Original Article

Structural and functional assessment of ganglion cell complex in patients on long-term hydroxychloroquine

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Purpose: Long-term hydroxychloroquine (HCQ) treatment has been shown to be associated with structural changes (reduced thickness) of ganglion cell complex (GCC). This study evaluated if these structural changes of GCC translate to functional deficits and if they represent true retinal toxicity. **Methods:** This was a cross-sectional study. Fifteen patients aged ≥18 years who had been on HCQ treatment for >5 years were recruited as cases, and 15 age- and gender-matched healthy individuals were recruited as controls. All cases underwent visual fields (central 10-2 SITA standard), pattern electroretinogram (ERG), spectral-domain optical coherence tomography (OCT), and widefield autofluorescence. Controls underwent pattern ERG and spectral-domain OCT. **Results:** A significantly lower average (77.0 ± 5.5 µm vs. 82.0 ± 5.3 µm, *P* = 0.017) and minimum ganglion cell-inner plexiform layer (GC-IPL) thickness (71.0 ± 8.1 µm vs. 76.6 ± 6.3 µm, *P* = 0.041) were noted among cases compared to controls. Similarly, average retinal nerve fiber layer (RNFL) thickness (86.7 ± 9.6 µm vs. 94.8 ± 7.6 µm, *P* = 0.015) and superior quadrant RNFL thickness (105.2 µm ± 16.7 µm vs. 120.0 µm ± 15.6 µm, *P* = 0.018) were lower in cases than in controls. Average RNFL thickness and GC-IPL thickness were negatively correlated with the mean deviation (MD), the pattern standard deviation (PSD) scores, and implicit times of P50 and N95 waveforms, respectively, but none were statistically significant. **Conclusion:** Long-term HCQ use is associated with structural changes in the GCC, manifested as lower GC-IPL and RNFL thickness. Although there was a trend suggesting ganglion cell dysfunction (prolonged implicit times) and possible deficits in RNFL function (MD and PSD scores), statistically significant correlations could not be established with GC-IPL and RNFL thickness, respectively. GC-IPL/RNFL thickness assessment can be a part of the screening. Mere GC-IPL thickness reduction should not be a criterion to recommend HCQ cessation in the absence of abnormality on routinely recommended screening tests.

Key words: Drug toxicity, ganglion cell complex, hydroxychloroquine, pattern electroretinogram, retinopathy

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Hydroxychloroquine (HCQ) is an antimalarial drug, now commonly employed for treating various autoimmune conditions. Retinal toxicity is a potentially sight-threatening complication of HCQ, which is dose- and duration-dependent. Before the advent of advanced imaging, the prevalence of HCQ toxicity was considered very low (<0.5%).[1] Using currently recommended screening tools such as optical coherence tomography (OCT) and visual fields (VFs), the prevalence was reported to be 7.5% in patients on HCQ therapy for more than 5 years.[2] In a recent study from India, HCQ retinopathy was detected in 10% of patients with HCQ for >5 years and/or having received a cumulative dose of >400 g.[3] With the advent of better tools for retinal structure and function assessment, toxicity due to HCQ can be picked up at earlier stages.

Current guidelines suggest the use of spectral-domain OCT, fundus autofluorescence (FAF), automated VF, and multifocal electroretinogram (mfERG) for the detection of retinal toxicity in patients after 5 years of starting HCQ therapy.[4,5] All these recommended tests focus on detecting damage to retinal pigment epithelium and photoreceptors. However, animal histopathological studies and OCT structural studies have shown the involvement of retinal ganglion cells much before the involvement of photoreceptors, even when fundus appearance was normal.[6-8]

Ganglion cell complex (GCC) involves three innermost layers of the retina: the ganglion cell layer, the inner plexiform layer, and the nerve fiber layer.[9] Structural assessment of the GCC can be done using spectral-domain OCT, which assesses the thickness of the ganglion cell-inner plexiform layer (GC-IPL) and the retinal nerve fiber layer (RNFL).

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Functional assessment of GCC can be done by automated VF testing and electrophysiology (pattern electroretinogram). Though there are studies to assess the structural changes in GCC in patients on long-term HCQ, there are no studies to assess functional changes and to find the correlation with the structural changes.[7,8] As HCQ toxicity can progress even after cessation of the drug, it is critical to detect toxicity at the early stages.[10,11] With the current recommendations, early retinal toxicity can be missed. Hence, the assessment of GCC structure and function can help us to understand the effect of HCQ much before its effect on photoreceptors and retinal pigment epithelium. This will also help us to know if these structural defects translate to functional deficits and if they represent true retinal toxicity.

Methods

This was a cross-sectional study done at a tertiary care referral institution. The study was approved by the Indian Council of Medical Research (Reference ID: 2023-14218) and the institutional ethics committee (JIP/IEC-OS/289/2023). The study adhered to the tenets of the Declaration of Helsinki.

The sample size was calculated using the method of comparison of independent means based on a study by Kan *et al*.,[8] which found a mean difference of 6 µm for average GC-IPL thickness between patients on HCQ (cases) and controls and a pooled standard deviation of 5 µm. With a confidence interval of 95% and power of 80%, the sample size was calculated to be 15 in each group.

The study recruited 30 participants. Fifteen patients aged ≥18 years on HCQ treatment for more than 5 years for various rheumatological disorders were considered as cases, and 15 age- and gender-matched healthy individuals were recruited as controls. Exclusion criteria for both cases and controls included spherical refractive error ≥5 D, participants who could not cooperate for OCT testing (due to musculoskeletal problems), presence of visually significant cataracts and corneal opacities, and coexisting retinal or optic nerve pathologies (such as macular diseases, glaucoma, or any form of optic neuropathy). Written informed consent was obtained from all the study participants.

For cases, details collected included indication for HCQ use, duration of HCQ use, daily dose of HCQ, body weight (in kg), other medications currently in use, and history of known comorbidities (diabetes, hypertension, renal disease, etc.). For controls, history was taken to rule out the presence of any comorbidities and long-term medication use.

Cases underwent the following examinations. *A comprehensive ophthalmological examination* was done to note best-corrected distance visual acuity using Snellen's chart, best-corrected near visual acuity using Jaeger's chart, and anterior segment and fundus evaluation. *VFs* were performed using standard automated perimetry (Humphrey 860i, Carl Zeiss Meditec Inc., Dublin, CA). The central 10-2 program using the Swedish interactive threshold algorithm (SITA) standard protocol was used for testing. Only data from reliable VF tests was considered. *Pattern electroretinogram (ERG)* was performed as per the recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV). It was recorded binocularly using the RETI-port/scan 21 system (Roland Consult, Germany), using Dawson-Trick-Litzkow electrodes in the light-adapted state. An average of 100 responses was recorded and repeated at least twice. *Spectral-domain OCT* was performed using Cirrus HD OCT (OCT-500, version 10.0, Carl Zeiss Meditec Inc., Dublin, CA). Macular cube 51x128°CT protocol was used to perform GC-IPL analysis. Optic disc cube 200x200 was used to perform RNFL analysis. A signal strength of more than 7 was considered suitable for reporting. *Widefield autofluorescence* was done using Clarus 700 (Carl Zeiss Meditec Inc., Dublin, CA) to look for areas of abnormal hypo/hyper autofluorescence.

Controls underwent the following examinations: *comprehensive ophthalmological examination, pattern ERG,* and *spectral-domain OCT.*

Data generated were analyzed using the statistical software STATA version 17.0. The continuous variables were represented by mean, and standard deviation or median with a range per normality based on the distribution of the data, and categorical variables were represented by frequency or percentage. Analytical statistics were applied to compare the dependent variables as appropriate to the data.

Results

This cross-sectional study included 30 participants (15 cases and 15 controls). There were one male and 14 female participants in each group. Data from the right eyes of all the participants were considered for analysis. The average age of cases was 42.8 ± 6.5 years, and the average age of controls was 45.4 ± 6.3 years, with no significant difference between the groups. Table 1 summarizes the baseline characteristics of the study participants in each group. The average duration of HCQ use among cases was 10.6 ± 4.2 years (range: 5–20 years), and the cumulative dose of HCQ was 871.2 ± 589.5 g (range: 365.2–2812.7 g). All 15 cases had a systemic diagnosis of systemic lupus erythematosus for which HCQ was indicated. Four patients had lupus nephritis, two had mucocutaneous lupus, two had musculoskeletal lupus, and one had autoimmune hemolytic anemia as predominant features of their lupus manifestations. None of the cases had detectable structural defects on fundus autofluorescence [Fig. 1a and b] or outer retinal/pigment epithelium changes on spectral-domain OCT [Fig. 1c and d]. In addition, none of the cases had significant scotomas on the 10-2 pattern deviation plots.

A comparison of the structural parameters of GCC assessment between the two groups showed a significantly lower average and the minimum GC-IPL thickness among the cases compared to controls. Similarly, the average RNFL and superior quadrant RNFL thicknesses were lower in the case group than in the control group. RNFL thickness of other quadrants (temporal, nasal, and inferior), though lower in the cases group, was not statistically significant [Table 1 and Fig. 2].

None of the functional parameters of GCC assessment (i.e., implicit times of P50 and N95 waves and amplitudes of N35-P50 and P50-N95 waveforms) showed any statistically significant difference between the two groups [Table 1].

In the cases group, as shown in Table 2, average GC-IPL thickness and average RNFL thickness showed a negative correlation with the duration of HCQ use and the cumulative dose of HCQ but were not statistically significant.

Correlation analysis between the structural and functional parameters in the cases group was done using the Spearman correlation test. Average RNFL thickness and average and minimum GC-IPL thickness were negatively correlated with

Table 1: Baseline characteristics of the two groups (cases and controls)

	Cases Mean±SD	Controls Mean±SD	*P*
Age	42.8±6.5	45.4±6.3	0.279
Weight	60.4±9.6	-	-
Gender (Female and Male)	14 and 1	14 and 1	0.999
Duration of HCQ use (years)	10.6±4.2	-	-
Cumulative dose (g)	871.2±589.5	-	-
Structural assessment of GCC			
Average GC-IPL thickness (μm)	77.0±5.5	82.0±5.3	0.017
Minimum GC-IPL (μm)	71.0±8.1	76.6±6.3	0.041
Average RNFL thickness (μm)	86.7±9.6	94.8±7.6	0.015
Temporal RNFL thickness (μm)	55.6±9.3	59.8±8.6	0.205
Superior RNFL thickness (μm)	105.2±16.7	120.0±15.6	0.018
Nasal RNFL thickness (μm)	69.2±8.6	73.5±8.0	0.172
Inferior RNFL thickness (μm)	118.1±16.8	127.4±12.4	0.097
Functional assessment of GCC			
P50 implicit time (ms)	57.1±9.3	56.5±4.4	0.846
N95 implicit time (ms)	96.3±10.1	95.9±6.5	0.878
N35-P50 amplitude (μv)	4.9±1.4	5.3±2.6	0.617
P50-N95 amplitude (μv)	7.6±2.9	8.7±3.4	0.318
Mean deviation 10-2 (dB)	-3.2±2.1	-	
Pattern standard deviation 10-2 (dB)	2.2±1.9	-	

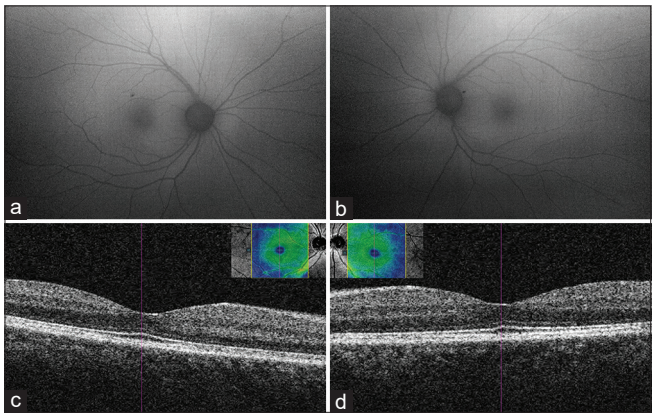

Figure 1: Images of a representative case – a 43-year-old lady on HCQ treatment for systemic lupus erythematosus for the past 7 years and having received a cumulative dose of 511.20 g. Fundus autofluorescence (1a and 1b) and spectral-domain OCT (1c and 1d) images show no evidence of damage to photoreceptors or retinal pigment epithelium

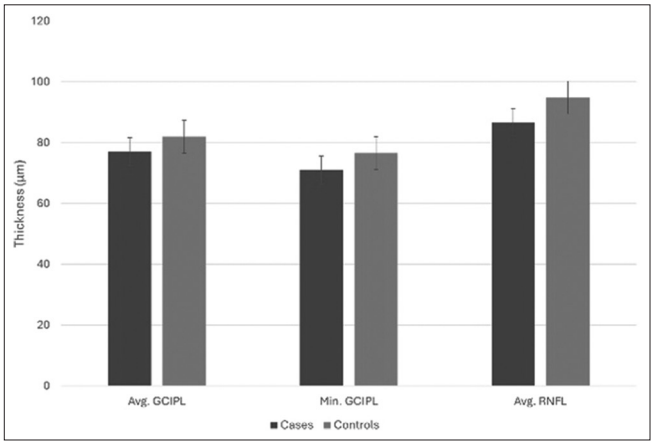

Figure 2: Difference in average and minimum GC-IPL thickness and average RNFL thickness between cases and controls

the implicit times of P50 and N95 waveforms, but none were statistically significant. In addition, average RNFL thickness showed a negative correlation with the mean deviation (MD) and a positive correlation with the pattern standard deviation (PSD) of 10-2 VFs, but statistical significance was not noted [Table 3]. Images of a representative case showing structural and functional analysis of GCC are shown in Fig. 3.

Discussion

Current guidelines on screening for HCQ toxicity focus on early detection of toxicity to the macular photoreceptors. This is done by various structural and functional tests, including macular OCT, fundus autofluorescence, VFs, and multifocal ERG.[4,5] Few animal histopathological studies suggest that chloroquine and its derivative HCQ can affect choroid and various retinal layers. In addition, ganglion cells are affected much before photoreceptors are affected.[6]

Studies have shown that GCC thickness is reduced in patients on long-term HCQ use, even without evident changes on clinical examination or on OCT and VFs.[7,8,12] Our study similarly found that the average and minimum GC-IPL thickness was reduced without evident autofluorescence changes or outer retinal changes on OCT. Pasadhika *et al*.[12] found that the perifoveal GCC is thinned even before the changes are evident in the central area. They utilized a complex protocol to identify the central macular area (area with a diameter of 2.8 mm) and perifoveal area (ring of 1.2 mm width surrounding the central macular area). Such complex analysis is unnecessary these days as the current-generation OCT scans

give an automated analysis of the GCC in an area between 1.2 mm and 4.8 mm from the center of the macula. This gives a fair idea of both central and perifoveal GCC.

Pattern ERG is an electrophysiological test that primarily assesses the function of macular ganglion cells. Several studies have found its utility in diagnosing and managing glaucoma,[13,14] optic neuropathies, and macular pathologies.[15,16] Among the two waveforms (P50 and N95), the N95 waveform is considered better to represent the ganglion cell function. It has been shown that in patients with glaucoma, pattern ERG may detect ganglion cell dysfunction (increased N95 implicit time) much before their death (decreased N95 amplitude) occurs.[17]

To the best of our knowledge, there are no studies assessing the functions of GCC in patients with long-term HCQ. Our study results showed that though there were significant differences between the cases and control groups in terms of GCC structural parameters (GC-IPL and RNFL thickness), there were no significant differences in the functional (pattern ERG) parameters between the two groups.

Subgroup analysis of the cases group showed a negative correlation between the P50 and N95 implicit times and the GC-IPL thickness, indicating the possibility of ganglion cell dysfunction with progressive loss of GCC structure. MD and PSD scores also pointed toward possible deficits of RNFL function but could not be statistically correlated with average RNFL thickness.

Bulut *et al*.[7] had shown a statistically significant negative correlation between the average GC-IPL thickness and cumulative dose of HCQ as well as the duration of use. Similarly. a negative correlation was seen between the duration of HCQ use and the average RNFL and GC-IPL in our study. A similar trend was noted between the cumulative dose and the average RNFL and GC-IPL. However, neither correlation was statistically significant. A small sample size in our study could have contributed to this. A larger sample size with different durations of HCQ use and different age groups of patients will give a better idea of the effect of HCQ on the structure and function of GCC.

Table 2: Correlation of HCQ parameters (duration of HCQ use and cumulative dose) with RNFL and GC-IPL thickness among cases

	Cases	
	Correlation coefficient	*P*
Duration of HCQ use and average RNFL thickness	-0.42	0.110
Cumulative dose and average RNFL thickness	-0.42	0.110
Duration of HCQ use and average GC-IPL thickness	-0.09	0.733
Cumulative dose and average GC-IPL thickness	-0.09	0.733

Conclusion

Long-term HCQ use is associated with definite structural changes in GCC manifested as lower RNFL and GC-IPL thicknesses. The structural changes, however, did not translate into significant functional changes (i.e., pattern ERG/VF changes). Screening for HCQ retinal toxicity should continue with routine recommended tests (SD-OCT, VFs, mf-ERG, and FAF). GC-IPL/RNFL thickness assessment can be a part of the screening. Mere GC-IPL thickness reduction should not be a criterion for HCQ cessation in the absence of abnormality on routinely recommended screening tests. Longitudinal studies with larger sample sizes will help in better understanding the functional implications of reduced GC-IPL thickness.

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Ethical approval and informed consent statements

The study was approved by the institute ethics committee. Written informed consent was obtained from all the study participants.

Table 3: Correlation of structural and functional tests of Ganglion cell complex among cases

	Cases	
	Correlation coefficient	*P*
P50 implicit time and average RNFL thickness	−0.18	0.502
N95 implicit time and average RNFL thickness	−0.24	0.388
N35-P50 and average RNFL thickness	−0.29	0.293
P50-N95 and average RNFL thickness	−0.09	0.724
P50 implicit time and average GC-IPL thickness	−0.10	0.712
N95 implicit time and average GC-IPL thickness	−0.07	0.784
N35-P50 and average GC-IPL thickness	−0.15	0.579
P50-N95 and average GC-IPL thickness	−0.27	0.322
P50 implicit time and minimum GC-IPL thickness	−0.13	0.624
N95 implicit time and minimum GC-IPL thickness	−0.10	0.708
N35-P50 and minimum GC-IPL thickness	−0.19	0.490
P50-N95 and minimum GC-IPL thickness	−0.32	0.236
Mean deviation 10-2 and average RNFL thickness	−0.22	0.418
Pattern standard deviation 10-2 and average RNFL thickness	+0.68	0.005

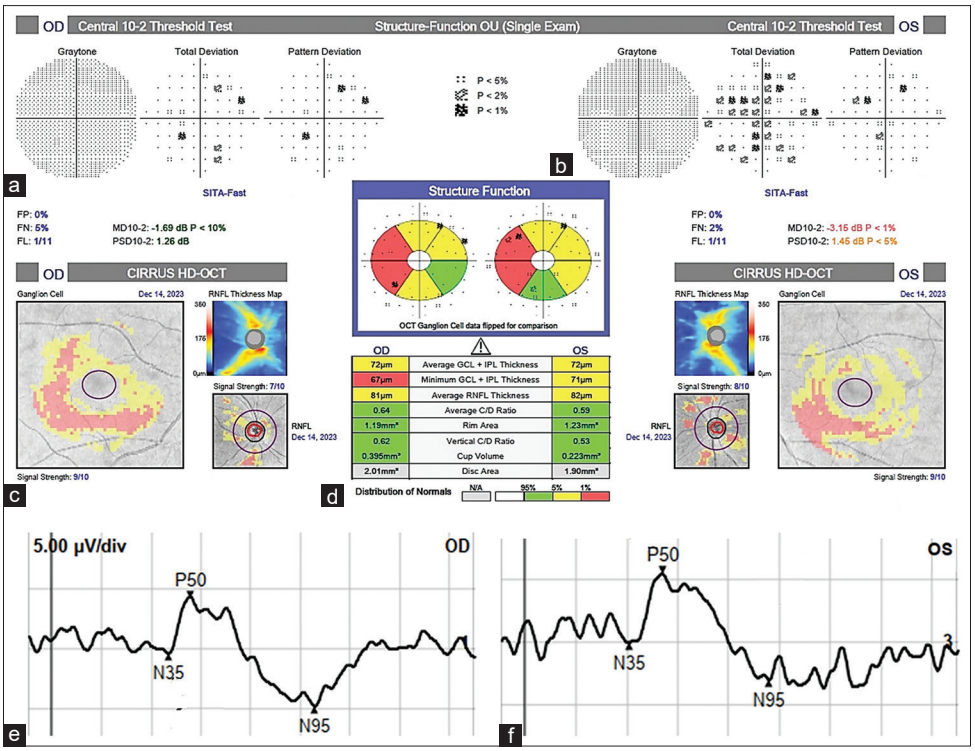

Figure 3: Structure and function correlation of the same patient described in Fig. 1. Central 10-2 visual fields showing no significant scotomas (3a and 3b). OCT structural analysis showed a reduction in average and minimum GC-IPL and average RNFL thicknesses (3c and 3d). Pattern ERG, however, showed implicit times and amplitudes of P50 and N95 waveforms to be within the normal range (3e and 3f)

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest: There are no conflicts of interest.

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