Retinal Damage in Chloroquine Maculopathy, Revealed by High Resolution Imaging: A Case Report Utilizing Adaptive Optics Scanning Laser Ophthalmoscopy

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A 53-year-old Asian woman was treated with hydroxychloroquine and chloroquine for lupus erythematosus. Within a few years, she noticed circle-shaped shadows in her central vision. Upon examination, the patient's visual acuity was 20 / 25 in both eyes. Humphrey visual field (HVF) testing revealed a central visual defect, and fundoscopy showed a ring-shaped area of parafoveal retinal pigment epithelium depigmentation. Fundus autofluorescence imaging showed a hypofluorescent lesion consistent with bull's eye retinopathy. Adaptive optics scanning laser ophthalmoscope (AO-SLO) revealed patch cone mosaic lesions, in which cones were missing or lost. In addition, the remaining cones consisted of asymmetrical shapes and sizes that varied in brightness. Unlike previous studies employing deformable mirrors for wavefront aberration correction, our AO-SLO approach utilized dual liquid crystal on silicon spatial light modulators. Thus, by using AO-SLO, we were able to create a photographic montage consisting of high quality images. Disrupted cone AO-SLO images were matched with visual field test results and functional deficits were associated with a precise location on the montage, which allowed correlation of histological findings with functional changes determined by HVF. We also investigated whether adaptive optics imaging was more sensitive to anatomical changes compared with spectral-domain optical coherence tomography.

Key Words: Adaptive optics scanning laser ophthalmoscopy, Bull's eye maculopathy, Chloroquine maculopathy, Hydroxychloroquines, Photoreceptor

Chloroquine and its analogue hydroxychloroquine have been used successfully for the treatment and prophylaxis of malaria. The indications for chloroquine and hydroxychloroquine have since been expanded to successfully and safely treatment rheumatic diseases including rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, dermatomyositis, Sjögren's syndrome, and chronic juvenile arthritis [1]. At present there are three forms of chloroquine drug in use,

namely, chloroquine sulphate (Nivaquine; Rhone Poulenc, Paris, France), chloroquine phosphate (Aralen; Sanofi-Synthelabo, New York, NY, USA) and hydroxychloroquine sulfate (Plaquenil, Sanofi-Synthelabo).

Visual loss associated with chloroquine is generally irreversible. In its advanced stages, chloroquine-associated visual loss is characterized by a bull's eye maculopathy due to degeneration of the retinal pigment epithelium (RPE) and neurosensory retina [2,3]. Histological sections of the retina from both animals and humans treated with chloroquine revealed disruption of the outer retinal layer [4,5]. Although multiple imaging methods including scanning laser ophthalmoscope (SLO) and spectral-domain (SD) optical coherence tomography (OCT) have been used to

study chloroquine maculopathy, currently available methods are unable to characterize photoreceptor microarchitecture. Aberrations in ocular optics distort SLO and OCT images, and thus in vivo imaging of photoreceptor damage associated with chloroquine has not yet been achieved [6,7]. However, recently developed adaptive optics (AO) imaging can correct ocular aberrations to allow for unprecedented visualization of the photoreceptor mosaic *in vivo*, with resolutions of ≤ 2 μm [6,8].

In the present study, we evaluated a patient with bull's eye maculopathy that developed as a result of chronic use of an antimalarial agent. The patient was studied with various imaging techniques, including AO-SLO. In addition to describing the disrupted microarchitecture of the photoreceptors of the patient, we also demonstrated a correlation between functional and structural defects associated with chloroquine retinopathy. The results of this study may provide a better understanding of antimalarial agent-associated retinopathy.
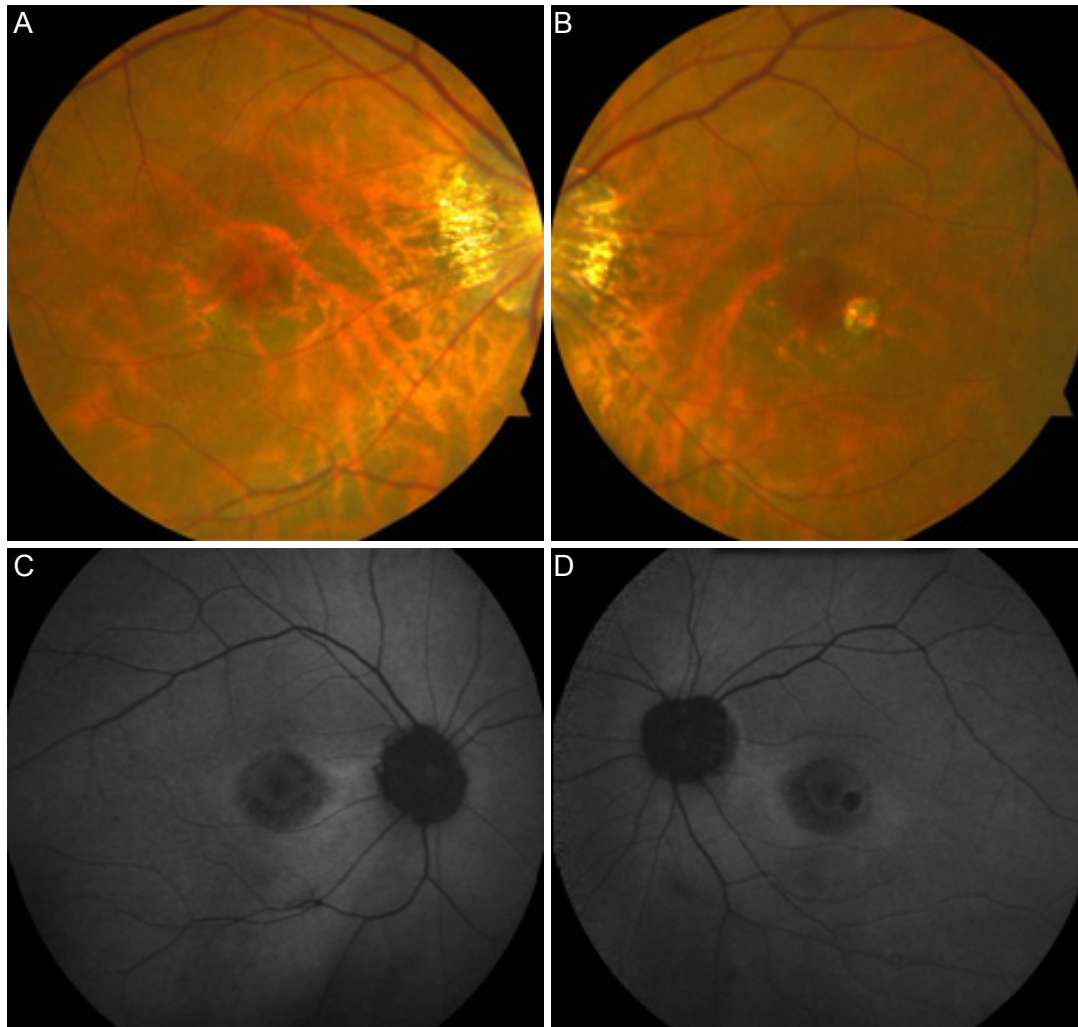
Case Report

A 53-year-old Asian woman with a history of lupus erythematosus was initially treated with hydroxychloroquine (Plaquenil, unknown dose) for 5 years. Approximately 3 years ago, the patient's medication was changed to chloroquine (400 mg daily). Within a few years of taking chloroquine, she noticed a circular shadow in her central vision, which she described as an arch surrounding her central vision. At first, she only noticed a superiorly oriented half-moon shadow that lasted for a few seconds during reading. The shadow was seen while both eyes were open and disappeared after a few seconds. Because of these symptoms and the presence of bull's eye maculopathy on fundus examination, chloroquine was discontinued and the patient's medication was changed to dapsone (unknown dose).

The best-corrected visual acuity (corrected with current spectacles) of the highly myopic patient during her most recent examination was 20 / 25 in both eyes, with no evidence of an afferent pupillary defect. Slit-lamp examination indicated that the anterior segment was unremarkable and Goldmann applanation intraocular pressure was normal (16 mmHg) in both eyes. However, a significant central defect was noted on Humphrey visual field (HVF) examination (Humphrey Visual Field Analyzer 10-2; Humphrey Instruments, San Leandro, CA, USA) in both eyes. Dilated fundoscopy revealed tilting of the optic nerves in both eyes, consistent with myopia, and a ring-shaped area of RPE depigmentation in the parafoveal area of the left eye (Fig. 1). Fundus autofluorescence (FAF) imaging revealed a hypofluorescent lesion in the foveal and perifoveal areas correspond with the bull's eye retinopathy. In the left eye, a prominent hypofluorescent lesion was also noticed on FAF imaging, reflecting marked atrophy of the RPE layer (Fig. 1).

Cross-sectional retinal scans, obtained with SD-OCT (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany) showed obvious thinning of the macula and disruptions in the outer retina. More specifically, loss of the photoreceptor inner/outer segment (IS/OS) junction corresponded to the location of the maculopathy as identified on fundus photography, FAF, and HVF 10-2. The outer retinal layer had a moth-eaten appearance at the IS/OS junction, but the thickness and appearance of the inner retina were within normal limits. Additionally, a downward displacement of the overlying retina was seen in perifoveal areas and corresponded to the hypofluorescent lesion in FAF images. Unlike the anatomically disrupted areas, which corresponded to the bull's eye lesion, the central fovea had normal thickness and structure on SD-OCT in both eyes (Fig. 2).

Instead of using a deformable mirror to correct for wavefront aberrations, the AO system used in the current study utilized a dual liquid crystal on silicon spatial light modulators (LCOS-SLMs; X10468-02, Hamamatsu Photonics, Morimoto, Japan) first introduced by Hirose et al. [8]. Although deformable mirrors have been widely used, their performance is limited with respect to compensating large amounts of aberration of human eyes. Meanwhile, although LCOS-SLM is able to compensate large amounts of aberration by the phase wrapping method utilizing a continuity of the wavefront of a laser beam, it is only capable of compensating a particular polarization component. Most AO systems simultaneously process the measurement/compensation of aberration and SLO imaging. This system is very efficient at following changes in aberration of a subject according to change in imaging location, but is not suitable for obtaining high contrast images because the power of the lights used for simultaneous imaging and measurement/compensation of time is limited for safety. In
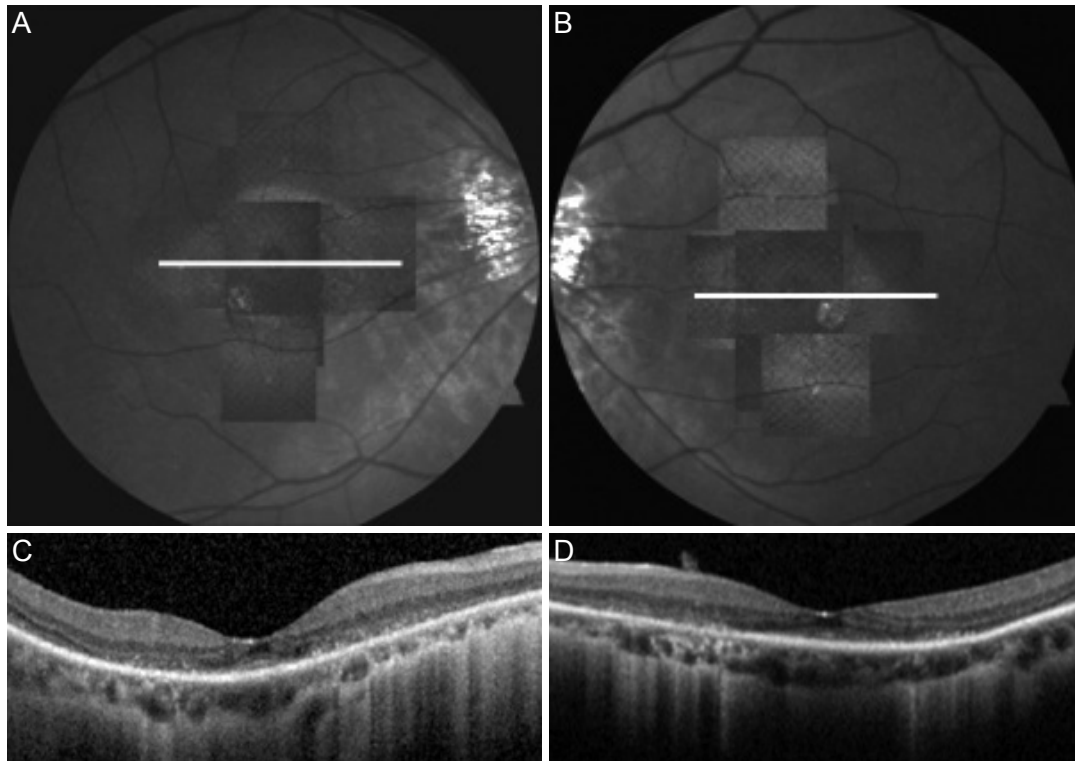
Fig. 1. Photographs of the right (A) and left (B) eyes. Fundus autofluorescent (FAF) images of the right (C) and left (D) eyes are also shown. The FAF image shows a hypofluorescent lesion in the foveal and perifoveal areas consistent with bull's eye retinopathy. A prominent hypofluorescent lesion is visible in the left eye, indicating a marked atrophy of the retinal pigment epithelium layer. The bull's eye pattern of depigmentation is also evident on fundus photography and fundus autofluorescent images.

this study, we overcame these problems by adopting dual LCOS-SLM to compensate the two orthogonal polarization components and sequential processing of SLO imaging and measurement/compensation of aberrations [8].

AO-SLO images were obtained and cone density was measured 0.5, 1.0, and 1.5 mm from the center of the fovea in each of the 4 major directions (superior, inferior, nasal, and temporal). A montage was then created and verified by checking the correspondence between the AO-SLO and wide-field images. Cone density was calculated using photoreceptor analysis software (Canon, Tokyo, Japan). To obtain an accurate cone density, the axial length, anterior chamber depth, and keratometry values were all consid-

ered when counting cone cells, which were represented in images as bright spots 2 to 5 μm in size. We manually selected areas of interest, and excluded retinal vessels and artifacts that obscured the underlying cone mosaic. AO-SLO images showed patch cone mosaic lesions, indicating abnormal regions of missing or lost cones. In addition, the observed cones were asymmetrical in shape and size and varied in brightness (Fig. 3).

Cone densities in the right eye were 14,927, 10,377, and 11,853 cones/mm^2 at 0.5, 1.0, and 1.5 mm from the center of the fovea, respectively. The corresponding cone densities in the left eye were 16,692, 12,564, and 13,100 cones/mm^2, respectively. The cone density at 1.5 mm from the center of

Fig. 2. Adaptive optics scanning laser ophthalmoscope (AO-SLO) montage (A,B) and spectral-domain optical coherence tomography (SD-OCT) (C,D) images of both eyes. The vertical SD-OCT images from both the right (A) and left (B) eyes show loss of photoreceptor inner segment/outer segment junctions (moth eaten appearance).

the fovea was closer to normal than the other distances; however, the photoreceptors located 1.5 mm from the foveal center exhibited asymmetrical shapes and sizes, and varied in brightness in AO-SLO images. Additionally, SD-OCT images showed an abnormal IS/OS cytoarchitecture 1.5 mm from the foveal center. When we matched the disrupted areas on the AO-SLO images with the visual field test, the areas with abnormal cone mosaic patterns and low cone density corresponded with the location of visual field defects on HVF (Fig. 4).
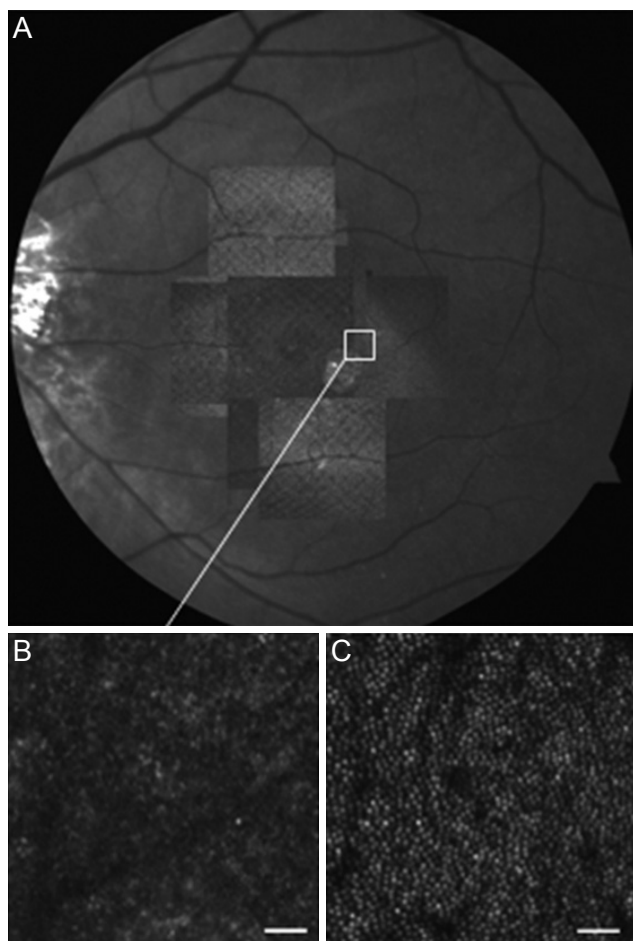
Discussion

Ocular side effects associated with chloroquine can be primarily divided into keratopathy, ciliary body changes, and retinopathy. Bull's eye maculopathy, a type of retinopathy, is associated with the use of antimalarial agents and is relatively rare. However, bull's eye maculopathy is the most serious of the ocular adverse effects and is of serious pathologic concern, as the associated visual changes can be severe and there is little chance of visual recovery. In addi-

tion, visual loss associated with bull's eye maculopathy can progress even after the drug treatment is terminated [9]. Patients with retinopathy usually present with central visual loss, visual field defect, color vision deficiency, photoaversion, night blindness, and entopic phenomenon [3].

Easterbrook reported that patients with minimal paracentral scotomas do not complain of symptoms and have normal color vision. In these patients, retinopathy does not progress after cessation of chloroquine therapy. However, in nearly 70% of patients with ocular symptoms, abnormal color vision, and fundus changes, retinopathy progresses even after termination of drug use [9]. Together, these studies illustrate the importance of early detection, ideally before the onset of visual symptoms. Various modalities are used for chloroquine retinopathy detection and classification. Fluorescein angiography (FA) can reveal the bull's eye pattern of granular hyperfluorescence, but is not useful for the diagnosis of early retinopathy. Most patients with relative scotomas have negative FA results [2]. In full-field electroretinography (ERG), there may be a deepening of the a-wave [10] or an amplitude reduction in the scotopic b-wave [11]. However, in most cases, ERG findings are nor-

Fig. 3. Adaptive optics scanning laser ophthalmoscope (AO-SLO) montage from the left eye (A) matched with the corresponding red free image. Magnified AO-SLO images (B,C) are also shown. (B) shows the area indicated by the white box on the montage. For comparison, (C) shows an age-matched normal retina in the same location. As shown in (B), disruptions in the cone mosaic, where cones were missing or lost, is apparent. These disruptions were not present in the normal subject. Additionally, in (B), cones appear to be asymmetrical in shape and size with variable brightness. Scale bar in (B) and (C) = 25 µm.

lopathy, and that further degeneration occurs despite cessation of medication [2,3]. Additionally, Rosenthal et al. [4] showed that use of chloroquine in rhesus monkeys causes significant changes in both the inner and outer retinal layers. Exposure to ultraviolet radiation causes chloroquine to fluoresce, and based on this property, clinical studies have shown that a widespread, severe reduction of rod and cone processes occur in the outer nuclear and plexiform layers [5]. Still, because of aberrations of ocular optics, none of the currently available imaging methods are capable of observing cells with disrupted photoreceptors in living human eyes. Recently, AO technology has been developed that allows for individual cones to be clearly visualized *in vivo* by compensating for aberrations in ocular optics [6-8]. Using AO-SLO technology, several studies have shown that successful visualization of photoreceptor microstructure is possible in both normal eyes and eyes with various retinal diseases [13,14].

Consistent with previous AO studies, our AO-SLO images from normal eyes showed a regular cone mosaic pattern, in which the cone density decreased with increasing distance from the center of the fovea. In the patient with bull's eye maculopathy, we observed a disrupted cone mosaic pattern with individual cones having irregular shapes and sizes. Additionally, compared with normal patients, patients with bull's eye maculopathy can exhibit irregular cone brightness as well as dark and patchy lesions where cones are either missing or lost. In the present study, along with the disrupted cone mosaic pattern, measured cone densities were diminished in all areas. To better interpret the findings in our study, comparative data was obtained from both previous studies and historical controls. In normal eyes the average cone densities 0.5 and 1.0 mm away from the foveal center are approximately 30,000 and 15,000 cones/mm^2, respectively. These values were in agreement with histological studies reporting cone densities of 37,000, 16,000, and 14,000 cones/mm^2 at 0.5, 1.0, and 1.5 mm away from the foveal center, respectively [15].

Stepien et al. [16] previously reported an AO image of bull's eye maculopathy associated with antimalarial agent use. Although similar abnormal images of cones have been obtained using their AO system, they were not as high quality as the images obtained in the present study. Specifically, we were able to achieve improved image quality and contrast because our AO-SLO utilized a dual liquid crystal on LCOS-SLMs to correct for wavefront aberrations, while
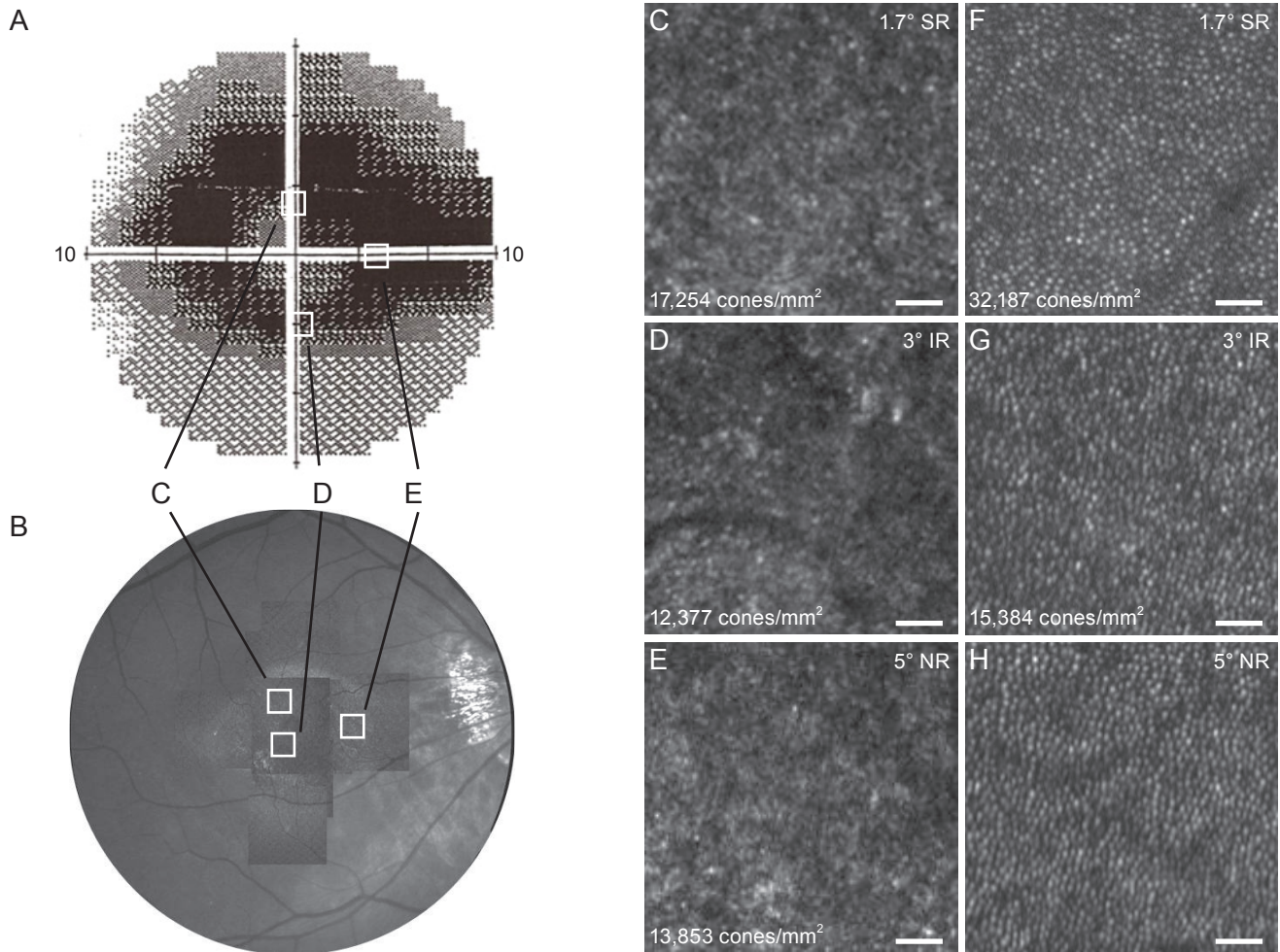
mal with minimal macular damage and decrease only after diffuse retinal damage has occurred [3,11]. A recent report revealed a typical depression of paracentral amplitude in multifocal electroretinograms. It was also reported that SD-OCT images are able to show the perifoveal interruption of the photoreceptor IS/OS junction in eyes with chloroquine retinopathy [12]. However, despite this new information, there is currently no established criteria for diagnosing chloroquine retinal toxicity prior to the development of irreversible visual loss [3].

Studies performed in mice have shown that photoreceptors are the primary targets affected by chloroquine macu-

Fig. 4. Correlation of structural and functional defects. Humphrey visual field (A) revealed a significant central defect. The adaptive optics scanning laser ophthalmoscope (AO-SLO) montage from the right eye (B) was matched with the infrared image. Images (C), (D), and (E) are magnified AO-SLO images of the locations of visual field defects (white boxes). Images (F), (G), and (H) are images in the same location from an age-matched normal retina. The location and cone density for each figure is shown. The AO-SLO images (C), (D), and (E), show the cone mosaic disruption and dark patchy lesions where cones are missing or lost. Image D had the lowest cone density, and was lower than observed in a normal subject (F,G,H). Cones in images (C), (D), and (E) were asymmetric in shape and size and exhibited variable brightness. Scale bar in (C), (D), and (E) = 25 μm. SR = superior retina; IR = inferior retina; NR = nasal retina.

the system used by Stepien et al. [16] relied upon a deformable mirror. Moreover, creating a montage of AO-SLO images made it possible to compare each AO-SLO image to visual field test results. Using this technique, we were able to demonstrate that areas with visual defects could be matched with cone pattern and density abnormalities. Because AO-SLO can image the retina with such high resolution, it is more sensitive to photoreceptor abnormalities than SD-OCT [6,7]. In our study, SD-OCT images revealed equal disruption of the outer retinal layer at 1.0 and 1.5 mm away from the fovea. However, AO-SLO images revealed a higher level of cone pattern disruption and a lower cone density at 1.0 mm away from the fovea, compared with 1.5 mm away from the fovea.

There were several limitations of the present study that should be mentioned. Because the central foveal structure was conserved, our patient had a visual acuity of 20 / 25. However, we could not obtain images of the central fovea for reasons described in previous studies [6-8]. Unfortunately, the inability to obtain these images made it impossible to compare cellular images of the fovea with those obtained from the bull's eye lesion. Another limitation of our study was that the AO-SLO system could not acquire images from the central fovea or the peripheral retina. Therefore, we only investigated cone properties at 0.5, 1.0, and 1.5 mm away from the center of the fovea. Be-

cause of this limitation, we were unable to compare preserved areas adjacent to the bull's eye lesion, which may possibly have contained cones in the preclinical stage of the disease.

As described above, the visual acuity of the patient was 20 / 25, and we hypothesized that the reason for the relatively mild visual decrease was because the structure of the central fovea remained relatively preserved. However, we could not confirm this possibility because the center of the fovea could not be precisely imaged by AO-SLO. Similarly, because the AO-SLO cannot obtain images from the peripheral retina, it was not possible to compare the disrupted macula to the relatively preserved peripheral retina [6,7,15].

Depending on the specific disease, patients may be treated with either a chronic maintenance or acute high-level dose of chloroquine. A daily dose of chloroquine >3 mg/kg and a treatment duration of >5 years are considered important risk factors for the development of retinopathy [3]. Therefore, patients who are taking chloroquine or hydroxychloroquine should have regular ophthalmic examinations to detect retinal changes as early as possible in order to minimize retinal toxicity. In the present study, we showed that AO-SLO can image cellular structures of the retina in living eyes in order to detect preclinical stages of chloroquine associated retinal damage that may not be apparent on standard clinical tests (e.g., fundus photography, FA, HVF, and electrophysiological testing). Importantly, the ability to identify photoreceptor disruptions in the preclinical stages of toxicity should allow for chloroquine withdrawal during the reversible stages of maculopathy. Despite the limitations of our AO system, our results suggest that AO-SLO should provide a non-invasive, quantitative, high-resolution modality for imaging chloroquine retinopathy patients. Likewise, the abilities of AO-SLO have the potential to provide a new approach for the diagnosis and monitoring of chloroquine toxicity.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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