A Pilot Optical Coherence Tomography Angiography Study on Superficial and Deep Capillary Plexus Foveal Avascular Zone in Patients With Beta-Thalassemia Major

Ilias Georgalas,1 Georgios Makris,2 Dimitrios Papaconstantinou,1 Petros Petrou,1 Evangelia Chalkiadaki,1 Konstantinos Droutsas,1 Konstantinos Andreanos,1 and Menelaos Kanakis3

1First Department of Ophthalmology, National and Kapodistrian University of Athens, G. Gennimatas Hospital, Athens, Greece
2Department of Ophthalmology, G. Gennimatas Hospital, Athens, Greece
3Department of Ophthalmology, Patras University School of Medicine, Rion, Patras, Greece

Correspondence: Ilias Georgalas, First University Eye Clinic, National and Kapodistrian University of Athens, G. Gennimatas General Hospital of Athens, 154 Messorhion Avenue, Athens 11527, Greece; igorgalas@yahoo.com.

Submitted: April 14, 2019
Accepted: August 12, 2019

Purpose. To investigate foveal avascular zone (FAZ) changes in the superficial (SCP) and deep (DCP) capillary plexuses in beta-thalassemia major (BTM) patients, as shown in optical coherence tomography angiography.

Methods. Nonrandomized, comparative case series of 54 eyes of 27 BTM patients and 46 eyes of 23 healthy controls, utilizing an automated FAZ detection algorithm. Measurements included FAZ area and FAZ shape descriptors (convexity, circularity, and contour temperature). Results were compared between the two groups, and correlated to iron load and chelation therapy parameters.

Results. SCP and DCP FAZ area were not significantly different between the control and BTM groups (P = 0.778 and P = 0.408, respectively). The same was true regarding SCP FAZ convexity (P = 0.946), circularity (P = 0.858), and contour temperature (P = 0.907). In contrast, a statistically significant difference was detected between controls and BTM group regarding DCP FAZ convexity (P = 0.013), circularity (P = 0.010), and contour temperature (P = 0.014). Desferrioxamine dosage was strongly correlated to the DCP area (r = 0.650, P = 0.05) and liver magnetic resonance imaging/T2-star to DCP circularity (r = −0.492, P = 0.038). Correlations were also revealed between urine Fe excretion and DCP convexity (r = 0.531, P = 0.019), circularity (r = 0.661, P = 0.002), and contour temperature (r = −0.591, P = 0.008).

Conclusions. Retinal capillary plexuses and especially DCP seem to present unique morphologic changes in BTM patients, not in the FAZ area, but in specific shape descriptors, indicating minor but detectable FAZ changes. These changes correlate well with iron load and chelation therapy parameters. Their clinical importance and pathophysiologic implications remain to be elucidated through further studies.

Keywords: β-thalassemia major, foveal avascular zone, shape descriptors, iron load, chelation therapy

Beta-thalassemia (BT) is a severe genetic autosomal recessive hemoglobinopathy leading to defective β-chain production, imbalance in α/β-globin chain synthesis, ineffective erythropoiesis, and anemia.1–3 Homozygous beta-thalassemia major (BTM) requires regular lifelong red blood cell (RBC) transfusions. Without transfusions, BTM leads to death before the age of three years old.3 Heterozygous patients present with milder symptoms, at an older age, and do not require transfusions. Every year, more than 42,000 newborns are affected by BT worldwide.4 Areas with high prevalence include the Mediterranean Basin, Central Asia, Transcaucasia, the Indian subcontinent and the Far East. BT is not uncommon in people of African descent.5

Transfusion therapy, along with iron chelation (the gradual accumulation of iron due to multiple transfusions can lead to organ failure), is the cornerstone of BTM treatment, preventing death and decreasing mortality.5–7 The whole spectrum of BTM ocular complications includes those due to iron overload,2,6 but also those related to chelation therapy.6 The chelation agent more extensively used clinically worldwide is desferrioxamine. Other agents include the orally administered deferiprone and deferasirox.2

The majority of BTM retinal lesions fall into the category of pseudoxanthoma elasticum-like (PXE-like) lesions, a term introduced by Acssopos et al.,10 to differentiate them from true pseudoxanthoma elasticum (PXE).11 PXE-like retinal lesions include angioid streaks, peau d’orange, and pseudopaillitis (optic disc drusen).12,13

Retinal venous tortuosity (RVT) is considered the primary non-PXE-like retinal abnormality, found in 4% to 24% of BTM patients, and attributable to chronic anemia.14–16 Although RVT is well documented, the possibility of microvascular anomalies in BTM has not yet been explored.

The purpose of this pilot study was to investigate the presence of macular microvascular alterations in BTM patients. Foveal avascular zone (FAZ) is the most sensitive index of insult
to the retinal capillary network, providing early and accurate data regarding possible pathologic processes. Under this notion, we utilized optical coherence tomography angiography (OCTA) to investigate probable changes in both the superficial (SCP) and deep (DCP) capillary plexuses.

**METHODS**

**Study Population**

We conducted a nonrandomized, comparative case series of 54 eyes of 27 BTM patients and 46 eyes of 23 healthy controls. Eyes with previous intraocular surgery, high myopia, or retinal vascular disease, or patients with difficulty regarding cooperation, were excluded from the analysis. Institutional approval was obtained and the study procedures conformed to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

Collected data included demographic characteristics, best-corrected visual acuity (BCVA), and refractive error (Table 1). All patients (both eyes) were examined clinically and with OCTA (DRI Triton swept-source OCT; Topcon, Oakland, NJ, USA). Three-dimensional OCT angiograms of SCP and DCP were obtained over a 3 × 3-mm square, and images (in lossless format) were exported for postprocessing. Images were obtained using the native segmentation algorithm of the DRI Triton OCT. Thus, in our study, DCP incorporates the intermediate capillary plexus (ICP). The whole structure is sometimes called deep vascular complex (DVC), depending on the preferred terminology.

**FAZ Shape Analysis**

The FAZ outline was determined automatically: After contrast enhancement with ImageJ software, images were imported to the Icy software to detect both SCP and DCP FAZ, utilizing the Active Contours plugin, after appropriate parameter adjustment. The evolution of the curve to fit the outer segmentation borders of the FAZ is presented in Figure 1.

The automated analysis was able to accurately depict DCP FAZ outline avoiding projection artifacts from the overlying SCP, as presented in Figure 2. The resulting regions of interest (ROIs), representing the FAZ outline, were then imported to ImageJ (using the incorporated in the Icy function to directly import the image and the segmentation results to ImageJ) for further analysis. This included not only the calculation of the FAZ area, but also several shape descriptors as included in the ImageJ Shape Filter plugin. The notion behind this was that area alone is insufficient to completely describe FAZ. The wide variation of FAZ area between normal individuals suggests that area is a more appropriate index to evaluate the progression of a disease in consecutive measurements on the same individual, rather than discriminate healthy individuals from patients. Shape descriptors were chosen for their ability to depict not only the overall shape and area of FAZ, but also to elucidate fine details of the FAZ outline. Many descriptors are not dependent on the shape’s area (scale independent) and describe only the shape’s outline, detecting changes with minor impact on the overall FAZ area but with evident impact on capillary elasticity and integrity, as is the case with focal loss of capillaries. These are described in detail in the Shape Filter documentation (https://imagej.net/Shape_Filter; in the public domain) and include the following:

- **FAZ Area (A).** The area of the FAZ.
- **FAZ Convex Hull (C).** The area enclosed by the convex hull of the outer contour of the FAZ. A simplified definition of convex hull is the smallest convex polygon (a polygon which has no corner that is bent inward) that encloses (envelops) the shape under consideration (in our case the FAZ outline).
- **FAZ Perimeter (P).** The perimeter of the outer FAZ contour.
- **Perimeter of FAZ Convex Hull (H).** The perimeter of the convex hull of the FAZ.

- **Feret Diameter.** The maximum distance between two parallel tangents touching the FAZ outline in all directions. The feret diameter is sometimes called the caliper diameter, as it is the larger measurable dimension of the shape if we use a caliper to measure its dimensions in all directions.
- **Minimum Feret Diameter.** The minimum distance between two parallel tangents touching the FAZ outline in all directions. It is the smallest measurable dimension of the shape if we use a caliper to measure its dimensions in all directions.
- **Maximum Inscribed Circle Diameter.** The diameter of the maximum inscribed in the FAZ circle.
- **Area-to-Perimeter Ratio.** Defined as $A/P$.

- **Circularity.** Is defined as $H/P$ and describes how convex the FAZ shape is, by comparing the convex hull perimeter to that of the FAZ. The more inward-bending angles in the FAZ outline, the greater the FAZ perimeter and the lower the ratio. For a perfect convex polygon, the ratio would be 1.
- **Solidity.** Defined as $A/C$, this is a measurement of how solid and uniform the area of a shape is, compared to the shape’s convex hull. It is complementary to the convexity, but less sensitive to boundary irregularity. A ratio of 1 signifies a solid object, and a ratio <1, an object with irregular boundary, or containing holes.
- **Circularity.** This is the general definition of normed circularity; defined as $4\pi A/P^2$, originally described in Shape Filter documentation as “Thimmes ratio.” Circularity is a measure of FAZ compactness relative to a circle. By definition, the circularity index of a circle is 1.0. Thus, a ratio closer to 0 indicates an irregular shape, and a ratio closer to 1.0 indicates a circular shape. Circularity is an established shape descriptor in many fields of biology and medicine.
- **Contour Temperature.** Defined as $(\log_2(2P/(P - H)))^{-1}$. The definition is based on thermodynamics. The entropy of a plane curve is defined in terms of the number of intersection points with a random line. Gibbs distribution (which maximizes the entropy) allows the conjunction of the entropy to a certain temperature. At temperature 0 the curve is reduced to a straight line, while at high temperatures the curve becomes more and more complex and chaotic and behaves like a perfect gas. The simplest way to visualize contour temperature is to imagine the points forming the FAZ contour as molecules of a perfect gas. As we “heat” the curve to a
FIGURE 1. An overview of the procedure to define SCP and DCP boundaries (ROIs). Top row images describe the procedure for the SCP, while bottom row images describe the same procedure for the DCP. (A) In the Icy software, an initial seed in the form of a circular ROI inside the SCP FAZ is required to start the procedure. This initial ROI is gradually expanded to cover the area of interest, in our case the FAZ. (B) The initial seed grows, tending to the SCP FAZ outline. (C) The initial seed has grown to reach the SCP FAZ outline. The growth then stops, and the FAZ area and outline are defined. Notably, no matter the exact location and size of the initial seed, the final ROI is always the same. (D) The SCP FAZ ROI has been imported to ImageJ software for further processing, using the incorporated in the Icy function. (E) Initial circular seed phase. (F) Growing of the seed, tending to the DCP FAZ outline. (G) DCP FAZ outline is defined. (H) Importing DCP FAZ ROI to ImageJ software.

FIGURE 2. The proposed algorithm is capable of defining the true boundaries of DCP, avoiding projection artifacts. (A) Deep capillary plexus boundaries (ROI) seem not to include a certain portion of DCP (yellow arrows). (B) The vascular outline in the aforementioned area is marked red. (C) Comparative projection on the DCP image of both DCP (green) and SCP (blue) ROIs. (D) The SCP image of the same patient. (E) The vascular outline of the DCP as depicted in (B), projected on SCP. It is obvious that the vascular structures outlined in red represent SCP vessels. This proves that the algorithm is capable of accurately defining the true DCP outline avoiding projection artifacts. DCP images are amenable to projection artifacts, due to the location of DCP and the different nature and distribution of its constituent capillaries (vortex-like vascular loops surrounding central seed points). (F) Comparative projection on the SCP image of both DCP (green) and SCP (blue) ROIs. As in (C) above, it is evident that DCP is larger than the SCP, and the DCP outline is not concentric with SCP. It seems that in certain areas DCP boundaries coincide with those of SCP, while elsewhere DCP seems enlarged.
higher temperature, the more vigorous the movement of the “molecules” would be. A snapshot of the curve would present increasing irregularity with increasing temperature.

**Fractal Box Dimension.** Estimated fractal dimension by the standard box count algorithm. We opted to use the Shape Filter default box sizes 2, 3, 4, 6, 8, 12, 16, 32, 64 as the goodness of fit index; also calculated by Shape Filter, in all cases was >0.995.

We also calculated two other possibly useful indexes: the ratio (feret diameter) / (minimum feret diameter), a measure of the elongation of FAZ and the ratio area / (maximum inscribed circle area), a measure of the irregularity of the FAZ outline.

**Iron Load Indices**

To investigate a possible correlation between SCP and DCP FAZ morphology and iron overload or chelation therapy, data already available from the previous standard patient’s follow-up were gathered. These included heart and liver magnetic resonance imaging/T2-star (MRT1/T2*) and current serum ferritin levels, but also mean serum ferritin levels and mean desferrioxamine monthly dosage, as well as mean urinary iron excretion (UIE) (all over the last three years).23

Iron detection in MRI is used as an effective alternative to the more accurate but invasive liver biopsy (liver contains >70% of the total body iron). The interaction of ferritin and hemosiderin with adjacent hydrogen nuclei in tissue H2O results in a significant reduction in signal intensity due to shortening of T1, T2, and T2* relaxation times, producing image darkening.24 T2* is fast, robust, and extremely sensitive to liver iron deposition.25 MRI myocardial T2* has also been used as a sensitive and easy-to-acquire index of myocardial iron levels.26

Serum ferritin level has been used for iron load monitoring in BTM. Repeated ferritin measurements at regular intervals are more accurate in predicting iron overload complications than a single liver biopsy. There is a significant correlation between serum ferritin (past year average) and liver MRI/T2*.27,28

Urinary iron excretion is used to estimate the efficacy of chelation therapy. Chelation promotes UIE, toward ideally a excretion mechanism in humans).29 The usual DSF dosage (as in our patients) is 5 gms/24 hours, 5 days per week, resulting in approximately 64 gms desferrioxamine monthly.29 The excretion rate increases with increased total iron load and increased DSF dosage.30,31

**Statistical Analysis**

Parametric statistical tests depend on the assumption that data are sampled from normal distributions. We analyzed quantitative variables (FAZ size, FAZ perimeter, shape descriptors, ferritin, 3-year average ferritin, heart MRI/T2*, liver MRI/T2*, UIE, and DSF mean monthly dosage). Between these only UIE followed a normal distribution (Shapiro-Wilk test for composite normality). Thus, all comparisons followed the nonparametric statistical scenario (comparison of medians), and Mann-Whitney U test was applied. The Spearman’s Rho nonparametric statistical correlation test was used to measure the strength of association between FAZ shape descriptors and parameters related to iron load and chelation therapy. Statistical significance was set at an initial P value < 0.05. Hochberg’s step-up procedure22,23 was used to control for multiple comparisons (Type I error). Under Hochberg’s procedure the P value for the comparisons between the FAZ shape descriptors was set as P < 0.016. Statistical analysis was performed in SPSS, version 20 (Chicago, IL, USA).

**RESULTS**

We separately compared area and shape descriptor parameters for both SCP and DCP FAZ. The level of statistical significance according to Hochberg’s step-up correction for multiple comparisons was set at 0.016.

In SCP there was no significant difference between the control and BTM groups regarding the FAZ area (μm²) (303.42 ± 118.73 ± vs. 312.22 ± 120.450, P = 0.778), the area of the FAZ convex hull (μm²) (338.13 ± 155.507 vs. 344.97 ± 153.560, P = 0.763), and the FAZ perimeter (μm) (2301 ± 544 vs. 2302 ± 527, P = 0.884).

Also, the perimeter of SCP FAZ convex hull (μm) did not differ significantly between the control and the BTM groups (2087 ± 584 vs. 2095 ± 390, P = 0.99). Elongation indices such as the SCP FAZ feret diameter (μm) (738 ± 125 vs. 735 ± 138, P = 0.661), the minimum feret diameter of FAZ (μm) (584 ± 125 vs. 582 ± 110, P = 0.961), and the ratio FAZ feret diameter/minimum FAZ feret diameter (1.278 ± 0.116 vs. 1.267 ± 0.104, P = 0.861) were not different between the control and BTM groups.

The same was true regarding the SCP maximum inscribed circle diameter (μm) (472 ± 111 vs. 479 ± 116, P = 0.661), the FAZ area-maximum inscribed circle area (1.87 ± 0.91 vs. 5.84 ± 11.60, P = 0.984), and the area/perimeter index (128.40 ± 22.10 vs. 130.38 ± 23.35, P = 0.765).

No statistically significant difference was detected between controls and BTM patients regarding SCP FAZ convexity (0.918 ± 0.050 vs. 0.919 ± 0.047, P = 0.946), solidity (0.901 ± 0.054 vs. 0.905 ± 0.032, P = 0.676), circularity (0.720 ± 0.108 vs. 0.728 ± 0.099, P = 0.838), FAZ contour temperature (0.211 ± 0.043 vs. 0.210 ± 0.039, P = 0.907), and FAZ fractal dimension (1.725 ± 0.040 vs. 1.722 ± 0.050, P = 0.800). All the above are summarized in Table 2 and Figure 3.

Regarding DCP, the same parameters as with SCP were measured and compared between the control and BTM group. In DCP there was no significant difference between the control and BTM groups regarding the FAZ area (μm²) (564.368 ± 180.227 vs. 508.743 ± 127.250, P = 0.408) and the area of the FAZ convex hull (μm²) (652.366 ± 211.692 vs. 576.295 ± 141.311, P = 0.298).

Also, the DCP FAZ perimeter (μm) (3668 ± 735 vs. 3289 ± 474, P = 0.089) and the perimeter of FAZ convex hull (μm) (2905 ± 433 vs. 2726 ± 351, P = 0.224) were not different between the two groups. As in SCP above, the DCP feret diameter (μm), the minimum feret diameter of FAZ, and the ratio FAZ feret diameter/minimum FAZ feret diameter were all within the same range between the DCP control and BTM groups: 1029 ± 142 vs. 957 ± 105 (P = 0.113), 814 ± 139 vs. 767 ± 102 (P = 0.376), and 1.274 ± 0.096 vs. 1.255 ± 0.065 (P = 0.592), respectively.

Other indices tested for the DCP included the maximum inscribed circle diameter (μm) (580 ± 183 vs. 619 ± 109, P = 0.606), the FAZ area-maximum inscribed circle area (6.91 ± 22.00 vs. 1.75 ± 0.62 P = 0.113), and the area/ perimeter index (151.28 ± 21.37 vs. 151.46 ± 19.00, P = 0.763), all failing to reveal any statistically significant difference. The same was true for shape descriptors, solidity (0.869 ± 0.024 vs. 0.883 ± 0.021, P = 0.059), and FAZ fractal dimension (1.728 ± 0.048 vs. 1.731 ± 0.046, P = 0.992).

Interestingly, a statistically significant difference was detected between controls and BTM patients regarding DCP FAZ convexity (0.804 ± 0.044 vs. 0.856 ± 0.037, P = 0.013), circularity (0.535 ± 0.068 vs. 0.588 ± 0.065, P = 0.010), and DCP FAZ contour temperature (0.298 ± 0.029 vs. 0.276 ± 0.025, P = 0.014). The results are summarized in Table 3 and Figure 4.
We subsequently investigated if iron load and chelation therapy indices were related to the FAZ area and/or FAZ shape parameters in the BTM patients. FAZ area correlation to DSF dosage was moderate for the SCP ($r=0.541$, $P=0.025$), and strong for the DCP ($r=0.650$, $P=0.05$). There was also a moderately strong negative correlation between liver MRI/T2* and DCP circularity ($r=-0.492$, $P=0.038$). As lower liver MRI/T2* values represent increased iron load in the liver, the correlation indicates a positive correlation between liver iron load and DCP circularity. The strongest correlations as a whole were revealed between UIE and DCP convexity ($r=0.531$, moderate correlation, $P=0.019$), DCP circularity ($r=0.661$, strong correlation, $P=0.002$), and DCP contour temperature ($r=-0.591$, strong correlation, $P=0.008$). Spearman’s correlation results for all comparisons are presented in detail in Table 4.

**TABLE 2.** Foveal Avascular Zone Shape Descriptors—Superficial Capillary Plexus

<table>
<thead>
<tr>
<th>Shape Descriptors</th>
<th>Controls, Mean ± SD</th>
<th>BTM, Mean ± SD</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, $\mu m^2$</td>
<td>303.425 ± 118.753</td>
<td>312.224 ± 120.450</td>
<td>0.778</td>
</tr>
<tr>
<td>Area convex hull, $\mu m^2$</td>
<td>358.138 ± 135.507</td>
<td>344.970 ± 133.560</td>
<td>0.763</td>
</tr>
<tr>
<td>Perimeter, $\mu m$</td>
<td>2.301 ± 544</td>
<td>2.302 ± 527</td>
<td>0.884</td>
</tr>
<tr>
<td>Perimeter convex hull, $\mu m$</td>
<td>2.087 ± 384</td>
<td>2.095 ± 390</td>
<td>0.992</td>
</tr>
<tr>
<td>Feret diameter, $\mu m$</td>
<td>758 ± 125</td>
<td>755 ± 138</td>
<td>0.661</td>
</tr>
<tr>
<td>Minimum feret diameter, $\mu m$</td>
<td>584 ± 125</td>
<td>582 ± 110</td>
<td>0.961</td>
</tr>
<tr>
<td>Feret diameter/minimum feret diameter</td>
<td>1.278 ± 0.116</td>
<td>1.267 ± 0.104</td>
<td>0.861</td>
</tr>
<tr>
<td>Maximum inscribed circle diameter, $\mu m$</td>
<td>472 ± 111</td>
<td>479 ± 116</td>
<td>0.661</td>
</tr>
<tr>
<td>Area/maximum inscribed circle area</td>
<td>1.870 ± 0.915</td>
<td>3.846 ± 11.604</td>
<td>0.984</td>
</tr>
<tr>
<td>Area/perimeter</td>
<td>128 ± 22</td>
<td>130 ± 23</td>
<td>0.763</td>
</tr>
<tr>
<td>Convexity</td>
<td>0.918 ± 0.050</td>
<td>0.919 ± 0.047</td>
<td>0.946</td>
</tr>
<tr>
<td>Solidity</td>
<td>0.901 ± 0.034</td>
<td>0.905 ± 0.032</td>
<td>0.676</td>
</tr>
<tr>
<td>Circularity</td>
<td>0.720 ± 0.108</td>
<td>0.728 ± 0.099</td>
<td>0.838</td>
</tr>
<tr>
<td>Contour temperature</td>
<td>0.211 ± 0.043</td>
<td>0.210 ± 0.039</td>
<td>0.907</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.725 ± 0.040</td>
<td>1.722 ± 0.050</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Shape descriptors of the FAZ (mean values ± SD) and level of statistical significance ($P$) for the superficial capillary plexus of both the BTM and the control group. The level of statistical significance was set at 0.016 based on Hochberg’s step-up correction for multiple comparisons.

**FIGURE 3.** Box plots of the area and circularity index for both SCP and DCP, for the control and BTM groups. (A) Box plot of SCP area ($\mu m^2$) for the control and BTM groups. (B) Box plot of SCP circularity for the control and BTM groups. (C) Box plot of DCP area ($\mu m^2$) for the control and BTM groups. (D) Box plot of DCP circularity for the control and BTM groups.

**DISCUSSION**

Although the impact of BTM on the retina has been extensively investigated, possible microvascular changes in BTM patients were revealed between UIE and DCP convexity ($r=0.531$, moderate correlation, $P=0.019$), DCP circularity ($r=0.661$, strong correlation, $P=0.002$), and DCP contour temperature ($r=-0.591$, strong correlation, $P=0.008$). Spearman’s correlation results for all comparisons are presented in detail in Table 4.
have never been evaluated. Although significant FAZ size variability has been reported, FAZ outline was the parameter of choice in this pilot study, as it is obtained in all OCTA machines, can be readily evaluated for artifacts, and does not carry the burden of the various vascularity indices that vary widely across different manufacturers.

SCP and DCP are fundamentally different. DCP represents an area of slow circulation and relatively low intravascular hydrostatic pressure, more susceptible to ischemia and oxidative stress. FAZ alterations seem to be evident in the DCP, as it represents the most remote and vulnerable part of the retinal capillary plexus. DCP capillaries form spider-like "vortices," revealing an entirely different structure than that of SCP. DCP capillaries are perfused through other small-diameter vessels, either directly from SCP arterioles, or indirectly through anastomotic branches from the SCP itself.

SCP and DCP FAZ in Patients With Beta-Thalassemia Major

<table>
<thead>
<tr>
<th>Shape Descriptors</th>
<th>Controls, Mean ± SD</th>
<th>BTM, Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, µm²</td>
<td>564,368 ± 180,227</td>
<td>508,743 ± 127,250</td>
<td>0.408</td>
</tr>
<tr>
<td>Area convex hull, µm²</td>
<td>652,366 ± 211,692</td>
<td>576,295 ± 141,311</td>
<td>0.298</td>
</tr>
<tr>
<td>Perimeter, µm</td>
<td>3,668 ± 735</td>
<td>3,289 ± 474</td>
<td>0.089</td>
</tr>
<tr>
<td>Perimeter convex hull, µm</td>
<td>2,905 ± 433</td>
<td>2,726 ± 331</td>
<td>0.224</td>
</tr>
<tr>
<td>Feret diameter, µm</td>
<td>1,029 ± 142</td>
<td>957 ± 105</td>
<td>0.113</td>
</tr>
<tr>
<td>Minimum feret diameter, µm</td>
<td>814 ± 139</td>
<td>767 ± 102</td>
<td>0.376</td>
</tr>
<tr>
<td>Feret diameter/minimum feret diameter</td>
<td>1.274 ± 0.096</td>
<td>1.253 ± 0.065</td>
<td>0.592</td>
</tr>
<tr>
<td>Maximum inscribed circle diameter, µm</td>
<td>580 ± 183</td>
<td>619 ± 109</td>
<td>0.606</td>
</tr>
<tr>
<td>Area/maximum inscribed circle area</td>
<td>6.91 ± 22.0</td>
<td>1.75 ± 0.62</td>
<td>0.113</td>
</tr>
<tr>
<td>Area/perimeter</td>
<td>151 ± 21</td>
<td>151 ± 19</td>
<td>0.763</td>
</tr>
<tr>
<td>Convexity</td>
<td>0.804 ± 0.044</td>
<td>0.836 ± 0.037</td>
<td>0.013</td>
</tr>
<tr>
<td>Solidity</td>
<td>0.869 ± 0.024</td>
<td>0.883 ± 0.021</td>
<td>0.059</td>
</tr>
<tr>
<td>Circularity</td>
<td>0.535 ± 0.068</td>
<td>0.588 ± 0.065</td>
<td>0.010</td>
</tr>
<tr>
<td>Contour temperature</td>
<td>0.298 ± 0.029</td>
<td>0.276 ± 0.025</td>
<td>0.014</td>
</tr>
<tr>
<td>Fractal dimension</td>
<td>1.728 ± 0.048</td>
<td>1.731 ± 0.046</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Shape descriptors of the FAZ (mean values ± SD) and level of statistical significance (P) for the DCP of both the BTM and the control group. The level of statistical significance was set at 0.016 based on Hochberg’s step-up correction for multiple comparisons. Statistically significant values are in bold.

FIGURE 4. Box plots of the convexity and contour temperature indexes for both SCP and DCP for the control and BTM groups. (A) Box plot of SCP convexity for the control and BTM groups. (B) Box plot of SCP contour temperature for the control and BTM groups. (C) Box plot of DCP convexity for the control and BTM groups. (D) Box plot of DCP contour temperature for the control and BTM groups.
Using shape descriptors, we demonstrated a more circular, convex, and less complex (with less multilobular contour) DCP FAZ in BTM patients than in normal controls. This seems to be paradoxical, as the notion is that the more circular, the more normal FAZ appearance is. Increased circularity could be an equivalent to RVT, which is not uncommon in BTM patients. RVT in BTM patients increases with age and is attributed to the mild chronic anemia between transfusions, resulting in tissue hypoxia. RVT is also well documented in chronically anemic patients (inverse relationship with hematocrit).

RVT can be evaluated as a function of venous length. For a tortuous vein the distance between two points in its course is by definition longer than that of a less tortuous path between the same points. If a similar effect could be expected in FAZ, it would be expressed as an arcuate or multilobular deformation of the FAZ defining vessels. This deformation could be expressed only as a centrifugal bending of FAZ capillaries leading to an overall increase in circularity. A centripetal deformation is rather unlikely, as the retinal tissue structure in the foveola is arranged in a centrifugal manner, and is incompatible with a centripetal or even randomly tortuous deformation.

Both venous tortuosity and DCP changes could be attributed to the hyperdynamic circulation due to chronic anemia. BTM patients usually have an increased cardiac index, heart rate and stroke volume, lower systolic blood pressure, and a blunted blood pressure temporal variability, all consistent with a markedly decreased systemic vascular resistance.55,56 DCP changes could be related to unique compensatory/autoregulatory responses to these systemic hemodynamic alterations. DCP’s autoregulatory response seems to be independent of that of the SCP, further supporting the notion that DCP is a discrete, sensitive, and accurate index of vascular changes.47

The observed DCP changes could also be viewed in the context of local vascular remodeling, including capillary dropout, arteriolar-venular anastomoses, and sea fan neovascularization. These changes are thought to be due to repeated episodes of vascular closure and reopening, or increased oxidative stress and ischemia in poorly perfused areas, especially between transfusions.

In order to correlate our findings with the known causative agents of BTM retinal abnormalities, namely, the iron accumulation and chelation therapy, we further investigated a possible correlation between FAZ shape descriptors and parameters related to the iron load. We found a moderate negative correlation ($r = -0.492$) between DCP circularity and liver T2* values, suggesting increased circularity with increasing liver iron load (as lower liver T2* values are associated with increased iron load).

There was also a moderate positive correlation between mean values of UIE during the last three years and DCP convexity ($r = 0.531$), and a strong positive correlation to DCP circularity ($r = 0.661$) and DCP contour temperature ($r = 0.650$). A moderate positive correlation ($r = 0.541$) was revealed between DSF mean dosage (mg/month) and SCP area, and a strong correlation to the DCP area (r = 0.650).

Urinary iron excretion values depend on both iron load (total body iron) and DSF dosage. The higher the iron load and/or DSF dosage, the more elevated the mean iron levels in the urine. Thus, it seems probable that both iron overload and DSF dosage contribute to the observed DCP changes.

Iron accumulation results in endothelial dysfunction due to oxidative stress, by catalyzing the conversion of oxygen into highly reactive free radicals (superoxide-dioxide Haber-Weiss reaction). Superoxide alone is capable of producing damaging effects in mitochondria.50 The highly reactive hydroxyl radical is produced in the presence of ferrous iron ($Fe^{2+}$) by the decomposition of hydrogen peroxide (Fenton reaction).51,52 Experimental evidence suggests that iron has the potential to reduce endothelium-derived nitric oxide bioactivity either directly (decreased nitric oxide synthase activity) or indirectly (increased membrane lipid peroxidation generating lipid peroxyl radicals).53,54 Microcirculatory hemodynamics in the retina seem to be affected by the nitric oxide synthase/nitric oxide pathway.55,56 Nitric oxide level is well documented to be decreased in BTM patients.57–59

Human vascular endothelial cells incubated with thalassemic serum present functional disturbances including increased levels of soluble adhesion molecules, reduced mitotic potential, and morphologic changes reminiscent of apoptosis.60,61 Subsequent in vivo studies confirmed the presence of endothelial dysfunction in BTM patients.62,63

Interestingly, no correlation was found between DCP and serum ferritin levels. It is, though, known that ferritin levels do not always reflect correctly the iron load, and are not always good predictors of iron load–related complications.64,65 Ferritin trends, rather than absolute values, provide useful information. Other measurements such as non-transferrin

<table>
<thead>
<tr>
<th>Shape Descriptors</th>
<th>Heart MRI/T2*</th>
<th>Liver MRI/T2*</th>
<th>Ferritin</th>
<th>Ferritin 3-Year Avg.</th>
<th>UIE 3-Year Avg.</th>
<th>DSF 3-Year Avg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial capillary plexus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>0.364</td>
<td>0.150</td>
<td>0.088</td>
<td>0.729</td>
<td>-0.008</td>
<td>0.975</td>
</tr>
<tr>
<td>Perimeter</td>
<td>0.395</td>
<td>0.116</td>
<td>0.166</td>
<td>0.510</td>
<td>-0.062</td>
<td>0.796</td>
</tr>
<tr>
<td>Convexity</td>
<td>-0.266</td>
<td>0.301</td>
<td>-0.276</td>
<td>0.268</td>
<td>0.124</td>
<td>0.602</td>
</tr>
<tr>
<td>Circularity</td>
<td>-0.147</td>
<td>0.573</td>
<td>-0.332</td>
<td>0.178</td>
<td>0.248</td>
<td>0.291</td>
</tr>
<tr>
<td>Contour temperature</td>
<td>0.281</td>
<td>0.275</td>
<td>0.316</td>
<td>0.201</td>
<td>-0.155</td>
<td>0.514</td>
</tr>
<tr>
<td>Deep capillary plexus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td>0.017</td>
<td>0.948</td>
<td>0.015</td>
<td>0.951</td>
<td>0.083</td>
<td>0.729</td>
</tr>
<tr>
<td>Perimeter</td>
<td>-0.070</td>
<td>0.790</td>
<td>0.123</td>
<td>0.627</td>
<td>-0.065</td>
<td>0.787</td>
</tr>
<tr>
<td>Convexity</td>
<td>0.259</td>
<td>0.316</td>
<td>-0.384</td>
<td>0.115</td>
<td>0.138</td>
<td>0.563</td>
</tr>
<tr>
<td>Circularity</td>
<td>0.325</td>
<td>0.203</td>
<td>-0.492</td>
<td>0.038</td>
<td>0.290</td>
<td>0.214</td>
</tr>
<tr>
<td>Contour temperature</td>
<td>-0.259</td>
<td>0.316</td>
<td>0.392</td>
<td>0.108</td>
<td>-0.178</td>
<td>0.453</td>
</tr>
</tbody>
</table>

Statistically significant values are in bold. Heart MRI/T2*, MRI myocardial T2* relaxation time; Liver MRI/T2*, MRI myocardial T2* relaxation time; Ferritin, current serum ferritin level; Ferritin 3-Year Avg., average ferritin level for the last three years; UIE 3-Year Avg., average urinary iron excretion for the last three years; DSF 3-Year Avg., desferrioxamine mean monthly dosage during the last three years; r, Spearman’s regression coefficient; P, level of statistical significance.
bound iron and labile plasma iron, are alternatives to ferritin serum markers but are not routinely available or completely validated.65,66

Another possible explanation for the DCP alterations includes changes in RBC deformability, an essential feature of RBCs enabling them to pass through the smallest capillaries, as retinal capillary diameter is smaller than that of the RBCs (6–8 μm). Deformability depends on the integrity of cytoskeletal proteins (spectrin, ankyrin, band 3), intracellular ion concentrations, intracellular water (viscosity), and membrane surface-to-volume ratio,67 factors that may be altered in BTM patients. Of particular interest is the increase in intracellular Ca2+ in BTM patients, which has been documented to induce RBC membrane deformations,68,69 RBC stiffness could probably act as an internal splint, the column of RBCs pushing like a ram and blunting the angles of FAZ outline.

DSF is capable of reducing iron levels, alleviating the detrimental effects of iron accumulation. On the other hand, a higher mean DSF dosage is mandated by high iron levels. It is possible that in our cases, the correlation between DSF dosage and SCP and DCP area may in part be indirect and related to elevated primary iron levels.

Regarding the possibility that DSF treatment may influence SCP and DCP changes, one should mention the many and well-documented DSF retinal side effects, including night blindness, impaired color vision, loss of visual field, reduced visual acuity, and retinal pigment epithelium (RPE) degeneration. DSF toxicity is attributed to the iron chelation (iron, copper, aluminum) on RPE, resulting in dysfunction or due to defective vasoregulation.3,70,71

DSF may also directly influence SCP and DCP by upregulating the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF).72–74 VEGF has been associated with capillary enlargement and efficient response to vasodilators as is nitric oxide.75–76 In vitro studies revealed mRNA levels of nitric oxide synthase to be profoundly increased by DSF.77 An enlargement of the SCP and DCP capillaries could lead to outward “bending” (inward bending is prevented by the retinal structure at the foveola). It is possible that an initial phase of oxidative stress-induced capillary damage is followed by a second phase of DSF-induced capillary deformation.

Our study has several limitations, including the relatively small number of patients and the lack of analysis of the SCP and DCP vascular plexus itself, rather than SCP and DCP FAZ. Nevertheless, we believe that given the nature of a pilot study we should concentrate on robust and uniform measures such as those regarding FAZ, rather than more complex and not uniformly defined quantities such as vascular density or vascular patterns.

Another limitation is that the axial length of the eye was not used for scale correction of the FAZ size. This, however, is in part balanced by the minor refractive error in both BTM and control groups. Notably, scale-independent shape descriptors, such as ratios (feret diameter/minimum feret diameter, area/maximum inscribed circle area, area/perimeter), solidity, fractal dimension, circularity, convexity, and contour temperature are not influenced at all.

Regarding the advantages of our study, one should mention the novelty of the approach to the FAZ characteristics by the utilization of shape descriptors that have not been commonly used in other studies, and the automated detection of the FAZ. Also, our study is, to the best of our knowledge, the first study of FAZ in BTM. Further studies should be conducted to elucidate the complex relationship between SCP, DCP and the iron load and chelation therapy.

In conclusion, retinal capillary plexuses and especially DCP seem to present unique morphologic changes in BTM patients.

These are not changes regarding the SCP and DCP FAZ area, but specific FAZ shape descriptors, indicating minor but detectable changes in the FAZ of patients with BTM. Moreover, the aforementioned changes correlate well with iron load and chelation therapy parameters of BTM patients. The clinical importance and pathophysiologic implications of these changes remain to be elucidated through further studies.

Acknowledgments

Disclosure: I. Georgalas, None; G. Makris, None; D. Papaconstantinou, None; P. Petrou, None; E. Chalkiadaki, None; K. Droutas, None; K. Andreasos, None; M. Kakakis, None

References


