CHOROIDAL VASCULARITY INDEX IN HYDROXYCHLOROQUINE TOXIC RETINOPATHY

A Quantitative Comparative Analysis Using Enhanced Depth Imaging In Spectral Domain Optical Coherence Tomography

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Purpose: To investigate choroidal involvement in eyes of patients treated with hydroxychloroquine (HCQ), by quantifying the choroidal vascularity index (CVI) and other choroidal biomarkers.

Methods: Vertical enhanced depth imaging spectral domain optical coherence tomography (SD-OCT) scans were performed in eyes with either advanced-stage or mild HCQ toxic retinopathy, as well as in healthy age-matched and sex-matched controls. Based on SD-OCT scans, the subfoveal and mean choroidal thickness (ChT) was measured. The CVI, total choroidal area (TCA), luminal choroidal area (LCA), and stromal choroidal area (SCA) were calculated based on a binarization image process. These variables were computed and compared between the three groups (i.e., advanced stage, mild toxicity, and healthy controls).

Results: Forty-eight eyes of 47 patients under HCQ (26 eyes presented with advanced stage HCQ toxicity and 22 eyes with mild toxicity) and 34 eyes of 31 healthy controls were included. Both CVI and ChT were significantly different between the three groups ($P < 0.001$, $P < 0.001$). When comparing the advanced stage toxicity group to healthy controls, both the subfoveal and the mean ChT were diminished ($P < 0.001$). The CVI, TCA, LCA, and SCA were significantly lower in the advanced stage of toxicity group when compared with controls ($P < 0.001$, <0.00001, <0.0001, and $P = 0.0094$, respectively).

Conclusion: Our study suggests that eyes with HCQ toxic retinopathy, especially at advanced stages, present with choroidal impairment, giving further pathophysiological insights into the unfolding of this retinal toxicity.

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Hydroxychloroquine (HCQ) is a long-term treatment with several indications in autoimmune diseases, given its anti-inflammatory, antithrombotic, and recently identified immunomodulatory properties.[1,2] As of its recent indications in pediatric inflammatory disorders, neurology, and oncology, a large and growing cohort of long-term users is predicted.[2–4] Retinal dysfunction is the main ocular side effect of HCQ, occurring after long-term use of the drug. Nevertheless, the retinal toxicity of HCQ has initially been considered rare (estimated occurrence in 0.5%–2.0% of long-term users), as the diagnosis was based on late-stage bull eye maculopathy.[5,6] However, without an early diagnosis and without treatment cessation, this toxic retinopathy can lead to severe bilateral visual loss and a subsequent loss of autonomy for the patient. The American Academy of Ophthalmology,[6] the Royal College of Ophthalmology,[7] and more recently the American College of Rheumatology, American Academy of Dermatology, Rheumatologic Dermatology Society, and American Academy of Ophthalmology 2020 Joint Statement recommendations have all highlighted the importance of strict monitoring in patients undergoing treatment by HCQ.[8] Regarding

the retinal toxicity, modern screening tools allow the detection of early-stage toxicity, with at least one objective structural test and one subjective functional test to be performed to make a diagnosis.[6] In detail, the recommended tests for primary routine screening are automated visual fields and spectral domain optical coherence tomography (SD-OCT) given their wide availability.[9,10]

High-resolution SD-OCT has allowed to demonstrate that a significant outer retinal layer volumetric thinning, affecting the photoreceptor layer, the interdigitation zone, and the ellipsoid zone (EZ), were present from the early stages of retinal involvement.[9,10] These structural abnormalities were followed by retinal pigment epithelium (RPE) damage and choroidal thinning in the late stages of HCQ toxicity.[10,11] Interestingly, besides the retinal involvement, recent literature suggests that choroidal thinning also plays a role in the physiopathology of HCQ toxicity.[12,13]

First introduced in 2016, the choroidal vascularity index (CVI) is a relatively new OCT-based biomarker allowing a quantitative analysis of the vascular structure of the choroid.[14] CVI has been shown to be a useful tool in assessing various retinal diseases, such as ocular inflammatory disease[15] and for monitoring geographic atrophy progression in age-related macular degeneration.[16] Recent studies suggested that CVI is a more reproducible and accurate biomarker than choroidal thickness, as it takes into account the morphological and vascular changes in the choroid.[14,15]

In this study, we aim to investigate choroidal involvement in eyes of patients treated with HCQ, by quantifying the CVI and comparing the CVI between eyes with advanced stages of HCQ toxicity, eyes of patients treated by HCQ with mild retinal toxicity, and healthy controls.

Methods

Study Participants

In this study, we retrospectively collected data of subjects from the Department of Ophthalmology of University Paris Est, in Creteil, France, and from IRCCS Ospedale San Raffaele, Milan, Italy. The study was conducted in agreement with the Declaration of Helsinki for research involving human subjects and was approved by the Ethics Committee.

Inclusion criteria were (1) patients having undergone a treatment with HCQ for at least one year; (2) mild to advanced stages of retinal toxicity, as diagnosed on multimodal imaging and functional tests; and (3) clear ocular media to ensure proper image quality. In addition, a sex-matched, age-matched control group was included.

Two groups were distinguished: the HCQ group, called group 1, and the healthy sex-matched and age-matched control group (group 2).

Within group 1, two subgroups were identified, according to the degree of retinal toxicity: (a) mild toxicity (group 1a) and (b) advanced-stage toxic retinopathy (group 1b).

For group 1, the disease for which the treatment was prescribed, the daily dose in milligrams per kilogram of weight (mg/kg), the treatment duration in years, the body weight, and the cumulative dose in grams were recorded.

Accordingly with Marmor definition and grading of toxicity,[11,17] diagnostic criteria for HCQ retinopathy were based on the presence of parafoveal or bull eye retinal damage confirmed by at least three different tests, including fundus examination, macular visual fields, multifocal electroretinogram (mfERG), SD-OCT, and fundus autofluorescence (FAF).

Eyes included in group 1a showed patchy parafoveal damage in SD-OCT or macular field. As for eyes included in the advanced stage of retinal toxicity group (group 1b), they had either moderate retinal toxicity, defined as ring damage sparing the RPE, or severe bull eye damage on fundus examination or FAF, with RPE involvement on SD-OCT (visible retinopathy). Figure 1 shows an example of multimodal imaging of patient with an advanced-stage toxic retinopathy included in group 1b.

If both eyes were corresponding to the inclusion criteria and to rule out any selection bias, the eye with the higher quality of SD-OCT images was selected as the study eye.

The exclusion criteria were (1) poor-quality of SD-OCT, including a poor framing and illumination, and (2) other concomitant macular diseases in the study eye.

All subjects included in group 1 underwent a complete ophthalmologic examination, including Snellen BCVA, that was converted to logarithm of the minimum angle of resolution (LogMAR) for statistical analysis and slit-lamp biomicroscopy. All patients underwent multimodal imaging including infrared reflectance (IR), FAF, macular visual field,

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The authors have no competing interests to declare.

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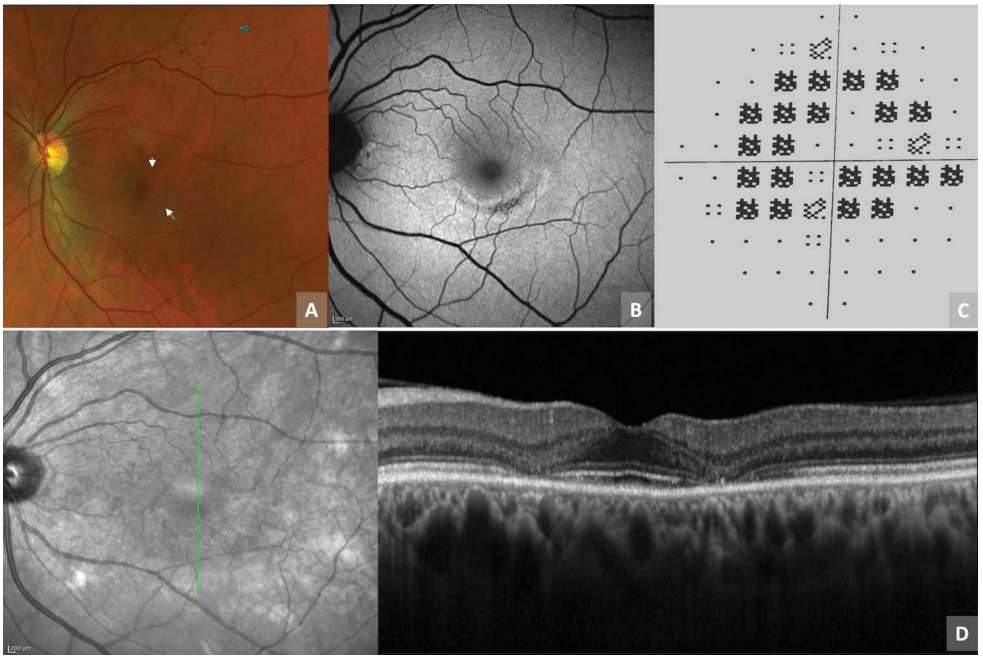


Fig. 1. Multimodal imaging of a left eye of patient presenting with hydroxychloroquine retinopathy with typical flying saucer sign: (**A**) Fundus photography showing discreet retinal pigmentary change at the paracentral area. **B.** Fundus autofluorescence revealing a circular inferior hyper autofluorescent corresponding to the outer retinal and retinal pigment epithelium lesions. (**C**) Humphrey macular visual field examination showing paracentral ring-shaped scotoma. **D.** Enhanced depth imaging mode in spectral domain OCT vertical B scan showing outer retinal layers thinning and complete ellipsoid zone disruption defining the typical "flying saucer" sign.

mfERG, and structural spectral domain OCT (Spectralis HRA + OCT system, Heidelberg Engineering, Heidelberg, Germany). Subjects included in group 2 were healthy controls with no abnormalities on ophthalmological examination, no/minimal refractive errors, and no previous/current ophthalmological and systemic disease and having undergone BCVA testing and multimodal imaging, including enhanced depth Imaging (EDI) SD-OCT as part of this study.

Imaging Analysis

The macular region was scanned using a horizontal and vertical line scan (30° × 5°) centered on the fovea, with 100 frames averaged in each B-scan.

On vertical EDI SD-OCT scans, choroidal thickness (ChT) was manually measured by two independent readers (SH and HML) as the distance between the Bruch membrane interface and the sclerochoroidal interface under the fovea and at 500 μm inferior and superior to the fovea. The mean value of ChT (i.e., the arithmetic means between the three ChT measurements) and the subfoveal (SFchT) measures were considered for statistical analysis.

CVI was computed using image binarization of the vertical EDI SD-OCT foveal scan (FIJI, National Institute of Health, Bethesda, MD, USA). As described previously,[16,18–20] the OCT image is opened in FIJI, and the polygon tool is used to select a region of interest (ROI) of 1,500 μm wide, centered on the fovea. The upper boundary of the ROI is traced along the choroidal-RPE junction and the lower boundary along the sclerochoroidal junction and thus defining the total choroidal area (TCA). Image brightness is adjusted based on the average value obtained from the luminal choroidal area (LCA) of three choroidal vessels selected using the oval selection tool. After conversion to an 8-bit image, Niblack local threshold is applied to binarize the image and to demarcate the LCA and the stromal choroidal area (SCA). The image is converted to a red, green, and blue image, and the color threshold tool is used to select the dark pixels, representing the LCA. The CVI was calculated by dividing the LCA by the TCA expressed as a percentage. Figure 2 shows an example of EDI-OCT before and after binarization in the three groups.

The TCA was converted from pixels^2 to μm^2 using the following formula (16):

"TCA μm^2"= "TCA pixel^2" * "x-scale (μm/pixel)" * "z-scale (μm/pixel)"

"x-scale" and "z-scale" were reported in each acquisition by the Spectralis software (Heidelberg Eye Explorer, Version 1.9.14.0, Heidelberg, Germany).

Statistical Analysis

Statistical analyses were performed using StataCorp 13/SE (STATA, Statacorp, USA).

Quantitative variables were reported as means ± standard deviations, whereas qualitative variables were expressed as counts and percentages.

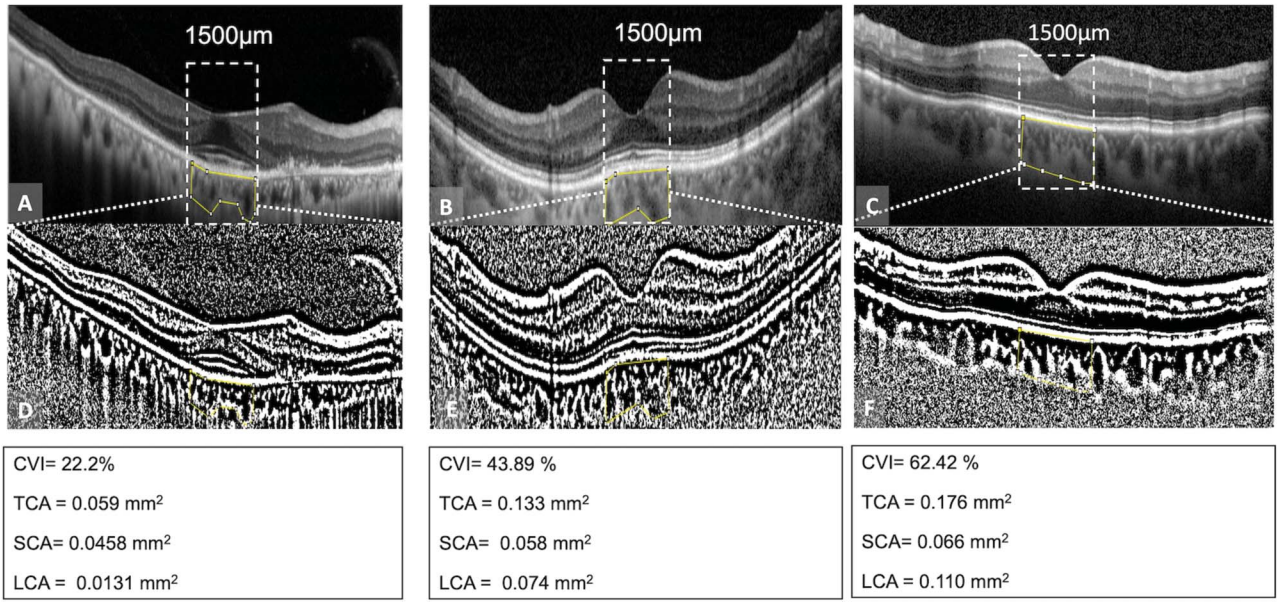


Fig. 2. EDI-OCT scans examples before and after binarization in the three groups. A, B, C. Vertical structural OCT passing through the fovea in an eye with advanced stage of hydroxychloroquine (HCQ) toxicity, mild HCQ toxicity and in healthy control respectively. The region of interest (ROI) of 1,500 μm centered to the fovea was selected and binarized using the Niblack autolocal threshold to calculate the CVI in the examples. **D, E, F.** Dark pixels represent the luminal area, whereas white pixels the stromal area. CVI was calculated as the ratio between the luminal area and the total choroidal area. **A** and **C.** The results of binarization in an eye with advanced-stage hydroxychloroquine (HCQ) toxicity showing outer nuclear layer thinning and ellipsoid zone fragmentation. **B** and **E.** The results of binarization in an eye with mild HCQ toxicity showing discreet ellipsoid zone disruption without outer nuclear layer thinning. **C** and **F.** The results of binarization in a healthy control.

Comparison of qualitative variables was performed with chi2 test. Comparison of quantitative variables was performed using analysis of variance (ANOVA) for repeated measures with the Bonferroni post hoc analysis or by the nonparametric method (Mann–Whitney tests, Kruskal Wallis) in case of inequality of variances.

For interrater reliability analysis for ChT measurements, the intraclass correlation coefficient was used. The relationship between choroidal biomarkers CVI, TCA, LCA, and SCA (considered as dependent variables) and the age, treatment duration, daily dose in milligrams per kilogram, and the cumulative dose was investigated using multiple linear regression analysis.

In all analyses, $P < 0.05$ were considered statistically significant.

Results

Patient Demographics and Main Clinical Features

Forty-eight eyes of 47 patients undergoing HCQ treatment (group 1) and 34 eyes of 31 healthy controls (group 2) were included in this study. Among the eyes included in group 1, 22 eyes (22 patients) had mild signs of HCQ toxicity (subgroup 1a), whereas 26 eyes (25 patients) presented with advanced stages of HCQ toxicity (subgroup 1b).

Within group 1, the mean age within the mild toxicity subgroup (1a) was 54.09 ± 15.59 years, whereas the mean age was 54.78 ± 10.39 years within the advanced stages of toxicity subgroup (1b). Most patients were female (90.91% in subgroup 1a and 80.77% in the subgroup 1b). The mean BCVA (logMar) for group 1a was 0.013 ± 0.071, whereas the mean BCVA for group 1b was 0.064 ± 0.124.

For subgroup 1a (mild toxicity), the mean treatment duration was 4.65 years ± 3.27, the mean daily dose in mg/kg was 4.95 ± 1.31, and the mean cumulative dose was 592.40 g ± 481.27. For group 1b (advanced stages of toxicity), treatment duration was 13.22 years ± 8.86 and the mean daily dose in mg/kg was 6.35 ± 1.94 and the cumulative dose was 1825 g ± 1,026.98. The treatment duration, the daily dose, and the cumulative dose were statistically different between the two subgroups ($P = 0.0002$, $P = 0.015$, and $P < 0.001$, respectively).

For group 1a, HCQ was mainly prescribed for undifferentiated connective tissue disease in 27.08% of cases, Gougerot Sjogren syndrome in 18.75%, and systemic lupus erythematous (SLE) in 16.66%. As for group 1b, the main diseases for which HCQ was prescribed were SLE in 29.16%, sharp syndrome in 8.3% of cases, and Gougerot Sjogren syndrome in 8.3%.

As for group 2 (healthy age-matched and sex-matched controls), the mean age was 49.88 ± 11.05 years, with a mean BCVA (logMar) of 0.009 ± 0.059. Of note, there was no significant difference with regards to age between the three groups ($P = 0.187$). With regards to gender matching, when comparing the groups two by two (1a and 2, 1b and 2, and 1a and 1b), the P was consistently nonsignificant ($P = 0.168$, $P = 0.688$, and $P = 0.321$, respectively, Chi2 test).

Although there was no significant difference in BCVA between the three groups ($P = 0.1896$), BCVA was significantly lower among eyes in subgroup 1b compared with those included in subgroup 1a ($P = 0.0092$).

All patient demographics and clinical data are summarized in Table 1.

Choroidal Analysis

Choroidal thickness measurements. There was no significant difference in mean ChT and SFchT between the two independent readers. The interrater reliability was excellent. The ICC was 0.989 for SFchT and 0.994 for mean ChT.

The mean ChT was 397.64 μm ± 88.81 for group 2 (healthy controls) and 255.030 μm ± 73.11 for group 1a (mild toxicity) and 229.12 μm ± 93.20 for group 1b (advanced stages of toxicity).

SFchT was 389.68 μm ± 84.82 for the controls included in group 2 while it was 250.27 μm ± 75.05 for subgroup 1a and 228.19 μm ± 91.54 for subgroup 1b.

The difference between the three groups was statistically significant for both the mean ChT and SFChT ($P < 0.001$, $P < 0.001$). The ChT was significantly lower in group 1 (patients under HCQ) compared with group 2 ($P < 0.001$). Within group 1, no significant difference was found between patients presenting with mild (1a) and advanced stages (1b) toxicity.

Image binarization variables. After the binarization of the vertical structural EDI-OCT, we calculated the CVI, TCA, LCA, and SCA. In detail, the mean CVI was 55% ± 6.23 in group 2, 41.96% ± 6.93 in subgroup 1a, and 38.34% ± 8.74 in subgroup 1b. The CVI was significantly different between the three groups ($P < 0.0001$) and was significantly lower in group 1 compared with controls ($P < 0.001$).

The TCA, LCA, and SCA were significantly different between the three groups ($P < 0.001$, <0.001, and 0.01 respectively). The TCA and LCA were significantly lower in group 1b compared with 1a and group 2.

Coefficients of variation. For subgroup 1a, the coefficient of variation of CVI was 16.51%, and for mean ChT, the coefficient of variation was 28.66%. For subgroup 1b, the coefficient of variation of CVI was 22.79% while it was 40.64% for the mean ChT. For group 2, the coefficient of variation of CVI was 11.32% while it was 22.33% for the mean ChT. Figure 3 summarizes main differences between CVI and SFChT in the three groups.

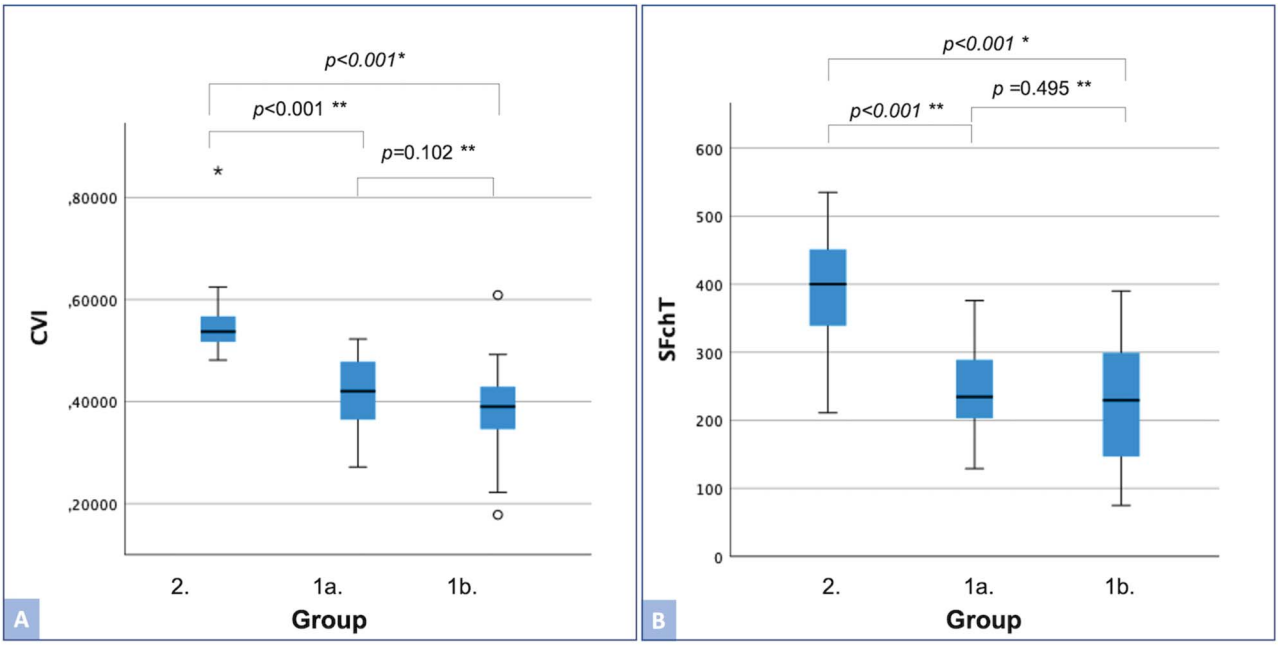
Multiple linear regression analysis. There was no significant correlation between SFchT, CVI, TCA, LCA, and SCA and patient's age, treatment duration, daily dose of treatment, and cumulative dose.

The main choroidal variables and the comparative analysis are summarized in Table 2.

Table 1. Main Clinical and Demographic Features of Study Group

	Group 1a (n = 22 patients) (mean ± SD) or n (%)	Group 1b (n = 25 patients) (mean ± SD) or n (%)	Group 2 (n = 31 patients) (mean ± SD) or n (%)	Significance
Age (years)	54.09 ± 15.59	54.78 ± 10.39	49.88 ± 11.05	0.187*
Sex (male %)	9.09%	19.23%	23.52%	0.168†, 0.321‡, 0.688§
Treatment duration (years)	4.659 ± 3.27	13.222 ± 8.86	NA	<0.001¶
Daily dose (mg/kg)	4.95 ± 1.31	6.35 ± 1.94	NA	0.015¶
Cumulative dose (g)	592.40 ± 481.27	1825 ± 1,026.98	NA	<0.001¶

*: *Kruskal Wallis.*
[a,b,c]: *Chi2 test*.
†Comparison between group 1a versus group 2 using the Chi2 test.
‡Comparison between group 1a versus group 1b using the Chi2 test.
§Comparison between group 1b versus group 2 using the Chi2 test.
¶Mann–Whitney; Group 1a: eyes of patients under HCQ presenting with mild toxicity; Group 1b: eyes of patients under HCQ presenting with advanced stage of toxicity; Group 2: eyes of healthy controls.



*Kruskal wallis, **Mann–Whitney

Fig. 3. Comparison of choroidal vascularity index and subfoveal choroidal thickness between the three groups. A. Choroidal vascularity index (CVI) comparison between group 1: Patients under hydroxychloroquine (HCQ) presenting with mild retinal toxicity (1a), and advanced stage of retinal toxicity (1b) healthy controls (group 2). The CVI was statistically different between patients from group 1a and group 2 ($P < 0.001$) and between patients from group 1b and group 2 ($P < 0.001$). **B.** Subfoveal choroidal thickness (SFChT) was statistically different between group 1a (mild toxicity) versus controls (group 2) and group 1b (advanced stage of toxicity) versus group 2.

Discussion

In our study, we investigated choroidal changes in eyes with advanced stages and mild HCQ toxicity by analyzing multiple choroidal biomarkers, including CVI, TCA, LCA, SCA, and ChT measurements. Overall, we observed that eyes with advanced stages of HCQ toxicity have significant choroidal alterations compared with healthy controls. Indeed, the CVI was significantly lower in eyes from group 1b (i.e., advanced stages of toxicity) compared with the healthy controls included in group 2 (38.34% ± 8.74 in group 1b vs. 55% ± 6.23 in group 2, $P < 0.001$). Furthermore, eyes in group 1a, i.e., mild toxicity, also had a significantly lower CVI (41.96% ± 6.93) compared with controls ($P < 0.001$). Interestingly, the more advanced the toxicity stage was, the lower the CVI (38.34% ± 8.74 in advanced stages of toxicity vs. 41.96% ± 6.93 in mild toxicity vs. 55% ± 6.23 in controls). The ChT was significantly different between the three groups. In the advanced stages of toxicity group compared with controls, both the SFCT (228.19 ± 91.54 μm vs. 389.68 ± 84.82 μm) and the mean ChT (229.12 ± 93.20 μm vs. 397.64 ± 88.81 μm) were lower ($P < 0.001$).

Our study raises a pivotal question regarding the pathophysiology of HCQ retinal toxicity. In detail, the choroidal impairment, found in advanced stages of HCQ toxicity, may be either secondary to the loss of photoreceptors and RPE or the choroidal involvement seen in HCQ toxic retinopathy may precipitate photoreceptor and RPE loss. The fact that, in our series, the group treated by HCQ but with mild HCQ toxicity (group 1a) had lower CVI than the control group would support the latter hypothesis, but the absence of a dose response association with choroidal thinning in the multiple linear regression analysis further weakens the argument that the choroidal impairment is related to a direct toxic effect of HCQ on the choroid.

Our results are consistent with recent literature investigating choroidal and choriocapillaris involvement in HCQ retinopathy, by means of both OCT and OCT angiography.[12,13] Ahn et al[12] have previously demonstrated choroidal thinning in HCQ retinopathy. Their study showed a significant decrease in total choroidal thickness and choriocapillaris-equivalent thickness in eyes with HCQ retinopathy compared with eyes without retinopathy. Moreover, previous reports have demonstrated, by means of qualitative analysis of OCT angiography, that there is a choriocapillaris involvement in HCQ retinopathy using OCT angiography.[13,21] The signal void locations corresponded to areas of RPE defect. The choroidal involvement continued to progress even after drug cessation.[13]

Table 2. Choroidal Variables and Comparative Analysis Between Eyes With Varying Staging of Hydroxychloroquine Stages and Healthy Controls

	Group 1a	Group 1b	Group 2	*P**	*P*† Group 1a versus 1b	*P*† Group 1a versus 2	*P*† Group 1b versus 2
Mean ChT	255.030 ± 73.11	229.12 ± 93.20	397.64 ± 88.81	<0.001	0.321	<0.001	<0.001
SFChT	250.27 ± 75.05	228.19 ± 91.54	389.68 ± 84.82	<0.001	0.495	<0.001	<0.001
CVI (%)	41.96 ± 6.93	38.34 ± 8.74	55 ± 6.23	<0.001	0.102	<0.001	<0.001
TCA (mm^2)	0.2457 ± 0.0576	0.1857 ± 0.1043	0.4576 ± 0.3739	<0.001	0.0025	0.0062	<0.00001
LCA (mm^2)	0.1039 ± 0.0322	0.0732 ± 0.0442	0.2513 ± 0.2033	<0.001	0.004	<0.0001	<0.0001
SCA (mm^2)	141,738 ± 33,521.83	112,569.7 ± 62,813.43	206,248.6 ± 175,122.8	0.011	0.0097	0.4916	0.0094

*Kruskal Wallis.
†Mann–Whitney.
Mean ChT: mean choroidal thickness; SFChT: subfoveal choroidal thickness; CVI: choroidal vascularity Index; TCA: total choroidal area; LCA: luminal choroidal area; SCA: stromal choroidal area; Group 1a: eyes of patients under HCQ presenting with mild toxicity; Group 1b: eyes of patients under HCQ presenting with advanced stages of retinal toxicity; Group 2: eyes of healthy controls.

Most studies confirm that the damage caused by HCQ starts at the outer retina[14,15,22] and that the inner retina is relatively intact in eyes with HCQ retinopathy.[14,23] The choroid, however, has not been addressed as a damage site of HCQ.

The pathogenesis of HCQ retinal toxicity is still unclear. The primary site of drug toxicity is known to be the photoreceptor layers, with possible secondary involvement of the RPE.[6,24,25] Choroidal alterations are reported in the pathogenesis of several retinal and choroidal diseases,[15,16,26] as the choriocapillaris is the source of nutrients for the outer retinal layers, including the RPE and photoreceptor layers. The choriocapillaris, the RPE, Bruch membrane, and photoreceptors form indeed a symbiotic unit, and the thinning of outer nuclear layer may also reflect a damage of this unit, ultimately resulting in large regions of RPE atrophy and choroid alterations.[25–27] Moreover, two particularities of the choroid may make it more vulnerable to HCQ toxicity: its rich vasculature making it easily exposed to systemic drugs[12] and the presence of melanin pigment. Previous reports have suggested that melanin may contribute to the development of HCQ retinopathy by concentrating the drug and, therefore, prolonging toxic effects.[6,12,23]

In terms of structural choroid analysis, previous reports tried to define a specific structural analysis to isolate and quantify the individual choroidal components, highlighting the challenges of accurately measuring ChT.[18,28,29] However, ChT does not specifically reflect the perfusion and functional status of the choroid. More specific and reproductible biomarkers are required to describe a choroidal dysfunction and correlate the latter with retinal findings. Betzler et al[15] suggested that, compared with CTh, CVI had a lower covariance and was not associated with patient factors such as age, systolic blood pressure, axial length, or intraocular pressure. A similar finding was described in the report by Zhou et al.[30] In our report, the coefficient of variance was lower for CVI than ChT in all groups. This finding is consistent with the CVI being a more reproductible biomarker to analyze the vasculature of the choroid.

There are different CVI measurement techniques described in the literature using different imaging processing methods.[14,29] Thus, CVI measurements may have some challenges, and a consistent methodology is important to avoid pitfalls. Regardless of the chosen algorithm, the accuracy of measurements is highly dependent on the type of instrument for OCT B-scan acquisition, the ROI selection, the presence/absence of contrast enhancement, and other image processing variables. A previous study by Koçak et al[19] proved that the 1.5-mm subfoveal area had a higher CVI compared with that of total choroidal area in a single B-scan OCT. As previously described, it is also essential to accurately use an autolocal threshold.[14] We adopted the Niblack autolocal threshold as it takes into consideration the mean and SD of all the pixels in the ROI, as well as to circumvent the possible influence of backscattering and shadowing effects secondary to the RPE alterations.[16] Finally, image illumination is partially related to pupillary dilatation, frame averaging, image contrast variation, and poor image resolution, so these additional factors contribute to enhanced image quality.[14]

Our study has several limitations, including the retrospective design, as well as the small sample size. Moreover, inherent subjectivity in manual measurements may have induced differences in the results. The lack of a reproducible, universally applied algorithm for CVI measurement, leading to differences in results, is also to be noted.

In conclusion, this study reports on the choroidal changes occurring in different stages of HCQ retinal toxicity. Eyes with advanced stages of HCQ toxicity are featured by a greater amount of choroidal hypoperfusion,

as proven by the decrease of CVI and derived biomarkers. Although the pathogenesis of HCQ retinopathy remains unclear, our results expand the current knowledge on HCQ choroidal involvement.

Key words: hydroxychloroquine, toxicity, choroidal vascularity index.

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