thalassemia major; retinal nerve fiber layer; hemoglobin; iron chelation therapy
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Слой нервных волокон сетчатки при большой бета-талассемии: сравнительное исследование

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Бета-талассемия — наследственное заболевание крови, возникающее из-за дефектного синтеза гена бета-глобина. Характеризуется дефицитом крови, обусловленным неэффективным эритропоэзом, гемолизом и повышенным уровнем железа вследствие повторных трансфузий. ***Цель*** *исследования — оценить изменения толщины слоя нервных волокон сетчатки (СНВС) при большой бета-талассемии в сравнении со здоровыми людьми.* ***Материал и методы.*** *Поперечное исследование, проведенное в период с декабря 2023 г. по июнь 2024 г., включало 56 случаев тяжелой бета-талассемии и 64 здоровых человека. Средний возраст больных (18,42 ± 4,05 года) и лиц контрольной группы (17,45 ± 4,02 года) был практически одинаковым (p = 0,190). Всем проведено стандартное офтальмологическое обследование, а также измерение толщины СНВС с использованием оптической*

когерентной томографии (Heidelberg Spectralis). ***Результаты.*** *Толщина СНВС значительно различалась в основной и контрольной группе. Значение p для общей, нижней, назальной и височной толщины СНВС составляло < 0,001, тогда как значение p для СНВС верхнего отдела составляло 0,006. Обнаружена значительная корреляция показателей глобальной толщины, а также толщины нижнего и назального СНВС с продолжительностью талассемии (p = 0,01, p = 0,01, p < 0,001). Значения глобальной и назальной толщины СНВС оказались ниже при монотерапии хелаторами железа (p = 0,048, p = 0,006).* ***Заключение.*** *СНВС при большой бета-талассемии значительно тоньше, чем у здоровых людей. Глобальная толщина и толщина СНВС в нижней и назальной области значимо коррелировали с продолжительностью талассемии и с терапией хелатированием железа.*

Ключевые слова: большая бета-талассемия; слой нервных волокон сетчатки; гемоглобин; терапия хелатированием железа
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Thalassemia is one of the most common global public health problem [1]. According to World Health Organization (W.H.O.), thalassemia is found to be the most common hereditary chronic blood disorder that impacts the lives of 10,000 people each year [2, 3]. Beta-thalassemia occurs due to defective beta globin genes synthesis, which in turn reduces the functional ability of hemoglobin. Beta-thalassemia can have one of the two variants, either complete absence or reduced synthesis of beta-chains that leads to ineffective red blood cells formation and their destruction [4]. Beta-thalassemia Major is a serious illness that needs the patient to get regular blood transfusions due to reduced hemoglobin [5, 6]. The routine blood transfusions increases the serum ferritin levels due to iron overload that results in various organ dysfunction. This makes it necessary for those individuals to take iron chelating agents which also have their own complications [5–7].

The measurement of RNFL thickness gives an important insight in understanding sight-threatening optic nerve disorders which can be helpful in early diagnosis and management of such diseases [8]. There are a number of studies conducted in the past to evaluate the visual functions and clinical changes in thalassemia individuals [2, 9–11], however, a few studies reported structural ocular changes in these patients. The literature showed sufficient gap to provide a room for further research as there was a conflict in the findings of already published data [3, 8, 10, 12–16].

PURPOSE AND OBJECTIVES.

This study was performed to evaluate the Retinal Nerve Fiber Layer (RNFL) among cases with Beta-thalassemia Major using Heidelberg Spectralis Optical Coherence Tomography (OCT), and to compare the values with healthy age-matched controls.

MATERIALS AND METHODS

This comparative cross-sectional study was carried out on a total of 56 cases with Beta-thalassemia Major and 64 healthy controls for a duration of seven months from December 2023 to June 2024. The cases were recruited from Jamila Sultana Foundation, Rawalpindi, Pakistan whereas controls were recruited from the General Outdoor Patient Department (OPD) of Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan. The inclusion criteria was beta-thalassemia major cases and healthy age-matched controls of more than 10 years having Spherical Equivalent Refraction (SER) of < ± 6.00 D. The cases with duration of thalassemia > 10 years, those who were regularly receiving blood transfusions twice or more each month to maintain their Hemoglobin (Hb) levels more than 7 g/dl, those who were taking iron chelators including deferiprone, deferasirox, or deferoxamine (either one or combination). Any other hemoglobin disorder, anemia, systemic diseases, SER of > ± 6.00 D, ocular infections, other ocular abnormalities (congenital or acquired), glaucoma, ocular trauma, intraocular surgery or history of contact lens use were excluded from the study.

The written and verbal informed consent was taken from each participant and/or their guardians followed by complete history regarding onset of thalassemia, iron chelating agents used, number of transfusions per month, other systemic history, ocular surgical and trauma history. A comprehensive ocular examination was carried out for all the subjects that included distance vision assessment on Early Treatment Diabetic Retinopathy Study vision chart (ETDRS), measurement of intraocular pressure (IOP) with Goldmann applanation tonometer. Anterior segment evaluation was carried out by an ophthalmologist using slit-lamp biomicroscope. The participants were then evaluated for RNFL on Heidelberg Spectralis OCT by a trained optometrist. The peripapillary RNFL thickness was noted globally along with all four quadrants including superior, inferior, nasal, and temporal. Only the high quality images were captured for inclusion in the study. Slit-lamp examination was again carried out for evaluating posterior segment of eye after mydriasis. The participants then undergone hematological examination including Hb levels prior to transfusion and serum ferritin.

The study was performed according to the ethical considerations of Institutional Review Board and Declaration of Helsinki. The study plan was approved by Ethical Review Committee of Lincoln University College, Malaysia (LUC/CPGS/FOS/20230517/002) and Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan (ERC-09/AST-24). An informed consent (written and verbal) was taken from the participants or their guardians where needed that ensured the confidentiality of data and its sole use for research purposes and publication.

Data Analysis. The data was analyzed using JASP (Jeffreys's Amazing Statistics Program) software with $p < 0.05$ accounted to as significant. The Shapiro-Wilk test was used to check normality of data with $p > 0.05$ considered significant, showing normal distribution of data. The mean and standard deviations were reported for quantitative data, while, percentages and frequencies for categorical data were detailed. The data distribution was normal, so, to compare mean values of the cases and controls independent samples t-test was applied. To find the correlation of RNFL measurements with duration of thalassemia, number

of transfusions, hemoglobin, and serum ferritin levels Pearson's correlation was used ($p < 0.05$ as significant). The RNFL measurements in the cases with combined and monotherapy were compared using independent samples t-test ($p < 0.05$ significant).

RESULTS

The study was performed on a total of 56 cases (beta-thalassemia major) and 64 healthy controls. The mean age of cases was 18.42 ± 4.05 years and controls was 17.45 ± 4.02 years. The individuals of the two groups showed significant similarity in their age group ($p = 0.190$). Twenty six out of fifty six cases were males whereas in control group twenty nine out of sixty four individuals were males and remaining were females. The details are shown in Table 1.

The refraction of the two study groups was comparable in terms of SER (Cases: median ± IQR = -0.625 ± 1.31, mean SD = -0.67 ± 1.78; Controls: median ± IQR = -0.65 ± 1.39, mean ± SD = -0.68 ± 1.82; $p = 0.31$ for median, $p = 0.21$ for mean). Moreover, the IOP among both groups was comparable ($p = 0.076$). The mean years of thalassemia duration in the cases was 17.48 ± 4.01 years, while the mean number of transfusions received by thalassemia cases was 2.35 ± 0.67 per month. The details of the clinical and laboratory parameters are shown in Table 2. 25% of the cases were using iron chelating treatment as a combined therapy (n = 14) while remaining 75% were receiving monotherapy (n = 42).

The RNFL thickness measurements in the cases were less in comparison to control group. The two groups showed significant difference as to global RNFL and RNFL in all the four quadrants (superior, inferior, nasal, and temporal) ($p < 0.05$) (Table 3).

The cases showed a significantly positive correlation of thalassemia duration with global, inferior, and nasal RNFL ($p < 0.05$). The global RNFL and inferior RNFL showed weak positive correlation (global RNFL: $r = 0.320$, $p = 0.01$; inferior RNFL: $r = 0.338$, $p = 0.01$), however nasal RNFL was moderately positively correlated to thalassemia duration ($r = 0.527$, $p < 0.001$). The RNFL was not significantly correlated to the frequency of transfusions per month, hemoglobin, and serum ferritin ($p > 0.05$) (Table 4). Regarding iron chelation therapy, global and nasal RNFL were significantly different between cases on monotherapy and combined therapy ($p_{global} = 0.048$, $p_{nasal} = 0.006$) (Table 5).

DISCUSSION

In the current study, beta-thalassemia major cases revealed thinner RNFL measurements in comparison to healthy age-matched control group (global, inferior, nasal, temporal RNFL: $p < 0.001$, superior RNFL: $p = 0.006$)

The results of current study were much the same as reported by A. El Sehmawy et al. (2019). The RNFL (global and in four quadrants) was less in cases than in controls ($p = 0.000$) [15]. F. Uzun et al. in another cross-sectional study conducted in the year 2017 reported similar results. The results revealed significant difference in the global ($p = 0.003$), inferior, nasal and temporal RNFL among the cases and controls ($p < 0.001$) [8]. Likely results were detailed by A. Aksoy et al. (2014), with significant difference of cases and controls with regard to RNFL thickness (global and in quadrants) ($p < 0.01$) [3]. A. Basiony et al. (2022), reported similar findings regarding RNFL thickness in nasal quadrant ($p = 0.029$) [17].

The results of this study were unalike from those of B. Koctekin et al., in a study conducted in the year 2023. The cases were having no significant difference in their global ($p = 0.802$), superior ($p = 0.824$), inferior ($p = 0.963$), nasal ($p = 0.325$), and temporal RNFL thickness ($p = 0.341$) from the control group [12]. Similar results were found by S. Haghpanah et al. in a study conducted in the year 2022. No significant difference in RNFL measurements between thalassemia patients and the control group was reported ($p_{global} = 0.322$, $p_{superior} = 0.72$, $p_{inferior} = 0.196$, $p_{nasal} = 0.817$, $p_{temporal} = 0.607$) [10]. A. Basiony et al. also reported no notable difference in

Table 1. Demographic characteristics
Таблица 1. Демографические характеристики

Study Groups Группы исследования	n	Age, yrs Возраст, лет			Gender Пол			
					males мужчины		females женщины	
		mean среднее	SD станд. отклонение	p-value значимость различий	f	%	f	%
Beta-thalassemia major cases Случаи большой бета-талассемии	56	18.42	4.05	0.190	26	46.42	30	53.57
Controls Контроль	64	17.45	4.02		29	45.31	35	54.68

Table 2. Distribution of clinical and laboratory parameters
Таблица 2. Клинические и лабораторные показатели в группах

Parameters Показатели	Beta-thalassemia major Большая бета-талассемия	Controls Контроль	p-value Значимость различий
	Mean ± SD	Mean ± SD	
SER Сферический эквивалент рефракции	-0.67 ± 1.78	-0.68 ± 1.82	0.21
	Median ± IQR = -0.625 ± 1.31	Median ± IQR = -0.65 ± 1.39	0.31
IOP, mm Hg ВГД, мм рт. ст.	14.90 ± 2.05	15.1 ± 2.04	0.076
Hb, g/dl Гемоглобин, г/дл	9.05 ± 0.68	13.12 ± 0.98	0.039
Ferritin, ng/dl Ферритин, нг/дл	5640.55 ± 3264.22	22.67 ± 18.50	< 0.001
Duration of thalassemia, yrs Длительность талассемии, лет	17.48 ± 4.01	Non applicable Неприменимо	
Number of transfusions per month Число трансфузий в месяц	2.35 ± 0.67	Non applicable Неприменимо	

the temporal RNFL between thalassemia cases and controls (p = 0.89) [17]. S. Bayramoglu et al. (2022) [13] and F. Tsapardoni et al. (2020) [14] also found similar results. No particular difference in the values of global RNFL thickness was reported among cases and controls (p = 0.474) (p = 0.658). In another study by M. Ulusoy et al. (2019), the mean values of RNFL thickness (global and all quadrants) were found to have no significant difference among cases and controls (global RNFL: p=0.073, superior RNFL: p = 0.067, inferior RNFL: p = 0.39, nasal RNFL: p = 0.18, temporal RNFL: p = 0.99) [16].

The duration of thalassemia was positively correlated with thinning of the global, inferior, and nasal retinal nerve fiber layers (pglobal = 0.01, pinferior = 0.01, and pnasal < 0.001). The correlation was observed to be weakly positive with global and inferior RNFL thinning, and moderately positive with nasal RNFL thinning. The RNFL thickness was not correlated with frequent transfusions, hemoglobin, and serum ferritin levels (p > 0.05). A significant difference was noted in global and nasal RNFL between the cases receiving iron chelation monotherapy and those undergoing combined therapy (p < 0.05). However, no differences were found between superior, inferior, and temporal RNFL measurements (p > 0.05).

Our results were in accordance to those reported by S. Haghpanah et al. (2022), who reported no significant correlation of RNFL with hemoglobin and ferritin (p > 0.05), except for superior RNFL which was negatively correlated with ferritin levels (r = -0.328, p = 0.042). The correlation of superior RNFL and ferritin levels was however weak. The results of this study were different from ours in term of iron chelation therapy, in which they found no significant correlation with RNFL thickness [10]. Our results were likely to those reported by A. Basiony et al. (2022). There was no correlation of hemoglobin and ferritin with average, temporal, temporal inferior, and nasal inferior quadrants RNFL (p > 0.05), however, naso-superior RNFL was correlated with hemoglobin whereas nasal and supero-temporal RNFL was correlated with ferritin (p < 0.05) [17]. The findings of this study were comparable with results of a study by M. Ulusoy et al. (2019), who found no correlation of RNFL (average and in various quadrants) with hemoglobin and ferritin (p > 0.05) [16]. Our results were supported by the findings of another study conducted by F. Uzun and colleagues, who reported no correlation in RNFL measurements with ferritin and hemoglobin levels [8].

The RNFL thickness is affected in a number of ocular diseases, mainly being glaucoma [18, 19] and axial myopia [20, 21]. Other systemic problems that cause changes in RNFL thickness include Multiple sclerosis, Parkinson's disease, Alzheimer's disease [22], and hypercholesterolemia [23]. Possible factors contributing to changes in the RNFL in individuals with thalassemia include prolonged anemia caused by hemoglobin deficiency, subsequent tissue hypoxia, and iron excess caused by repeated blood transfusions, oxidative stress due to iron excess, and iron chelation toxicity [3, 8, 10, 24]. Iron is an crucial component that helps in many cell reactions in neural tissue, however, too much iron can be threatening due to production of reactive oxygen species that can lead to destruction of retinal layers [24].

Although this study was performed on a sample of 56 cases and 64 healthy controls which makes the greatest number of cases taken in any study so far, but still larger sample sizes are required with community controls to reduce the risk of bias in future studies. Moreover, we also found the correlation of duration of thalassemia and number of transfusions received per month with RNFL measurements (global and in quadrants) which is not detailed in any previous study. The study has a limitation in terms of study design,

Table 3. Comparison of RNFL thickness measurements between beta-thalassemia major patients and healthy controls
Таблица 3. Толщина СНВС у пациентов с большой бета-талассемией и в группе контроля

RNFL, μm СНВС, квадрант, мкм	Beta-thalassemia major Большая бета-талассемия	Controls Контроль	p-value Значимость различий
	Mean ± SD	Mean ± SD	
Global В целом	105.25 ± 8.86	132.98 ± 10.96	< 0.001
Superior Верхний	132.46 ± 16.52	139.29 ± 9.71	0.006
Inferior Нижний	134.48 ± 16.58	148.09 ± 9.97	< 0.001
Nasal Назальный	80.94 ± 13.54	112.70 ± 9.03	< 0.001
Temporal Темпоральный	73.25 ± 11.33	93.48 ± 7.83	< 0.001

Table 4. Correlation of RNFL measurements with duration of thalassemia, number of transfusions, hemoglobin, and serum ferritin levels
Таблица 4. Корреляция толщины слоя нервных волокон сетчатки (СНВС) с длительностью талассемии, количеством трансфузий, уровнем гемоглобина и сывороточного ферритина

RNFL, μm СНВС, квадрант,мкм	Duration of thalassemia, yrs Длительность талассемии, лет		Number of transfusions, per month Число трансфузий в месяц		Hemoglobin, g/dl Гемоглобин, г/дл		Ferritin, ng/ml Ферритин, нг/дл	
	correlation coefficient коэффициент корреляции	p-value значимость различий	correlation coefficient коэффициент корреляции	p-value значимость различий	correlation coefficient коэффициент корреляции	p-value значимость различий	correlation coefficient коэффициент корреляции	p-value значимость различий
Global В целом	0.320	0.01	-0.058	0.67	0.00	1.00	-0.3	0.82
Superior Верхний	-0.029	0.83	-0.184	0.17	0.05	0.70	-0.04	0.73
Inferior Нижний	0.338	0.01	-0.086	0.52	0.068	0.61	-0.140	0.30
Nasal Назальный	0.527	< 0.001	0.150	0.27	-0.09	0.49	0.025	0.85
Temporal Темпоральный	-0.095	0.48	-0.069	0.61	-0.13	0.32	0.23	0.07

which was Cross-sectional. Further studies should be conducted with follow-up research design to evaluate the prolonged effects of the disease itself as well as the chelation therapy on the RNFL.

CONCLUSION

The study concludes that there is significant RNFL thinning in beta-thalassemia major cases in comparison to controls. The global and nasal RNFL measurements were significantly correlated to the duration of thalassemia, and significantly thinner among the cases receiving iron chelation monotherapy compared to combined therapy.

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Table 5. RNFL thickness measurements in relation to iron chelation therapy
Таблица 5. Толщина СНВС при терапии хелатированием железа

RNFL, μm СНВС, квадрант, мкм	Iron chelation therapy Терапия хелатированием железа		
	monotherapy монотерапия	combined therapy комбинированная терапия	p-value значимость различий
Global В целом	103.90 ± 7.58	109.28 ± 11.31	0.048
Superior Верхний	132.45 ± 17.02	132.50 ± 15.51	0.99
Inferior Нижний	132.31 ± 14.92	141.00 ± 20.19	0.09
Nasal Назальный	78.14 ± 12.13	89.35 ± 14.5	0.006
Temporal Темпоральный	72.31 ± 12.14	76.07 ± 8.15	0.28

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