Evaluation of retinal, choroidal thickness and retinal pigmented epithelium using Cirrus SD-OCT in Portuguese children with history of preterm birth

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Abstract

Purpose: To determine choroidal thickness in preterm children with or without history of Retinopathy of Prematurity (ROP) with spectral domain optical coherence tomography (SD-OCT) system.

Methods: In a cross-sectional study, 39 eyes of premature patients (Group 1) and 39 eyes of children with history of ROP (Group 2) between 9 and 17 years old were evaluated with Cirrus Zeiss 500 SD-OCT after cycloplegia. Choroidal thickness was measured from the fovea to 1500µm nasal and temporally with intervals of 500 µm using HD1 line raster program of SD-OCT.

Clinical history of gestational age at birth, birth weight, time of oxygen exposure, stage of ROP in maximal severity of acute disease and ROP treatment were inquired.

Results: Subfoveal thickness was lower in Group 2 (296,85±60,11µm) than in Group 1 (301,82±30,63µm). The mean choroidal thickness in all retinal tested points was not statistically different between groups.

Conclusions: Choroidal thickness can be measured using SD-OCT high definition raster scans in the majority of children. Comparing macular choroidal thickness in both groups, we found that it was thinner nasally, subfoveal and temporally in group 2, with no statistical significance. This can suggest a probable delayed choroidal development in preterm infant due to ROP which can be attenuated with age.

Key words: Choroidal thickness, preterm children, Retinopathy of Prematurity, optical coherence tomography.

Introduction

Retinopathy of Prematurity (ROP) is one of the leading cause of blindness in children. It contributes also for a higher incidence of other ocular disorders, including glaucoma, cataracts, retinal detachment and strabismus.

Patients with history of ROP have lower visual acuity associated with optical components, macular and retinal nerve fiber layer (RNFL) thickness alterations. In previous studies, children with ROP showed steeper corneas, shallower anterior chambers, thicker lenses, higher incidence of absence of foveal depression, and lower RNFL. These changes were associated with lower visual acuities and a higher spherical equivalent.

The choroid is the most important supplier of nutrients and oxygen to the outer retina and its development begins prior to retinal vasculature and persists after birth. Oxygen-induced retinopathy is associated with lower circulation in choroid, decreased nutrients delivery to outer retinal layers, photoreceptor layer damage and decreased, persistent visual function.

Abnormal choroidal vasculature development and consecutive retinal pigmented epithelium dysfunction could also contribute to photoreceptor damage and dysfunction in this oxygen-induced retinopathy. Photoreceptors damage is also a possible explanation for lower visual acuity in children after ROP resolution. Because choroidal vasculature is not yet fully developed in preterm birth and ROP is an oxygen-induced retinopathy, deficient choroidal vasculature development, retinal-pigmented epithelium (RPE) and photoreceptor damage could be cause of lower visual acuity in these children. However, existing studies on choroidal thickness and function in children with history of ROP are limited.

Adequate structural examination of choroid was not possible through spectral domain OCT (SD-OCT) until recently due to retinal pigmented epithelium, which attenuates the incident light, and also due to lower depth penetration associated with SD-OCT instruments. Nowadays, there are new SD-OCT software with high penetration and swept-source longer-wavelength OCT (SS-OCT), that provide higher penetration through the RPE, allowing choroidal thickness evaluation. Cirrus SD-OCT is more commonly used in clinical practice and has new software that allows a higher choroid penetration and the visualization of choroid-sclera transition through high definition 1 line raster image from 20 B-scans taken at a single
location. HD 1 line raster is a 6mm line consisting of 4096A-scans.

Imaging of the choroid in preterm children may provide important features about the role of the choroid in ROP and visual acuity. For this reason, the aim of this study was to determine choroidal thickness in preterm children presenting with or without history of ROP using the Cirrus Zeiss SD-OCT 500.

To the best of our knowledge, this is the first report of evaluation of macular RPE through measurement of autofluorescence in patients with ROP history.

Methods

A cross-sectional study was performed at the Ophthalmology Department of Garcia de Orta Hospital, Portugal. This study adhered to the tenets of the Declaration of Helsinki.

Macular choroidal thickness and autofluorescence were evaluated in all children included in this study, after their parents and older than 9-year-old children provided informed consent. All examinations were obtained in early afternoon to avoid choroidal thickness diurnal variations.

In total, 40 children between 9 and 17 years-old were included in this study: 20 with history of ROP and 20 children with history of preterm birth. Both eyes of each child were evaluated and included. The eyes of children were divided in 2 Groups: eyes with history of preterm birth without ROP (n=39; Group 1) and eyes with history of ROP (n=39; Group 2).

The preterm children (Group 1) were an age-matched group of healthy children with gestational age< 37 weeks and birth weight< 2500g without ROP.

Children with ROP, who had history of ROP spontaneous regression or history of laser/ cryotherapy treatment, were included in Group 2.

These children were recruited from the Pediatric Ophthalmology consultation in the Ophthalmology Department of the Garcia de Orta Hospital in 2014.

Patients were excluded if they had history of neurological damage or congenital defects that contributed to their non-cooperation to ophthalmological exams. Patients with residual alterations of ROP (such as macular fold, retinal detachment, macular dragging, and history of ocular surgery due to ROP) or other ocular pathologies (e.g. cataracts, inflammatory diseases, glaucoma) were also excluded.

Other medical data was obtained through the review of clinical files: gestational age, weight birth, time of oxygen exposure, stage of maximal severity of ROP in the acute disease, other complications developed in neonatal stage.

Evaluation of visual acuity and refractive errors

Uncorrected visual acuity and best-corrected visual acuity (BCVA) were assessed in all children. Automated refraction was performed under cycloplegia followed by manual refraction.

Evaluation of macular autofluorescence and choroidal thickness through SD-OCT

Macular autofluorescence and choroidal thickness were evaluated with SD-OCT Cirrus Zeiss 500 in the two groups. The exams were performed under cycloplegia and by a qualified technician.

Lipofuscin is a bioproduct of phagocitated external segments of photoreceptors, which accumulates in RPE with age and in certain retinal diseases. When exposed to visible light with short and medium wavelengths, lipofuscin will present autofluorescence.

Autofluorescence of retinal fundus studies the accumulation of lipofuscin in all fundus, helping to understand the physiopathology and progression of retinal diseases.

Autofluorescence images in this study were obtained with Cirrus Zeiss SD-OCT 500, with a resolution of 5 megapixels and were taken in a sequence from 1, 5 seconds. The quantification of autofluorescence in macular area was performed through Matlab software (7.0 R14; Mathworks Inc, Natick, MA, USA). The intensity values had a minimum of 0 (black) and a maximum of 255 (white).

Choroidal thickness was evaluated through HD1 line raster program from SD-OCT Cirrus Zeiss 500. Images had at least 6 out of 10 in intensity and taken as close to the fovea as possible, by choosing the image of the thinnest point of the macula. Images were taken with the vitreoretinal interface adjacent to the zero-delay with no inversion of macular images.

Using the Cirrus linear measurement tool, two independent observers measured choroidal thickness perpendicularly from the outer edge of the hyper-reflective RPE to the inner sclera at 500 μm intervals temporal and nasal from the fovea, up to 1500 μm. Furthermore, the central foveal thickness was also measured at this time in order to determine the correlation between retinal thickness and choroidal thickness.
Statistical analysis

Numerical variables are presented as the mean and standard deviation. The normality of variable distribution was tested through Komolgorov-Smirnov test.

To evaluate autofluorescence, continuous variable with normal distribution, a T-student test was performed.

To evaluate association between time of oxygen exposure, ROP stage, birth weight, gestational and actual age, macular thickness, subfoveal choroidal thickness, macular autofluorescence and visual acuities, correlation of Spearman were applied.

Linear regression test was performed to evaluate the importance of gestational and actual age, birth weight, gender, ROP stage in the other factors.

The software SPSS program version 22 was used for statistical analysis. P<0.05 was considered to be of statistical significance through this study.

Results

A total of 40 patients met the inclusion criteria and entered in this study. There were 20 children (n=39 eyes) in Group 1 and 20 children in Group 2 (n=39 eyes). Group 2 included 18 eyes with history of non-treated ROP and 21 eyes with history of treated ROP. In Group 2, there were 8 eyes with ROP stage 1, 2 and plus disease and 15 eyes with ROP stage 3.

Mean gestational age in the 2 groups were 29,95±2.76 and 25,33±1.42 weeks, respectively. Mean birth weight was 1142,10±238,36 and 838,28±77,76 grams, respectively. Mean actual age was 12,69±1,6 and 12,33±2,93 years in both groups with no statistical difference (p=0,541).

There was no significant difference in gender distribution between the groups (p=0.80) (Table 1).

Both uncorrected and BCVA were significant lower in Group 2. (p=0.008 and p=0.048, respectively) (Table 1).

Mean spherical power of patients in Groups 1 and 2 were -0,85±1,57 and -0,897±3,16 diopters, respectively (p=0.174). Mean cylindrical power in each group was -0,63±1,53 and -1,13±0,975 diopters (p=0.332). Spherical equivalent was not significantly different in the two groups (-1,15±1,79 and -1,35±3,00, respectively; p=0.651).

There were no statistical differences in the two groups regarding spherical, cylindrical powers. However, Group 2 showed a discrete higher refraction error than Group 1.

In the SD-OCT analysis, both groups revealed similar total retinal volumes (10,23±0,44 and 10,23±0,52, respectively).

Mean foveal thickness was found to be higher in Group 2 (289,00±31,53 µm and 298,38±23,23µm, respectively; p=0,041). In the same group, eyes with treated ROP had higher mean foveal thickness than eyes with non-treated ROP with values of 308,85±18,81µm. (Graphic 1).

We found a significant and positive correlation between foveal thickness and the ROP stage (r²=0,286, p<0,05) and negative correlation between foveal thickness and birth weight (r²=0,241, p<0,05).

Almost all eyes from Group 2 revealed foveal dysplasia. Foveal contour was abnormal, with foveal depression either absent or shallow, with preservation of multiple inner retinal layers (Figure 1). The absence of foveal depression and maintenance of multiple inner retinal layers were correlated with higher foveal thickness. Foveal dysplasia was not detected in eyes from Group 1 patients (Figure 2).

Macular autofluorescence

Macular autofluorescence between both groups revealed no statistical significance with values of 92,61±12,77 e 92,12±12,75, respectively (p=0.314).

Macular autofluorescence is correlated positively with actual age of the children included in this study (r²=0,286;p=0,011). Using linear regression, actual age of children was the most relevant positive factor influencing autofluorescence; however, not statistically significant (ß=0,257 , p=0,081). There was no statistically significant correlation between autofluorescence and visual acuity, mean foveal thickness and mean subfoveal choroidal thickness.

Choroidal Thickness

In this study, the temporal choroid showed a higher thickness than the nasal choroid in both Groups (Table 2). The subfoveal choroidal thickness was lower in Group 2 than in Group 1, with values of 301,82±30,64µm and 296,85±60,12µm, respectively. Nasal and temporal choroid of patients of Group 2 showed also lower values than patients of Group 1. However, differences in temporal, subfoveal and nasal choroid were not statistically significant between both Groups.

When the influence of all factors in the choroidal thickness was evaluated, the time of oxygen exposure was considered the most significant negative predictor of choroidal thickness (ß=0,420;p=0,032).

The subfoveal choroidal thickness was found to be positively associated with spherical power (r²=0,476, p=0,002) and spherical equivalent (r²=0,513, p=0,001) in Group 2 (Figure 3). There were no associations between spherical power and spherical equivalent in Group 1. Actual age, gestational age, birth weight, other optical components and foveal thickness were not
found to be correlated to choroidal thickness in both Groups. BCVA was correlated positively with subfoveal choroidal thickness in Group 1 and Group 2 ($r^2=0.135$, p=0.042 and $r^2=0.321$, p=0.03), respectively.

However, when evaluating the influence of different factors in BCVA, there was a tendency of gestational age ($\beta=0.542$, p=0.067) and foveal thickness ($\beta=-0.269$, p=0.081) to be the most significant predictors of BCVA.

**Discussion**

In our study, foveal thickness was higher in eyes with history of ROP comparing with premature eyes without history of ROP. Foveal thickness had ROP stage and birth weight as the most relevant predictors, being gestational age also a relevant predictor; however not statistically significant.

In the literature, ROP is associated with foveal dysplasia. ROP is also described as associated with macular alterations such as retention of ganglionar cell layer, internal plexiform layer and internal nuclear layers. The SD-OCT images present with absence of foveal depression, more common from normal eyes than in eyes with treated ROP, as described by Wu et al. In this study, total foveal thickness in a group of children with treated and not treated ROP, with history of prematurity and with normal eyes was $281.03\pm22.0\mu m$, $266.0\pm7.6\mu m$, $264.3\pm25.8\mu m$, and $247.1\pm21.3\mu m$, respectively. Foveal thickness correlated significantly and negatively with gestational age. Considering foveal thickness and morphology, our results were similar to those presented in the above described study.

The reason for this elevated foveal thickness is not well known. Macular development is associated with centrifugal migration of the retinal internal layers and centripetal migration of photoreceptor layer towards the macula. This phenomenon ends at 3-4 years of age, being the fovea the last structure to be formed. The absence of foveal depression is related to an anomalous retinal development in children with history of ROP and is presented by absence of completely migration of internal layers of retina towards the periphery.

Williams and Trese associated ROP to other macular alterations such as disruption of RPE with the documentation of a case of a child with non-treated ROP and macular lesion simulating a cryotherapy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Foveal choroidal thickness</th>
<th>Choroidal thickness 500µm nasally from the fovea</th>
<th>Choroidal thickness 1000µm nasally from the fovea</th>
<th>Choroidal thickness 1500µm nasally from the fovea</th>
<th>Choroidal thickness 500µm temporally from the fovea</th>
<th>Choroidal thickness 1000µm temporally from the fovea</th>
<th>Choroidal thickness 1500µm temporally from the fovea</th>
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</thead>
<tbody>
<tr>
<td>Group 1 (n=39)</td>
<td>301.82±30.64</td>
<td>292.72±3.73</td>
<td>281.97±29.24</td>
<td>269.59±31.89</td>
<td>294.46±31.34</td>
<td>286.85±32.88</td>
<td>v275.56±38.65</td>
</tr>
<tr>
<td>Group 2 (n=39)</td>
<td>296.85±60.11</td>
<td>285.56±61.26</td>
<td>277.56±59.61</td>
<td>266.56±60.75</td>
<td>286.33±57.89</td>
<td>275.49±54.72</td>
<td>269.43±50.75</td>
</tr>
<tr>
<td>Total (n=78)</td>
<td>299.33±47.47</td>
<td>289.4±48.60</td>
<td>279.62±46.70</td>
<td>268.08±48.22</td>
<td>290.39±46.42</td>
<td>281.17±45.21</td>
<td>272.50±44.92</td>
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Table 2. Choroidal thickness values in the 2 Groups.
lesion. ROP could be related to RPE alterations and, consecutively, to retinal autofluorescence.

RPE is a monolayer of pigmented cells, which interacts closely with photoreceptors layer. It is responsible for its nutrition, phagocytosis of photoreceptors outer segments and plays a relevant role in visual cycle and light adaptation.

In the literature, patients with ROP are associated with lower BCVA with no correlation to foveal alterations. The physiopathological mechanism for lower BCVA plays a key role.

To the best of our knowledge, this is the first study to evaluate macular autofluorescence in children with history of ROP, comparing mean foveal and perifoveal autofluorescence. Our study included 16 eyes with ROP stage 1 and 2, without posterior pole involvement. In these cases, hypoxia and rod dysfunction is predominant in periphery, with probably no dysfunction in macular RPE. It would also be interesting to test autofluorescence of temporal RPE (the first location affected by ROP) in future studies.

Andreson et al recently evaluated choroidal thickness in patients with ROP vs. a control group. They concluded that the mean subfoveal choroidal thickness in patients with ROP and in control group was 271.1 µm and 327.4 µm, respectively (p=0.008).9 Patients with history of ROP also presented a temporal choroid thinner than in the control group.9 We obtained lower mean values of choroidal thickness in the different points tested until 1500 µm from fovea of patients with history of ROP. However, there were no statistically differences between the two groups.

Ruiz-Moreno et al, found a choroidal thickness of 285.2±67 µm in normal patients with a mean age of 10±3 years.9 Considering this mean value, we notice that the mean choroidal thickness for both groups were lower than for eyes of patients with full term birth and similar mean actual age.

Moreno et al evaluated choroidal thickness in premature newborns andnormal full term newborns. They found that choroidal thickness in premature newborns was statistically lower than in normal newborns of full term. However, in a second ophthalmological examination, there was an increase in subfoveal choroid in preterm newborns, maintaining lower mean values than normal newborns. We can conclude from the study that prematurels present a delayed choroidal development10 and that, after birth, choroid is still in development.

Anderson et al.7 compared choroidal thickness in patients with regressed ROP stage 3 with healthy controls using enhanced depth imaging optical coherence tomography. They obtained a mean subfoveal choroidal thickness, adjusted for refraction, of 271.1 µm in the ex-ROP group, which was significantly thinner than 327.4 µm in controls (p=0.008). Temporal choroidal thickness was also thinner than in controls with values of 257.2 µm (p=0.001). They concluded that there is choroidal involvement in the pathogenesis of this disease. In our study, we obtained inferior values for choroidal thickness in patients with history of ROP, however with no statistical significance. This could be due to our inclusion of all stages of ROP in group 2 and of treated and non-treated ROP in this same group. We also compare patients with history of prematurity vs. eyes with history of ROP, instead of comparing eyes with ROP vs. eyes of patients with full term birth.

In the literature, RPE underdevelopment in preterm birth is associated with a delayed development of choroidal vasculature and could result in a thinner subfoveal choroid in these patients.12 Robb showed the ability of RPE to stimulate choroidal vasculature development.12 Other study that corroborates the relevance of RPE in choroid development, revealed absence of choriocapillaris in absence of VEGF expression by RPE.13

The time of oxygen exposure was the most relevant and negative predictor for choroidal thickness in our study. As we know, exposure to high levels of oxygen leads to less VEGF expression by RPE, which can contribute to a slower choroidal development and a thinner choroid in preterm newborns. After exposure to lower oxygen levels, choroidal vasculature continues to develop, and the choroid becomes thicker and closer to choroid of normal infants.11 Lower expression of VEGF RPE and high levels of oxygen could be the reason for a thinner choroid in patients with ROP comparing with preterm children.

There were no statistically significant differences in choroidal thickness in every point tested between the two groups in our study. This could be related to the continued choroid development through childhood after high levels of Oxygen exposure. This also contributes to the theory that choroid development continues after birth.

To our knowledge, this is the first study to evaluate RPE through autofluorescence in children with history of prematurity.

This study also demonstrates a persistent thin choroid in children with history of ROP, representing a probable delay in choroid development. This highlights the relevance of not only retinal, but also choroid vasculature, in infant ocular development and a possible important role of choroid in ROP.

To a better future knowledge of RPE function in ROP, autofluorescence of the temporal RPE should be tested, as this is the most common region affected by the disease.

References