Evaluation of Corneal and Retinal Toxicity in Rheumatoid Arthritis Patients Treated with Hydroxychloroquine

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Keywords
Hydroxychloroquine · Toxicity · Confocal microscopy · GDxVCC · Multifocal electroretinography

Abstract

Introduction: The study aimed to assess the ocular toxicity of hydroxychloroquine (HCQ) by confocal microscopy, multifocal electroretinography (mfERG), and scanning laser polarimetry with variable corneal compensation (GDxVCC) in patients with rheumatoid arthritis (RA). Methods: A cross-sectional, comparative case series study was retrospectively conducted on 61 patients under HCQ treatment for RA without fundoscopic anomalies (group 1), 65 RA patients with no HCQ treatment (group 2), and 27 normal subjects (group 3). A comprehensive ophthalmological examination, including confocal microscopy, mfERG, and GDxVCC, was performed in the three groups. Results: In group 1, the duration of treatment ranged from 19 to 96 months (54.9 ± 15.2 months). The mean cumulative dose of HCQ was 446.1 ± 164.0 g (range 114–864 g). Confocal microscopy revealed hyper-reflective abnormal particles in 45 patients (73.8%) and beaded, tortuous fibers in 34 patients (55.7%) in group 1. No corneal change was observed in the other two groups. The mfERG responses in the 6 concentric rings (R1–R6) among the three groups differed except at R3 (all \( p \) < 0.05), and data from R1–R6 were not significantly different between groups 2 and 3. The retinal nerve fiber layer thicknesses were statistically thinner in group 1 than in groups 2 and 3 (all \( p \) < 0.05). Conclusions: Early signs of corneal and neural retina structure changes were detected in patients with RA treated with HCQ. Whether these findings should be a mark of drug recession still needs further study and more evidence.

Introduction

Hydroxychloroquine (HCQ) sulfate is a derivative of chloroquine (CQ), which was initially used as an antimalarial agent. HCQ is also used for the treatment of rheumatoid arthritis (RA) and other autoimmune disorders since the early 1950s [1]. However, HCQ can affect the lens, vitreous body, uvea, and especially the cornea and retina [2]. Although the pathophysiology of HCQ toxicity is not well understood, animal models of CQ toxicity have suggested that the earliest detectable changes occurred in the ganglion cell and ultimately progressed to lysosomal disruption in the photoreceptors or retinal pig-
ment epithelium [3, 4]. The risk factors for HCQ retinopathy included high daily dosage relative to body weight, high cumulative dose of HCQ, long duration of use, diminished renal function, liver disease, concomitant tamoxifen use, advanced age, female sex, and/or retinal disease [5].

In 2016, the American Academy of Ophthalmology published the “Recommendations on Screening for Chloroquine and Hydroxychloroquine Retinopathy,” in which it was acknowledged that no anatomic features of the retina and RPE are known to correlate specifically with CQ damage [5]. The association recommended automated visual fields plus spectral-domain optical coherence tomography as the primary screening tests, which could be a sign for drug withdrawal. In 2020, American College of Rheumatology (ACR), American Academy of Dermatology (AAD), Rheumatologic Dermatology Society (RDS), and American Academy of Ophthalmology (AAO) issued a joint statement on HCQ use with respect to retinal toxicity [6]. A baseline retinal exam should be performed within the first few months of HCQ usage, and annual screening with sensitive modalities should begin no more than 5 years later or in the presence of major risk factors.

Recently, several other instruments, including scanning laser polarimetry with variable corneal compensation (GDxVCC), multifocal electroretinography (mfERG), fundus autofluorescence, and micro-perimeter, have been applied to identify the early stage of HCQ-related retinal toxicity [7–11]. The focus of this study is not to suggest a drug withdrawal mark but to investigate the in vivo microscopic changes in the cornea and fundus of patients treated with HCQ that occur before abnormalities become apparent by slit lamp microscopy or fundus imaging. We wanted to detect the differences and correlations between corneal and retinal sensitivity to HCQ toxicity by comparing corneal and retinal changes in RA patients.

Materials and Methods

Patients

One hundred and twenty-six patients with RA were included in this retrospective, nonrandomized study, which was conducted at the Guanghua Hospital. The inclusion criteria were RA patients aged 18–70 years old, with a history of medical treatment for more than 12 months. The exclusion criteria included a history of taking any other medication that could cause keratopathy or retinopathy according to the literature, a history of ocular trauma or ocular disease, concomitant tamoxifen use, advanced age, female sex, and/or retinal disease [5].

Sixty-one RA patients received HCQ therapy (group 1) (75.4% female, mean age 49.8 ± 8.5 years, range 33–65 years), 65 RA patients received a treatment other than HCQ (group 2) (78.5% female, mean age 48.8 ± 8.6 years, range 35–64 years), and twenty-seven normal subjects (group 3) (77.8% female, mean age 47.9 ± 10.2 years, and an intraocular pressure higher than 21 mm Hg [12]. The protocol used was approved by the Ethics Committee of Guanghua Hospital (application number 2014-K-06), and all patients provided written informed consent. A complete ophthalmic examination was performed on each participant, including best corrected visual acuity, intraocular pressure, slit lamp microscopy, confocal microscopy, mfERG, and GDxVCC.

In vivo Confocal Microscopy

All participants were examined with an in vivo corneal confocal microscope (Confoscan 3; Nidek Technologies, Gamagori, Japan) using an Achroplan nonaplanating ×40 immersion objective lens (Zeiss, Oberkochen, Germany), as previously described [13]. For each participant, the thickness of the corneal sublayers (epithelium, stromal layer, and whole cornea), the cell density of the subepithelium, anterior stromal keratocytes, posterior stromal keratocytes and endothelium, the number of sub-basal nerves and nerve branches, and the density of abnormal particles were recorded and analyzed. Keratocyte density and deposit areas were measured and calculated using built-in analysis software (Navis; Lucent Technologies, Murray Hill, NJ, USA). The average value from the three repeated images was obtained.

Multifocal Electroretinography

mfERG was performed using the VERIS 4.8 clinic system (Electro-Diagnostic Imaging, San Mateo, CA, USA) following the recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV), as previously described [14]. The response amplitudes (nV) and latencies (ms) of the first negative peak (N1) and the first positive peak (P1) of each individual ring were measured.

Scanning Laser Polarimetry (GDxVCC)

The peripapillary retinal nerve fiber layer (RNFL) thickness evaluations were performed using GDxVCC (Laser Diagnostic Technologies, Inc., San Diego, CA, USA). Three measurements with a clear image (quality > 8) were obtained and used in analyses. The temporal-superior-nasal-inferior-temporal average (TSNITave), superior average (Superiorave), inferior average (Inferiorave), TSNIT standard deviation (TSNITSD), and nerve fiber indicator (NFI) were calculated.

Data Analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). One-way ANOVA was used to compare values between groups. Pearson correlation was used for the correlation analysis. All tests were two-tailed, and \( p < 0.05 \) was considered statistically significant.

Results

Sixty-one RA patients received HCQ therapy (group 1) (75.4% female, mean age 49.8 ± 8.5 years, range 33–65 years), 65 RA patients received a treatment other than HCQ (group 2) (78.5% female, mean age 48.8 ± 8.6 years, range 35–64 years), and twenty-seven normal subjects (group 3) (77.8% female, mean age 47.9 ± 10.2 years,
range 34–66 years) were also enrolled in this study. In group 1, RA patients were treated with HCQ 200–300 mg/day and iguratimod 50 mg/day. The duration of treatment ranged from 19 to 96 months (54.9 ± 15.2 months). The mean cumulative dose of HCQ was 446.1 ± 164.0 g (range 114–864 g). In group 2, RA patients were treated with methotrexate 7.5 mg/week and iguratimod 50 mg/day. The duration of treatment ranged from 25 to 76 months (48.9 ± 12.2 months).

There were no significant differences in age or gender among the three groups (p = 0.523 and 0.625, respectively). The mean best corrected visual acuity of patients was 77.0 ± 17.5 (group 1), 78.7 ± 18.4 (group 2), and 79.1 ± 7.2 (group 3) ETDRS letters, respectively (p = 0.799). There was no significant difference in the duration of treatment between group 1 and group 2 (p = 0.081).

**Table 1.** Confocal microscopy measurements among the three groups

| Measurement parameters | Group 1 | Group 2 | Group 3 | p value†   |
|------------------------|--------|--------|--------|-----------|
|                        | group 1 vs. 2 | group 1 vs. 3 | group 2 vs. 3 |
| Corneal thickness, μm  | 516.5±7.5 | 516.1±11.7 | 511.0±9.5 | 0.062     |
| Epithelial thickness, μm | 72.6±7.8 | 72.3±9.1 | 74.8±7.8 | 0.404     |
| Sub-epithelial cell density, cells/mm² | 1,058.4±102.1 | 1,006.7±83.2 | 1,035.2±86.0 | 0.057     |
| Stroma thickness, μm  | 420.4±11.1 | 420.5±11.2 | 416.5±11.2 | 0.251     |
| Anterior keratocyte density, cells/mm² | 1,035.3±58.1 | 1,142.1±87.2 | 1,086.9±57.0 | <0.001    |
| Posterior keratocyte density, cells/mm² | 701.7±55.4 | 741.8±93.3 | 701.4±29.2 | <0.001    |
| Endothelial cell density, cells/mm² | 2,646.0±275.0 | 2,665.9±192.6 | 2,694.6±135.0 | 0.633     |
| Sub-basal nerves, n   | 4.4±0.8 | 5.1±1.0 | 3.9±0.8 | 0.059     |
| Sub-basal nerve branches, n | 6.4±1.1 | 5.4±1.4 | 3.5±0.6 | <0.001    |
| Abnormal particle density, particles area/0.12 mm²* | 0.029±0.014 | 0 | 0 | <0.001 |

* 0.12 mm²: the area of the mono visual field of the confocal microscope. † SNK-q test was used for pairwise comparison when the variance analysis results showed significant differences at 0.05 level.
Abnormal Deposits

HCQ deposits were identified by confocal microscopy in 45 patients (73.8%) in group 1 (Fig. 1a, b). Of these, 37 patients (82.2%) had deposits in the superficial epithelium layer; 18 patients (40.0%) had deposits in the basal epithelium; 6 patients (13.3%) had deposits in the anterior stroma; 4 patients (8.88%) had deposits in the posterior stroma; and no HCQ deposits were observed within the endothelium layer in group 1 (Table 1). In the other two groups, no HCQ deposits were found.

Corneal Nerves

The anterior keratocyte density in group 1 was significantly lower compared to those of groups 2 and 3 (both \( p < 0.001 \)). Group 2 had a higher posterior keratocyte density and number of sub-basal nerves compared to groups 1 and 3 (both \( p = 0.035 \) and 0.002, respectively). The sub-basal nerve branch number was significantly higher in group 1 than in group 2, and both groups had higher number than group 3 (both \( p < 0.001 \) ) (Fig. 1c, d). Abnormal particles only existed in group 1. The epithelial thickness, stroma thickness, corneal thickness, sub-epithelial cell density, and endothelial cell density in the three groups were not significantly different.

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**Table 2. N1 response amplitudes according to mfERG for the three groups**

| Parameters (nV/deg²) | Group 1 | Group 2 | Group 3 | \( p \) value¹ |
|---------------------|---------|---------|---------|----------------|
|                     |         |         |         | group 1 vs. 2 | group 1 vs. 3 | group 2 vs. 3 |
| R1                  | 37.0±7.9| 40.2±1.6| 37.7±3.6| 0.002         | 0.549         | 0.044         |
| R2                  | 20.8±5.3| 25.6±1.7| 25.9±2.0| <0.001        | <0.001        | 0.719         |
| R3                  | 17.4±2.5| 17.5±3.7| 16.6±1.5| 0.391         | 0.391         | 0.391         |
| R4                  | 13.7±3.6| 12.9±4.5| 14.1±3.7| 0.344         | 0.344         | 0.344         |
| R5                  | 13.4±3.3| 13.0±2.1| 13.2±4.5| 0.773         | 0.773         | 0.773         |
| R6                  | 9.8±1.8 | 10.2±3.2| 9.4±3.2 | 0.426         | 0.426         | 0.426         |
| R1/R2               | 1.9±0.7 | 1.6±0.1 | 1.5±0.2 | <0.001        | <0.001        | 0.304         |
| R1/R3               | 2.2±0.5 | 2.4±0.6 | 2.3±0.3 | 0.016         | 0.232         | 0.344         |
| R1/R4               | 2.3±0.8 | 4.3±1.9 | 2.9±0.8 | <0.001        | 0.095         | <0.001        |
| R1/R5               | 2.9±1.0 | 3.2±0.6 | 3.2±1.1 | 0.152         | 0.152         | 0.152         |
| R1/R6               | 3.5±1.0¹ | 3.9±1.1 | 4.6±2.0 | 0.147         | <0.001        | 0.011         |

¹SNK-q test was used for pairwise comparison when the variance analysis results showed significant differences at 0.05 level.

**Table 3. P1 response amplitudes according to mfERG for the three groups**

| Parameters (nV/deg²) | Group 1 | Group 2 | Group 3 | \( p \) value¹ |
|---------------------|---------|---------|---------|----------------|
|                     |         |         |         | group 1 vs. 2 | group 1 vs. 3 | group 2 vs. 3 |
| R1                  | 83.7±7.0| 90.8±7.7| 89.8±4.4| <0.001        | <0.001        | 0.527         |
| R2                  | 60.5±5.3| 69.1±16.5| 67.5±4.4| <0.001        | 0.008         | 0.532         |
| R3                  | 44.3±6.2| 45.5±6.8| 43.0±4.9| 0.194         | 0.194         | 0.194         |
| R4                  | 36.7±2.8| 39.1±2.9| 38.7±4.5| <0.001        | 0.007         | 0.594         |
| R5                  | 29.0±2.2| 31.3±3.8| 31.9±3.8| <0.001        | <0.001        | 0.427         |
| R6                  | 24.3±3.1| 26.4±2.6| 26.6±3.2| <0.001        | 0.001         | 0.686         |
| R1/R2               | 1.4±0.2 | 1.4±0.3 | 1.3±0.1 | 0.564         | 0.564         | 0.564         |
| R1/R3               | 1.9±0.2 | 2.0±0.3 | 2.1±0.3 | 0.014         | 0.003         | 0.166         |
| R1/R4               | 2.3±0.2 | 2.7±0.2 | 2.4±0.3 | <0.001        | 0.013         | <0.001        |
| R1/R5               | 2.9±0.3 | 2.9±0.3 | 2.9±0.3 | 0.552         | 0.552         | 0.552         |
| R1/R6               | 3.5±0.4 | 3.5±0.4 | 3.4±0.4 | 0.737         | 0.737         | 0.737         |

¹SNK-q test was used for pairwise comparison when the variance analysis results showed significant differences at 0.05 level.

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**In vivo Confocal Microscopy**

Abnormal Deposits

HCQ deposits were identified by confocal microscopy in 45 patients (73.8%) in group 1 (Fig. 1a, b). Of these, 37 patients (82.2%) had deposits in the superficial epithelium layer; 18 patients (40.0%) had deposits in the basal epithelium; 6 patients (13.3%) had deposits in the anterior stroma; 4 patients (8.88%) had deposits in the posterior stroma; and no HCQ deposits were observed within the endothelium layer in group 1 (Table 1). In the other two groups, no HCQ deposits were found.

Corneal Nerves

The anterior keratocyte density in group 1 was significantly lower compared to those of groups 2 and 3 (both \( p < 0.001 \)). Group 2 had a higher posterior keratocyte density and number of sub-basal nerves compared to groups 1 and 3 (both \( p = 0.035 \) and 0.002, respectively). The sub-basal nerve branch number was significantly higher in group 1 than in group 2, and both groups had higher number than group 3 (both \( p < 0.001 \) ) (Fig. 1c, d). Abnormal particles only existed in group 1. The epithelial thickness, stroma thickness, corneal thickness, sub-epithelial cell density, and endothelial cell density in the three groups were not significantly different.
Multifocal Electroretinography

Tables 2 and 3 show the values for each ring in the eyes of three groups. The N1 amplitudes at R2, and the P1 amplitudes at R1, R2, and R4–R6 were lower in the HCQ-treated group compared to the non-HCQ-treated and normal groups (all \( p < 0.05 \)). A comparison of mfERG ring ratio data revealed that R1/R2 (N1 amplitudes), and R1/R3 and R1/R4 (P1 amplitudes) were significantly different in the HCQ-treated group compared to the non-HCQ-treated and normal groups (all \( p < 0.05 \)).

Scanning Laser Polarimetry (GDxVCC)

The RNFL thickness was statistically thinner in group 1 than in groups 2 and 3 in all retinal quadrants examined (all \( p < 0.05 \)), whereas there were no significant differences between groups 2 and 3 (Table 4). Representative GDxVCC images of three groups are shown in Figure 2.

Correlations between Corneal and Retinal Changes

In group 1, 45 patients (73.8%) had hyper-reflective abnormal particles and 34 patients (55.7%) had beaded, tortuous fibers in the cornea, as observed via confocal microscopy. Sixteen patients (26.2%) showed corneal hyper-reflective abnormal particles and beaded, tortuous fibers but normal RNFL thickness was observed via GDxVCC. Five patients (8.19%) had thinning RNFL thicknesses, but no corneal changes were detected via confocal microscopy. Eleven patients (18.0%) were normal in all examinations (Table 5).

Correlations between Corneal Manifestation and RNFL Thickness

The results above showed that patients under HCQ treatment had different presentations in GDxVCC and confocal microscopy examination. To investigate whether this observation had a quantitative correlation among the two kinds of examination results, we conducted further analysis of the correlations among GDxVCC measurements, keratocyte density, nerve number, and particle density (Table 6). In Table 6, we found that keratocyte density, abnormal particle density, and number of sub-basal nerves and branches significantly correlated with TSNITave and/or NFI. There was no significant correlation between confocal microscopy measurements and the other three RNFL parameters (Superiorave, Inferiorave, and TSNIT SD, all \( p > 0.05 \)).

Discussion

HCQ has been widely used for the treatment of RA and other inflammatory diseases since the early 1950s [1]. HCQ has a long half-life (30 days for HCQ in plasma) and can persist for months after the medication is discontinued. It has been reported the incidence of HCQ retinopathy to be 0.38–7.5% in patients, and the toxicity varies with daily dosage and duration of use [15, 16]. The HCQ-induced keratopathy has been also observed in patients with RA treated with HCQ [17, 18].

We examined 2 patients treated with CQ who suffered from gradually decreasing visual acuity and visual field [19]. In these patients, FFA, OCT, and electroretinography all suggested severe CQ-related retinopathy, but the RNFL thicknesses around the optic nerve (measured by OCT and GDxVCC) were normal. However, another GDxVCC study with 60 RA patients suggested a significant positive correlation between the cumulative dose of CQ and RNFL loss, and a positive correlation with the NFI [20]. In the present study, we also used in vivo confocal microscopy to investigate corneal morphology chang-

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Table 4. RNFL thickness and comparison of parameter measurements among the three groups

| Parameters          | Group 1 | Group 2 | Group 3 | \( p \) value‡ |
|---------------------|---------|---------|---------|----------------|
| TSNITave            | 53.5±5.8| 57.8±2.6| 57.0±3.0| <0.001         |
| Superiorave         | 64.6±2.8| 68.2±3.7| 66.5±4.2| <0.001         |
| Inferiorave         | 63.1±9.1| 68.4±3.4| 67.8±3.9| <0.001         |
| TSNITSD             | 25.6±1.9| 20.3±2.5| 21.4±3.3| <0.001         |
| NFI                 | 22.7±7.6| 16.3±5.0| 16.5±5.2| <0.001         |

TSNITave, temporal-superior-nasal-inferior-temporal average retinal nerve fiber layer (RNFL) thickness; Superiorave, superior average RNFL thickness; Inferiorave, inferior average RNFL thickness; TSNITSD, temporal-superior-nasal-inferior-temporal average RNFL thickness standard deviation; NFI, nerve fiber indicator. ‡ SNK-q test was used for pairwise comparison when the variance analysis results showed significant differences at 0.05 level.
(For legend see next page.)
es in HCQ-treated patients with normal slit lamp microscopy results. The cumulative dosage was significantly correlated with the density of abnormal particles, anterior keratocyte density, and the number of sub-basal nerves [13]. The existence of morphological changes in the cornea might suggest irreversible retinal toxicity, but there was no clear conclusion regarding the chronological order of HCQ-related keratopathy and maculopathy.

There have been relatively few reports regarding confocal microscopy findings of HCQ-related keratopathy. Dosso et al. [18] reported on a patient with vortex keratopathy after 1 year of HCQ treatment [17]. In the present study, we observed hyper-reflective particles, beaded and tortuous nerve fibers, and increased nerve branches in the corneas. The in vivo confocal microscopy identified highly reflective, dot-like intracellular inclusions concentrated in the basal epithelial layer, anterior stroma, and posterior stroma. In previous studies, hyper-reflective abnormal particles in different layers of the cornea were considered to be CQ deposits in CQ-treated patients [17, 21]. Corneal deposits result from binding to cellular lipids and deposition of the drug in the cornea. Therefore, we think that these abnormal deposits should be HCQ deposits, not a metabolite accumulation. The most specific features of HCQ-related keratopathy are corneal deposits, which we observed only in the HCQ treatment group in this study. Further, corneal deposits were more prevalent than neurological changes, which may be attributed to the phagocytosis of Langerhans cells, which are similar to the findings regarding amiodarone-induced keratopathy [22, 23]. Although it has been reported that patients may complain of halos around a light source and photophobia, corneal deposits very rarely impair vision. In most cases, corneal deposits

| Table 5. Correlations between HCQ dose, GDxVCC, and confocal microscopy in Group 1 |
|-----------------------------------------------|---------------------------------|----------------|-----------------|-----------------|
| GDxVCC      | Confocal microscopy    | Patients,  | HCQ duration, | Cumulative HCQ |
|             | deposits                  | n (%)        | months     | dose, g         |
| Abnormal    | Yes                      | Abnormal*    | 22 (36.1)  | 60.6±10.3       | 504.3±121.9    |
| Abnormal    | Yes                      | Normal       | 7 (11.5)   | 44.8±8.0        | 240.4±80.1    |
| Normal      | Yes                      | Abnormal*    | 12 (19.7)  | 60.3±13.9       | 494.8±147.3   |
| Normal      | Yes                      | Normal       | 4 (6.6)    | 61.4±10.6       | 713.3±104.3   |
| Abnormal    | No                       | Normal       | 5 (8.2)    | 50.9±12.9       | 391.2±113.5   |
| Normal      | No                       | Normal       | 11 (18.0)  | 47.0±12.2       | 335.2±102.4   |

* Abnormal: beaded and tortuous nerve fibers.

| Table 6. Correlations between corneal manifestation and RNFL thickness in Group 1 |
|-----------------------------------------------|----------------|-----------------|
| TSNITave | Spearman’s p | p value | Spearman’s p | p value |
| Anterior keratocyte density               | 0.226           | 0.080          | 0.319         | 0.012         |
| Posterior keratocyte density             | 0.419           | 0.001          | 0.339         | 0.008         |
| Number of sub-basal nerves               | 0.416           | 0.001          | 0.334         | 0.009         |
| Number of sub-basal nerve branches       | 0.560           | 0.000          | 0.175         | 0.178         |
| Abnormal particle density                | 0.308           | 0.017          | 0.189         | 0.149         |

TSNITave, temporal-superior-nasal-inferior-temporal average retinal nerve fiber layer thickness; NFI, nerve fiber indicator.

**Fig. 2.** Examples of RNFL thickness maps measured by scanning laser polarimetry (GDxVCC) in RA patients received HCQ therapy (a), RA patients received a treatment other than HCQ (b), and normal subjects (c). A thinner RNFL was found in the HCQ-treated patients compared to the non-HCQ-treated patients and normal subjects.
are reversible on drug discontinuation [18]. In addition, previous studies have reported decreased corneal endothelial cell density, increased central corneal thickness, and other changes in corneal parameters in patients with long-term HCQ use [24–26]. Our results also showed decreased corneal endothelial cell density and increased central corneal thickness in the HCQ treatment group, although they were not statistically significant. Further studies are required for the possible association of HCQ on the cornea.

Retinal toxicity is a very rare side effect of HCQ therapy, but when it occurs, vision loss may be permanent and may progress even years after the cessation of medication. Therefore, more sensitive testing techniques, including mfERG, may reveal the early deposition of debris in the outer segments of photoreceptors. mfERG may be more sensitive to early paracentral functional loss than the white 10-2 field [27]. In this study, the P1 amplitudes at R1, R2, and R4–R6 were lower in the HCQ-treated group compared to the non-HCQ-treated and normal groups (all significantly different). We speculate that pericentral amplitude loss was most specific to HCQ toxicity, which is consistent with the findings reported by Maturi et al. [28]. Ring ratios may also be a sensitive measure of dysfunction in ocular HCQ toxicity [29]. It is clinically important to clarify the association between different mfERG abnormalities and different HCQ dosages and treatment duration. However, our data did not reveal any significant correlation. This may be due to the small sample size and the retrospective study design of the current study. We hope that further research will provide new insights into mfERG abnormalities in screening for HCQ-related ocular toxicity.

Identifying anatomical changes prior to the occurrence of functional damage would be ideal. Thus, we compared the results of confocal microscopy and GDx-VCC. However, Spearman’s correlations between the pathological changes were not strong, which indicates that the first symptom of HCQ-related optical toxicity could appear in either the cornea or retina. However, the chronological order of optical toxicities remains unclear. Enlarging the sample size may be helpful. Therefore, for patients treated with HCQ, it may be considered to increase test frequency using sensitive modalities on the basis of current AAO screening recommendations, especially those with risk factors. The optimal screening strategy requires further investigation. The risk estimated in the present study was much higher than prior estimations because the use of more sensitive screening techniques can detect retinopathy earlier. Once the toxicity is confirmed, the physician should be notified, and the use of HCQ should be discontinued unless it is medically critical and the patient has been informed of the visual risk. We also found that keratocyte density, abnormal particle density, and the number of sub-basal nerves and branches had significant correlations with TSNITave and/or NFI. In particular, the posterior keratocyte density and number of sub-basal nerves were related to TSNITave and NFI, indicating that attention should be paid to both the damage of HCQ to the retina and cornea in patients in clinic. Cornea and retina could be the “target” of HCQ at the same time, and this could be another important finding based on our previous and present studies [20].

In conclusion, thinning the RNFL may be a useful sign for detecting early HCQ retinopathy. HCQ optical toxicity can first appear in the cornea or retina, and both GDx-VCC and mfERG may reveal early HCQ oculopathy. Both techniques have high reliability, and the confocal microscopy results correlate with the GDxVCC results. The limitations of this study include the small sample size and the retrospective study design. We do not know whether HCQ-related retinopathy or keratopathy would appear first. We suggest that both techniques should be used during the screening procedure, and further follow-up should be conducted in these patients.

Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study protocol was reviewed and approved by the Ethics Committee of Guanghua Hospital, approval number 2014–K-06. Written informed consent was obtained from all individual participants included in this study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by Leading Talent Training Program of Health System in Pudong New Area (PWR2020-01) and Project of Shanghai Science and Technology (19ZR1443500).
Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Yiyong Qian, Dan Zhou, Dan Zhu, Tingli Shi, and Xiaoyun Ma. The first draft of the manuscript was written by Jun Zou and Xiaoyun Ma, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

All data generated during this study are included in this article. Further inquiries can be directed to the corresponding author.