Evaluation of changes in the stromal and vascular structure of the choroid in patients with iron deficiency and iron deficiency anemia

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ABSTRACT

Purpose: To objectively measure the possible changes in the vascular structure of the choroid in patients with iron deficiency and iron deficiency anemia (IDA) using choroidal vascularity index (CVI) and to compare with healthy control group

Materials and Methods: The study included 74 eyes of 74 female subjects without any ocular disease. Of the participants, 25 had IDA (group 1) and 23 had iron deficiency (group 2). 26 subjects were determined as healthy control group (group 3). Ophthalmological examination findings and laboratory tests were recorded. Subfoveal choroidal thickness (SFCT) was measured by swept source optical coherence tomography (OCT) images. The central 1000 µm choroidal area and luminal area were determined by binarising the OCT image. The CVI result was obtained by proportioning luminal area to total choroidal area.

Results: Mean SFCT was significantly lower in group 1 compared to both other groups ($p<0.001$). It was similar in groups 2 and 3. Mean CVI was significantly different between all groups. It was lowest in group 1 and highest in group 3 ($p=0.007$). Statistically significant positive correlation existed between ferritin level and SFCT and CVI in groups 1 and 2.

Conclusion: The findings of our study show that choroidal thickness decreased only in IDA, whereas CVI is decreased in both IDA and iron deficiency. The decrease in CVI in iron deficiency suggests that the choroidal structure is affected in early period before development of anemia.

Keywords: Iron deficiency, Iron deficiency anemia, Choroidal vascularity index.

INTRODUCTION

Iron deficiency anaemia (IDA) has been a common nutritional disorder for many years, affecting the health of millions of individuals worldwide.[1] Iron deficiency is also one of the underlying causes of various metabolic disorders that cause morbidity and mortality in susceptible populations around the world.[2] The eye is one of the organs affected by iron deficiency and IDA. IDA is known to increase the risk of optic neuropathy and retinopathy due to low iron and haemoglobin levels.[3-6] Fundus findings are thought to be related to changes in blood flow and hypoxia, although the exact cause is unknown. Iron is one of the most abundant metals in the retina and plays a critical role in retinal physiology and pathologies.[7]

The pathological effects of many systemic diseases on the retina, choroid and optic disc can be evaluated by examination and in vivo imaging methods. In previous studies, changes in retinal vascular and choroidal thickness in patients with IDA have been investigated with imaging modalities such as optical coherence tomography (OCT) and OCT-angiography (OCTA).[8-10] The choroidal vascularity index (CVI) is a marker that shows the ratio of the luminal (vascular) area of the choroid to the total choroidal area (vascular and stromal), thus providing more specific information about vascular changes.[11] Since many factors can affect choroidal thickness, CVI is more sensitive for the assessment of vascular changes. Although retinal vascular changes have been described in detail in IDA, choroidal vascular changes have not been evaluated.

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Received: 13.06.2024
Accepted: 14.09.2024

J Ret-Vit 2024; 33: 260-265

DOI:10.37845/ret.vit.2024.33.42

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In our study, we aimed to objectively measure the possible changes in the vascular structure of the choroid in patients with iron deficiency and IDA with CVI and to compare them with the healthy control group.

MATERIALS AND METHODS

Approval of the study was granted by the Clinical Research Ethics Committee following the acquisition of data use permission for the examination of the medical records of patients who had applied to the ophthalmology outpatient clinic between January 2020 and December 2022. The study was conducted in accordance with the ethical standards set in the Declaration of Helsinki and the principles of publication ethics.

The medical records of the patients who were examined in ophthalmology and internal medicine outpatient clinics of our hospital and who had macular imaging with OCT were analysed. Between 18 and 40 years of age, female patients with IDA (group 1), iron deficiency (group 2) and healthy female subjects (group 3) were identified by an internal medicine specialist. Patients with any known eye disease (glaucoma, uveitis, amblyopia or strabismus, etc.), history of ocular surgery, and additional systemic diseases (diabetes mellitus, hypertension, autoimmune or autoinflammatory diseases, etc.) were excluded. Patients with hypermetropia greater than 1 diopter and myopia greater than 2 diopters in terms of spherical refraction were excluded from the study. Patients with serum ferritin 15 mg/dl, serum iron 50 µg/dl, mean red blood cell volume (MCV) 80, mean red blood cell haemoglobin (MCHC) 30 and transferrin saturation less than 15% were included in the iron deficiency group.[12] Among the patients who were found to have iron deficiency, those with haemoglobin below 12 g/dl according to the WHO (World Health Organization) classification were categorised as the IDA group.[13] Patients who had previously received treatment for iron deficiency or IDA and smokers were excluded from the study.

Haemoglobin (Hb), haematocrit (HCT), MCV, MCHC, red blood cell count (RBC), ferritin, serum iron, total iron binding capacity and transferrin saturation values were recorded. Best corrected visual acuity (BCVA), biomicroscopic examination findings, intraocular pressure and fundoscopic examination findings were recorded by randomly selecting one eye of all patients included in the study. Foveal thickness (FT) was measured from swept source OCT (SS-OCT) images (DRI OCT Triton, Topcon Inc., Tokyo, Japan) and subfoveal choroidal thickness (SFCT) was measured from a single scan passing through the centre of the fovea. For CVI measurement, a horizontal OCT scan passing through the foveal center was selected and ImageJ software (Version 1.53, NIH, Bethesda, MD, USA; https://imagej.nih.gov/ij/) was used. The selected OCT image was opened with ImageJ software and binarised using a semi-automatic method. A 1000 µm wide area in the central subfoveal region was marked (Figure 1). After the image was converted to 8-bit, Niblack autolocal threshold was applied to sharpen the choroid-sclera intersection region. The selection of the subfoveal choroidal area was performed more precisely in this way. Using the polygon tool with ROI Manager, the borders of the choroidal region were determined in a 1000 µm wide area with the upper border of the retinal pigment epithelium and the lower border of the choroid-sclera intersection. The image was converted to RGB colour and the colour threshold tool was used to reveal the dark pixels. The total area of the selected region and the area of dark pixels were calculated. Lumen area was defined as the area of dark pixels. Stromal area was calculated by subtracting the lumen area from the total area. The ratio between the lumen area and the total area was determined as CVI. All measurements from OCT images were performed by one person (MFKD) and group information was hidden.

Statistical analysis

Statistical Package for the Social Sciences software version 20 (IBM, SPSS version 20.0; IBM, New York, NY, USA) was used for statistical analysis of the data. The distribution of quantitative data was determined by Shapiro-Wilk test. Descriptive statistics were expressed as mean ± standard deviation for normally distributed variables. Student t test was used for intergroup comparisons and Pearson

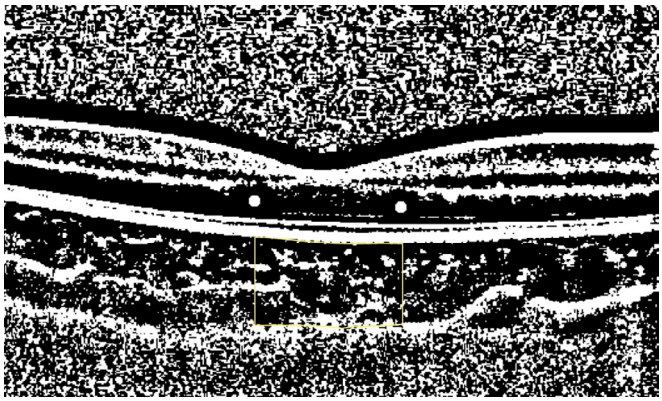

Figure 1: *Semi-automatic binarisation of optical coherence tomography image in a healthy eye using ImageJ software and marking of 1000 µm wide choroidal area in the central subfoveal region*

correlation test was used for within-group correlation analyses. The results were evaluated within the 95% confidence interval and the significance level was set as 0.05 in all statistical tests.

RESULTS

The study included 74 eyes of 74 female patients. The mean age was 31.56 ± 6.2 years in group 1 (n=25), 31.41 ± 7.3 years in group 2 (n=23) and 30.68 ± 8.2 years in group 3 (n=26). The mean age was similar between the groups (p=0.777). Laboratory measurements and comparisons of the groups are summarised in Table 1.

BCVA was 10/10 in all cases. There was no significant difference between the groups in terms of refractive error (spherical equivalent) and IOP measurements (p=0.543 and p=0.422, respectively). Mean SFCT was similar in groups 2 and 3 (p=0.093). In group 1, it was significantly lower than the other two groups (p<0.001). Mean CVI was significantly different in all three groups (p=0.007). The lowest was in group 1 and the highest was in group 3 (Table 2).

In the within-group correlation analysis, no significant correlation was found between spherical equivalent and IOP and laboratory measurements. Similarly, there was no significant correlation between spherical equivalent and IOP and OCT measurements. The correlation findings between OCT and laboratory measurements of all groups are shown in Table 3. There was a statistically significant positive correlation between ferritin and SFCT and CVI in both group 1 and group 2. In group 3, there was no significant correlation between OCT and laboratory findings.

DISCUSSION

Compared to its area, the choroid is the tissue with the highest blood flow in the body. It supplies oxygen and nutrients to the outer layers of the retina and ensures the removal of metabolic residues.[14] In addition to many ocular diseases, changes in the structure of the choroid were observed in systemic diseases due to its dense vascular structure.[15] In OCTA studies performed in IDA cases, a decrease in capillary plexus was reported.[9,16] In previous years,

Table 1: *Laboratory results and comparisons of iron deficiency anaemia (group 1), iron deficiency (group 2) and healthy control (group 3) subjects*

	Group 1	Group 2	Group 3	p value
Hb	10.54 ± 1.03	13.48 ± 1.34	14.28 ± 1.04	<0.001
HTC	34.06 ± 3.14	40.70 ± 3.31	41.75 ± 3.24	<0.001
MCV	74.21 ± 5.34	82.35 ± 3.98	81.94 ± 2.82	<0.001
MCHC	23.19 ± 2.71	27.34 ± 2.73	28.02 ± 0.83	<0.001
RBC	4.56 ± 0.29	4.97 ± 0.43	5.10 ± 0.48	<0.001
Ferritin	6.89 ± 2.90	13.60 ± 5.28	55.01 ± 30.36	<0.001
Serum iron	28.88 ± 19.91	49.40 ± 18.21	75.09 ± 14.12	<0.001
TIBC	476.96 ± 68.41	418.66 ± 40.03	388.63 ± 26.46	<0.001
TS	6.54 ± 4.75	13.92 ± 8.25	19.45 ± 2.70	<0.001

Hb: haemoglobin, HTC: haematocrit, MCV: mean red blood cell volume, MCHC: mean red blood cell haemoglobin, RBC: red blood cell count, TIBC: total iron binding capacity, TS: transferrin saturation

Table 2: *Comparison of ophthalmic examination and optical coherence tomography findings between groups*

	Group 1	Group 2	Group 3	p* value	p$^{\#}$ value	p$^{\%}$ value	p$^{\&}$ value
SE	- 0.95 ± 1.12	- 2.34 ± 1.19	- 1.43 ± 1.94	0.442	0.654	0.487	0.543
IOP	16.5 ± 2.4	14.9 ± 2.1	15.6 ± 3.0	0.532	0.353	0.498	0.422
SFCT	229.12 ± 14.04	248.25 ± 19.58	251.18 ± 18.01	0.002	0.093	<0.001	<0.001
CVI	0.64 ± 0.02	0.66 ± 0.01	0.69 ± 0.03	0.023	0.030	0.005	0.007

SE: spherical equivalent, IOP: intraocular pressure, SFCT: subfoveal choroidal thickness, CVI: choroidal vascularity index
* p value for group 1 vs group 2, $^{\#}$ p value for group 2 vs group 3, $^{\%}$ p value for group 1 vs group 3, $^{\&}$ p value between all groups

Table 3: *In-group comparisons of laboratory and optical coherence tomography findings*

	Group 1 (p value)	Group 2 (p value)	Group 3 (p value)
Hb vs SFCT	0.095	0.177	0.789
Hb vs CVI	0.841	0.744	0.994
HTC vs SFCT	0.358	0.075	0.622
HTC vs CVI	0.365	0.354	0.771
MCV vs SFCT	0.066	0.850	0.199
MCV vs CVI	0.969	0.596	0.465
MCHC vs SFCT	0.093	0.415	0.205
MCHC vs CVI	0.258	0.670	0.534
RBC vs SFCT	0.522	0.312	0.900
RBC vs CVI	0.238	0.682	0.609
Ferritin vs SFCT	0.012*(r=0.443)	0.023*(r=0.298)	0.074
Ferritin vs CVI	0.009*(r=0.480)	0.019*(r=0.309)	0.103
Serum iron vs SFCT	0.115	0.801	0.279
Serum iron vs CVI	0.862	0.515	0.360
TIBC vs SFCT	0.103	0.381	0.856
TIBC vs CVI	0.987	0.852	0.639
TS vs SFCT	0.108	0.327	0.161
TS vs CVI	0.985	0.859	0.187

* comparisons with significant correlations
Hb: haemoglobin, SFCT: subfoveal choroidal thickness, CVI: choroidal vascularity index, HTC: haematocrit, MCV: mean red blood cell volume, MCHC: mean red blood cell haemoglobin, RBC: red blood cell count, TIBC: total iron binding capacity, TS: transferrin saturation

decreased choroidal thickness was shown in both adult and childhood age groups in IDA.[10,17] However, it is known that choroidal thickness is affected by many physiological and pathological factors. Therefore, CVI measurement has been performed in recent years to better evaluate not only the thickness of the choroid but also the vascular changes in its structure.[18] While choroidal thickness allows us to evaluate both the stroma and vascular structure together, CVI measurement allows us to evaluate only the changes in the vascular structure of the choroid independent of possible changes in the stroma. In addition, choroidal thickness measurement can be performed relatively easily and consistently. CVI measurement requires the use of an auxiliary software and can produce incorrect results if not performed carefully. Although choroidal thickness and retinal vascular changes have been investigated in IDA[9,10,16,17,19], there is no study in the literature evaluating CVI in these patients. In this study, choroidal thickness and CVI were evaluated in patients with iron deficiency and IDA and healthy subjects.

In our study, choroidal thickness was similar in patients with iron deficiency compared to healthy subjects, whereas it was significantly less in IDA patients compared to the other two groups. Previous studies have also shown that choroidal thickness decreased in IDA.[10,17] However, there is no clear explanation for the decrease in choroidal thickness. Iron deficiency has different pathophysiological consequences. At the tissue level, separation of oxygen from haemoglobin is facilitated by the local effects of substances secreted due to hypoxia. Systemically, decreased oxygen carrying capacity is compensated by increased cardiac output. Since the mechanism related to the above is multifactorial, it has not been clearly demonstrated. Another hypothesis is that blood flow is affected due to decreased deformability of erythrocytes and increased viscosity in iron deficiency and retinochoroidal circulation is affected as a consequence.[20]

The results of our study show that choroidal thickness was decreased only in the group with IDA, whereas CVI was also decreased in the iron deficiency group. These findings may be interpreted as a potential vascular constriction or

decrease in blood flow in the choroid before the development of anaemia, but iron deficiency results in a potential vascular constriction or decrease in blood flow. Previous OCTA studies in patients with IDA have reported decreased vascular density in the superficial and deep capillary plexus. Korkmaz et al. reported a significant decrease in vessel density in all areas of the retinal superficial capillary plexus and a significant decrease in some areas of the deep capillary plexus.[19] In another study, a significant decrease in vessel density was found in the superficial capillary plexus.[9] In the same study, choriocapillary vessel density was found to be lower. In a study in which vascular density was examined in the macula and peripapillary region, a similar decrease in vascular density was found and it was reported that retinal ischaemia could be demonstrated with OCTA before clinical retinopathy developed.[16] Although there is no publication in the literature in which CVI was examined, previous studies in which retinal vascular changes were examined, when evaluated together with the results of our study, suggest that both retinal and choroidal circulation is impaired before clinical findings occur in iron deficiency and IDA.

When the relationship between laboratory findings and OCT findings were analysed separately in all groups, it was observed that there was a significant positive correlation only between ferritin level and SFCT and CVI in the two patient groups with IDA and iron deficiency. No correlation was found in the control group. In previous studies in which choroidal thickness was analysed in patients with IDA, it was reported that ferritin level was significantly positively correlated with choroidal thickness.[10,17] Unlike in our study, ferritin level was correlated with CVI and this correlation was found to be higher compared to choroidal thickness. This may be interpreted that ferritin level has a predictive potential in terms of choroidal vascular changes in patients with iron deficiency and IDA and CVI may be a more significant marker compared to choroidal thickness.

Limitations of the study

The small number of patients and the retrospective nature of our study can be considered as the limitations of our study. In addition, since it is not possible to leave the patients who were found to have IDA without treatment, the time of onset and duration of the disease are other weaknesses of our study.

CONCLUSION

In summary, our study suggests that there is a reduction in the vascular structure before the choroidal thickness changes in iron deficiency before the development of anaemia. When considered together with the decrease in vascular density in retinal capillary plexuses shown in previous studies with OCTA, it is suggested that ocular blood flow may be decreased in these patients. However, prospective studies including larger patient groups and including post-treatment changes are needed to better evaluate the changes in retinal and choroidal circulation in this patient group.

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