The risk of Hydroxychloroquine retinopathy and the role of the multifocal electroretinogram in screening and monitoring retinal toxicity

by
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Abstract

Background: Hydroxychloroquine sulfate (Plaquenil) is a useful, inexpensive drug with a high systemic safety profile. Prolonged use of Plaquenil however causes toxic effects on ocular structures, including the retina. The aim of this study was to determine the role of the multifocal electroretinogram (mfERG) in screening and monitoring Plaquenil retinal toxicity by determining its sensitivity and specificity, assessing the changes in abnormal mfERG due to Plaquenil retinal toxicity after therapy cessation and identifying risk factors associated with Plaquenil retinal toxicity. **Methods:** 414 patients on Plaquenil therapy were retrospectively studied. Multifocal ERG, spectral domain-OCT and 10-2 Humphrey visual field chart images were reviewed. Clinical and demographic characteristics of the patients were also collected. COX univariate and multivariate regression analyses were used to determine risk. Relative sensitivity and specificity of mfERG, SD-0CT and 10-2 Humphrey visual field were determined using logistic regression and ROC curve analysis. One way-ANOVA with repeated measure and Post Hoc analysis was applied to assess change in abnormal mfERG response secondary to Plaquenil therapy cessation.

Results: The most significant predictors of Plaquenil toxicity were cumulative dose >1 kg (cumulative dose >1 kg, odd ratio (OR)= 0.21, 95% CI= 0.047-0.957, cumulative dose >2 kg, OR= 0.33, 95% CI= 0.127-0.875) , daily drug dose >5 mg/kg (OR= 0.19, 95% CI= 0.072-0.506) and duration of therapy >5 years (OR= 2.30, 95%C1= 0.06-0.30, P=0.028). mfERG, SD-OCT and 10-2 VFT share a commonality in their ability to correctly classify non-toxic patients (specificity >90%) but mfERG was the most sensitive of the three (sensitivity= 92%).

Conclusion: A combination of at least two of these screening tests yields higher sensitivity and specificity. Abnormal mfERG response in Plaquenil retinal toxicity could be recovered if therapy is discontinued before significant reduction in mfERG ring amplitudes (not >50%) occurs.

Preface

This thesis is an original work by Beatrice Adamptey. The research project, of which this thesis is a part, received ethics approval from the University of Alberta Health Research Ethics Board, under the Project Name "The role of the multifocal electroretinogram in screening for hydroxychloroquine retinopathy. The development of hydroxychloroquine screening guidelines for Edmonton", No. 72173, 05/04/2017.

I was responsible for data collection, analysis and composition of thesis. Dr. Ian MacDonald was the project supervisor and was responsible for conception of project, data editing and interpretation, as well as ensuring overall quality of the data and data analysis.

Acknowledgement

My sincerest gratitude is to Dr. Ian MacDonald for his tremendous support, guidance and encouragement throughout my graduate studies and for this research project in particular. I am grateful to Dr. MacDonald for his mentorship and the valuable lessons of integrity, discipline and hard work which I have learnt from being his student.

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Table of Contents

List of tables

List of figures

List of Abbreviations

ARMD	Age related macular degeneration
AUC	Area under the curve
BMI	Body mass index
BRVO	Branch retina vein occlusion
CD	Cumulative dose
CI	Confidence interval
CRAO	Central retina artery occlusion
CRF	Case report form
CSME	Clinically significant macula oedema
DDD	Daily drug dose
EIA	Eye Institute of Alberta
FN	False negative
FP	False positive
GCL	Ganglion cell layer
INL	Inner nuclear layer
IPL	Inner plexiform layer
IQR	Interquartile range
IS/OS	Photoreceptor inner segment/outer segment junction
mfERG	Multifocal electroretinogram
NFL	Nerve fibre layer
NPV	Negative predictive value
OD	Right eye
ONL	Outer nuclear layer
OPL	Outer plexiform layer
OR	Odds ratio
OS	Left eye
PDR	Proliferative diabetic retinopathy
PPV	Positive predictive value
RA	Rheumatoid arthritis
ROC	Receiver operating curve

ROP	Retinopathy of prematurity
RPE	Retina pigment epithelium
SD-OCT	Spectral domain optical coherence tomography
SLE	Systemic lupus erythematosus
TDD	Total daily dose
TN	True negative
TP	True positive
VA	Visual acuity
VFT	Visual field test

Statistical definitions

•False positive rate (α) = type I error = 1 − specificity = FP / (FP + TN) =

•False negative rate (β) = type II error = 1 − sensitivity = FN / (TP + FN) = Power= sensitivity = 1 − β

•Positive likelihood ratio = sensitivity / (1 − specificity)

•Negative likelihood ratio = (1 − sensitivity) / specificity

A likelihood ratio of greater than 1 indicates the test result is associated with the disease. A likelihood ratio less than 1 indicates that the result is associated with absence of the disease.

•True positive: Patients with Plaquenil toxicity (condition) correctly identified as having Plaquenil retinal toxicity

•False positive: Patients without Plaquenil retinal toxicity incorrectly identified as having Plaquenil retinal toxicity

•True negative: Patients without toxicity correctly identified as not having Plaquenil retinal toxicity

•False negative: Patients with Plaquenil retinal toxicity incorrectly identified as not having toxicity

•True positive = correctly identified

•False positive = incorrectly identified

•True negative = correctly rejected

•False negative = incorrectly rejected

1. INTRODUCTION

1.1 Hydroxychloroquine sulfate (Plaquenil)

Hydroxychloroquine sulfate (Plaquenil) (Figure 1) is originally an anti-malaria drug and a derivative of chloroquine (1). Plaquenil is a highly lipophilic base, more soluble and less toxic than chloroquine (1,2). The hydroxyl group of hydroxychloroquine reduces its ability to cross the blood brain barrier, thus reducing side effect on the central nervous system, including the retina as discussed in a review by Ding et al (3). It functions by interfering with immune response activation and antigen presentation by increasing lysosomal pH (1,4) . Primarily, it inhibits prostaglandin and cytokine production and controls signalling and leucocyte activation processes (3,5,6), thus resulting in immunosuppression, antithrombotic effects and control of inflammatory flares (3,4). Plaquenil metabolism takes place in the liver while the kidney serves as the primary organ responsible for its excretion as a metabolite or as intact drug (≈ 60%) (5). Plaquenil often has a slow onset of action varying between two to three months (5). It is widely used in rheumatologic care for the effective control of inflammatory, connective tissue and autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren syndrome (2,7–9). In North America, Plaquenil is part of the five classes of drugs used in managing rheumatoid arthritis (5) and is listed as a disease modifying drug for rheumatoid arthritis in the World Health Organization model list of essential medicines (10,11). The efficacy and safety profile of Plaquenil in managing rheumatoid arthritis and SLE are well documented in a systematic review by Gaujoux-Viala et al. (12). It's been found to be safe even in pregnancy where it is believed to confer some form of protection for the foetus at high risk of SLE when taken by a mother during pregnancy (13). Besides its high safety profile, it is also relatively cheaper with less adverse ocular side effect compared to chloroquine (5,10,14). Plaquenil is also often used across many disciplines in medicine including dermatology and oncology, with consideration for future use as a potential drug in managing diabetes mellitus, heart diseases and as an adjunct therapy in cancer treatment (9–11). In spite of its relatively safe profile, and cost effectiveness (that is both efficacious and less expensive with less adverse effect compared to other medications used for similar purpose), hydroxychloroquine sulfate still causes vision threatening side effects referred to as hydroxychloroquine or Plaquenil retinal toxicity/ retinopathy (15,16).

Figure 1 Molecular structure of hydroxychloroquine

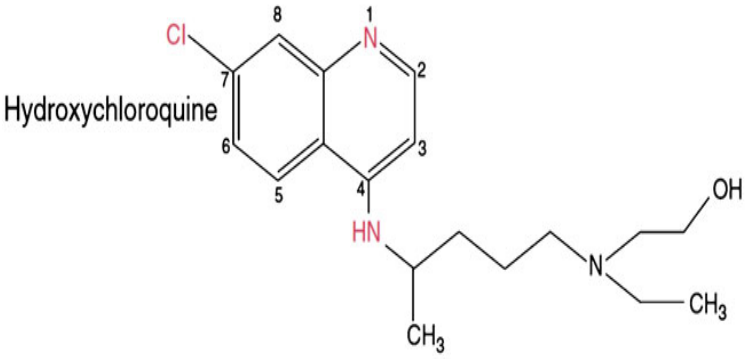
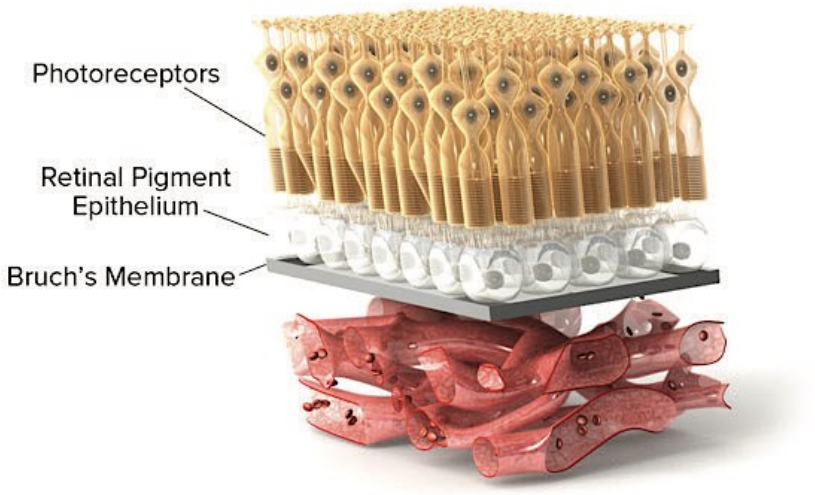


Image adopted and used with permission from Browning DJ (2014). Hydroxychloroquine and chloroquine retinopathy, pages 1-38 Springer publications, New York.

1.2 Hydroxychloroquine (Plaquenil) retinopathy

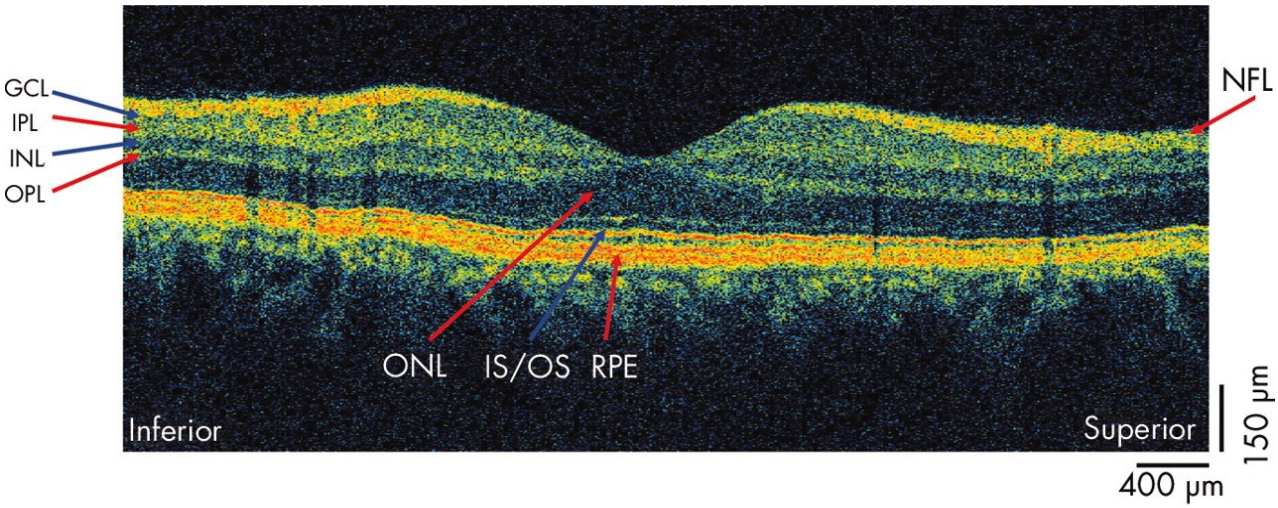
Hydroxychloroquine sulfate or Plaquenil retinopathy is a progressive, often irreversible vision loss due to persistent damage to photoreceptor cells and the retinal pigment epithelium (RPE) layer (Figure 2) (3,7,9). Plaquenil retinal toxicity is often observed as disintegration of the parafoveal ellipsoid zone at the junction of photoreceptor inner and outer segment (1,17) (Figure 3). This area of disruption (Figures 3, 4, 5), is approximately 0.5mm -1.0 mm (Figure 6) from the centre of the macular (fovea) (17) and corresponds to ring 2 amplitude on multifocal electroretinogram (mfERG) (Figures 6, 7), inner concentric ring on spectral domain optical coherence tomography (SD-OCT) (Figures 5, 7), and paracentral area on automated Humphrey visual field (VFT) (Figure 12) (16,18). Different definitions however exist (9,19,20).

Figure 2 Image of retina showing photoreceptor cells and RPE



Photoreceptor/RPE membranes adapted from scienceofamd.org

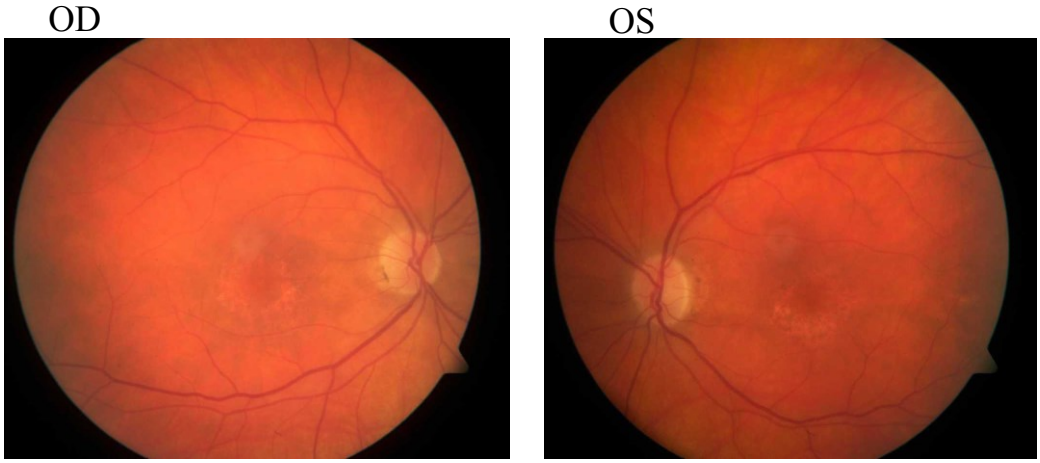
Figure 3 SD-OCT image of photoreceptor cells showing ellipsoid zone (junction between inner and outer segment of the photoreceptor cells (IS/0S)) in a normal eye



Adopted and used with permission from Witkin AJ et al (2005). ultrahigh resolution optical coherence tomography of birdshot retinochoroidopathy. Br J Ophthalmol 2005;89:1660-1

NFL: nerve fibre layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, IS/OS: photoreceptor inner/outer segment junction, RPE: retinal pigment epithelium.

Figure 4 Fundus image of Plaquenil retinal toxicity involving mainly parafoveal region



Fundus image of a 67year old patient being screened and monitored for Plaquenil retinal toxicity as captured in the secure diagnostic database of the EIA, Edmonton, AB, showing Bull's eye maculopathy due to Plaquenil use.

Figure 5 Macular area correlates with SD-OCT concentric rings

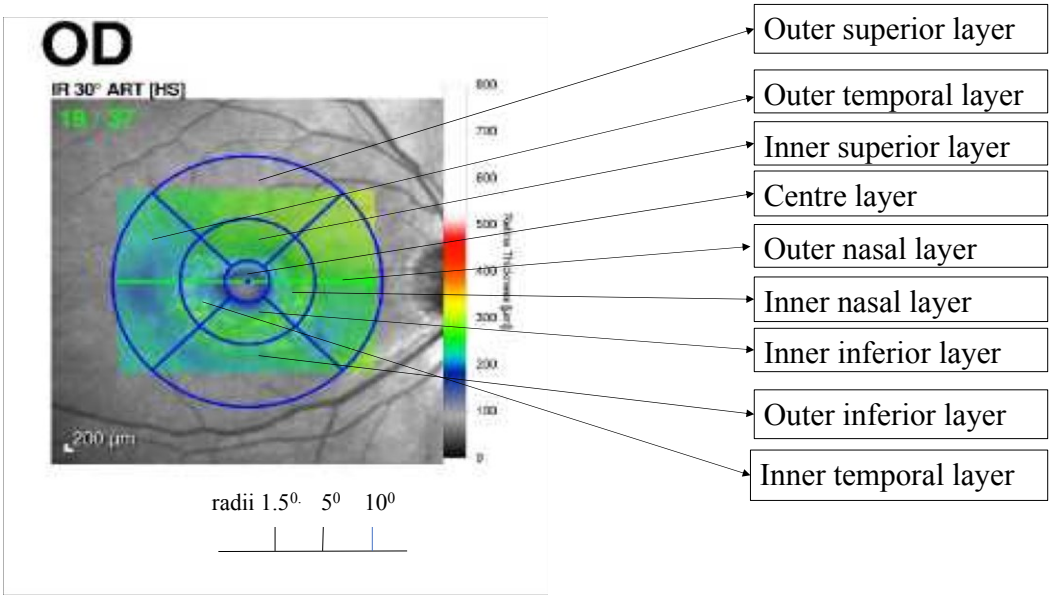


Figure 6 Macular correlates of ring amplitudes of the 61 hexagons of the multifocal electroretinogram.

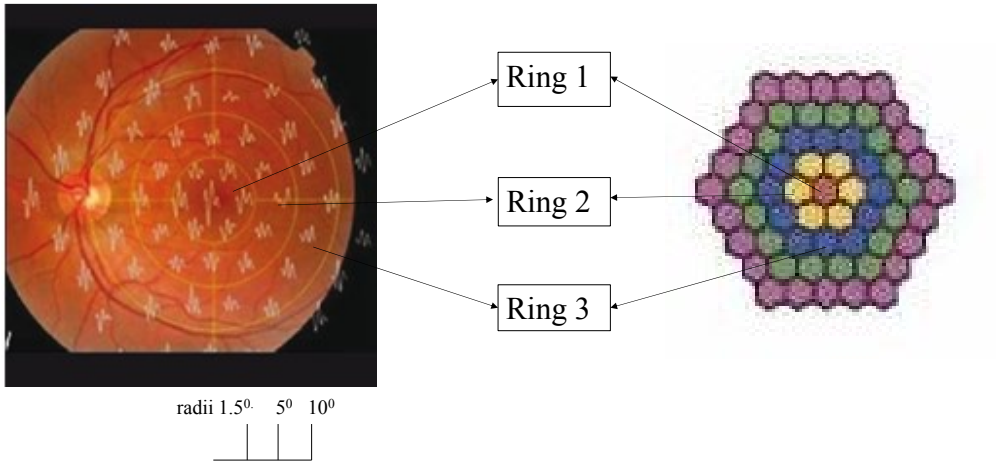
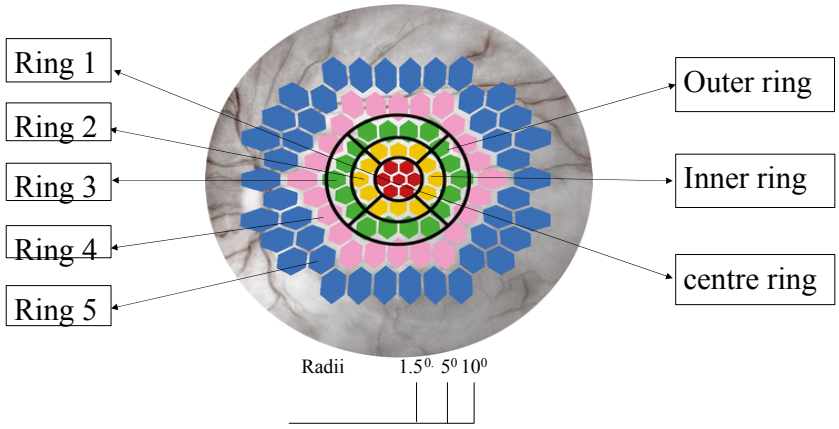


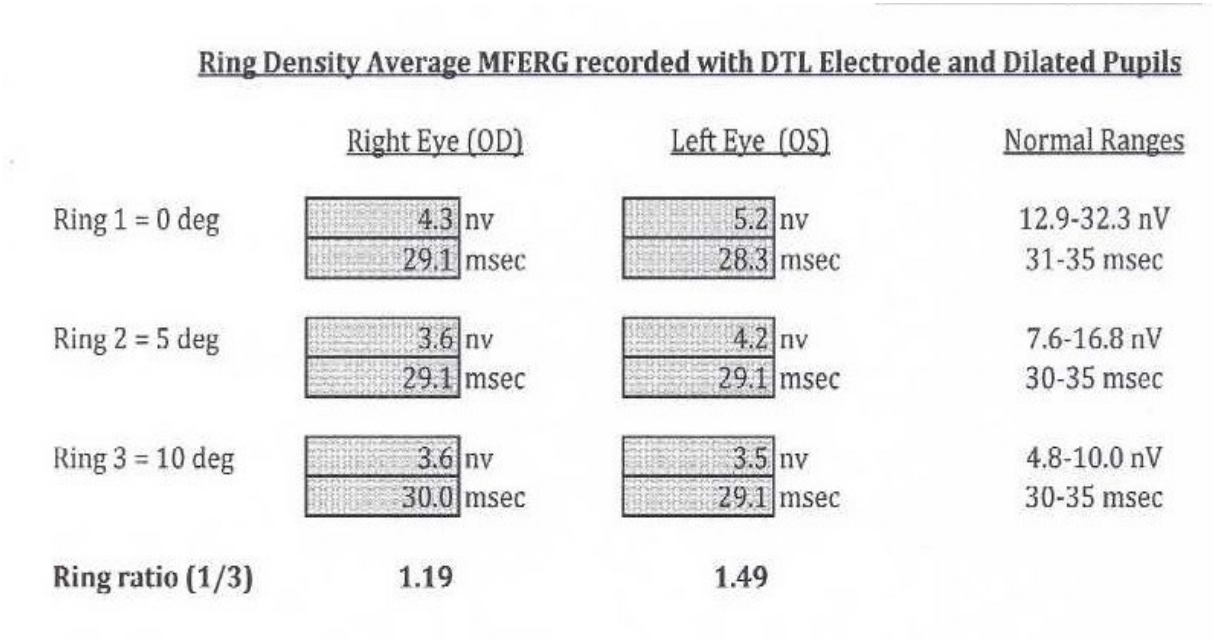
Figure 7 Macular correlates with SD-OCT concentric rings and mfERG ring amplitudes



Adopted and modified from Akerblom et al (2016). Macular function in preterm children at school age. Doc Ophthalmol. 2016;3:151-7

Plaquenil retinopathy presents as a partial or full ring parafoveal scotoma (Figure 12) which could be perceived by the patient or discovered by automated Humphrey visual field examination (16,21). On spectral domain optical coherence tomography (SD-OCT), hydroxychloroquine retinopathy presents as thinning of the parafoveal retina, (Figure 10) predominantly affecting photoreceptors (16,18,22,23). Plaquenil retinal toxicity is observed as a reduction in ring amplitudes (Figures 8, 9) involving particularly ring 2, with/without elevated ring 1/2 ratio or ring 1/3 ratio, and with/without delayed implicit time on mfERG (2,24). Plaquenil retinal toxicity progresses over time to a characteristic clinic feature known as bull's eye maculopathy (figure 4).

Figure 8 Abnormal mfERG response in Plaquenil retinal toxicity

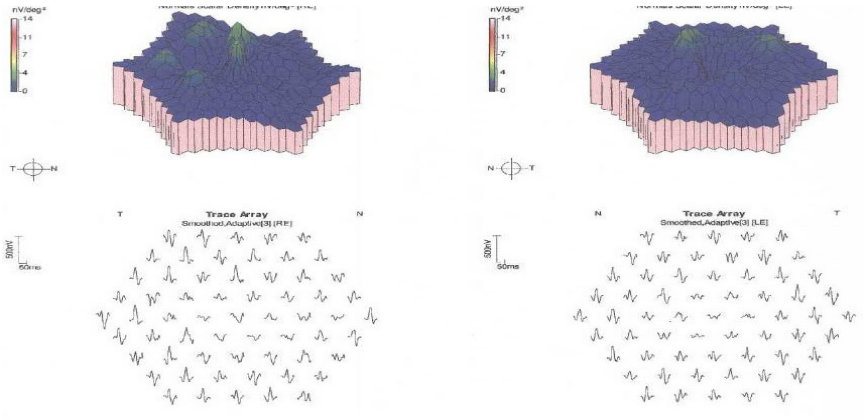


Ring Density Average MFERG recorded with DTL Electrode and Dilated Pupils

	Right Eye (OD)	Left Eye (OS)	Normal Ranges
Ring 1 = 0 deg	4.3 nv 29.1 msec	5.2 nv 28.3 msec	12.9-32.3 nV 31-35 msec
Ring 2 = 5 deg	3.6 nv 29.1 msec	4.2 nv 29.1 msec	7.6-16.8 nV 30-35 msec
Ring 3 = 10 deg	3.6 nv 30.0 msec	3.5 nv 29.1 msec	4.8-10.0 nV 30-35 msec
Ring ratio (1/3)	**1.19**	**1.49**	

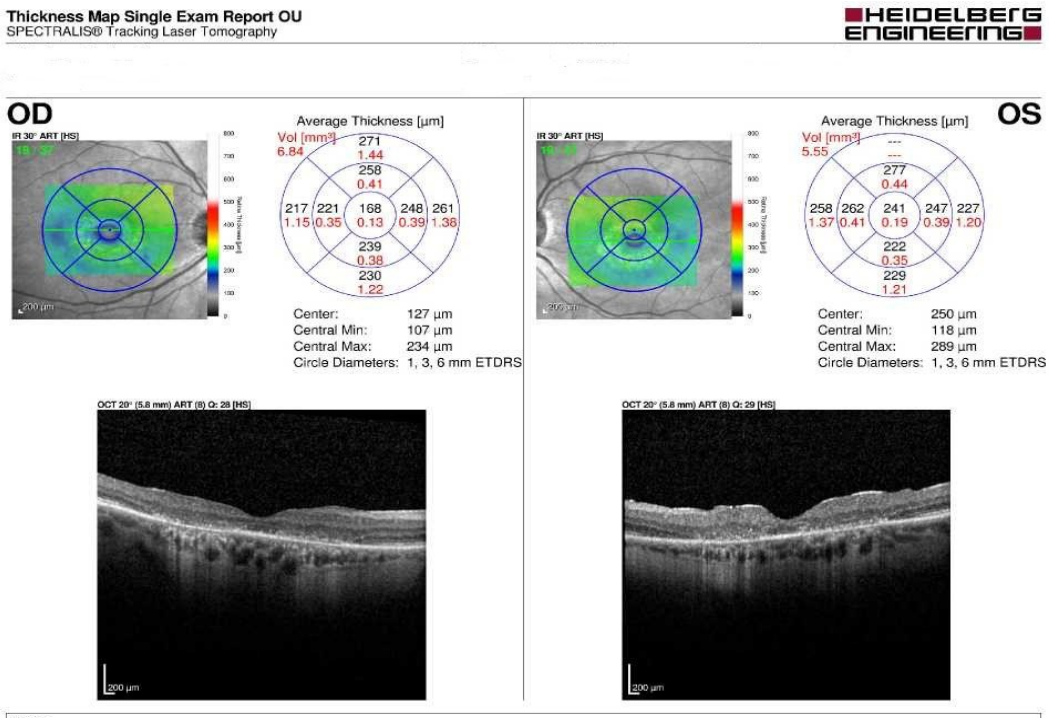
mfERG ring amplitudes response for a patient captured in the EIA secure electronic database with Plaquenil retinal toxicity shows severe reduction in amplitudes on all rings.

Figure 9 Distortion on mfERG waveform in Plaquenil retinal toxicity



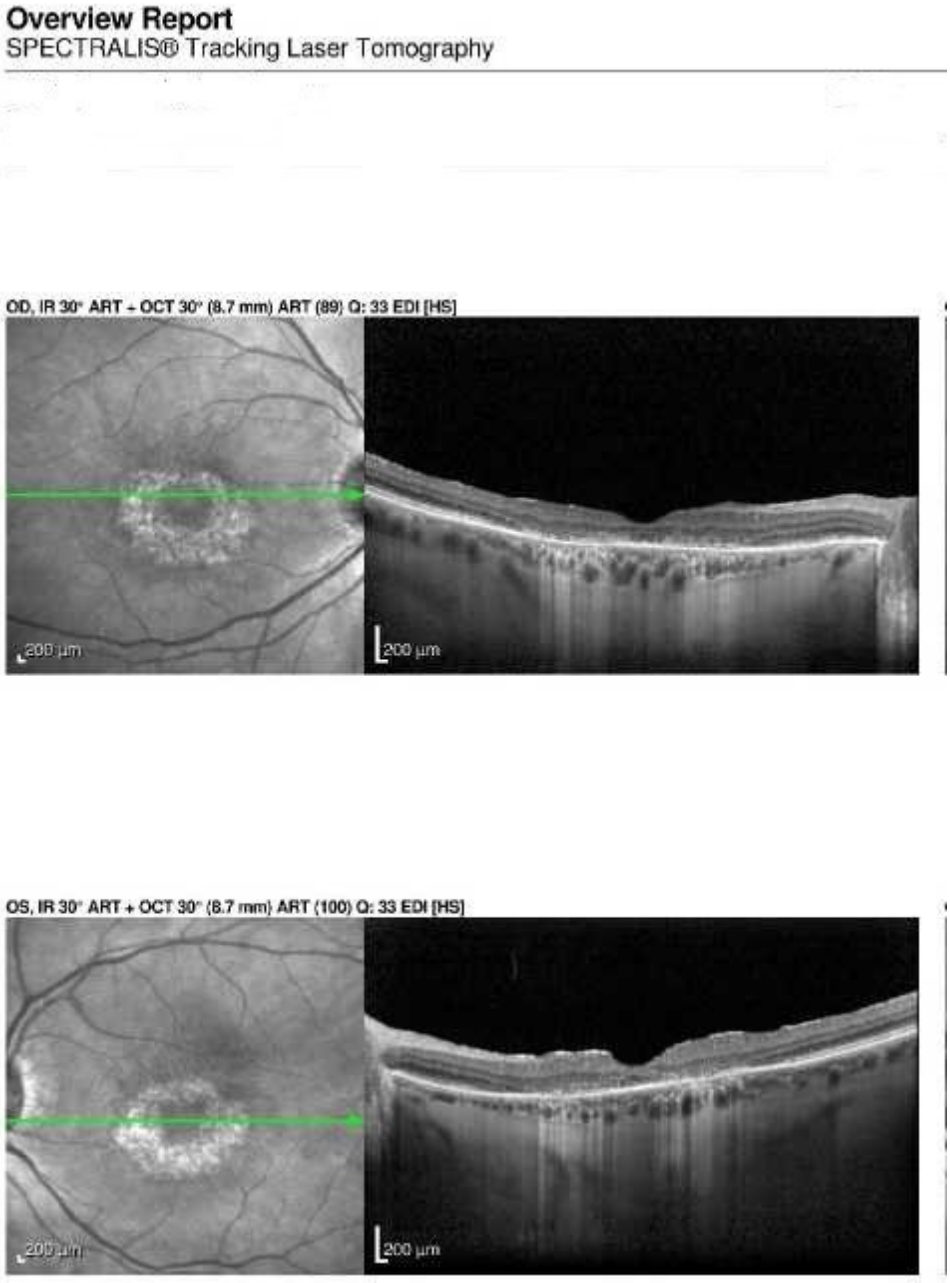
Abnormal waveform presentation for a patient with Plaquenil retinal toxicity whose mfERG ring amplitudes are shown in figure 8. This data was captured from the EIA electronic database.

Figure 10 Abnormal SD-OCT presentation in Plaquenil retinal toxicity



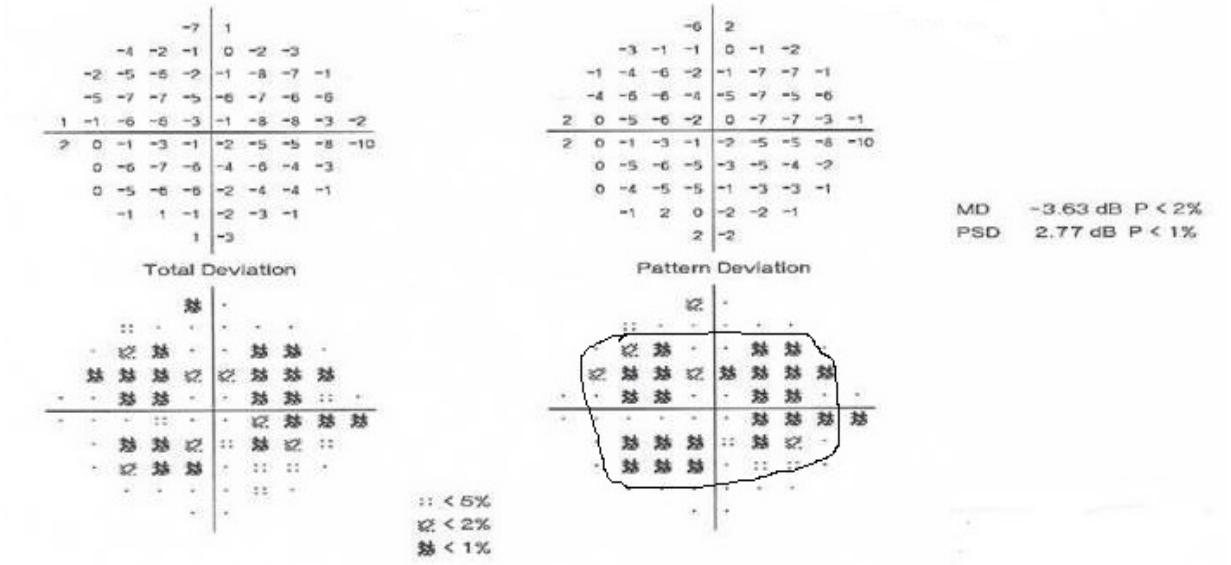
SD-OCT for a 67year old patient captured from the EIA database with Plaquenil retinal toxicity showing disruption in the photoreceptor and RPE layer of the retina with reduced retinal layer thickness in both eyes.

Figure 11 FAF image in Plaquenil retinal toxicity



Parafoveal area of hyperfluorescence in Plaquenil retina toxicity for 67 years old patient captured in the EIA database.

Figure 12 Abnormal 10-2 Humphrey visual field presentation of Plaquenil retinal toxicity



10-2 Humphrey automated visual field presentation of a 67year old patient at the point of Plaquenil retinal toxicity showing almost full ring paracentral scotoma captured from EIA database.

The patient with Plaquenil retinal toxicity may be asymptomatic in early disease stage. However, patients may present with visual symptoms such as decreased night vision, difficulty with reading or near activities, photopsia, metamorphosia, colour vision problems and possible peripheral field loss or blindness in advanced retinopathy (7,25). Plaquenil retinal toxicity is important because it is potentially blinding with no available treatment currently (9,16). Early detection on eye screening and discontinuation of treatment remains the main means of preventing vision loss (1,7,9). Blindness and low vision have implications not only on quality of life (26) but mortality, risk of fall and fracture, possibility of gainful employment, huge economic burden both on the patient and the health care system, and loss of productive human resource (27–30). The fear of developing retinopathy leads to non-compliance with treatment among patients and discontinuation of use of an otherwise useful, effective and cheaper medication (3,15,31).

1.3 Mechanism of hydroxychloroquine retinopathy

The biological and physiological processes that produce retinopathy following long term hydroxychloroquine therapy are not clearly understood (3,7). Different mechanisms have been proposed including;

- Lysosometropism (9,21)
- Toxic effect of metabolic by-products from photopigment recycling (21)
- Melanotropic nature of Plaquenil (21).
- Involvement of defective genes and light energy from the sun (9,32).

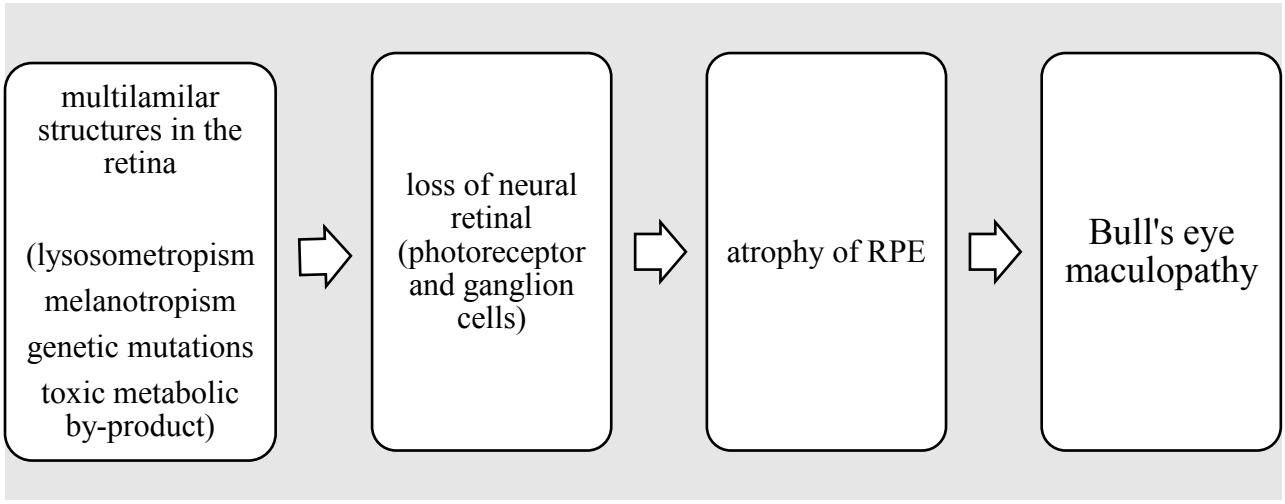
Lysosometropism involves altering of the lysosomal pH of intracellular structures due to accumulation of Plaquenil in phagosomes of macrophages which results in inhibition of lysosomal phospholipase. Lysosomes become swollen as they imbibe water. Swollen lysosomes combine with photoreceptor outer segments to form lamellar structures which leads to destruction of photoreceptor cells over time (1,14).

Photopigment recycling produces a toxic metabolic by-product called lipofusin. Lipofuscin is believed to have toxic side effect on photoreceptor cells (1).

Plaquenil is a melanotrophic drug that binds readily to cells and tissues with high melanin content such as the retinal pigment epithelium, photoreceptor cells and the skin. It is proposed that, Plaquenil readily accumulates in photoreceptor cells of the retina due to its affinity for melanin. High concentration of Plaquenil in the photoreceptor cells of the retina for a prolonged time period leads to cell disruption (1). Literature (3,33,34) is however divergent on the effect of the melanotrophic nature of Plaquenil in causing toxic effect on retinal layers. Some studies have suggested that the melanotrophic nature of Plaquenil could be responsible for accumulating high concentrations of the drug in the retina which leads to retinal toxicity while other studies have rather indicated that melanin could actually be protective against Plaquenil retina toxicity (1,9,34).

The effect of genetic mutation particularly in the *ABCA4* gene has also been cited as possible mechanism of hydroxychloroquine retinopathy (32). *ABCA4* protein is involved in the removal of one of the toxic substances called N-retinylidene-PE from photoreceptor cells which is a by-product of phototransduction processes. Mutation in *ABCA4* leads to accumulation of N-retinylidene-PE in photoreceptor cells, thus causing photoreceptor cell disruption. The Proposed mechanism for Plaquenil retinal toxicity is summarized in Figure. 13.

Figure 13 Flow chart of mechanism of Plaquenil retinal toxicity



Shroyer et al. (2001), Am J Ophthalmol, Marmor et al (2011), Ophthalmology, Michaelides et al (2011) Arch Ophthalmol.

1.4 Incidence/prevalence of hydroxychloroquine retinopathy

Hydroxychloroquine retinopathy was considered a rare condition with very low prevalence in the past (19,35). Screening for Plaquenil retinal toxicity was in the past deemed unnecessary in some countries such as the United Kingdom due to rarity of the condition (35–37). Earlier records of global prevalence of hydroxychloroquine retinopathy were between 0.1 - 0.4% among those on Plaquenil therapy (7,9,16). The estimated low prevalence in the past was attributed to less sensitive screening tools available at the time, lack of awareness and knowledge of the disease and less use of the drug across many different disciplines in medicine (9,11,19). Current global prevalence of hydroxychloroquine retinopathy is estimated between 0.4% and 1% (7,38) among patients on therapy. Melles and Marmor in 2014, recorded a 7% proportion of Plaquenil retinal toxicity among those on therapy (16).

1.5 Risk factors for hydroxychloroquine retinal toxicity

The risk, progression and severity of hydroxychloroquine retinopathy can be aggravated by some extraneous and internal factors related to the patient. The most recent statement on recommended screening protocols for Plaquenil retinal toxicity by the American Academy of Ophthalmology (AAO) (9) in 2016 indicates the following as risk factors;

- Main risk factors: daily drug dose, duration of therapy, cumulative dose, presence of renal disease, retinal and/ macular diseases and concomitant use of tamoxifen.
- Lesser risk factors: age, genetic factors and liver disease.
- Others: real/ideal body weight and body mass index (BMI)

1.5.1 Daily drug dose

A daily Plaquenil drug dose exceeding 5 mg/kg of real body weight is considered the most significant risk factor for hydroxychloroquine retinopathy (9,16). Melles and Marmor (16) in 2014 reported that the risk of toxicity in patients on a hydroxychloroquine daily drug dose of >5 mg/kg within ten 10 years of therapy was about 10%. However, for the same duration of Plaquenil therapy, the risk of retinal toxicity was just about 2% in patients using a daily drug dose of <5 mg/kg. In 2015, two separate studies (39,40) confirmed the relation between daily drug dose and risk of toxicity indicating that high daily drug dose exceeding 5 mg/kg of real body weight was associated with higher risk of toxicity. Leung et al. and Navajas et al. (39,40) in 2015 reported higher incidence of hydroxychloroquine retinopathy among patients on very high daily Plaquenil dose of > 10 mg/kg as part of cancer and chronic graft-versus-host disease to be 25%, with retinal toxicity occurring within 1-2 years of Plaquenil therapy. There may exist, however, no safe Plaquenil dose and toxicity could still occur even among patients on long-term Plaquenil daily drug dose of <5 mg/kg of (3,7,9,16,41). The 2016 AAO revised recommendation on screening advises that, patients should be administered not more than 5mg/kg of daily dose of Plaquenil to decrease their risk of developing toxic retinal defect unless giving a higher dose is necessary to avert a life threatening condition (9).

1.5.2 Cumulative Plaquenil dose as a risk factor for Plaquenil retinal toxicity

Cumulative Plaquenil dose (kg) is another important risk factor for toxic retinopathy among patients on Plaquenil therapy (2,7,41). It is sometimes considered a better predictor of Plaquenil retinal toxicity than daily drug dose as it takes into account therapy duration (42). The risk of Plaquenil retinal toxicity increases with a cumulative drug dose of >1000 g/ 1.0 kg (2,7,24). Marmor and Wolfe in 2010 found the risk for toxicity for patients on >1000 g cumulative dose to be 4.5 (95% CI 1.4 –14.5) times compared with patients on <1000 g cumulative dose. Lyons and Severns (2,24) reported ≈41% incidence of Plaquenil retinopathy in patients who had accumulated a dose of >1250 g and 10% incidence in those patients on <1250 g. Like daily drug dose, patients on <1000 g of Plaquenil cumulative dose could still be susceptible to toxicity (41), while other patients are able to tolerate a high cumulative dose of up to approximately 4000 g without any sign of retinal toxicity (43,44).

1.5.3 Duration of therapy as risk for Plaquenil retinal toxicity

Duration of Plaquenil therapy is an important risk factor for Plaquenil retina toxicity (7,9,15,16,45–47). The risk of retinal toxicity among patients on Plaquenil therapy is almost non-existent or very minimal at the recommended daily dose of <5 mg/kg for a duration of <5 years. The risk of toxicity increases after 5 years of therapy (7,9). The risk of hydroxychloroquine retinal toxicity was estimated to be 0.29% at the fifth year of therapy , 0.33% in the 7th year, 1.0% in the 10th year, 2.1% at the 15th year and 3.1% at the 20th year of use (7). Comparing the incremental risk of Plaquenil retinal toxicity for three categories of patients based on their daily drug dose, Melles and Marmor (16) in 2014 stated that the risk of toxicity per annum was <1.0% in the first ten years of therapy for patients taking </=5 mg/kg of drug, the risk increased to 4% at the same drug dose after 10 years of therapy and even higher after 20 years of therapy. Ding et al. (3) in a systematic review in 2016 noted that estimates of the risk of retinal toxicity for patients on hydroxychloroquine therapy is uncertain after very long duration of therapy (>20 years) due to small sample size of patients who are using the medication for long periods.

1.5.4 Presence of liver and renal diseases as risk factors for Plaquenil retinal toxicity

Renal disease affects Plaquenil excretion because ≈40% to 50% of Plaquenil is excreted as unchanged drug by the kidneys and to some extent by the liver (5,20). Renal dysfunction thus leads to accumulation of Plaquenil in the body, causing an increase in serum concentration of the drug (1). Higher drug concentrations have toxic effects on photoreceptor and RPE cells of the retina (20). Renal impairment is therefore considered a critical risk factor for increasing the risk of hydroxychloroquine retinopathy (9,48–50). Melles and Marmor (16) in 2014 reported that a 50% reduction in kidney function increased the risk of toxicity by approximately two fold. The effect of hepatic diseases on the risk of Plaquenil retinal toxicity is either minimal or non- existent although the liver is involved in partial clearance of the drug (4,9,16).

1.5.5 Pre-existing retinal/macular disease as risk factor for Plaquenil retinal toxicity

The relation between pre-existing retinal or macular disease and increased susceptibility to hydroxychloroquine retinopathy is not documented (9). The AAO however indicated that it is reasonable to assume that any existing macular/retinal disease may predispose a person to developing toxicity as the pre-existing macular/retina conditions would have already undermined the integrity of the retina (9,19,34).

1.5.5 Less important risk factors

1.5.5.1 Age of patient

Other less critical risk factors related to the patient include body weight (kg), age of the patient, and genetic factors. Elderly or aged patients have been considered to be at increased risk of Plaquenil retinal toxicity than younger patients when all other factors are constant (1). This reasoning was attributed to the knowledge of the debilitating effect of aging on a person's general eye health (19,51). Aging is associated with many ocular defects such as age related macular degeneration (ARMD) that may mask the correct diagnosis of Plaquenil retinopathy (42). The effect of age on Plaquenil retinal toxicity is debatable because recent studies with large sample size found no association between age and risk of hydroxychloroquine retinopathy (9,16).

1.5.5.2 Genetic mutations in the *ABCA4* gene

Mutations of the *ABCA4* gene are responsible for some macular diseases such as Age-related macular degeneration, cone rod dystrophy and Stargardt disease (32). Mutation in this gene is proposed to be implicated in Plaquenil retinal toxicity (32). The *ABCA4* provides instruction for the synthesis of a protein responsible for the removal of toxic by-products of phototransduction (1,32). A mutation in the gene leads to accumulation of these toxic substances that eventually damage photoreceptor cells (9,32). The AAO in 2016 suggested that genetic factors may be responsible not only for Plaquenil retinal toxicity but also for the existing difference in the presentation of Plaquenil retinopathy between Asian and European eyes (9).

1.5.5.3 Real body weight (kg)

Real body weight is considered a better predictor of Plaquenil retinal toxicity than ideal body weight (16). Plaquenil is thought not to distribute evenly (that is fatter patients may be overdosed) (16).This assumption has however not been proven. The America Academy of Ophthalmology (AA0) created a binomial (daily drug dose </>5 mg/kg/real body weight) to recognise a threshold for safety (9). The guiding principle is to make the calculation of drug dose simple and practical.

1.5.5.4 Concomitant use of tamoxifen

Concomitant use of tamoxifen increases the risk of Plaquenil retinal toxicity (9,16).

1.6 Summary of AAO 2016 revised recommended screening guideline (9)

- All patients must undergo baseline screening at the start of Plaquenil therapy. Subsequent screening could be deferred until 5 years following baseline screening. Screening should be based on the patient's risk of toxicity. Patients at greater risk (taking >5 mg/kg of daily Plaquenil dose for >5 years of therapy) should be screened more frequently.
- More sensitive and objective screening tests such as the multifocal electroretinogram should be used in Plaquenil screening.
- SD-OCT and 10-2 Humphrey VFT are useful in Plaquenil screening. SD-OCT is less sensitive but highly specific to Plaquenil toxicity. 10-2 Humphrey VFT has equal sensitivity as mfERG to Plaquenil retinal toxicity.

- Plaquenil retinal toxicity is irreversible and that disease progresses despite therapy cessation

1.7 Screening modalities for hydroxychloroquine retinopathy

Screening of Plaquenil retinal toxicity is a relevant safety factor for the patients on Plaquenil therapy (9,16). This is because, Plaquenil retinal toxicity is a potentially vision threatening condition with no available treatment (9). Blindness has many adverse implications; economically, for quality of life and productive human resources (52–54). Early detection through screening and discontinuation of therapy is the means of preventing disease progression and vision loss among patients on therapy. Screening methods often used include mfERG, SD-OCT and 10-2 Humphrey visual fields.

1.7 1 Multifocal electroretinogram (mfERG)

The multifocal electroretinogram (mfERG) was developed in 1992 by Sutter and Tran (55,56) as a tool for determining central retina function in a regional manner (specifically of the macula and up to about 30^0 from the centre of the fovea**).** mfERG is used for simultaneous photopic (cone driven) stimulation of many parts of the retinal, thus allowing for the assessment of the functional status of the fovea, parafoveal and near peripheral retina (57). The mfERG is a relatively new, objective, non-invasive and sensitive device, capable of detecting early retinal changes in hydroxychloroquine retinal toxicity before significant structural changes occur in the retina (9)**.** It measures the electrophysiological response obtained from focal stimulation of many retinal areas (58). It operates by stimulating the retina with an array of either 61 or 103 hexagonal stimuli that alternate between black and white in a random sequence (m-sequence) (57). Each hexagon generates a waveform (Fig 14, 15) of two components namely a negative trough (N1) and a positive peak (P1) (Fig 14), subsequently followed by another negative trough (N2). The responses generated by the 61 or 103 hexagons are then grouped into five concentric rings as ring 1, ring 2, ring 3, ring 4 and ring 5 (Fig 15). The value of N1 subtracted from that of P1 (P1-N1) for each ring gives the average response density or amplitude (in nanovolts/degree squared) (17) .

Figure 14 Negative trough and positive peak components of the waveform generated on mfERG

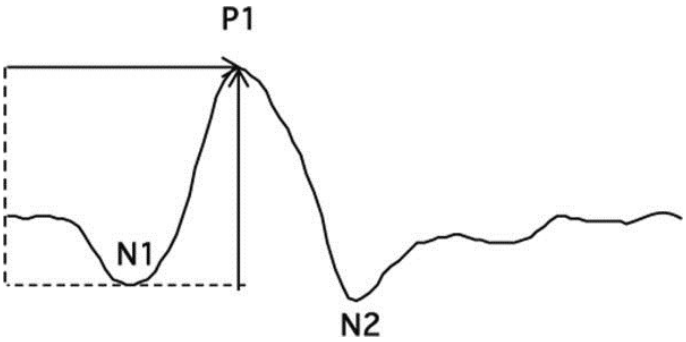
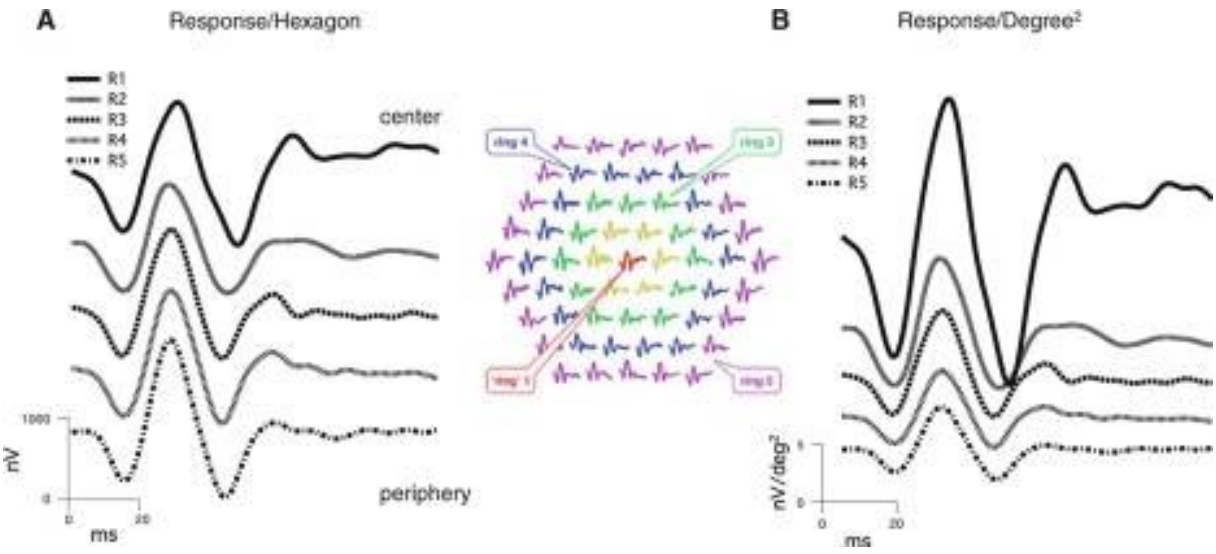


Image of negative trough (N1) and positive peak (P1) of the mfERG response. Horizontal arrow indicates implicit time while vertical arrows indicates the trough-to-peak measures of the mfERG Adopted and used with permission from Hood DC, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). Doc Ophthalmol. 2012;124:1–13.

Figure 15 Generation of mfERG ring amplitudes and waveform



Grouping of mfERG responses into rings adopted and used with permission from Hood DC, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). Doc Ophthalmol. 2012;124:1–13.

Ring 1 represents the electrophysiological response from the foveal region (0-3 degrees), ring 2 is representative of the parafoveal area (5 to 10 degrees), ring 3 represents perifoveal region (10 to 15 degrees), ring 4 represents the near periphery (15 to 20 degrees) and ring 5 is the central part of the middle periphery (20 to 30 degrees) (58,59). The waveform is distorted in a disease state of the retina such as in age related macular degeneration (ARMD), macular hole, retinoschisis, vitelliform maculopathy, other forms of maculopathy and in hydroxychloroquine retinopathy (59–61). Plaquenil retinal toxicity and its progression across time is observed as a decrease in the ring amplitudes (nV/degree^2) of the mfERG as the parafoveal responses become compromised (that is decreased response observed as decreased ring amplitude and/or delayed implicit time) (39,62). Early retinal toxicity is said to exist when there is a parafoveal loss in amplitude with/without a prolonged implicit time or elevated mfERG ring 1/2, 1/3 ratios or both (2,24).

1.7.2 Spectral domain optical coherence tomography (SD-OCT)

The spectral domain optical coherence tomography (SD-OCT) is an objective non-invasive method of detecting retinal defects due to prolonged use of hydroxychloroquine sulfate (9). SD-OCT operates on the principle of echo time delay of light by measuring the spectrum of interference between the tissue being scanned (in this case the retina layers) and that of a reference point that is not being scanned (63,64). Hydroxychloroquine retinopathy presents as a disruption of retinal morphology in the parafoveal ellipsoid zone (near the junction of the inner and outer segment of the photoreceptor cells) and thinning of foveal subfield thickness (Figure 10). This zone is situated approximately 0.5-1 mm from the centre of the macular (fovea) (17). The SD-OCT is quite specific to anatomic changes in Plaquenil retinal toxicity although relatively less sensitive to early toxicity (17,18).

1.7.3 10-2 Humphrey Automated visual fields test (VFT)

The 10-2 Humphrey automated threshold visual field test with standard pattern deviation plots and mean deviation values is a subjective test used in screening for hydroxychloroquine retinopathy (9). A visual field defect in Plaquenil retinal toxicity is seen as a partial/full ring scotoma (Figure 12) in the parafoveal region (16,65,66). The mean and standard pattern deviation plot and values enable discrimination of Plaquenil retinal toxicity defects from other forms of retinal abnormalities (17). It is specific to Plaquenil retinal toxicity in patients whose tests have high reliability indices

(9). It is readily available in most clinics and hospitals and can be used as a tool for detection of Plaquenil retinal toxicity (9). Wider field stimuli >10-2 (for example 24-2) may be advocated for patients of Asian origin as they are more likely to develop Plaquenil retinal toxicity in the peripheral retina (9,67,68).

1.7.4 Fundus autofluorescence (FAF) in Plaquenil screening

FAF is "the signal detected when the fundus is illuminated with light" (63). FAF takes advantage of the presence of lipofuscin in the RPE (63). Accumulation of lipofuscin at the level of the RPE due to alteration of photoreceptor outer segment in Plaquenil retinal toxicity is associated with mottled area of hyperfluorescence (61) (Figure 11). FAF gives a topographical view of the area of retina affected, its pattern and extent of damage in Plaquenil retinal toxicity (9). Early parafoveal or photoreceptor disruptions (Figure 11) are seen as areas of hyperfluorescence (9,14,61,68,69). In advanced disease, RPE defect or loss is observed on FAF as an area of hypofluorescence. FAF is especially valuable in detecting Plaquenil retinal toxicity in Asian eyes where defect is more peripheral than parafoveal (67,68).

Other recommended screening tests include microperimetry and colour vision assessment (9).

Table 1 Summary of Plaquenil screening tests, pros and cons

Test	Function	Merits (Pros)	Demerits (Cons)
mfERG	Simultaneous photopic stimulation of a local area of the macula to generate electrophysiological response. Retinal defects are observed as reduction in ring amplitudes	1. High sensitivity to diseased retina state 2. Objective, non-invasive	1. High cost of machine, 2. Unavailable in most clinics and hospitals 3. Requires high skilled technical know-how for interpretation of result
SD-OCT	Detects structural changes in Plaquenil retinal toxicity, observed as reduced retina nerve fiber layer thickness and disruption of photoreceptor inner/outer segment junction	1.Highly specific to Plaquenil retinal toxicity 2. Available in most clinics and hospitals 3. Gives objective assessment of retina state	1. less sensitive to early disease stage
10-2 VFT	Used in detection of Plaquenil retinal toxicity, observed as full or partial ring scotoma	1.Readily available in most clinics and hospitals Sensitive to Plaquenil retinal toxicity	1.Highly subjective 2. Can be unreliable in most patients with Plaquenil retinal toxicity
FAF	Detects Plaquenil retinal toxicity as area of hyper/ hypofluorescence	1.more useful in patients where toxicity presents in peripheral regions of the retina 2. gives pattern and an indication of extent of retinal damage	1. less sensitive to early Plaquenil toxicity

Marmor MF et al. Recommendations on screening for chloroquine and hydroxychloroquine retinopathy (2016 Revision). Ophthalmology 2016;123:1386–94.

1.8 The issues to be addressed

The 2012 and 2016 revised American Academy of Ophthalmology recommended Plaquenil screening guidelines (9,42) have significantly influenced the practice of many ophthalmologists and directed their screening protocols for Plaquenil retinal toxicity (38). Screening and monitoring protocols which require the use of sensitive and specific objective screening tests such as the multifocal electroretinogram (mfERG) (9) are associated with additional cost to the patients and health care system (38). Browning et al. in 2012 reported that mfERG was not superior to 10-2 Humphrey VFT or SD-OCT in terms of its ability to detect Plaquenil retinal toxicity (35). Additional challenges with mfERG include the cost of purchase, unavailability of the machine in most eye clinics and the requirement of a high level of expertise and technical know-how for correct interpretation) (9). There is a challenge in estimating which parameters (Rings 1, 2, 3, ½ and 1/3 ratios) are more predictive of toxicity and what should be the frequency of subsequent screening (9,38,70). While some clinicians consider the ring 1/2 ratio to be more diagnostic of Plaquenil retinal toxicity, others prefer to use the ring 1/3 ratio (2). Given the many unknown factors associated with mfERG, it ought to be highly sensitive and specific to Plaquenil toxicity in order to be considered in prognosis. There have only been a few studies (2,17,24,35) among few patients (small sample size) that have assessed the sensitivity and specificity of the mfERG in Plaquenil retinal toxicity, or addressed the challenge of standardization of parameters on mfERG associated with Plaquenil retinal toxicity.

The risk of Plaquenil retinal toxicity increases when daily drug dose exceeds 5 mg/kg of body weight over a long duration of >5 years and a cumulative dose of over >1.0 kg (9)**.** The AAO (9) recommends that screening be guided by the patients' risk based on their drug dose and duration of therapy. Screening was recommended to be deferred until after 5 years following baseline screening as patients are unlikely to develop toxicity. Risk assessment is thus important not only in informing screening intervals but also to serve as a guide to prescribing clinicians in ensuring a balance between drug use among patients and ocular safety, compliance and continuous use of a useful drug.

Also, the nature (progression, reversal/recovery or stable) of Plaquenil retinal toxicity after therapy discontinuation is not clearly understood (50,62). Insight into the clinical characteristics that inform the course of Plaquenil retinal toxicity following therapy cessation could guide physicians in ensuring timely intervention through therapy discontinuation to prevent vision loss.

1.9 Study aim, objectives and significances

1.9.1 Aim:

To investigate the prognostic value of the multifocal electroretinogram in screening and monitoring of Plaquenil retinal toxicity among patients on Plaquenil therapy in Edmonton, AB, Canada.

1.9.2 Objectives

- To determine the risk of Plaquenil retinal toxicity relative to daily drug dose (high dose of >5 mg/kg and low dose of <5 mg/kg) and duration (long duration of >5 years and short duration <5 years) of therapy
- To determine risk factors associated with Plaquenil retinal toxicity
- To determine the relative sensitivity, specificity, positive and negative predictive values of mfERG, SD-OCT and 10-2 Humphrey visual fields to Plaquenil retinal toxicity
- To determine parameters of the mfERG most significantly associated with Plaquenil retinal toxicity
- To evaluate longitudinal changes in mfERG abnormalities in individual patients followed for a period of 3 years secondary to Plaquenil therapy cessation

1.9.3 Study significance

The outcome of the study will inform drug use to ensure ocular safety by serving as a guide to both physicians and patients through risk assessment. It will inform timely intervention that results in **recovery** or prevents further progression of Plaquenil retinal toxicity. Finally, the outcome of the study will contribute to our understanding of the usefulness of multifocal electroretinogram in the assessment of patients on Plaquenil therapy.

2. METHODOLOGY

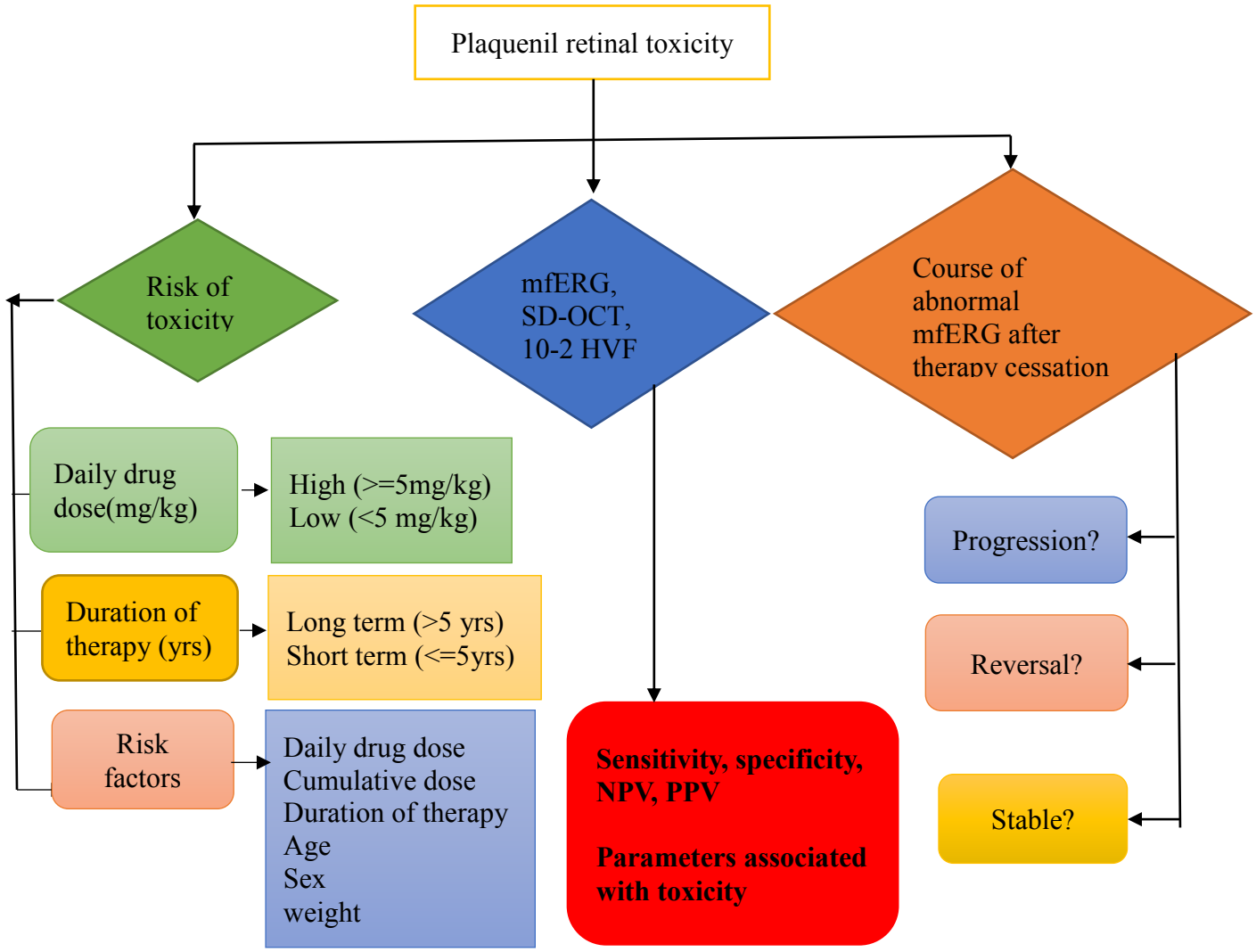
2.1 Study design and setting:

This was a retrospective case control study of 414 patients on Plaquenil therapy captured in the secure electronic diagnostic database of the Eye Institute of Alberta (EIA), in Edmonton, AB, Canada. Study sites included the Ophthalmology unit of the Royal Alexandra Hospital and Ophthalmology research office at the University of Alberta, Canada.

2.2 Conceptual framework

The study explored the risk of Plaquenil retinal toxicity based on drug dose and duration of therapy. It also assessed the relationship between risk factors (daily drug dose, cumulative drug dose, age, sex, body mass index, body weight and duration of therapy) and the risk of Plaquenil retinal toxicity. The conceptual framework also looked at the relative sensitivity and specificity of the three main tests used in screening for Plaquenil retinal toxicity namely mfERG, SD-OCT and 10-2 Humphrey VFT. Finally, it explored changes in abnormal mfERG responses among patients previously diagnosed as having Plaquenil retinal toxicity following Plaquenil therapy cessation.

Figure 16 Conceptual framework



The conceptual framework seeks to test the AAO 2016 revised recommendation for screening and monitoring of Plaquenil retinal toxicity. The study seeks to determine the associated risk based on drug dose (low dose of <5 mg/kg / high drug dose of >5 mg/kg, and/or therapy duration (short term based on AAO 2016 recommendation is <5 years, and long term is >5 years of Plaquenil therapy) toxicity.

2.3 Study subjects and sampling

A web-based database of patients referred for visual electrophysiology had been previously established. Within this database, patients were categorized as having been referred for mfERG testing to monitor them for Plaquenil toxicity. Some patients were sent for mfERG as part of a

series of tests for patients being routinely evaluated for Plaquenil toxicity, whereas others were sent as Plaquenil retinopathy suspects, with the hope that mfERG could clarify their status. Implicit in this exercise may be the inclusion of more patients in whom toxicity was suspected (selection bias).

From January 2008 to December 2016, 623 patients were screened for Plaquenil retinal toxicity with mfERG. The study received approval from the Health Research Ethics Board of the University of Alberta and operational approval from Alberta Health Services and the Royal Alexandra Hospital, Edmonton, AB, Canada.

2.3.1 Inclusion criteria

Patients included in the study were those who had:

- Legible, sharp and reliable mfERG, SD-OCT and 10-2 Humphrey VFT images of at least two-time points. False -positive and false negative rates on 10-2 VFT were to be <20%, fixation losses <10%. SD-OCT images had to show clear retinal layers without artefacts. mfERG images had no 60 cycle noise (35).
- Been on Plaquenil therapy
- information on age, sex, daily Plaquenil drug dose, start and end date of therapy

2.3.2 Exclusion criteria

Patients were excluded if they had any findings that could affect correct interpretation of test images or determine their Plaquenil retinal toxicity status or other factors that could be confound or impact the risk of Plaquenil retinal toxicity**.** These included:

- Patients with comorbid retinal disease, vitreous abnormalities, advanced glaucoma and/or cataract
- Disagreement in diagnosis by ophthalmologists and inconsistent mfERG grading
- Patients with only one time point mfERG test result
- Patients on chloroquine therapy

2.3.3. Sample size

A total of 414 patients were recruited and studied. Patients were classified as cases (patients with Plaquenil toxicity) and controls (patients on Plaquenil therapy without toxicity). Two hundred and nine (209) patients were excluded from the study**.**

Figure 17 Sampling of patients

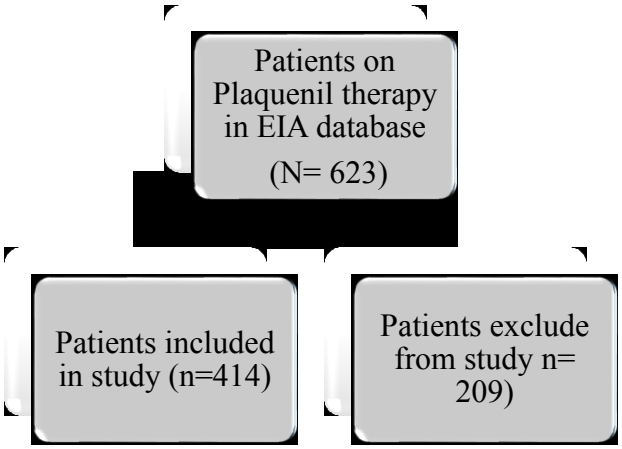
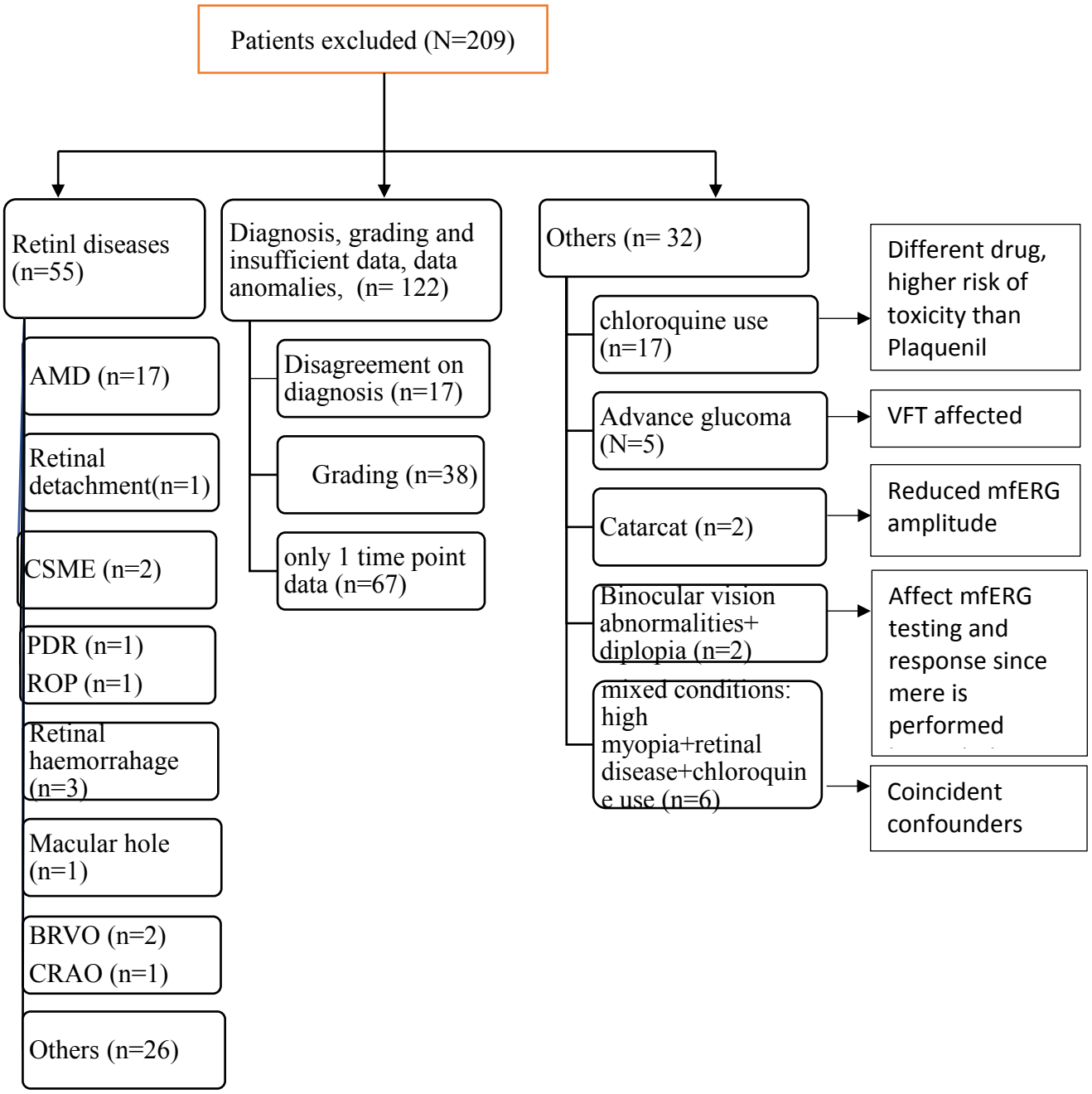


Figure 18 Categorization of patients excluded from study (n=209)



Retinal diseases: affect results of functional testing (HVF, mfERG, SD-OCT), making a categorical assignment difficult.

Grading anomalies: there were three incomparable grading of mfERG results. Only comparable data was used.

Diagnosis anomalies: made categorization of patients (cases or controls) difficult, unable to determine the Plaquenil status of the patient

Insufficient data: repeatability of functional test result could not be determined especially when the only test available is abnormal.
The most common reason for exclusion was insufficient data and only one time point of examination.

2.4 Data acquisition processes

A case report form (CRF) (Appendix 1) was generated to guide data collection. Patient information collected included age (years), sex (male/female), duration of therapy (years), weight (kg), height (m), BMI (kg/m^2), report of any systemic and ocular disease, cumulative drug dose (kg), daily drug dose (mg/kg per body weight). mfERG images of 414 patients, SD-OCT images of 242 patients and 10-2 Humphrey VFT images of 205 patients were gathered from the database.

2.4.1 Acquisition of mfERG images

Rings 1, 2, 3 amplitudes and rings 1/2 and 1/3 ratios were recorded from a second time point mfERG image of each patient. mfERG imaging was recorded in accordance with the standards of the International Society for Clinical Electrophysiology of Vision (ISCEV) (58) with DTL fibre electrodes using the Espion system (Diagnosys LLC, Lowell, MA, 2015 model), version 6+ software. Patients were dilated with 1% tropicamide to a pupil size of >8mm before image acquisition. The m-sequence controlled, 61-hexagon black and white stimulus pattern was used traversing a visual field of 30^0 from the point of fixation. The black and white hexagons had luminance of $1{,}000cd/m^2$ and $0cd/m^2$ respectively. The waveform (negative trough N1 and positive peak P1) generated from continuous stimulation of the retina by each of the 61 hexagons were processed through the 10-100 hertz bandpass filter. The Espion system Version 6+ software was used to analyse the response of the first order Kernel. The average rings 1-3 densities/ amplitudes ($nanovolts/degree^2$) were determined by the Espion software as P1-N1

within each concentric ring. Rings 1/2 and 1/3 ratios were calculated as the ratio of the central ring amplitude (ring 1) to the amplitudes generated on rings 2 and 3 respectively (17).

2.4.2 Acquisition SD-OCT images

Foveal centred volume scans with Spectralis tracking laser tomography (Heidelberg Engineering, Carlsbad, CA, USA) system were acquired by 4 ophthalmic technicians. The macular thickness maps generated were divided into three concentric circles using the EDTRS grading grids system as follows; a concentric fovea centred ring (0.5mm or 1.5^0 radius), inner ring (1.5mm or 5^0 radius) and outer ring (3mm or 10^0 radius). The 3 concentric circles were further divided into 9 ETDRS subfields at angles 45^0 and 135^0 radii (figure 5). The average retinal thickness in each of the 9 EDTRS subfields (outer superior, inner superior, outer inferior, inner inferior, outer nasal, inner nasal, outer temporal, inner temporal, and central) were generated using the manufacturer's software version 5.8.3 (Spectralis Heidelberg Engineering, USA).

2.4.3 Acquisition of 10-2 Humphrey visual fields

The Humphrey automated 10-2 program visual field analyser (Carl Zeiss Meditec, Dublin, CA, USA) with white stimulus test target, using the SITA- standard protocol was used for visual field assessment. 10-2 Humphrey visual field images used were those with high test reliability (false negative and false positive rates <20%, fixation losses <10%). Visual field images of 205 patients were collected. Mean deviation and pattern standard deviation values were recorded and used for data analysis. Scotoma point locations were also recorded.

2.5. Data analysis

The Statistical Package for Social Scientist (SPSS) Chicago, IL, version 24 was used in the analysis of data. Data was cleaned for abnormal inputs. Descriptive statistics were used to determine patients' background and clinical characteristics. Only data from the right eye (OD) was analysed as computed correlation coefficient values (appendix 3) showed high correlation (lack of independence) between the right (OD) and left (OS) eyes.

2.5.1 Risk assessment

COX univariate and multivariate regression models were applied to determine risk factors associated with Plaquenil retinal toxicity, while analysis of time-to-event (hazard/ survival function) was assessed using COX proportional hazard model. COX hazard function curves were used to graphically display the hazard (Plaquenil retinal toxicity) relative to daily drug dose (high or low), cumulative dose (<1 kg, >=1-2 kg and >2kg), sex, BMI and duration of therapy. COX regression models have the advantage over Kaplain Meier curves as they adjust for the effect of confounding factors such as age and sex (71). Mann-Whitney U analysis was employed to determine differences in background and clinical characteristics between cases and controls.

2.5.2. Relative sensitivity and specificity of mfERG, SD-OCT and 10-2 Humphrey VFT

The sensitivity and specificity of the three diagnostic tests were determined using logistic regression analysis and Receiver Operating Characteristic curves (ROC curves). ROC curves provide graphical presentation of the diagnostic ability of two or more diagnostic tests at varying thresholds of discrimination (72). ROC curves are created by plotting the true positive rates against false positive rates at varying threshold values (72,73) . The performance or diagnostic ability of a test is measured by observing the area under the curve (AUC). The larger the AUC, the greater the performance of the diagnostic test to correctly identify disease and non-disease persons (72,74–78). When two or more diagnostic tests have the same AUC, it indicates equal overall performance. ROC curves are useful in decision making in cost/benefit analysis of diagnostic tests. In this study, the diagnostic ability of three tests (mfERG, SD-OCT and 10-2 Humphrey VFT) was assessed. A gold standard against which the diagnostic tests are tested is required in relative sensitivity and specificity analysis (35). The gold standard in this study was that patients were diagnosed as having Plaquenil toxicity by two Ophthalmologists given the totality of clinical evidence including patients visual symptom presentation, colour vision test results, findings from fundus examination and a functional test (14,79) (Appendix 2).

Logistic regression analysis was applied to determine parameters of each of the three diagnostic tests (mfERG, SD-OCT and 10-2 Humphrey VFT) that were associated with Plaquenil retinal toxicity. The most strongly associated parameters on each of the three diagnostic tests were then selected and combined.

Stepwise logistic regression analysis was then used to identify the most strongly associated parameters to Plaquenil retinal toxicity from the combined parameters (17).

2.5.3 Data analysis for mfERG changes secondary to Plaquenil therapy cessation

One-way-ANOVA with repeated measure and post-hoc analysis was used to determine mfERG changes over a period of three years following therapy cessation among patients previously diagnosed as having developed Plaquenil retinal toxicity.

2.6 Definitions

- Plaquenil toxicity: A patient was classified as having Plaquenil retinal toxicity (case) based on diagnosis by two ophthalmologists given the totality of clinical evidence.

- mfERG abnormality was defined as reduced rings 1, 2 and 3 amplitudes below the lower limit of the 95% confidence interval of the age normative data, with/without elevated ring 1/2 ratio (>2. 61) or ring 1/3 ratio (> 3.20), with or without delayed implicit time (2,24,60,62,80).
- Spectral domain optical coherence tomography (SD-OCT) abnormality was defined as reduced paracentral retinal fibre thickness (parafoveal thinning and loss of photoreceptor ellipsoid zone or reduced macular thickness below age previously published normative data (22,35), reference values (17) were as following:

Table 2 SD-OCT nerve fibre layer thickness referenced normative data

9 EDTRS layers	Thickness (μm) mean±SD
Outer superior	277.6±15.7
Inner superior	316.4±17.8
Outer inferior	263.8±15.2
Inner inferior	311.1±19.3
Outer nasal	291.8±15.2
Inner nasal	317.3±20.1
Outer temporal	257.9±22.9
Inner temporal	302.5±19.5
Central	248.5±37.4

- 10-2 Humphrey automated visual field test abnormality was defined as partial or full ring scotoma mainly affecting the parafoveal region (16). The mean deviation (deviation from normative data) and pattern standard deviation values were also recorded (17)

- Sensitivity (true positive rate or probability of detection) was defined as a measure of the proportion of positives (disease state) that are correctly identified as positive. For instance the percentage of cases correctly identified as having Plaquenil retinal toxicity (81).
- Specificity (true negative rate) measures the proportion of negatives (without Plaquenil retinal toxicity) that are correctly identified as not having disease. An example is the percentage of patients on Plaquenil therapy without retinal toxicity correctly identified as such (81).
- Positive predictive values (PPV/precision) measures the proportion of positive results (Plaquenil retinal toxicity) in diagnostic tests that are true positives (82)
- Negative predictive values (NPV) predicts the proportion of negative results (no Plaquenil retinal toxicity) in diagnostic tests that are true negative results (81,82)

The PPV and NPV describe the performance of a diagnostic test or other statistical measure. A high result can be interpreted as indicating high accuracy. The PPV and NPV are not intrinsic to the test; they depend also on the prevalence (35).

Table 3 Calculation of sensitivity and specificity

		Predicted condition	
True Plaquenil retinal toxicity status	Positive	Positive	Negative
		True positive (A)	False negative (B)
	Negative	False positive (C)	True negative (D)

Sensitivity = number of true positives / (number of true positives) + (number of false negatives)
= A/A+B

Specificity = number of true negatives/ (number of true negatives) + (number of false positives)
= D/ D+C

PPV = number of true positive/ (number of true positives) + (number of false positives)
= number of true positives / number of positive calls
= A/A+C

Prevalence adjusted PPV=sensitivity X prevalence/ (sensitivity X prevalence) + ((1-specificity) X (1- prevalence))

NPV = number of true negatives/ (number of true negatives) + (number of false negatives)
= number of true negatives / number of negative calls
= D/ D+B

3. RESULTS

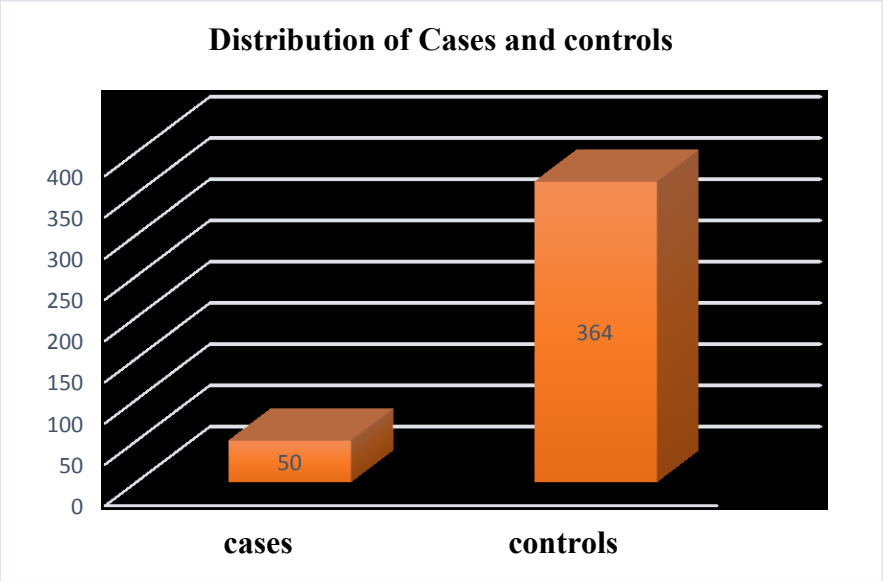
Four hundred and fourteen patients on Plaquenil therapy were studied. The median and interquartile range (IQR) of background characteristics of patients included and excluded from the study is summarized in Table 4.

Table 4 Background characteristics of included (n=414) and excluded (n=209) patients)

Variable	Included Median (IQR)	Excluded Median (IQR)	p-value
Age (years)	54.00 (45.00-63.00)	61.00 (52.00-71.00)	<0.001
Daily drug dose (mg/kg)	4.02 (2.95-5.19)	4.35 (2.62-5.15)	0.943
Cumulative dose (kg)	0.88 (0.37-1.53)	0.99 (0.27-1.46)	0.567
Duration of therapy (years)	7.00 (2.00-12.00)	9.00 (3.00-12.00)	0.123
BCVA (logMAR)	0.00 (0.00-0.10)	0.10 (0.00-0.18)	0.085

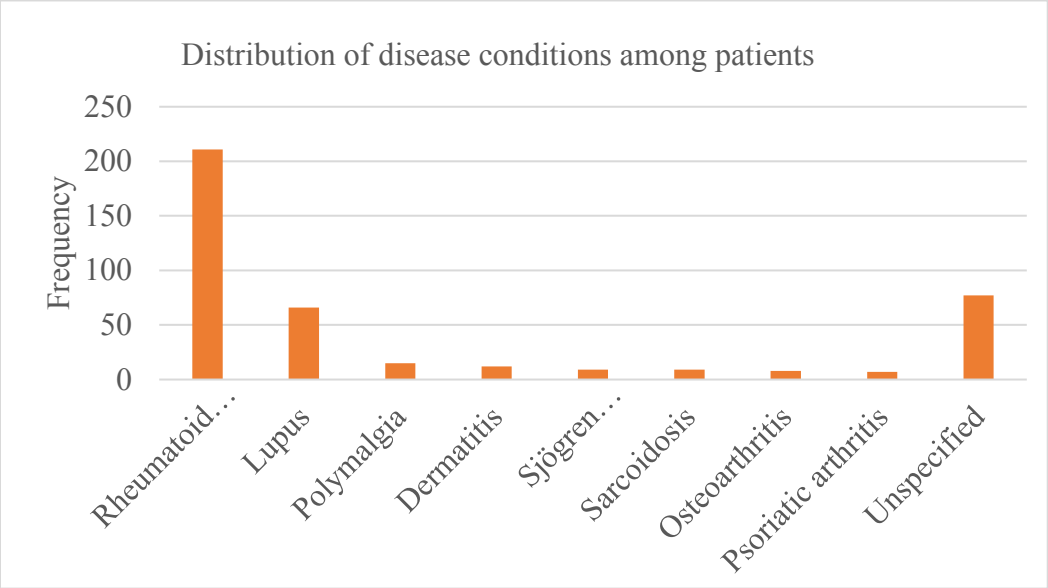
BCVA: Best corrected visual acuity. Background characteristics were not statistically different ($p>0.05$) between patients included and those excluded from the study except for age ($p<0.001$)

Figure 19 Distribution of cases and controls (N=414)



There were 50 cases and 364 controls (patients on Plaquenil therapy but without toxicity. The proportion (12%) of patients with cases among the sample is higher than that found in the general population of patients on Plaquenil therapy (16). This reflect higher drug dose and lengthier therapy duration (41% of the studied patients had been on Plaquenil therapy for <=5 years, while 59% of the sample were on Plaquenil treatment for >5 years) (Figure18, Table 4)

Figure 20 Distribution of disease conditions among studied sample (N=414)



Rheumatoid arthritis (≈51%) was the most common medical condition among the studied patients. SLE was the second most common condition among the patients.

Table 5 Distribution of diseases among cases and controls

Condition	Cases Frequency within case (%)	Controls Frequency within controls (%)
Rheumatoid arthritis	27 (12.80)	184 (87.20)
Systemic Lupus erythematosus	9 (13.60)	57 (86.40)
Sjögren syndrome	0 (0.00)	9 (100.00)
Polymyalgia	0 (0.00)	15 (1100.00)
Sarcoidosis	1 (11.10)	8 (88.90)
Psoriatic arthritis	0 (0.00)	7 (100.00)
Dermatitis	0 (0.00)	12(100.00)
Osteoarthritis	0 (0.00)	8 (100.00)

The proportion (72.97%) of cases with Rheumatoid arthritis is higher. However, the odds of having Plaquenil retinal toxicity was not significantly ($p>0.05$) associated with patient's disease condition.

Table 6 Distribution of background characteristics of cases (n=50) and controls (n=364)

Variable	**Cases (frequency within cases (%))**	**Controls (frequency within controls (%))**
Sex		
Male	8 (16.00)	82 (22.60)
Female	42 (84.00)	281 (77.40)
Daily drug dose (mg/kg)		
<5	10 (30.30)	140 (76.50)
>=5	23 (69.70)	43 (23.5)
Cumulative drug dose (kg)		
<=1	6 (15.00)	119 (60.70)
>1-2	16 (40.00)	58 (29.60)
>2	18 (45.00)	19 (9.7)
Duration of therapy		
(years)	3 (7.5)	136 (52.3)
<5	37 (92.5)	124 (47.87)
>=5		

Descriptive statistics of the distribution of background characteristics of cases and controls.

Table 7 Differences in background characteristics between cases (n=50) and controls (n=364)

Variable	Case (n=50) Median (IQR)	Controls (n=364) Median (IQR)	p-value
Age (years)	62.00 (49.75–67.00)	53.00 (49.75-67.00)	0.001
Daily drug dose (mg/kg)	6.00 (4.86-6.75)	3.81 (2.83–4.90)	<0.001
Total daily drug dose (g)	400.00 (300.00-400.00)	300.00 (200.00-400.00)	0.001
Cumulative dose (kg)	1.68 (1.17–2.25)	0.73 (0.29 – 1.28)	<0.001
Therapy duration (years)	12.00 (9.75 – 19.75)	5.00 (2.00- 11.00)	<0.001
Weight (kg)	65.77 (61.23-76.20)	74.84 (63.28-90.36)	0.010
Body mass index (kg/m^2)	28.78 (26.48-35.61)	31.61(26.50-38.71)	0.538
Visual acuity (logMAR)	0.10 (0.00 – 0.18)	0.00 (0.00 – 0.10)	<0.001

Differences in background characteristics between cases and controls were determined using Mann Whitney U analysis. There was statistically significant difference in age, daily drug dose, cumulative dose, total daily dose, weight and VA of cases and controls $p< 0.05$. Body mass index (BMI) was not different between cases and controls $p=0.538$).

Table 8 Univariate COX regression analysis of risk of Plaquenil retinal toxicity

Variable	β (95% CI)	p-value
Age (years)	1.02 (0.99 – 1.05)	0.135
Sex		
Male	Ref	
Female	1.30 (0.57-2.99)	0.539
Daily drug dose (mg/kg)		
<=5	Ref	
>5	0.23 (0.10-0.49)	<0.001
Cumulative dose (kg)		
<=1	Ref	Ref
>1-2	1.37 (0.50-3.73)	0.539
>2	1.29 (0.63-2.67)	0.486
Weight (kg)	0.98 (0.95-1.00)	0.072
BMI (kg/m^2)	0.98 (0.93-1.04)	0.548
BMI (kg/m^2)		
<18.5	Ref	Ref
18.5-24.99	0.00 (0.00-0.00)	0.986
25.00-29.99	0.63 (0.11-3.55)	0.602
30.00-34.99	2.66 (0.76-9.28)	0.126
35.00-39.99	0.86 (0.21-3.44)	0.826
40+	0.75 (0.08-7.13)	0.807
Duration of therapy (years)		
<5	Ref	Ref
5-10	13.66 (0.00-3.45)	0.553
>10	6.29 (5.74-51.13)	0.007

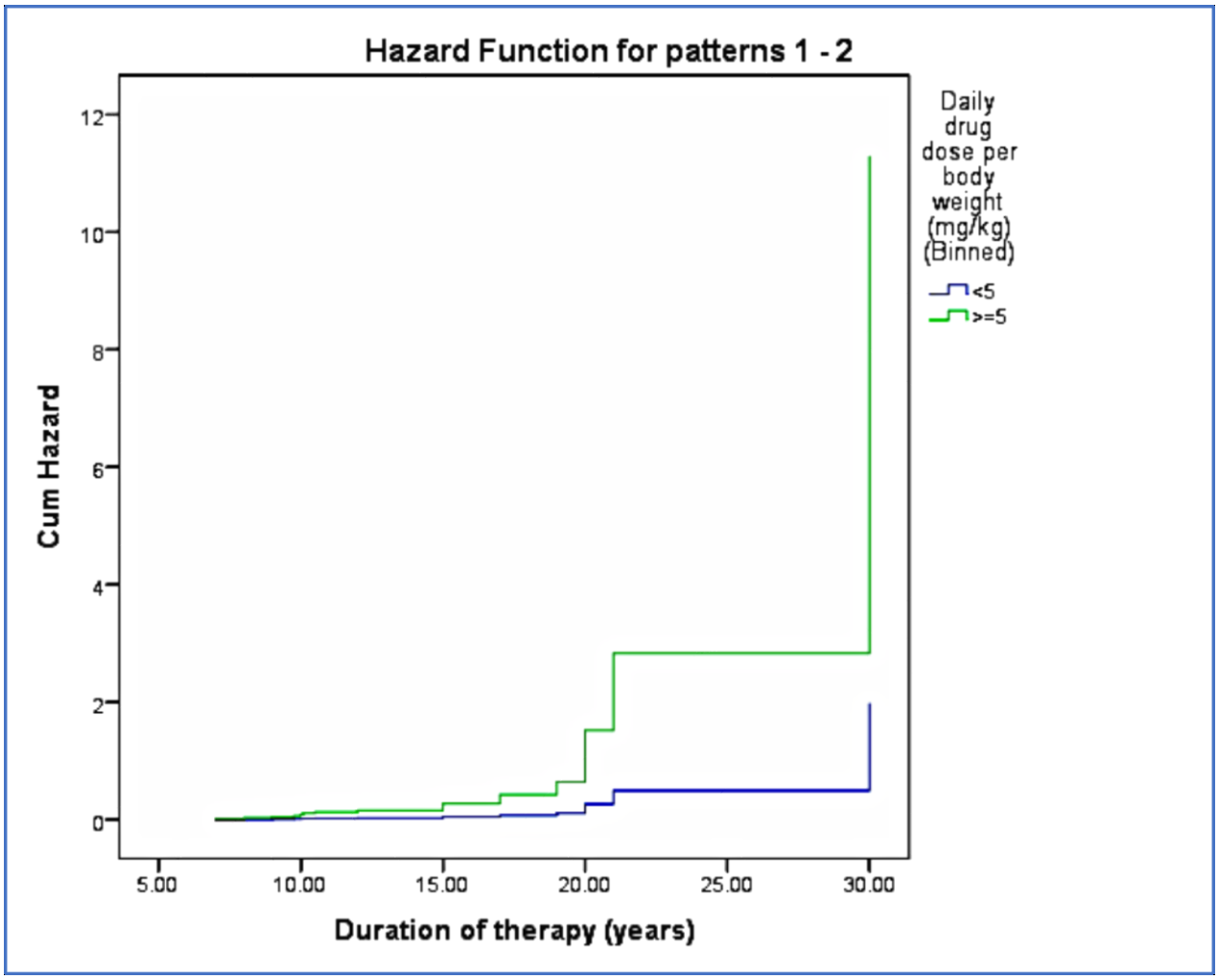
Univariate Cox regression analysis to determine the risk of Plaquenil retinal toxicity per each background characteristic. Duration of therapy of >10 years and DDD >5 mg/kg were significantly associated with Plaquenil retinal toxicity, $p<0.05$. weight (kg) showed almost borderline significance ($p=0.072$)

Table 9 Multivariate COX regression analysis of risk of Plaquenil toxicity

Variable	β (95% CI)	p-value
Age (years)	1.02 (0.0.98 – 1.06)	0.388
Sex		
Male	Ref	
Female	1.48 (0.37-5.81)	0.578
Daily drug dose (mg/kg)		
<5	Ref	
>=5	0.23 (0.08-0.65)	0.006
Cumulative dose (kg)		
<=1	Ref	Ref
>1-2	1.84 (0.44-7.53)	0.0.413
>2	2.83 (1.06-7.58)	0.039
Weight (kg)	0.99 (0.96-1.03)	0.634

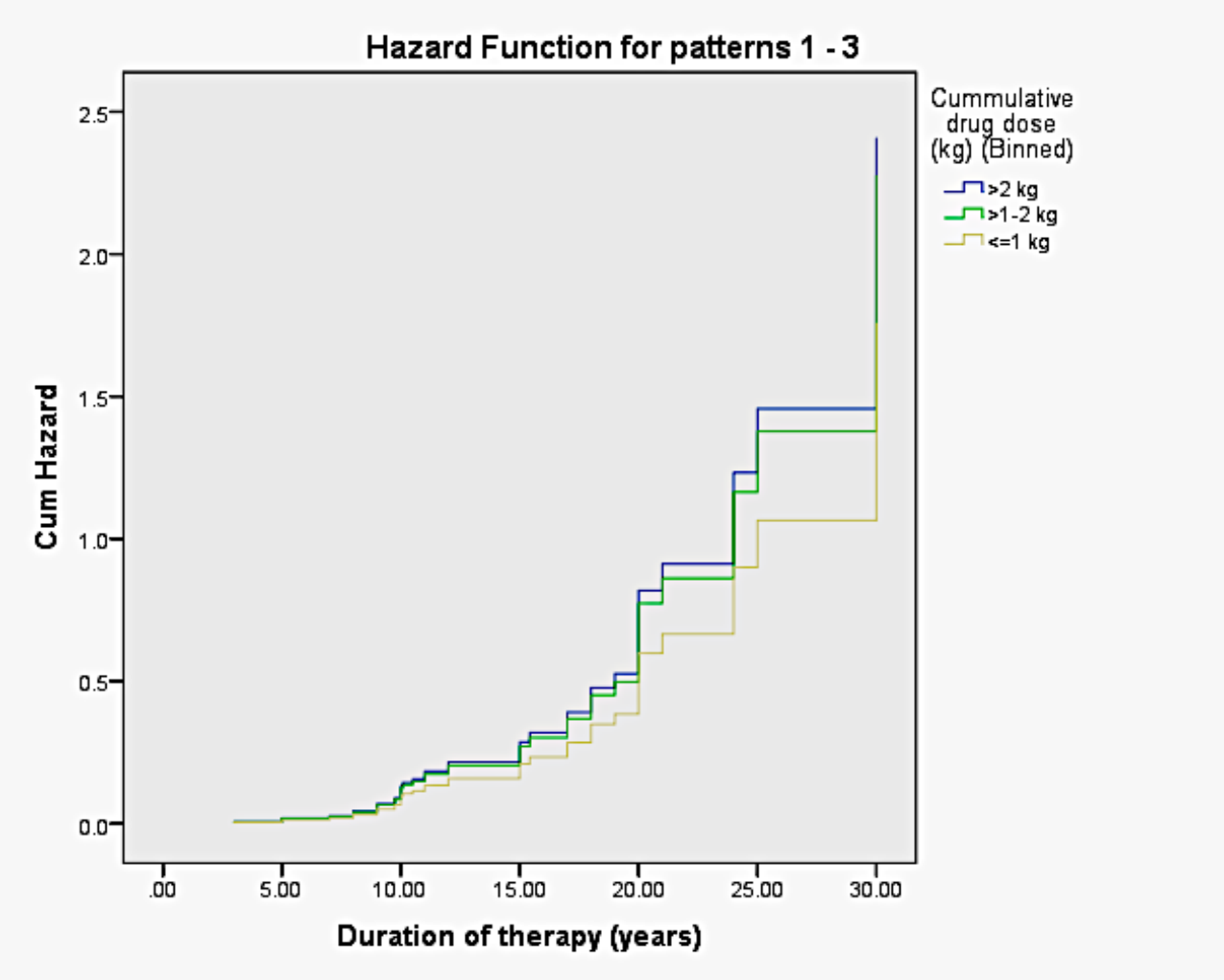
Multivariate COX regression analysis was used to determine the risk of Plaquenil retinal toxicity associated with patient characteristics. Factors included in the analysis were age, sex, daily drug dose, cumulative dose, BMI, weight, duration of therapy Factors that were predictors of Plaquenil retinal toxicity among these patients in the multivariate regression analysis were daily drug dose (DDD) >5 mg/kg, cumulative dose >2 kg, $p<0.05$.

Figure 21 COX hazard function curve of the risk of Plaquenil retinal toxicity based on daily Plaquenil dose



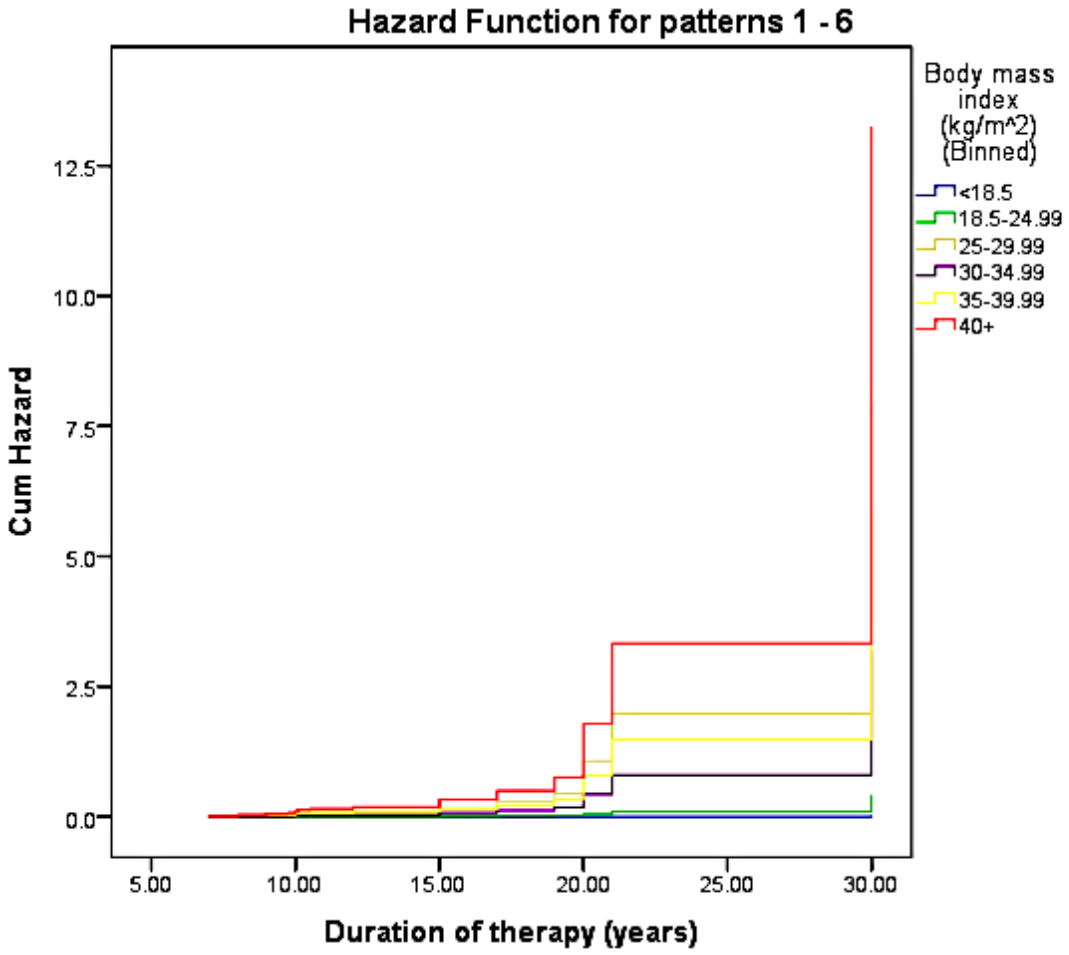
COX-hazard function curve shows risk of Plaquenil retinal toxicity per daily drug dose. Test of equality of survival distributions for the different levels of Daily drug dose per body weight (mg/kg) (Binned) showed significant difference, Log Rank (Mantel-COX) = $p<0.001$.

Figure 22 COX hazard function curve of the risk of Plaquenil retinal toxicity based on cumulative Plaquenil dose



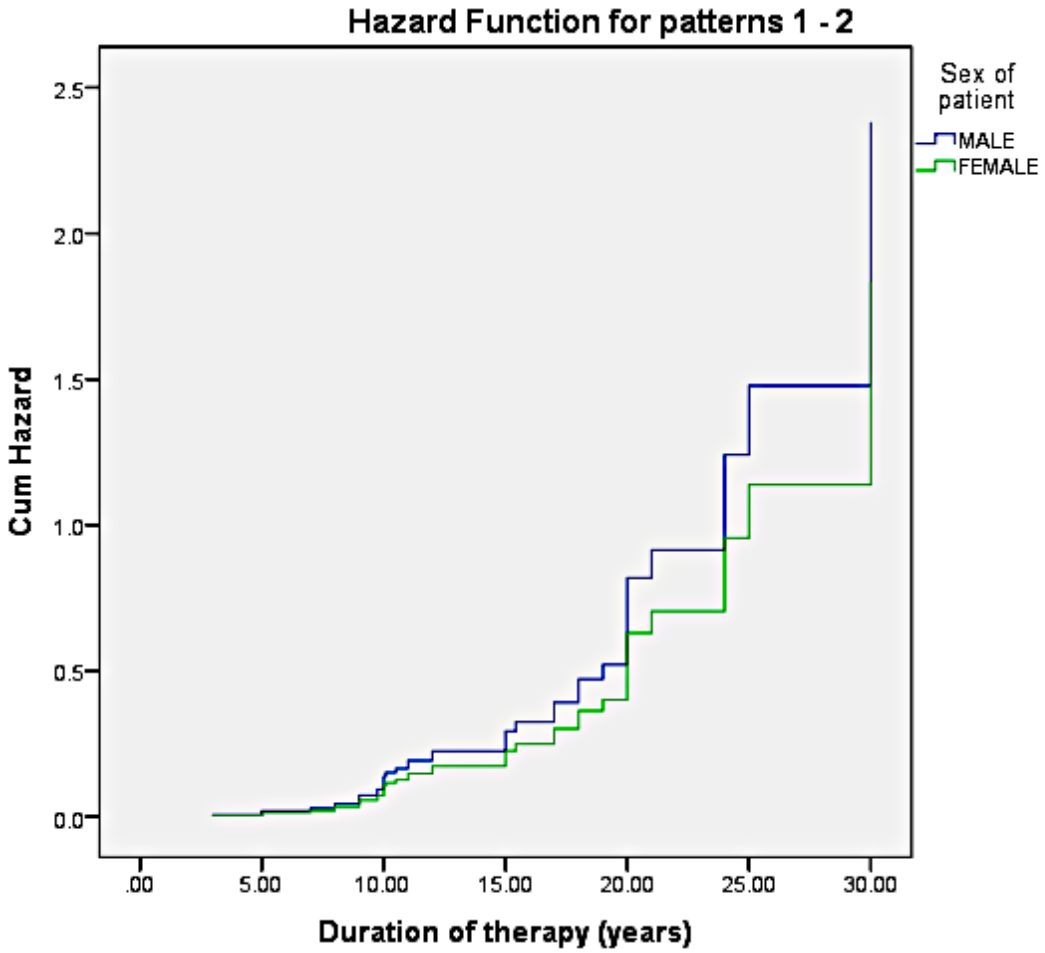
Test of equality of survival distributions for the different levels of cumulative dose (kg)were not significantly different in the univariate COX regression analysis Log Rank (Mantel-COX) p=0.727, in the univariate COX regression analysis. However, cumulative dose was a significant risk factor in the multivariate analysis.

Figure 23 COX hazard function curve of the risk of Plaquenil retinal toxicity at different levels of BMI (kg/m^2)



BMI at the different levels were not significant difference, Log Rank (Mantel-COX) p=0.462, in the univariate COX regression analysis.

Figure 24 COX hazard function curve of the risk of Plaquenil retinal toxicity by sex



Test of equality of survival distributions for the males and females showed no significant difference, Log Rank (Mantel-COX) p=0.939, in the univariate COX regression analysis.

Table 10 Life table showing the risk of Plaquenil retinal toxicity per duration (years) of therapy (n=300)

Interval start time (years)	Number entering interval	Number exposed to risk	Number of cases	Proportion with toxicity	Proportion without toxicity	Cumulative proportion without toxicity at end of interval	Hazard rate
0	300	240	1	0.00	1.00	1.00	0.00
5	178	153	10	0.01	0.93	0.93	0.01
10	117	92	10	0.11	0.89	0.83	0.02
15	56	45	9	0.20	0.80	0.66	0.05
20	24	19	8	0.42	0.58	0.38	0.11
25	6	5	1	0.22	0.50	0.30	0.15
30	2	2	1	0.67	0.33	0.10	0.20

Median survival time is 22 years. Life table shows the proportion of patients who developed Plaquenil retinal toxicity at each given time interval.

Table 11 Relative sensitivity, specificity, positive predictive value and negative predictive values of mfERG, SD-OCT AND 10-2 VFT

Test	Sensitivity %	Specificity %	PPV %	NPV %	FPR %	FNR %	PLR	NLR	F-score %	Accuracy (%)
mfERG	92.00	95.59	75.41	98.78	4.00	8.00	20.86	0.08	82.88	95.13
SD-OCT	68.00	97.24	73.91	96.34	2.76	32.00	24.64	0.33	70.80	94.21
10-2 VFT	80.95	94.57	60.71	97.75	5.43	19.05	14.91	0.20	70.83	93.17
mfERG+ SD-OCT	94.00	97.53	83.93	99.16	0.84	6.00	38.06	0.06	89.52	97.10
mfERG + 10-2 VFT	96.00	97.51	84.21	99.44	2.49	4.41	38.55	0.04	89.72	97.33
SD-OCT + 10-2 VFT	83.33	97.60	83.33	97.60	2.40	16.67	34.72	0.17	83.33	95.80
mfERG+ SD-OCT+ 10-2 VFT	98.00	99.45	96.08	99.72	0.55	2.00	178.18	0.02	97.03	99.28

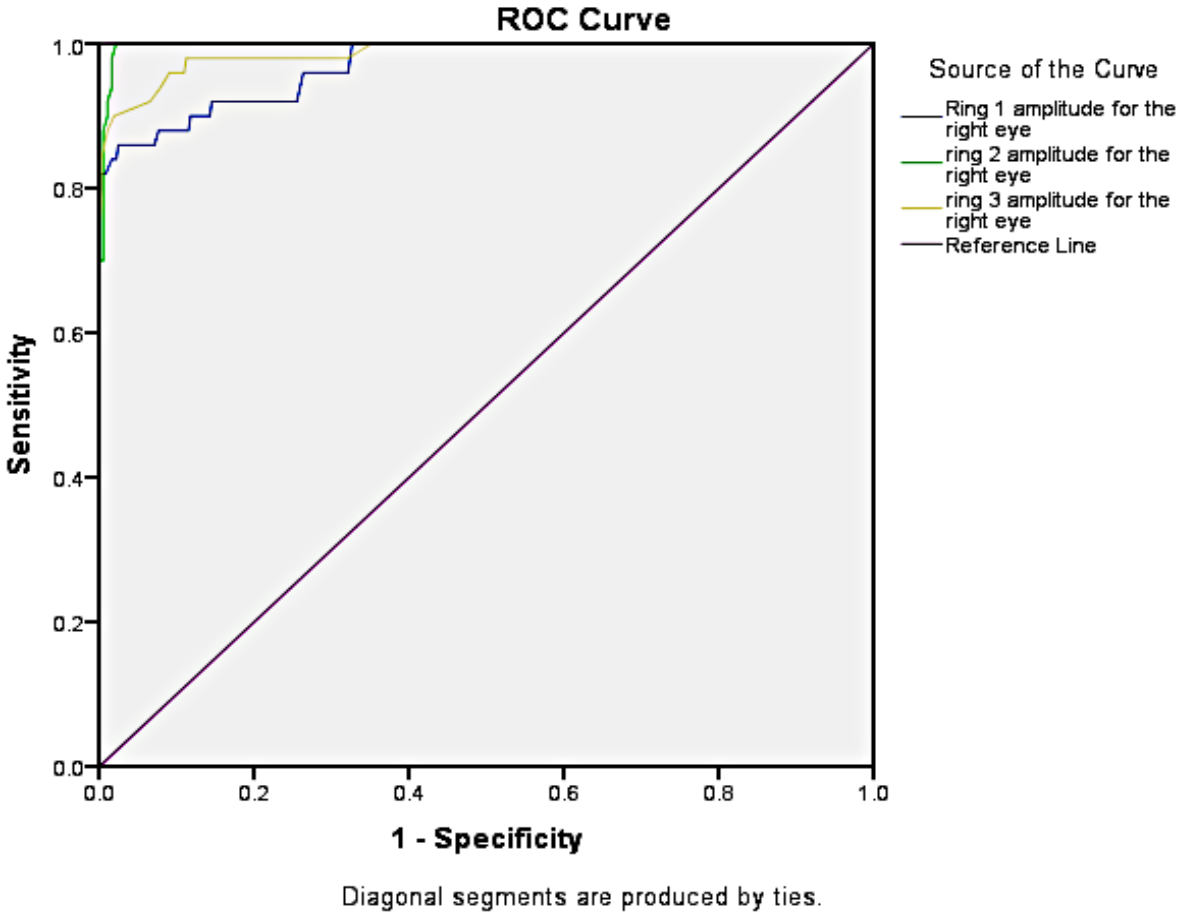
Logistic regression analysis was used to determine the sensitivity and specificity of the three screening tests. SD-OCT, mfERG and 10-2 Humphrey VFT have high specificity (>90%) but the mfERG recorded the highest sensitivity (92%).

Table 12 mfERG, SD-OCT and 10-2 VFT prevalence adjusted PPV and NPV

Estimated prevalence		Adjusted PPV (%)	Adjusted NPV (%)
	mfERG	0.63	0.82
	SD-OCT	0.62	0.80
	10-2 VFT	0.51	0.81
0.1%	mfERG + SD-OCT	0.70	0.83
	mfERG + 10-2 VFT	0.70	0.83
	SD-OCT + 10-2 VFT	0.69	0.81
	mfERG +SD-OCT +10-2 VFT	0.80	0.83
	mfERG	1.89	2.47
	SD-OCT	1.85	2.41
	10-2 VFT	1.52	2.44
0.3%	mfERG +SD-OCT	2.10	2.48
	mfERG + 10-2 VFT	2.11	2.49
	SD-OCT + 10-2 VFT	2.08	2.44
	mfERG +SD-OCT +10-2 VFT	2.40	2.49
	mfERG	3.14	4.12
	SD-OCT	3.08	4.01
	10-2 VFT	2.53	4.07
0.5%	mfERG +SD-OCT	3.50	4.13
	mfERG + 10-2 VFT	3.51	4.14
	SD-OCT + 10-2 VFT	3.47	4.07
	mfERG +SD-OCT +10-2 VFT	4.00	4.16
	mfERG	6.28	8.23
	SD-OCT	6.16	8.02
	10-2 VFT	5.06	8.15
1.0%	mfERG +SD-OCT	6.99	8.26
	mfERG + 10-2 VFT	7.02	8.29
	SD-OCT + 10-2 VFT	6.94	8.13
	mfERG +SD-OCT +10-2 VFT	8.01	8.31

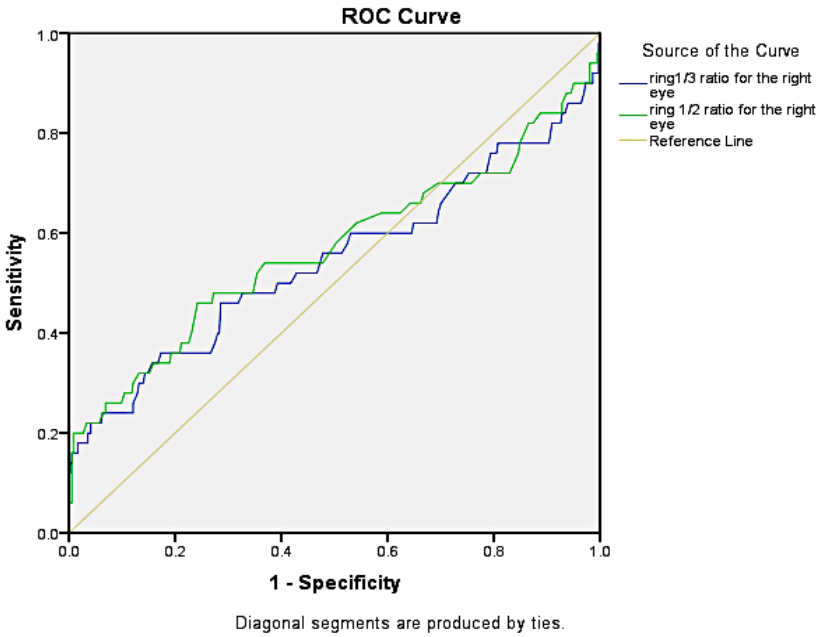
PPV and NPR are prevalence dependent. The PPR and NPR of mfERG, SD-OCT and 10-2 Humphrey VFT increases with increasing prevalence of Plaquenil retinal toxicity.

Figure 25 ROC curve of the predictability of the Plaquenil retinal toxicity using ring amplitudes on mfERG



Ring 2 amplitude is strongly associated with toxicity among the three ring amplitudes of mfERG.

Figure 26 ROC curves of the predictability of Plaquenil retinal toxicity using mfERG ring ratios



Both ring 1/2 and 1/3 ratios have approximately 50% discriminatory ability

Table 13 Area under the curve predict the diagnostic ability of mfERG parameters to Plaquenil retinal toxicity

Ring	**Area under the curve (AUC)**	**95% CI**	**P-value**
Ring 1	0.969	0.95-0.99	<0.001
Ring 2	0.997	0.99-1.00	<0.001
Ring 3	0.986	0.97-1.00	<0.001
Ring 1/2 ratio	0.568	0.47-0.67	0.118
Ring 1/3 ratio	0.545	0.44-0.65	0.297

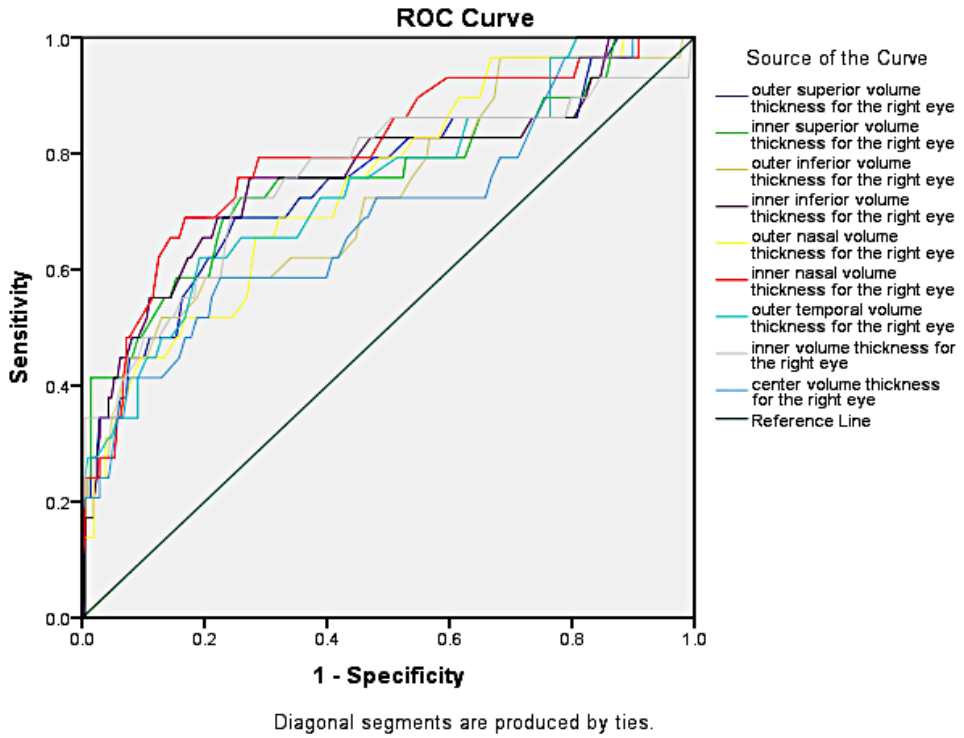
AUC is area under the curve. A larger area under the curve, that is AUC values closer to 1.0 indicates that the parameter or test is better able to correctly classify disease and non-diseases state. Reduction of absolute values of the individual ring amplitudes are more predictive of toxicity than the ring ratios

Table 14 Sensitivity and specificity of mfERG parameters at cut-off points, Area under the curve predicts the diagnostic ability of mfERG parameters to Plaquenil retinal toxicity

mfERG parameter	Youden's statistic	Optimal cutoff point (nV)	Sensitivity (%)	Specificity (%)
Ring 1	0.81	12.95	82.00	99.20
Ring 2	0.96	7.75	98.00	98.40
Ring 3	0.87	4.85	88.00	98.90
Ring 1/2 ratio	0.21	1.91	48.00	72.80
Ring 1/3 ratio	0.09	2.91	45.00	63.70

Ring amplitudes on mfERG are more sensitive and specific to Plaquenil retinal toxicity at the above cut-off points than ring ratios.

Figure 27 ROC curve of parameters on SD-OCT associated with Plaquenil retinal toxicity



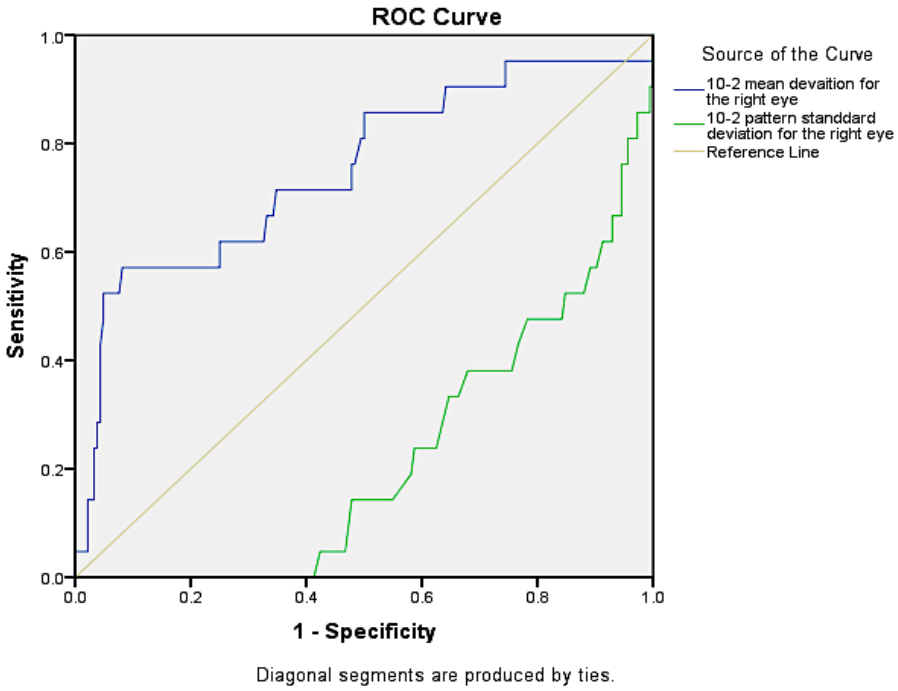
Decrease in inner concentric retinal nerve fibre layer thickness are associated with Plaquenil retinal toxicity. Reduction in inner nasal fibre thickness is most strongly associated with Plaquenil retinal toxicity on SD-OCT.

Table 15 Area under the curve predicts the diagnostic ability of SD-OCT parameters to Plaquenil retinal toxicity

SD-OCT parameter	AUC	95% CI	P-value
Outer superior layer	0.752	0.65-0.86	<0.001
Inner superior layer	0.760	0.65-0.87	<0.001
Outer inferior layer	0.729	0.62-0.87	<0.001
Inner inferior layer	0.765	0.66-0.87	<0.001
Outer nasal layer	0.746	0.65-0.84	<0.001
Inner nasal layer	0.802	0.71-0.90	<0.001
Outer temporal layer	0.743	0.64-0.85	<0.001
Inner temporal layer	0.759	0.65-0.87	<0.001
Central layer	0.690	0.58-0.81	0.001

Inner nasal and inner inferior retinal nerve fibre layers thickness of SD-OCT are more predictable of Plaquenil retinal toxicity.

Figure 28 ROC curve of 10-2 Humphrey VFT parameters associated with Plaquenil retinal toxicity



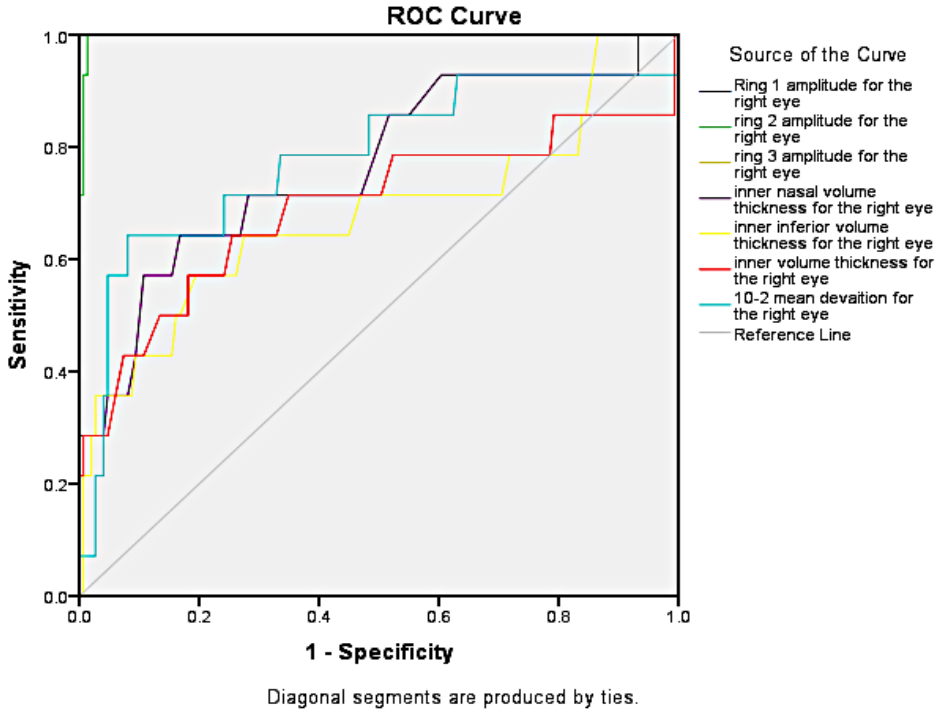
Mean deviation on 10-2 Humphrey VFT is highly sensitive and specific to Plaquenil

Table 16 Area under the curve of 10-2 VFT parameters predict Plaquenil retinal toxicity

10-2 VFT parameters	**Area under the curve (AUC)**	**95% CI**	**P-value**
Mean deviation	0.751	0.63-0.88	<0.001
Pattern standard deviation	0.220	0.130-0.311	<0.001

10-2 Humphrey visual field mean deviation is more predictive of Plaquenil retinal toxicity than pattern standard deviation.

Figure 29 mfERG, SD-OCT and 10-2 VFT parameters that are most strongly associated with Plaquenil retinal toxicity



mfERG ring 2 amplitude and SD-OCT inner nasal retinal layer are most predictive of Plaquenil retinal toxicity .

Table 17 Stepwise regression analysis of SD-OCT, mfERG, and 10-2 Humphrey VFT parameters associated with Plaquenil toxicity using all three tests.

Parameter	**β (95% CI)**	**P-value**
Ring 1 amplitude	0.54 (0.32-0.89)	0.016
Ring 2 amplitude	0.92 (0.89-0.98)	0.006
Inner nasal retina layer thickness	0.84 (0.80-0.90)	0.020
Inner inferior retina layer thickness	0.69 (0.49-0.96)	0.028
10-2 mean deviation	0.78 (0.63-0.98)	0.035

Stepwise logistic regression determined predictive ability of mfERG, SD-OCT and 10-2 Humphrey VFT parameters (How well the parameters predict Plaquenil retinal toxicity)

Inner nasal retinal nerve fibre layer thickness and ring 2 amplitude are the strongest predictors of Plaquenil retinal toxicity in stepwise regression analysis

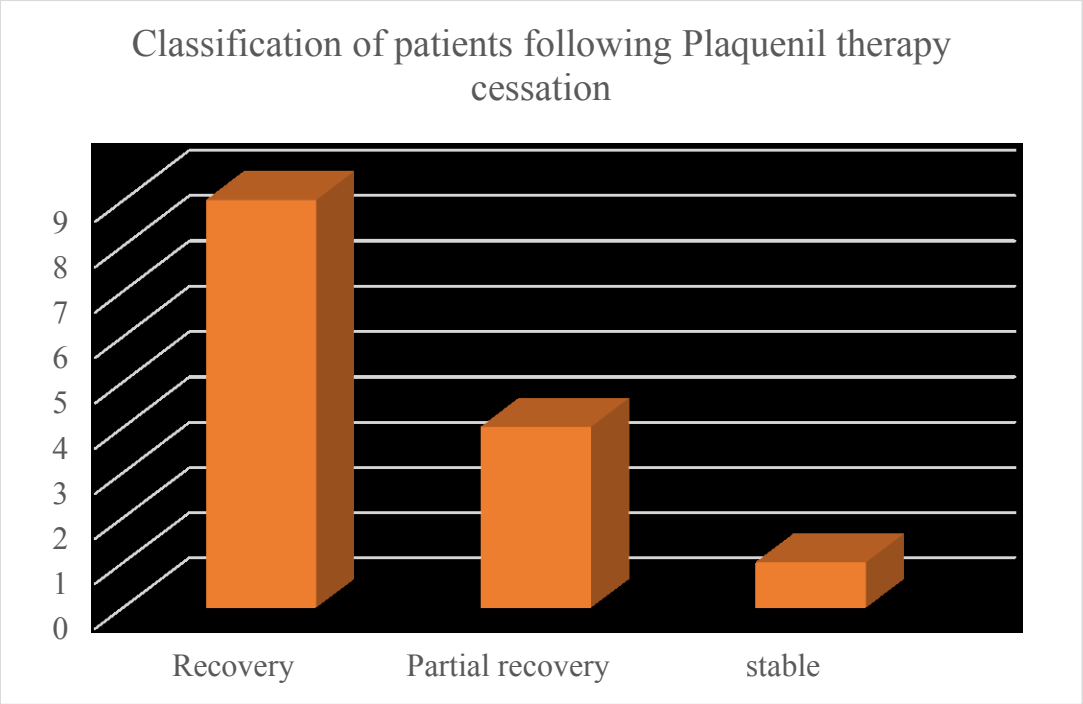
Table 18 Distribution of mfERG, SD-OCT and 10-2 VFT parameters between cases (n=50) and controls (n=364)

Variable	Cases Median (IQR)	Controls Median (IQR)	P-value	Effect size
mfERG parameters				
Ring 1 amplitude	10.40 (7.15-12.30)	21.15 (17.53-26.30)	<0.001	0.53
Ring 2 amplitude	5.25 (3.60-6.70)	11.88 (9.90-14.98)	<0.001	0.56
Ring 3 amplitude	3.50 (2.85-4.30)	7.75 (6.40-9.60)	<0.001	0.55
Ring 1/2 ratio	1.83 (1.56-2.28)	1.74 (1.61-1.93)	0.118	0.08
Ring 1/3 ratio	2.87 (2.41-3.37)	1.74 (1.61-1.93)	0.267	0.05
SD-OCT parameters				
Outer superior retina thickness	284.00 (264.00-298.00)	301.00 (290.50-312.00)	<0.001	0.29
Inner superior retina thickness	318.00 (295.50-335.50)	338.00 (328.00-353.00)	<0.001	0.29
Outer inferior retina thickness	276.00 (261.00-295.50)	294.00 (284.00-307.50)	<0.001	0.26
Inner inferior retina thickness	314.00 (297.50-329.50)	336.00 (325.00-347.50)	<0.001	0.30
Outer nasal retina thickness			<0.001	0.28
Inner nasal retina thickness			<0.001	0.34

Outer temporal retina thickness	292.00 (273.00-307.00)	309.00 (299.00-322.50)	<0.001	0.29
Inner temporal retina thickness	321.00 (302.00-333.00)	343.00 (331.50-353.00)	0.001	0.22
Centre retina thickness	266.00 (241.50-279.50)	281.00 (271.00-294.00)		
	306.00 (285.00-322.00)	328.00 (317.50-338.00)		
	262.00 (243.50-288.50)	281.00 (266.50-294.00)		
10-2 VFT parameters				
Mean deviation	-2.02 (-2.49- -0.29)	-0.21 (-0.93-0.45)	<0.001	0.27
Pattern standard deviation	1.44 (1.26-1.72)	1.21 (1.07-1.36)	<0.001	0.29

Mann-Whitney U analysis determined the difference in mfERG, SD-OCT and 10-2 Humphrey VFT parameters between cases and controls. There was statistically a significant difference between the two groups on all parameters except ring 1/2 and 1/3 ratios. Effect size quantifies the difference between the two groups being compared.

Figure 30 Classification of patients who discontinued Plaquenil therapy due to toxicity (N=14)



Of the 14 patients who discontinued Plaquenil therapy due to retinal toxicity, 9 experienced recovery (improvement in mfERG ring amplitudes to age normal response) of abnormal mfERG findings, 4 experienced partial recovery (improvement in mfERG ring amplitudes but still below the lower limit of the 95% CI of age normal response) and 1 patient experienced stable abnormal mfERG response (with no further depression nor improvement of mfERG ring amplitudes). Only the 13 patients who experienced changes in abnormal mfERG findings are included in the analysis (some patients were lost to follow up, that is data not available for some patients on subsequent testing after discontinuation of therapy)

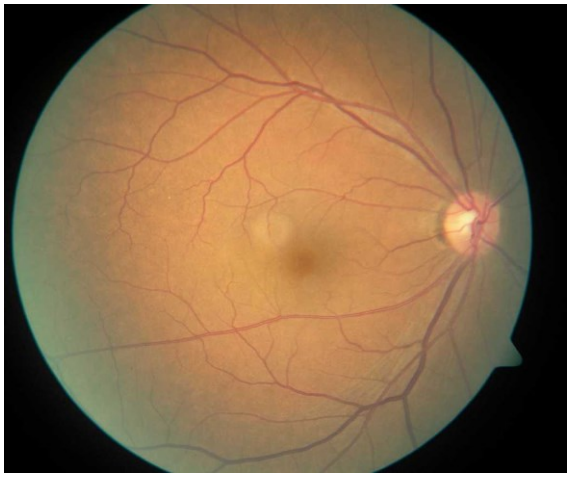
Table 19 Background characteristics of patients who discontinued Plaquenil therapy at the point of toxicity (N=13)

Case	Age (years)	Daily dose (mg/kg)	Total daily dose (g)	Cumulative dose (kg)	Duration of therapy (years)	Visual acuity (logMAR)	
						OD	OS
1	56	3.15	200	1.24	17	0.00	0.00
2	48	4.41	300	0.99	9	0.00	0.18
3	67	6.21	400	1.75	12	0.10	0.00
4	50	9.12	600	2.19	10	0.00	0.00
5	44	6.00	400	1.75	12	0.00	0.10
6	48	6.08	200	1.24	15	0.10	0.00
7	68	2.99	200	0.75	6	0.10	0.00
8	65	6.17	400	1.02	7	0.00	0.00
9	63	6.35	400	0.84	5.75	0.30	0.18
10	70	5.52	300	2.19	20	0.10	0.10
11	62	6.53	400	2.19	15	0.10	0.10
12	64	8.40	400	1.90	13	0.10	0.30
13	66	6.78	400	1.24	9	0.10	0.00

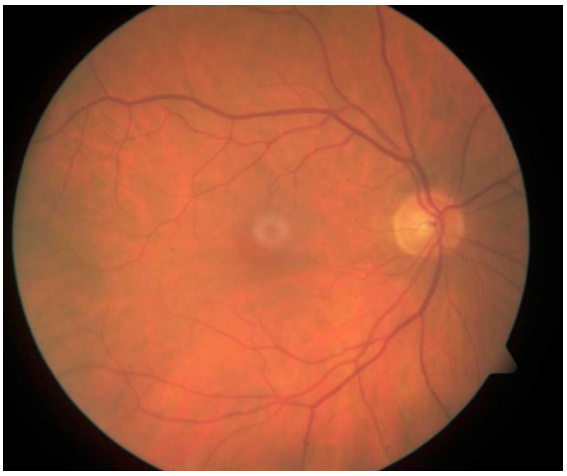
Background data on Patients that cessed Plaquenil therapy. Data captured at the point of abnormal mfERG response.

Figure 31 Fundus images at the point of toxicity (N=13)

P1



P2



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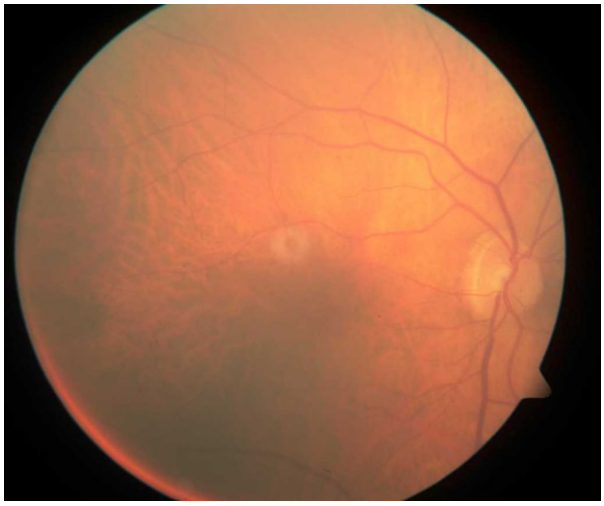

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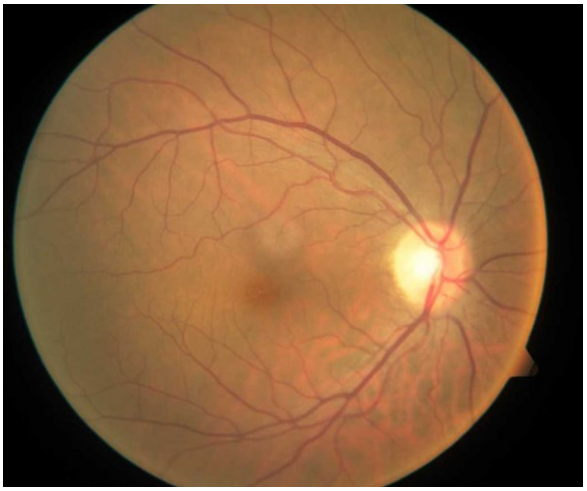

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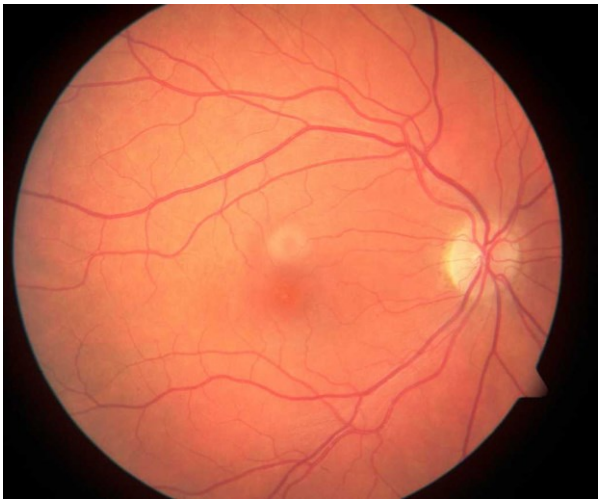

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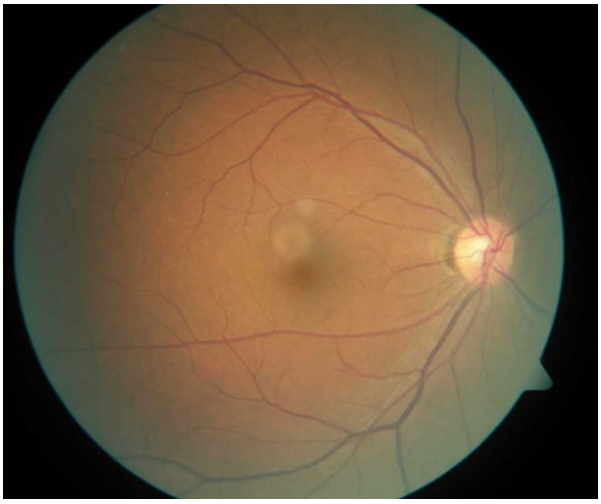
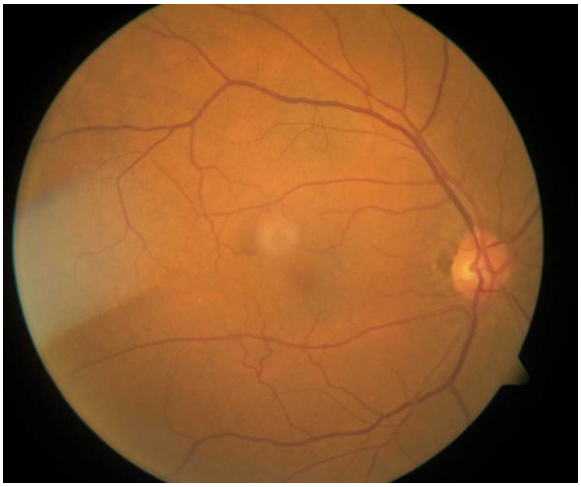
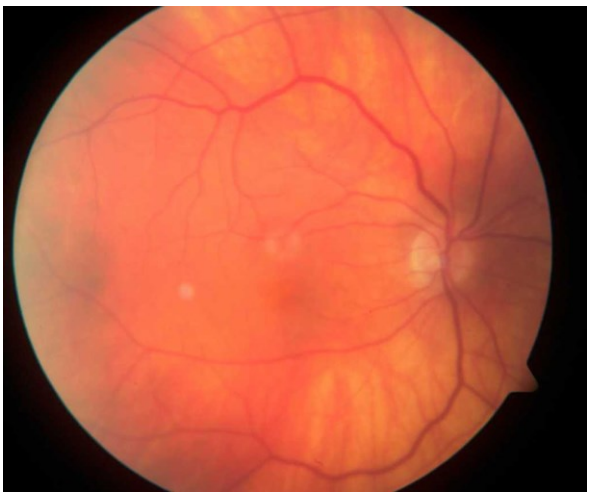


P7



P8

P9



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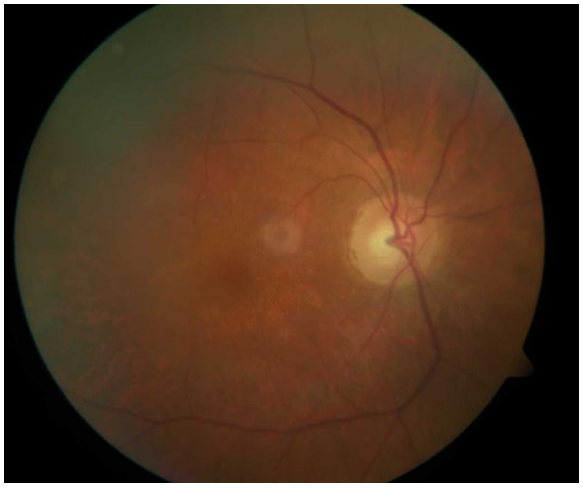
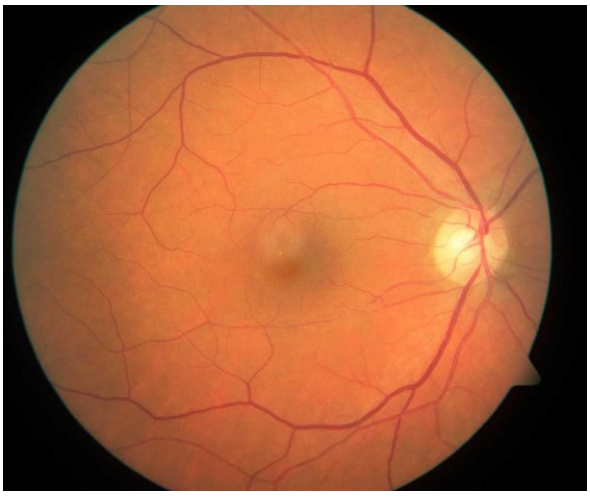
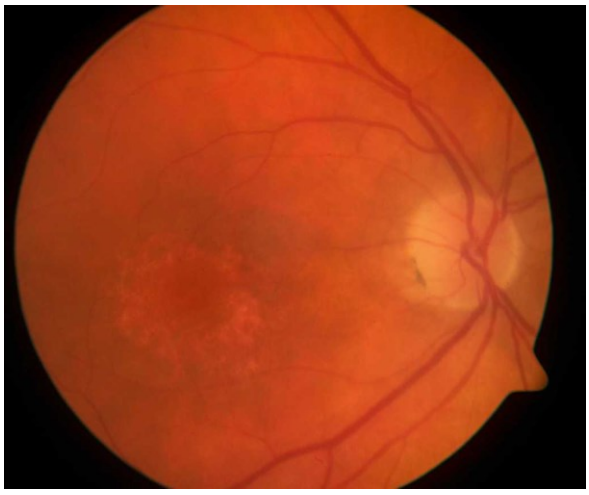


P11



P12



P13

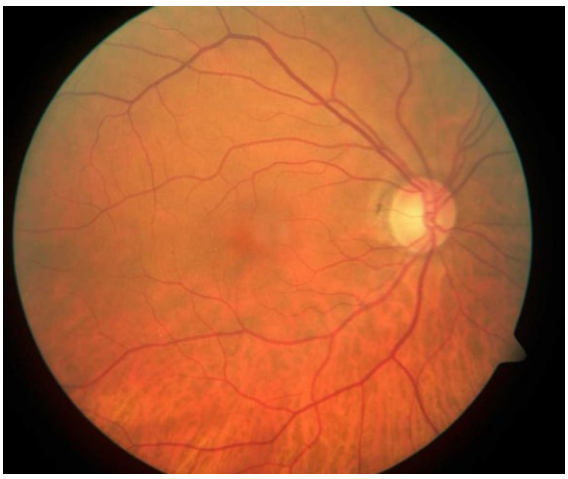


Figure 32 Case example of mfERG and waveform images at toxicity and after Plaquenil therapy cessation for patients experiencing recovery of abnormal mfERG findings

Ring amplitudes at toxicity

Ring Density Average MFERG Recorded with DTL Electrode and Dilated Pupils

	Right Eye 7-9 mm		Left Eye 7-9 mm		Normal Ranges
Ring 1 = 0 deg	10.2	nV	13.0	nV	12- 32.3 nV
	32.4	msec	33.3	msec	31 - 35 msec
Ring 2 = 5 deg	4.8	nV	4.8	nV	7.6-16.8 nV
	31.6	msec	30.8	msec	30 - 35 msec
Ring 3 = 10 deg	3.1	nV	3.5	nV	4.8-10.0 nV
	32.4	msec	32.4	msec	30 - 35 msec

Comment

Patient dilated at: 8:20 Am

Pupil size:

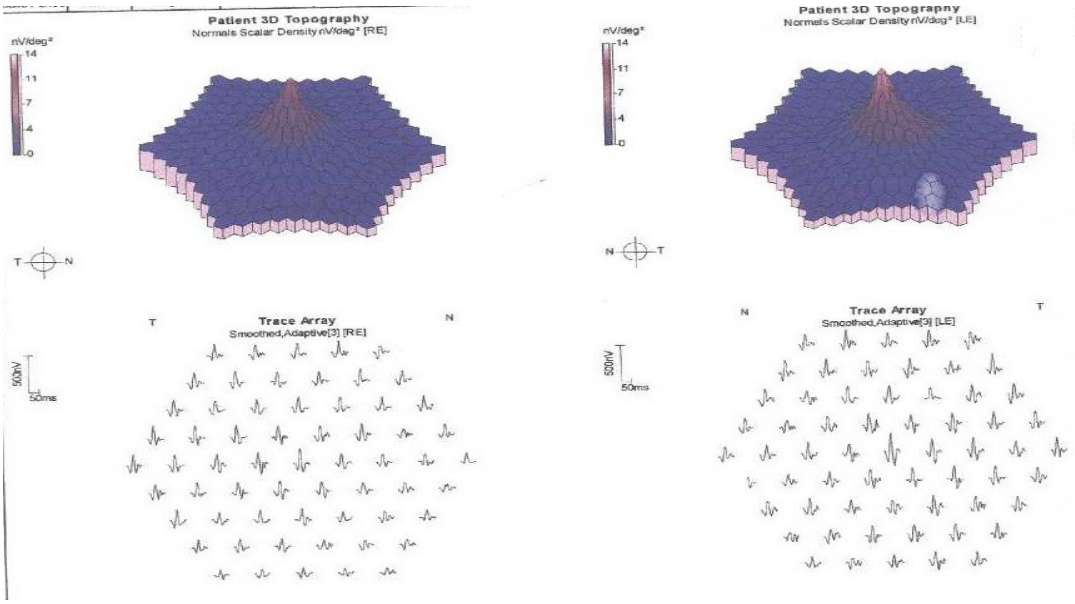
Plaquenil taken for:

Duration taken (months/years): 12 years

Dosage of Plaquenil: 2 tablets of 200 mg each = 400 mg / day

Patient's weight: 155 lbs.

Waveform at toxicity



Ring amplitudes at 1st point of examination after therapy cessation

Ring Density Average MFERG recorded with DTL Electrode and Dilated Pupils

	Right Eye (OD)	Left Eye (OS)	Normal Ranges
Ring 1 = 0 deg	20.9 nv 30.8 msec	20.0 nv 30.0 msec	12.9-32.3 nV 31-35 msec
Ring 2 = 5 deg	12.2 nv 30.8 msec	11.6 nv 30.0 msec	7.6-16.8 nV 30-35 msec
Ring 3 = 10 deg	7.3 nv 32.4 msec	7.3 nv 30.8 msec	4.8-10.0 nV 30-35 msec
Ring ratio (1/3)	**2.86**	**2.74**	

Comment

Patient dilated at: 13:20
Pupil Size: R-7, L-8 R dilated 2x

Plaquenil taken for: RA
Duration taken: 12 year(s)
month(s)
week(s) = 4380 days

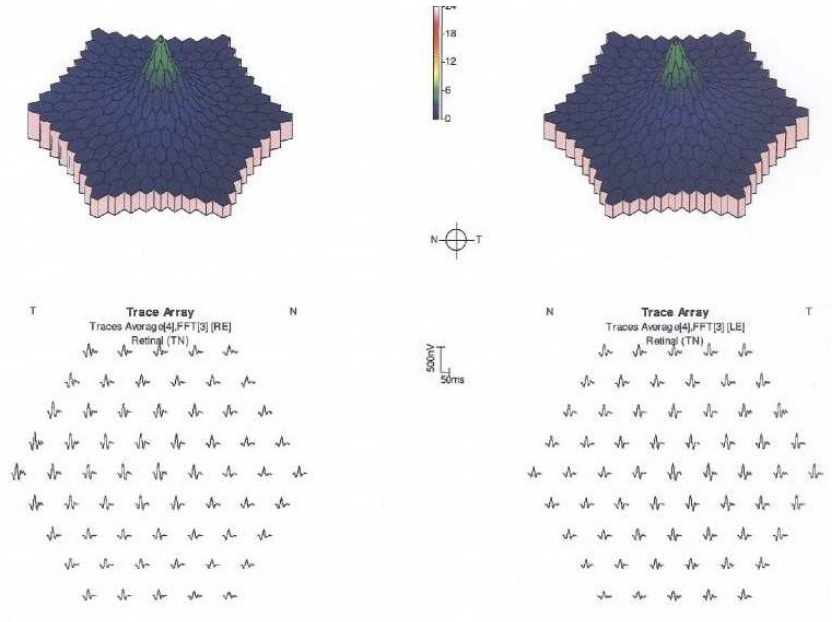
Dose of Plaquenil: 400 mg/day **OR** mg/week

Total Dose: 1.752 kg

Patients weight: 147 lbs
66.68 kg
Patients height: 5 ft 1 inc

NOTE: Pt stopped Plaquenil 11 month ago (April 2015)

Waveform at 1st point examination after therapy cessation



Ring amplitudes at 2nd time point after therapy cessation

Ring Density Average MFERG recorded with DTL Electrode and Dilated Pupils

	Right Eye (OD)	Left Eye (OS)	Normal Ranges
Ring 1 = 0 deg	16.3 nv 30.8 msec	27.1 nv 30.8 msec	12.9-32.3 nV 28-33 msec
Ring 2 = 5 deg	11.5 nv 30.8 msec	15.9 nv 30.8 msec	7.6-16.8 nV 28-33 msec
Ring 3 = 10 deg	8.0 nv 31.6 msec	9.4 nv 31.6 msec	4.8-10.0 nV 28-32 msec
Ring ratio (1/3)	**2.04**	**2.88**	2.5 -3.2

Comment

Patient dilated at: 837
Pupil Size: R=6 L=7

Plaquenil taken for: RA
Duration taken: 12 year(s) month(s) week(s) = 4380 days

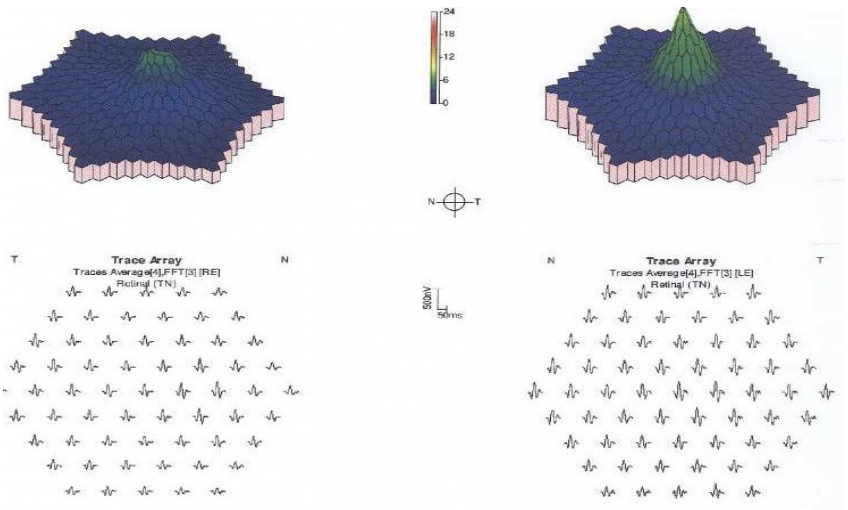
Dose of Plaquenil: 0 mg/day **OR** mg/week

Total Dose: 0.000 kg * Cumulitive dose on prev. ERG.

Patients weight: 142 lbs 64.41 kg Patients height: 5 ft 1 in

NOTE: **D/C Plaquenil 18/12 ago.** **DTL in good position.**

Waveform at 2nd point of examination after cessation of therapy



After therapy cessation, patient experience recovery of toxicity. Appendix 4 shows SD-OCT and 10-2 VFT images (where data is available) for all patients at the point of toxicity as captured in the EIA database.

Figure 33 Case example of mfERG ring amplitude changes of a patient experiencing partial recovery of abnormal mfERG findings

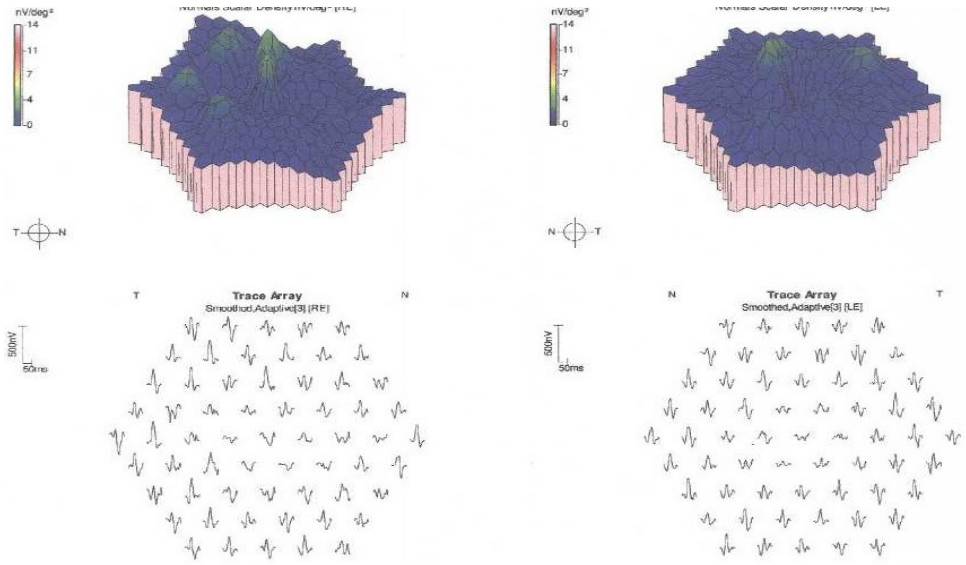
P12

mfERG at toxicity

RING DENSITY AVERAGE MF ERG RECORDED WITH DTL ELECTRODE AND DILATED PUPILS

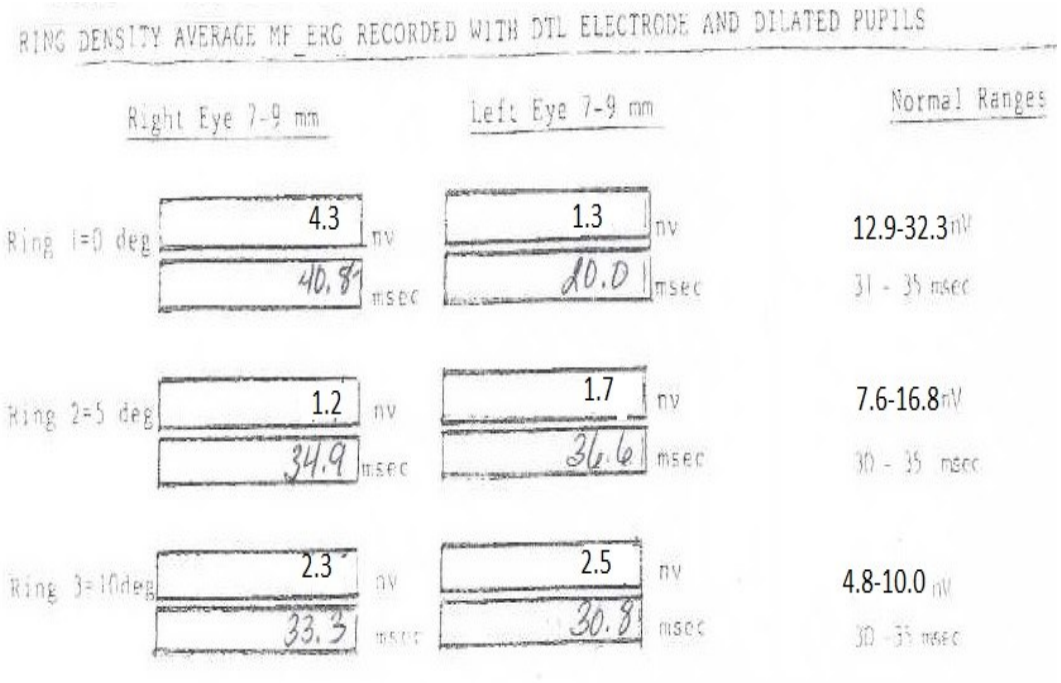
	Right Eye 7-9 mm		Left Eye 7-9 mm		Normal Ranges
Ring 1=0 deg	0.0	nv	1.7	nv	12.9- 32.3nV
	15.0	msec	40.8	msec	31 - 35 msec
Ring 2=5 deg	0.95	nv	1.5	nv	7.6- 16.8nV
	32.4	msec	38.3	msec	30 - 35 msec
Ring 3=10deg	2.0	nv	2.4	nv	4.8-10.0nV
	34.1	msec	34.1	msec	30 -35 msec

waveform

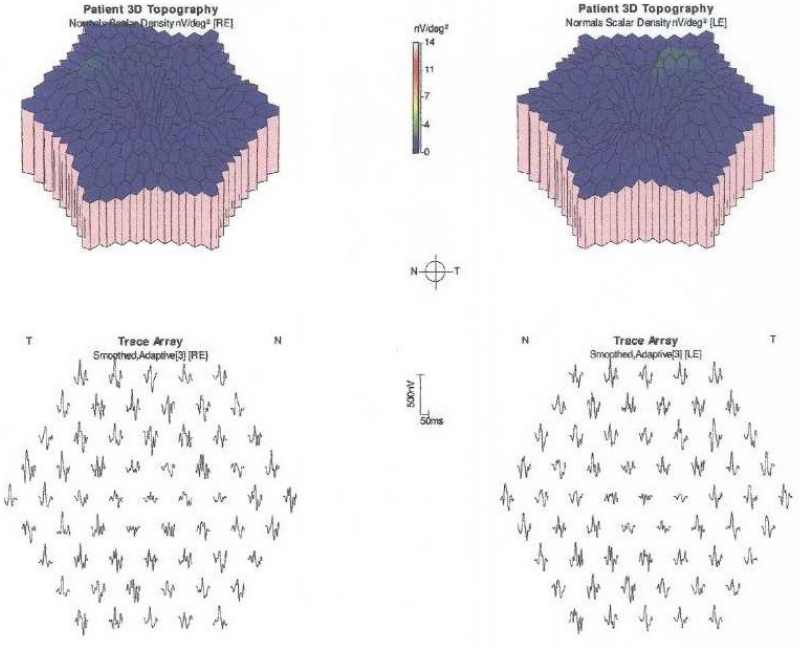


P12

mfERG after therapy cessation



Wave for after therapy cessation



P12

mfERG at 2nd time point after therapy cessation

Ring Density Average MFERG recorded with DTL Electrode and Dilated Pupils

	Right Eye (OD)	Left Eye (OS)	Normal Ranges
Ring 1 = 0 deg	4.3 nv 29.1 msec	5.2 nv 28.3 msec	12.9-32.3 nV 31-35 msec
Ring 2 = 5 deg	3.6 nv 29.1 msec	4.2 nv 29.1 msec	7.6-16.8 nV 30-35 msec
Ring 3 = 10 deg	3.6 nv 30.0 msec	3.5 nv 29.1 msec	4.8-10.0 nV 30-35 msec
Ring ratio (1/3)	**1.19**	**1.49**	

Comment

Patient dilated at: 1420
Pupil Size: 7 mm

Plaquenil taken for: RA
Duration taken: 10 year(s)
month(s)
week(s) = 3650 days

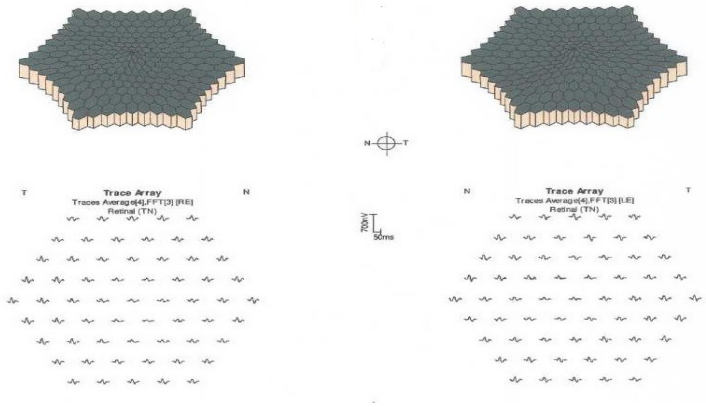
Dose of Plaquenil: 400 mg

Total Dose: 1.46 kg

Patients weight: 105 lbs
47.63 kg
Patients height: 4 ft 11 i

NOTE: Patient discontinued PLAQUENIL JUNE 2012

Wave for after therapy cessation



After therapy cessation, patient experienced partial recovery of toxicity. Appendix 4 shows SD-OCT and 10-2 VFT images (where data is available) for all patients at the point of toxicity as captured in the EIA database.

Table 20 Difference in background characteristics of patents who experienced partial recovery and those with recovery of Plaquenil retina toxicity after therapy cessation (N=13)

Variable	**Recovery (n=9) Mean±SD**	**Partial recovery (n=4) Mean±SD**	**p-value**
Age (years)	56.56±9.36	65.50±3.42	0.096
Daily drug dose (mg/kg)	5.51±2.00	6.35±1.97	0.508
Total daily drug dose (g)	366.67±122.47	375.50±50.00	0.900
Cumulative dose (kg)	1.40±0.62	1.88±0.44	0.192
Duration of therapy (years)	10.25±4.34	14.25±4.57	0.159
Visual acuity (logMAR)	0.84±0.18	0.77±0.07	0.446

Independent sample t-test analysis was done. There was no statistically significant ()$p>0.05$ difference in background characteristics between the two group.

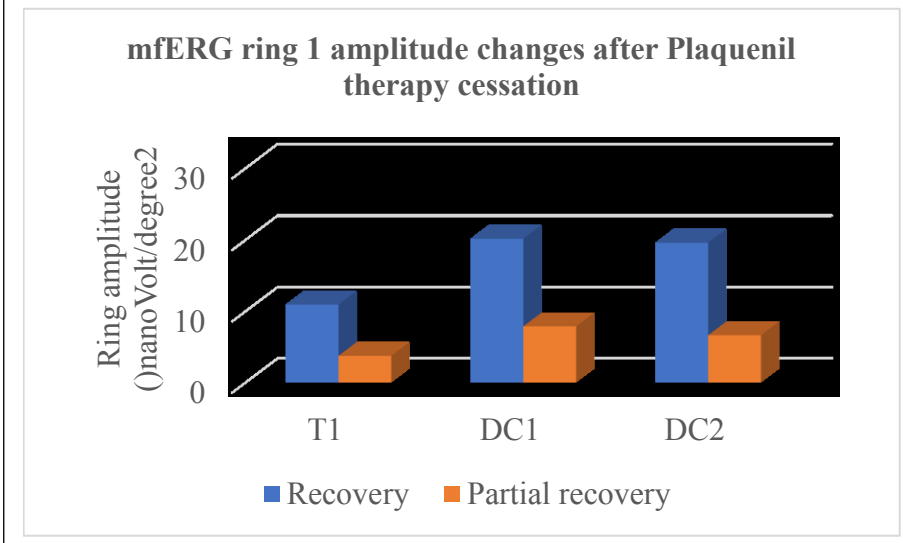
Table 21 Differences in ring amplitudes and ratios between the group that experienced recovery (n=9) and the group with partial recovery (n=4) of abnormal mfERG response after therapy cessation

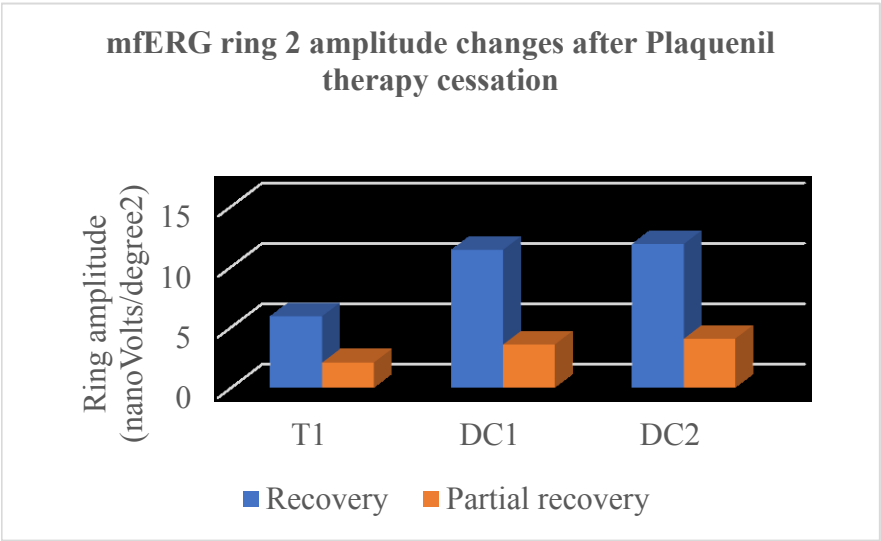
variable	Recovery mean±SD	Partial recovery mean±SD	p-value	Effect size
At toxicity				
R1 amplitude	10.90±4.27	3.71±3.16	0.004	0.55
R2 amplitude	5.89±2.35	2.06±1.45	0.013	0.45
R3 amplitude	3.72±1.22	1.88±1.02	0.024	0.38
R 1/2 ratio	2.26±0.92	1.75±1.30	0.427	
R 1/3 ratio	3.42±0.91	2.40±1.83	0.194	
First point of discontinuation				
Ring 1 amplitude				
Ring 2 amplitude	20.12±6.21	7.84±3.07	0.004	0.932
Ring 3 amplitude	11.38±3.05	3.55±2.12	0.001	0.681
Ring 1/2ratio	6.94±2.07	2.69±1.33	0.003	0.556
Ring 1/3 ratio	1.75±0.13	2.55±0.80	0.141	
	2.90±0.27	3.53±2.23	0.397	
At second point of discontinuation				
Ring 1 amplitude				
Ring 2 amplitude	19.57±3.35	6.60±2.84	0.003	0.737
Ring 3 amplitude	11.87±0.91	4.03±1.67	0.001	0.826
Ring 1/2 ratio	7.37±0.57	3.10±1.34	0.004	0.700
Ring 1/3 ratio	1.64±0.20	1.68±0.38	0.888	
	1.64±0.20	2.39±1.01	0.673	

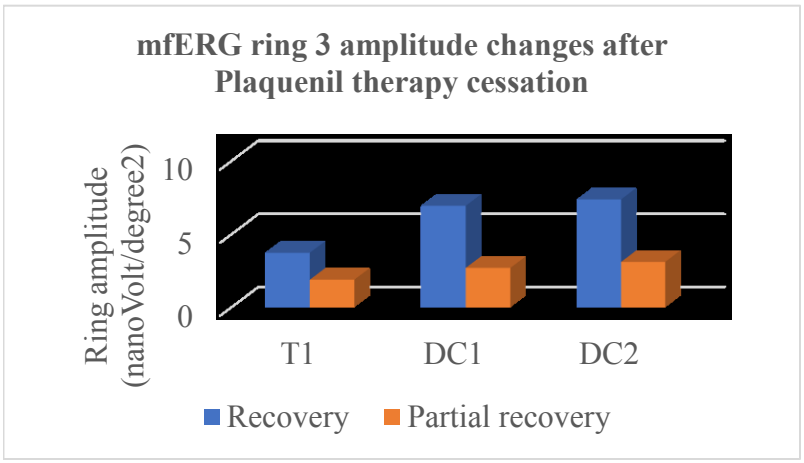
Independent sample t test that assessed differences between groups. There was a statistically significant difference in all ring amplitudes between the recovery group and partial recovery group at toxicity, and when discontinued. The ring 1/2 and ring 1/3 ratios did not differ between the two groups both at toxicity and when discontinued. For patients in both category, there was

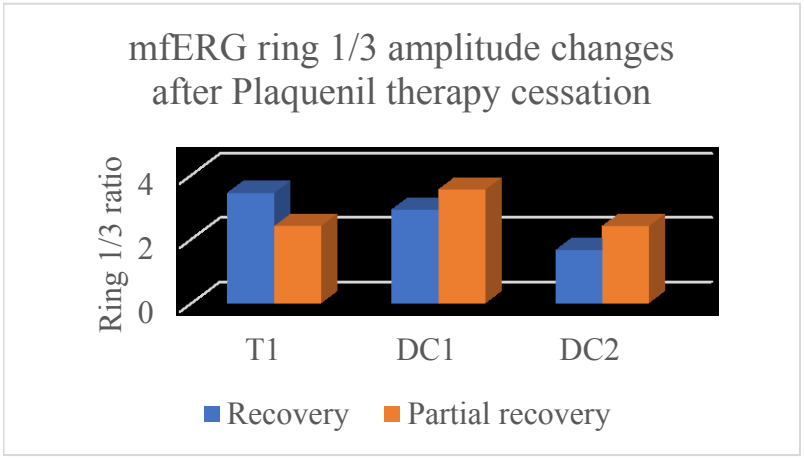
improvement in ring amplitudes at the first point of discontinuation but this was not significant for the group that experienced partial recovery of abnormal mfERG response.

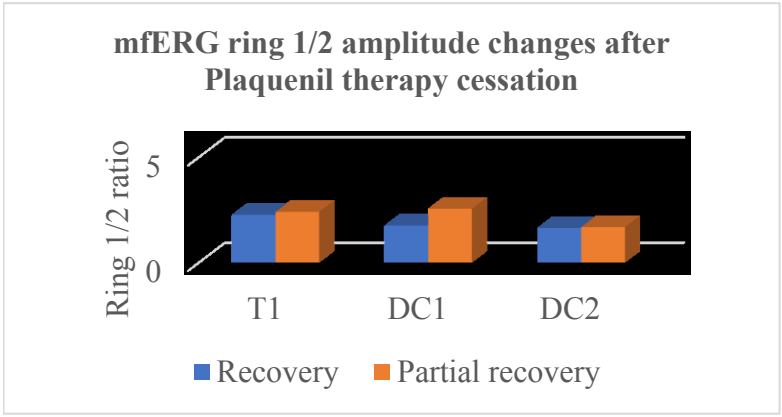
Figure 34 mfERG changes after therapy cessation











T1= mean ring amplitude at point of toxicity

DC1= mean ring amplitude at first point of examination after therapy cessation

DC2= mean ring amplitude at 2nd point of examination after therapy cessation

Table 22 Determining the change in ring 1 amplitude after therapy discontinuation

mfERG parameter	Ring1	Mean difference (std error)	p-value[b]	95% CI lower limit	upper limit
Ring 1					
T	DC1	-8.48 (1.36)	0.075	-18.91	1.94
	DC2	-7.42* (0.78)	**0.033**	-17.46	15.32
DC2	T	7.417* (0.78)	**0.033**	1.45	13.38
	DC1	-1.07 (2.14)	1.00	-17.46.	15.32

* Mean difference is significant at the 0.05 level, b. Adjustment for multiple comparison-Bonferroni.

One-Way ANOVA with repeated measure and post hoc analysis.

Discontinuation of Plaquenil therapy produced a significant change in ring 1 mfERG response from abnormal response to normal response, Wilks' Lambda= 0.01, F(2, 7.09), p= 0.011, effect size =0.98. The trend towards recovery occurs at DC2. T1= mfERG response at toxicity, DC1= mfERG response at first time point of examination following Plaquenil therapy cessation, DC2= 2nd time point of examination following therapy cessation.

Table 23 Determining the change in ring 2 amplitude after therapy discontinuation

Ring 2	Ring 2	mean difference	Std error	p-value[b]	95% CI lower limit	upper limit
T	DC1	-6.32*	0.65	**0.031**	-11.29	-1.35
	DC2	-6.28	1.09	0.087	-14.65	2.08
DC1	T	6.32*	0.65	**0.031**	1.35.	11.29
	DC2	0.03	0.82	1.00	-6.25.	6.32

* Mean difference is significant at the 0.05 level, b. Adjustment for multiple comparison-Bonferroni.

A significant change in ring 2 mfERG response from abnormal response to normal response occurred after therapy cessation, Wilks' Lambda= 0.65, F(2, 0.44), p= 0.003, effect size= 0.95 The trend towards recovery occurs at DC1.

Table 24 Determining the change in ring 3 amplitude after therapy discontinuation

Ring 3	Ring 3	Mean difference	Std error	p-value[b]	95% CI	
					lower limit	upper limit
T	DC1	-3.55*	0.36	**0.030**	-6.263	-0.84
	DC2	-4.18*	0.45	**0.034**	-7.61	-0.76
DC1	T	3.55*	0.18	0.030	1.84.	6.26
	DC2	-0.63	0.45	0.209	-1.98.	0.72

* Mean difference is significant at the 0.05 level, b. Adjustment for multiple comparison-Bonferroni.

A significant change in mfERG ring 3 response from abnormal response to normal response occurred after therapy cessation, Wilks' Lambda= 0.02, F(2, 1.13), p= 0.014, effect size= 0.98 The trend towards recovery occurs at both DC1 and DC2.

Table 25 Proportion of reduction in mfERG ring amplitudes among patients experiencing recovery of abnormal mfERG response from age normative data (n=9)

Ring	Recovery Group Mean	Normative data mean	Proportion of reduction (%)	p-value	Effect size
Ring 1	10.90	21.90	48.56	<0.001	1.10
Ring 2	5.00	11.74	57.41	<0.001	1.20
Ring 3	3.70	7.02	47.29	<0.001	1.10

Ring amplitudes were depressed by >40% on rings 1, 2, 3 among patients who experienced recovery of abnormal mfERG response at the point of toxicity compared to expected mean values among age normative data.

Table 26 Proportion of reduction in mfERG ring amplitudes among patients who experienced partial recovery of abnormal mfERG response from age normative data (n=4)

Ring	Partial recovery Group Mean	Normative data mean	Proportion of reduction (%)	p-value	Effect size
Ring 1	4.13	20.10	79.45	<0.001	5.8
Ring 2	1.98	11.33	83.26	<0.001	5.9
Ring 3	2.03	6.78	70.06	<0.001	5.8

Ring amplitudes are significantly reduced (>70%) among patients experiencing partial recovery of abnormal mfERG response from age normal data.

4. DISCUSSION

4.1. The risk of Plaquenil retinal toxicity

4.1.1. Background characteristics of patients studied

The patients studied (median age= 54.00, IQR: 45.00-63.00) were predominantly females (proportion of females n=324, 78.26%, males, n= 90, 21.74%) (Table 6. The commonest medical conditions for which patients were treated with Plaquenil included rheumatoid arthritis (51%) and SLE (15.9%) (Figure 19). Rheumatoid arthritis and SLE are immune regulated medical conditions (83). These diseases could be genetically inherited in humans (84,85) and are influenced by sex hormones and age (84–88). Patients mostly experience these autoimmune diseases in the fifth decade (88), a time when most females also begin to experience hormonal changes. It therefore stands to reason that the proportion of females to males (3.6:1) is higher for this elderly (median age >50 years) sample. The ratio of females to males (3.6:1) in this sample is very similar to that found in the general population of patients (3:1) with autoimmune conditions in North America (88). Cases recorded higher values (Table 5-6) on all background characteristics (age, daily drug dose, cumulative dose, duration of therapy) compared to controls except for weight (kg).

4.1.2. Risk factors for Plaquenil retinal toxicity

In both univariate and multivariate COX regression analyses, daily drug dose (mg/kg), cumulative dose (kg) and duration of therapy (years) were found to be the most significant risk factors for Plaquenil retinal toxicity (Tables 8-10, Figures 21-24) in this group. In a logistic regression model containing five independent variables (age, sex, daily drug dose, cumulative dose, duration of therapy), the full model containing all 5 predictors (variable) was statistically significant, X^2 (5, N=414) = 86.40, P<0.001, indicating that the model was able to distinguish between cases and controls. The model explained between 24.60% (COX and Snell R squared) and 42.60% (Nagelkerke R squared) of the variance in Plaquenil retinal toxicity status. The model correctly classified 86.40% of cases. The most significant predictors of Plaquenil toxicity among these patients were cumulative dose >1 kg (cumulative dose >1 kg, odds ratio (OR)= 0.21, 95% CI= 0.047-0.957, cumulative dose >2 kg, OR= 0.33, 95% CI= 0.127-0.875) , daily drug dose >5 mg/kg (OR= 0.19, 95% CI= 0.072-0.506) and duration of therapy >5 years (OR= 2.30, 95%C1= 0.06-0.30, P=0.028). Thus, patients on >5 years Plaquenil therapy were 2.3 times at greater risk of toxicity than those on <5 years of therapy. Similarly, patients on <5 mg/kg of Plaquenil daily dose

were 5.26 times less likely to have toxicity than those on >5 mg/kg of daily Plaquenil dose. Patients on <1 kg of cumulative Plaquenil dose were 4.76 and 3.03 times less likely to develop retinal toxicity than patients on >1-2 kg, and >2 kg respectively. Plaquenil daily drug dose, duration of therapy and cumulative dose have been considered by the AAO to be the most significant risk factors for retinal toxicity (9,16,43) as was the case in this study.

Age and sex were not found to be significant risk factors for Plaquenil retinal toxicity among the sample studied contrary to the AAO (9) suggestion that age may be associated with Plaquenil retinal toxicity due to its debilitating effect on retinal function, but in agreement with most current studies (7,16). As previously indicated (7,9,16), there may be no safe recommended Plaquenil dose as there were instances where some patients developed retinopathy while on <5 mg/kg daily dose, with <1 kg of cumulative drug dose and for < 5 years of therapy. On the contrary, there were patients who showed resilience to Plaquenil retinal toxicity even at >2 kg of cumulative drug dose for >20 years of therapy. Genetic, patient and environmental factors that influence resilience to toxicity even at such high cumulative doses or easy susceptibility to Plaquenil retinal toxicity at low cumulative dose or daily drug dose need to be explored further to inform clinical decisions. Table 8 provides the hazard rate or survival rate for each five-year interval of Plaquenil therapy. At zero to 5 years, the hazard rate is zero, thus the risk of toxicity is almost not present for most patients from start to 5 years of therapy, however, by 30 years, only 33% of the patients were surviving, that is, showing resilience to Plaquenil retinal toxicity. As recommended by the AAO, screening could be deferred till 5 years of therapy following baseline screening. However, Ophthalmologists may have to decide on screening intervals based on individual patient presentation.

4.2. Relative sensitivity and specificity of mfERG, SD-OCT and 10-2 Humphrey VFT to Plaquenil retinal toxicity and parameters associated with toxicity.

4.2.1. Relative sensitivity and specificity of mfERG, SD-OCT and 10-2 Humphrey visual fields to Plaquenil retinal toxicity

The relative sensitivity, specificity, positive and negative predictive values of all three screening tests are shown in Tables 11-12. All three screening tests (mfERG, SD-OCT, 10-2 Humphrey VFT) recorded a high specificity (mfERG= 95.59%, SD-OCT= 97.24%, 10-2 Humphrey VFT= 94.57%) which indicates the ability of all three to truly detect non-disease state (Table 11). The SD-OCT was most specific to Plaquenil retinal toxicity. However, in terms of sensitivity, mfERG recorded the highest value (sensitivity of 92.00%). High sensitivity of mfERG has been attributed to its ability to detect abnormal retinal response before obvious structural or anatomical changes occur on SD-OCT and 10-2 Humphrey VFT, thus mfERG is able to pick up early abnormal electrophysiological response in Plaquenil retinal toxicity in advance of abnormal morphological changes on SD-OCT or 10-2 Humphrey visual field (9,89). Marmor and Melles (90) in 2014 observed that patients with Plaquenil retinal toxicity had normal SD-OCT presentation even though visual fields for these patients showed prominent ring scotoma in the parafoveal regions**.** Tsang et al. (89) in a systematic review in 2015 discussed that mfERG recorded the most positive finding and highest sensitivity to Plaquenil retinal toxicity compared to SD-OCT or 10-2 Humphrey VFT even after controlling for such confounding factors such as age, and daily drug dose. Since the aim in Plaquenil retinal toxicity screening is to detect early disease to prevent vision loss or ensure possible reversal of disease, mfERG would play a significant role in that direction. Again, supposing the argument that functional changes precede structural changes in Plaquenil retinal toxicity stands, mfERG would be more valuable in screening for toxicity due to its high sensitivity to early functional changes (89). An abnormal mfERG test result at the second-time point following baseline screening among patients at high risk and with/without symptoms of visual disturbance, in the absence of any other ocular condition should warrant further investigation with SD-OCT or 10-2 Humphrey VFT to establish the presence of toxic retinopathy or otherwise. The sensitivity and specificity increase when at least two or all three of these screening tests are combined (Table 11). In hospitals where at least two of these screening tools are available, it may be useful to confirm toxicity when responses generated on one test shows

consistent decline over time (50). The decision to use two or all three screening tests should be weighed against cost/benefit to the patient and patient's risk of toxicity.

4.2.2 Parameters on mfERG, SD-OCT and 10-2 Humphrey VFT most specific and sensitive to Plaquenil retinal toxicity

ROC curve and logistic regression analysis were used to determine which parameters would be most predictive of Plaquenil retinal toxicity (Table 13-16, Figure 25-29).

***mfERG*:** mfERG Ring 2 amplitude was most predictable of Plaquenil retinal toxicity (AUC= 0.997, 95% CI= 0.95-0.99-1.00, $p<0.001$) (Table 13-14). The ability of mfERG ring ratios (ring 1/2, 1/3 ratios) to discriminate between Plaquenil retinal toxicity and non-toxicity state was ≈50% for each (Table 13, Figure 26). Therefore, depressed/reduced mfERG ring amplitudes, especially on ring 2 below the lower limit of the 95% CI of age normative data have greater predictive value in discriminating between toxic and non-toxic retinal state among patients on Plaquenil therapy compared to ring ratios. Although ring ratios may serve as a guide, their discriminating ability is less desirable. Normal ring amplitudes in the presence of elevated ring ratios (ring 1/2 ratio >2.91, ring 1/3 ratio >3.5) may not necessary suggest Plaquenil toxicity. However, normal ring 1/2 and 1/3 ratios with depressed ring amplitude below the lower limit of the 95% CI of age normative data should warrant further investigation, as that could be early indication of possible retinal toxicity.

***SD-OCT*:** Parameters associated with Plaquenil retinal toxicity were retinal layers of the inner concentric ring (Figure 27). Inner nasal and inner inferior retinal thickness were the parameters on SD-OCT most capable of discriminating between cases and controls (Table 15, Figure 27). Inner concentric rings on SD-OCT corresponds, anatomically to ring 2 amplitude on mfERG (Figure 7) and the parafoveal region on 10-2 Humphrey VFT (17,91). Effect of Plaquenil retinal toxicity on inner retina nerve fibre thickness has previously been studied (92–94). Pasadhika and Fisman (93,95) found 37.5% of their sample with Plaquenil retina toxicity to have significantly reduced retina nerve fibre thickness involving inner nasal and inner temporal retina layers and 87% of patients with early signs of possible toxicity had reduced inner retina layer thickness. Cukras et al. (17) and Marmor (69) found reduced inner retinal layer thickness to be associated with Plaquenil retinal toxicity. Cukras et al. (17) however found inner inferior retinal layer thickness to be the most significant variable associated with toxic or non-toxic status. In 2016, a study by Lius de

Sistern et al. (94) of 27 patients on short term Plaquenil therapy but without toxicity, followed over a short period (<5 years) found no significant change in both inner and outer retina nerve fibre layer thickness. Perhaps, Luis de Sistern et al. (94) found no change in both inner and outer retina nerve fibre layer thickness because their sample did not include patients with Plaquenil retinal toxicity.

VFT*:* Mean deviation on 10-2 Humphrey VFT was found to better discriminate between cases and controls (Figure 28, Table 16) than pattern standard deviation among the patients studied.

Stepwise regression analysis (Table 17) and ROC curve analysis (Figure 29) were then applied to determine the parameters from each screening test that best discriminate toxicity from non-toxicity. The parameters analysed included rings 1, 2 and 3 amplitudes of mfERG, inner nasal and inner inferior retinal nerve fibre thickness of SD-OCT and mean deviation on 10-2 Humphrey VFT. SD-OCT inner nasal retina nerve fibre thickness (β= 0.82, 95% CI= 0.80-0.99, P= 0.020) and mfERG ring 2 amplitude (β= 0.92, 95% CI= 0.89-0.98, P= 0.016) were the most significant parameters which best discriminate between cases and controls (Table 15).

An examination of ring 2 amplitude, inner nerve fibre thickness and mean deviation values (plus scotoma analysis) on mfERG, SD-OCT and 10-2 Humphrey VFT respectively should serve as a guide to clinicians in screening and monitoring Plaquenil retinal toxicity. Significant changes in responses of these parameters for each patient over time taking into account inter-visit and inter-test variability could direct diagnosis.

The distribution of mfERG, SD-OCT and 10-2 VFT findings between cases and controls is summarised in Table 16.

4.3. Changes in abnormal mfERG response among cases who discontinued Plaquenil therapy

4.3.1 Background characteristics

Fourteen patients previously diagnosed as having developed Plaquenil retinal toxicity discontinued therapy. These patients were aged between 47 to 71 years. The mean of the background characteristics for the patients are summarised in Table 19. These patients were followed for 4 years and the changes in mfERG response were observed. Of the 14 patients, 9 experienced recovery of abnormal mfERG response to normative responses (case sample as presented in Figure 32), 4 experienced partial recovery (less improvement in mfERG after therapy cessation (case sample as presented in figure 33), while 1 patient experienced stable abnormal mfERG response

(no improvement or further decline in mfERG response) after discontinuation of Plaquenil therapy (Figure 30). Medical records, SD-OCT and 10-2 Humphrey VFT images were reviewed were data was available (appendix 4). Patients who experienced recovery of abnormal mfERG (Figure 32) response following Plaquenil therapy cessation had normal SD-OCT images, 10-2 Humphrey VFT scotoma points between <5% and <1%. Patients who experienced partial recovery of abnormal mfERG response (appendix 4) secondary to therapy cessation had significantly reduced nerve fibre layer thickness and disruption of photoreceptor cells on SD-OCT, point scotoma of <1%, partial ring scotoma or full ring scotoma, delayed implicit time (appendix 4) on mfERG, and severe distortion on mfERG wave form (Figure 33 Appendix 4).

4.3.2. Long term changes after Plaquenil therapy cessation

Long term mfERG changes following Plaquenil therapy cessation was evaluated using One-Way ANOVA with repeated measure and post hoc analysis. Significant improvement occurred in ring amplitudes and not necessarily in ring ratios for patients who regained normal mfERG response following therapy cessation (Figure 34, Table 21-24). This further suggests that, ring amplitudes are more valuable in the assessment of Plaquenil retinal toxicity than ring ratios. The most significant change from abnormal to normal response occurred at the first point of examination (Figure 34) following cessation of therapy, which ranged between 1-1.5 years after therapy cessation. The mfERG response was normal and stable at the 2nd time point of examination for patients who experienced recovery of toxicity. This 2nd time point of examination ranged between 3.5-4 years following therapy discontinuation (Table 21-24, Figure 34). Among the patients who experienced partial recovery of abnormal mfERG changes, it was observed that, they had advanced disease stage with ring amplitudes reduced by >70% (Table 26) of age normative data and significant changes on SD-OCT including RPE and photoreceptor cell loss (Figure 33, appendix 4). Patients who show consecutive and consistent decline in mfERG ring amplitudes can have a recovery of abnormal response if Plaquenil therapy is discontinued before the ring amplitudes are reduced below 50% of age normative limit (Table 23). The possibility of recovery of abnormal mfERG response among patients diagnosed with Plaquenil retinal toxicity has also been shown by other studies (62,96,97). It can be inferred that once significant reduction (>70%) in mfERG ring amplitudes occur, the possibility of recovery is limited or almost impossible, and disease may progress despite therapy cessation (98,99), thus the utility of the mfERG will be less valuable once

SD-OCT and 10-2 Humphrey VFT shows visible structural changes consistent with Plaquenil retinal toxicity.

STUDY LIMITATIONS

1. Given the fact that a large proportion of the sample (59%) had been on Plaquenil therapy for a lengthier amount of time and on higher drug dose, it is likely that only patients in whom concern for Plaquenil toxicity is high may have been referred for mfERG testing. In other words, an unbiased sample of patients monitored for Plaquenil toxicity may have more patients at lower risk of toxicity.
2. A large number of 209 patients were excluded due to insufficient data, inconsistency in mfERG, 10-2 Humphrey VFT and SD-OCT grading and comorbid ocular conditions. Not only did this reduce the sample size but the findings in this study may not be as generalizable to a larger population given these patients' special circumstances. However, including these patients, who's ocular comorbidities confound the true performance of mfERG (and SD-OCT ABD 10-2 VFT) would degrade the ability to understand to understand how to use mfERG for the majority of patients on Plaquenil. Exclusion of patients with comorbidities is a reasonable exclusion criterion. Nevertheless, further study exploring the mfERG, SD-OCT and 10-2 VFT Plaquenil retinal toxicity findings is needed for this category of patients especially among patients with coincident

CONCLUSION

Plaquenil retinal toxicity is an important health concern due to the potential for blindness. Screening and monitoring that is informed by research provides understanding and direction that allows patients to continue to use an important and relatively safe drug while maintaining ocular health. I would like to convey the following key points:

- High Plaquenil daily and cumulative drug dose and long duration of therapy are significant risk factors for Plaquenil retinal toxicity.
- mfERG is a valid, sensitive test for screening and monitoring Plaquenil retinal toxicity and its use could be advocated for in hospitals where it is available.
- SD-OCT and 10-2 Humphrey VFT are also valid screening tests. A combination of any of these two tests will yield higher sensitivity and specificity to Plaquenil retinal toxicity.

- Ring amplitudes on mfERG, inner retinal nerve fibre layer thickness on SD-OCT and mean deviation plus scotoma presentation (points, partial or full ring scotoma) on 10-2 Humphrey VFT could be used in assessing Plaquenil retinal toxicity. Consistent decline in ring 2 amplitude on mfERG, inner nasal retina nerve fibre layer thickness on SD-OCT, mean deviation and partial or full ring scotoma at the parafoveal region on 10-2 Humphrey VFT on at least three consecutive examinations could guide diagnosis of Plaquenil retinal toxicity.
- Despite its limitations, this study provides evidence that discontinuation of Plaquenil therapy at a critical point can allow recovery of mfERG function.

FUTURE DIRECTION

1. The genetic, patient and environmental factors that determine resilience to high cumulative and daily drug dose among patients on long term Plaquenil therapy needs to be studied to inform clinical decisions.
2. An in-depth knowledge through a long term follow up prospective study of large samples of patients who discontinued Plaquenil therapy or received lesser drug dose due to toxicity would give further insight into the course of Plaquenil retinal toxicity after therapy cessation/ reduction of drug dose. The outcome of such a study will guide timely intervention that ensures preservation of vision or recovery of toxicity or halting of disease process and ensure better clinical practices)
3. A long term, multi-centre prospective study of a large sample of patients on Plaquenil therapy is needed to address issues of drug dose and efficacy, cost-benefit of Plaquenil retinal toxicity screening, cost effectiveness of mfERG, SD-OCT and Humphrey VF in Plaquenil retinal screening, screening intervals, classification of patients on Plaquenil therapy (no toxicity, suspect toxicity, early toxicity, definite toxicity).

retinal disorders. Exclusion of 209 patients may have created a selection bias, thus only Patients likely to give a desired end result and/or at high risk were included in the study.

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APPENDICES

Appendix 1 Case report form (CRF)

CASE REPORT FORM

THE ROLE OF THE MULTIFOCAL ELECTRORETINOGRAM IN SCREENING FOR HYDROXYCHLOROQUINE RETINOPATHY; THE DEVELOPMENT OF HYDROXYCHLOROQUINE RETINOPATHY SCREENING PROTOCOL FOR EDMONTON, AB

Reference number: **Pro00072173**

Study site	**Edmonton, Alberta**
Principal investigator	**Dr. Ian Macdonald**
Participant code	☐☐☐
Review date (month/day/year)	☐☐☐

DEMOGRAPHIC PROFILE

Age (years) ☐☐ Sex: Male ☐ Female ☐

Date of birth ☐☐☐☐☐☐☐☐☐

d d m m y y y y y

VISUAL ACUITY AND REFRACTIVE CORRECTION

Refractive correction:	OD		OS	
Best corrected vision:	OD		OS	

GENERAL INFORMATION

Height		m
Weight		kg
BMI		Kg/m^2

CONCOMITANT MEDICATION LOG

Medication	Indication	Site	Dosage/unit	Start date	Stop date
		Ocular ☐ Non-ocular ☐		—/ —/ —	—/ —/ —
		Ocular ☐ Non-ocular ☐		—/ —/ —	—/ —/ —
		Ocular ☐ Non-ocular ☐		—/ —/ —	—/ —/ —
		Ocular ☐ Non-ocular ☐		—/ —/ —	—/ —/ —
		Ocular ☐ Non-ocular ☐		—/ —/ —	—/ —/ —

HYDROXYCHLOROQUINE DOSAGE

Start date of therapy ☐☐/☐☐/☐☐☐☐ (D D M M Y Y Y Y)

End date of therapy ☐☐/☐☐/☐☐☐☐ (D D M M Y Y Y Y)

Daily dose ☐☐☐ mg/kg

Condition Being Treated For ☐☐☐

HYDROXYCHLOROQUINE TOXICITY SCREENING

1. MULTIFOCAL ELECTRORETINOGRAM

	Test date	Ring 1	Ring 2	Ring 3	Ring 1/3 , 1/2 ratios
1st exam					
2nd exam					
3rd exam					
4th exam					
5th exam					

Dilated pupil size: ☐ mm

Stimulus Pattern: 61 Hexagon ☐ Recording Details: Binocular ☐

2. Central 10-2 Humphrey VISUAL FIELD TEST

	TEST DATE	RELIABILITY INDICES			GHT	PATTERN DEVIATION
		False negatives	False positives	Fixation losses		
1st test						
2nd test						
3rd test						
4th test (if applicable)						

5th test (if applicable)						

3 SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY TEST

	9 EDTRS RETINA SUBFIELD THICKNESS								
test/ date	outer superior	inner superior	outer inferior	inner inferior	outer nasal	inner nasal	outer temporal	inner temporal	center
1st test									
2nd test									
3rd test									
4th test									
5th test									

OTHER DISEASE

Systemic	Specify Kidney ☐ Liver ☐
Ocular	Glaucoma ☐ Cataract ☐ AMD ☐
Other	

Appendix 2

DRAFT Reporting Structure for Plaquenil Toxicity based on mfERG recording and other testing

1. **Toxicity (at least one of the following ERG findings)**

- Clear reduction in the amplitudes of mfERG responses either for all rings (compared to normative data), OR
- Elevated ring 1/3 ratio with evidence of progression from previous examinations

AND at least one of the following:

- Abnormal fundus examination or photography
- Abnormal autofluorescence imaging
- Abnormal OCT with paracentral disruption of photoreceptors
- Abnormal Humphrey automated visual field testing (or other field testing)

Recommendation would be to halt Plaquenil

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2. **Likely toxicity**

- Clear reduction in the amplitudes of mfERG responses either for all rings (compared to normative data), OR
- Elevated ring 1/3 ratio with evidence of progression from previous examinations,

AND

- Total dose at or above the threshold of 1Kg

WITHOUT data on OCT or other test modalities

Recommendation would be to either re-test, or test more frequently or reduce dosage or acquire further test data. If addition testing reveals a normal fundus and no abnormality with autofluorescence, OCT or Humphrey field testing, then re-testing with mfERG would be recommended in 6 months.

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3. **Suspect toxicity**

- Elevated ring 1/3 ratio and/or evidence of progression from previous examinations

AND

- Total dose below threshold of 1 Kg

BUT WITHOUT

- Reduction in visual acuity
- Abnormal fundus examination or photography
- Abnormal autofluorescence imaging (or unavailable data)
- Abnormal OCT with paracentral disruption of photoreceptors (or unavailable data)

Recommendation would be to either re-test, test more frequently with mfERG or provide additional data from autofluorescence or OCT imaging.

4. **No evidence of toxicity**

- Normal amplitudes of mfERG

WITH

- Normal fundus examination or photography
- Normal autofluorescence imaging
- Normal OCT with paracentral disruption of photoreceptors

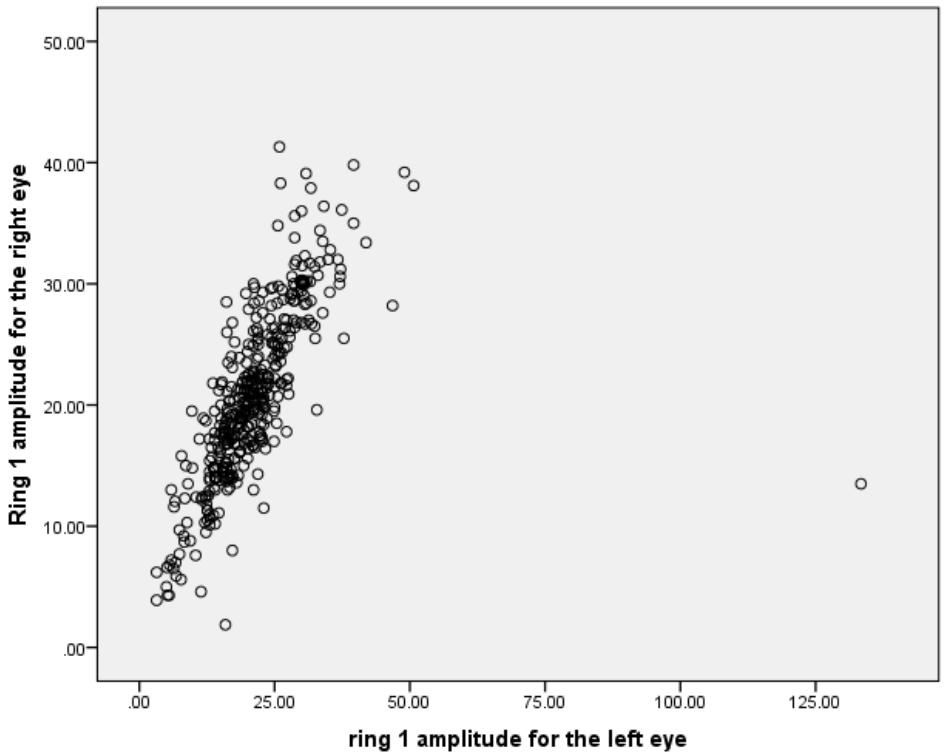
Recommendation would be routine follow-up

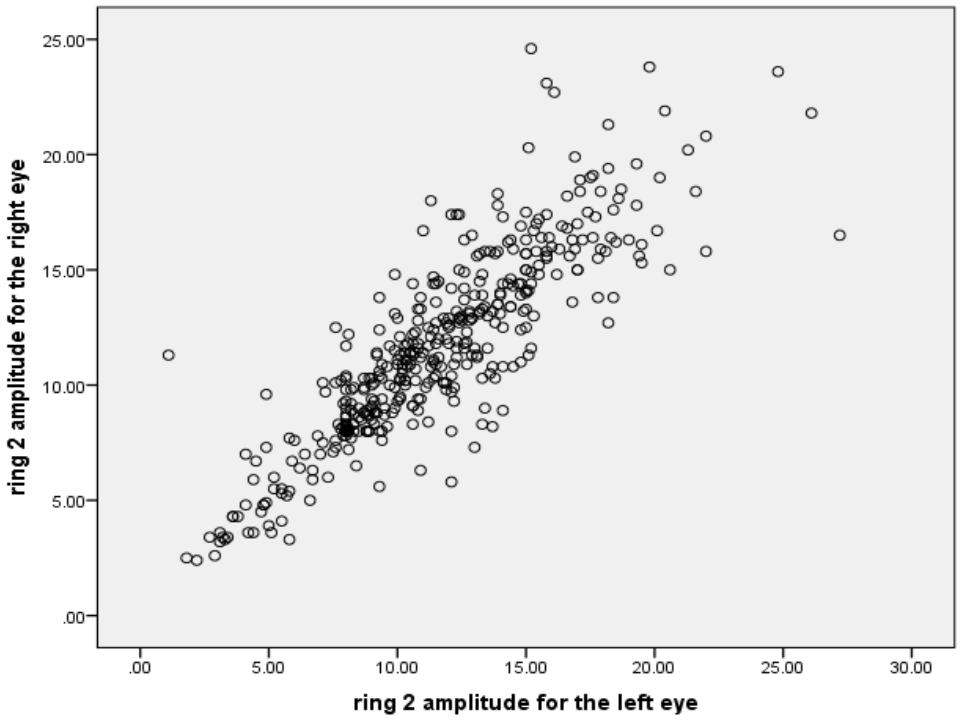
Appendix 3 Scatterplots showing correlation between right eye (OD) and left eye (OS) variables

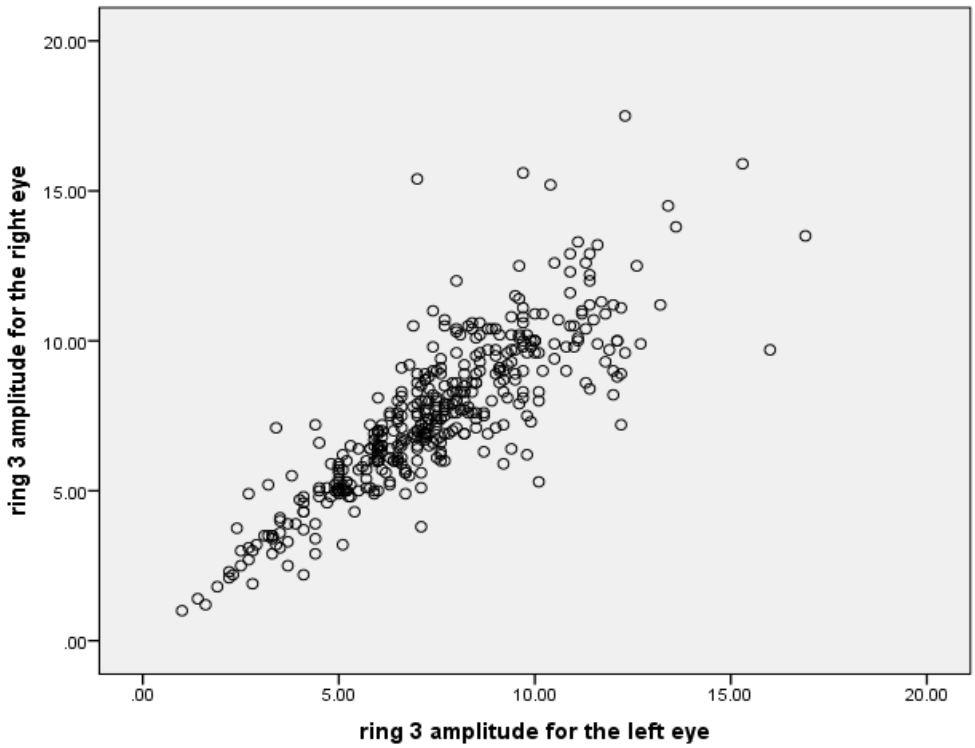
Variable	OS Correlation coefficient (rho)	p-value
Ring amplitudes OD		
R1	0.835**	<0.005
R2	0.870**	<0.005
R3	0.865**	<0.005
R1/2	0.679**	<0.005
R1/3	0.648**	<0.005
Retinal layer thickness OD		
Outer superior	0.910**	<0.005
Inner superior	0.917**	<0.005
Outer inferior	0.812**	<0.005
Inner inferior	0.891**	<0.005
Outer nasal	0.865**	<0.005
Inner nasal		

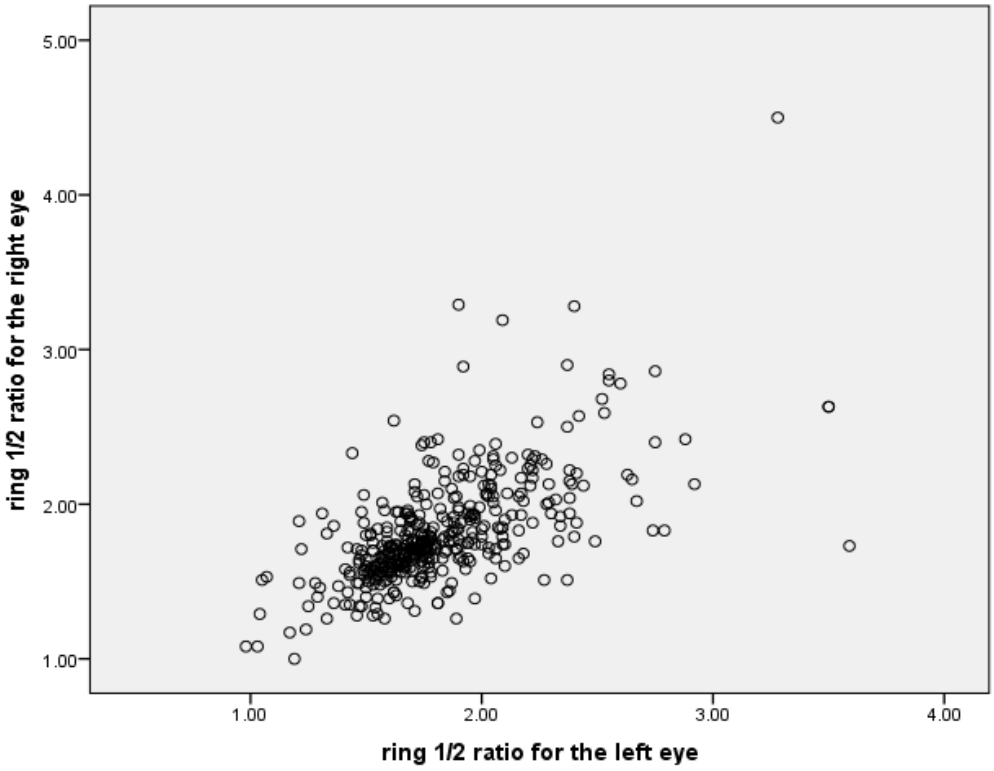
Outer temporal	0.896**	<0.005
Inner temporal	0.860**	<0.005
Central	0.892**	<0.005
	0.853**	<0.005
10-2 parameters OD		
Mean deviation	0.724**	<0.005
Pattern standard deviation	0.597**	<0.005

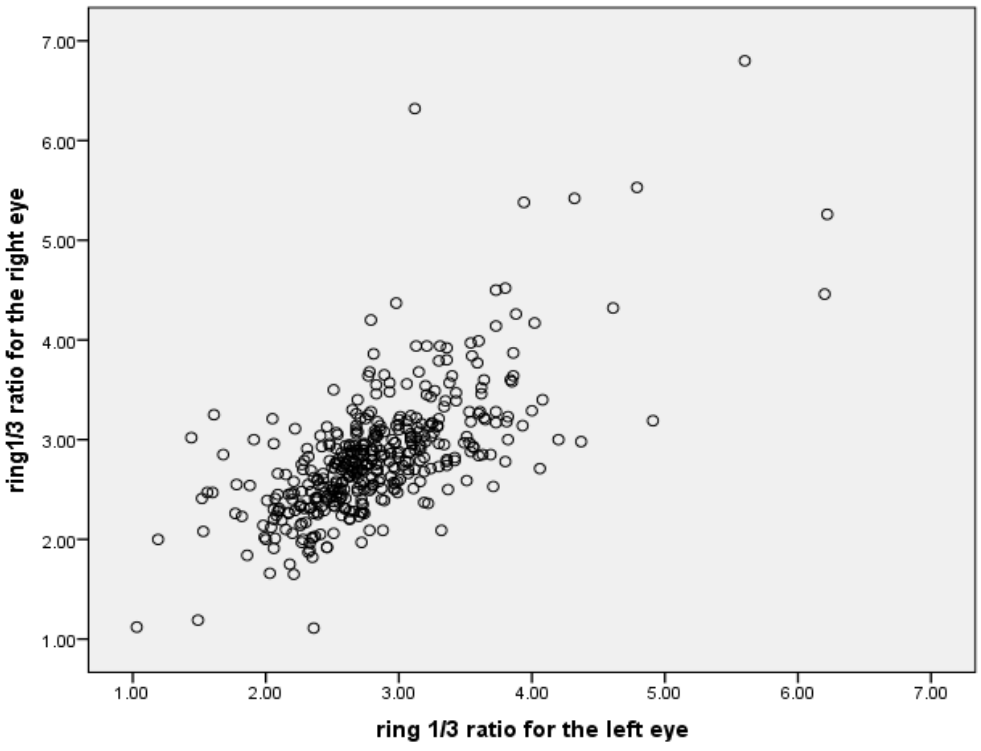
** correlation is significant at the 0.001 level (2-tailed)

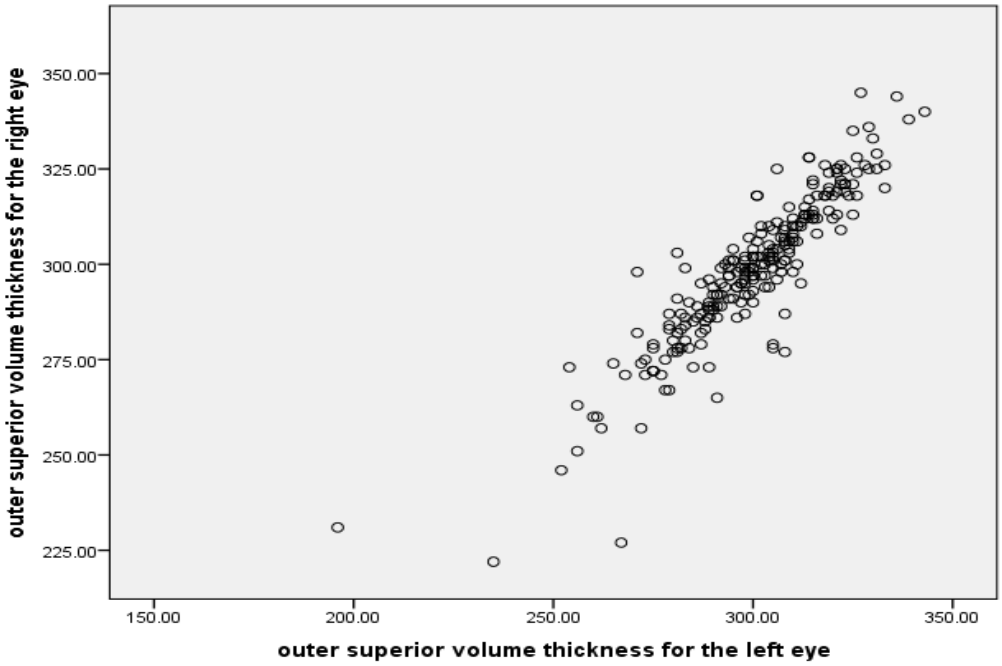


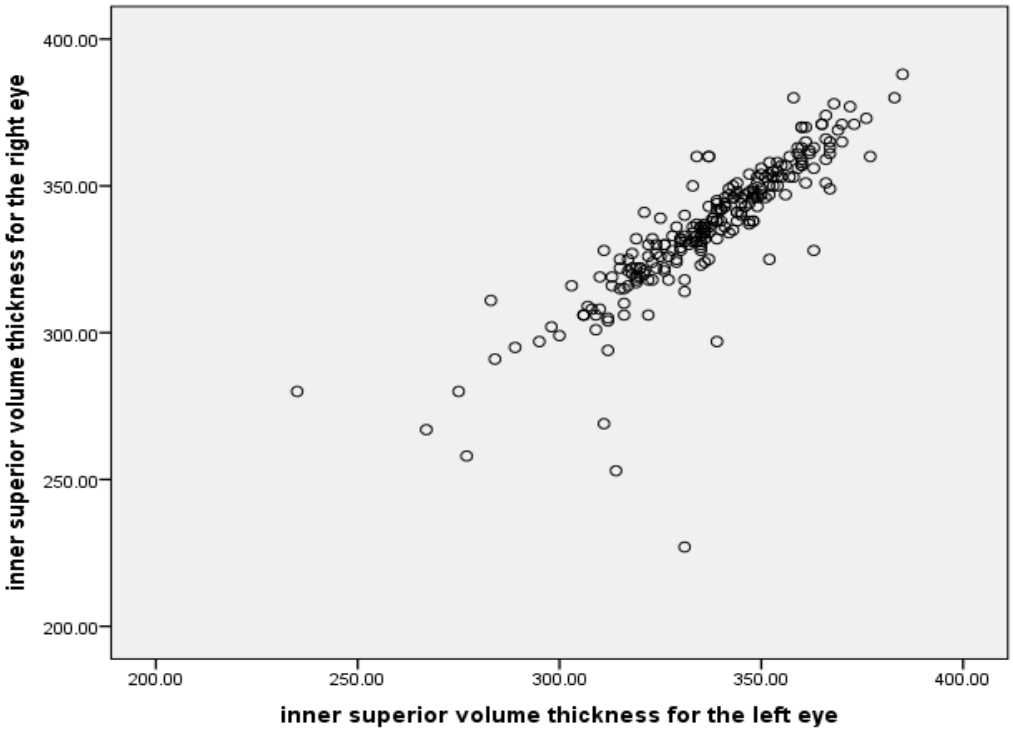

ring 2 amplitude for the right eye
25.00
20.00
15.00
10.00
5.00
.00
.00
5.00
10.00
15.00
20.00
25.00
30.00
ring 2 amplitude for the left eye

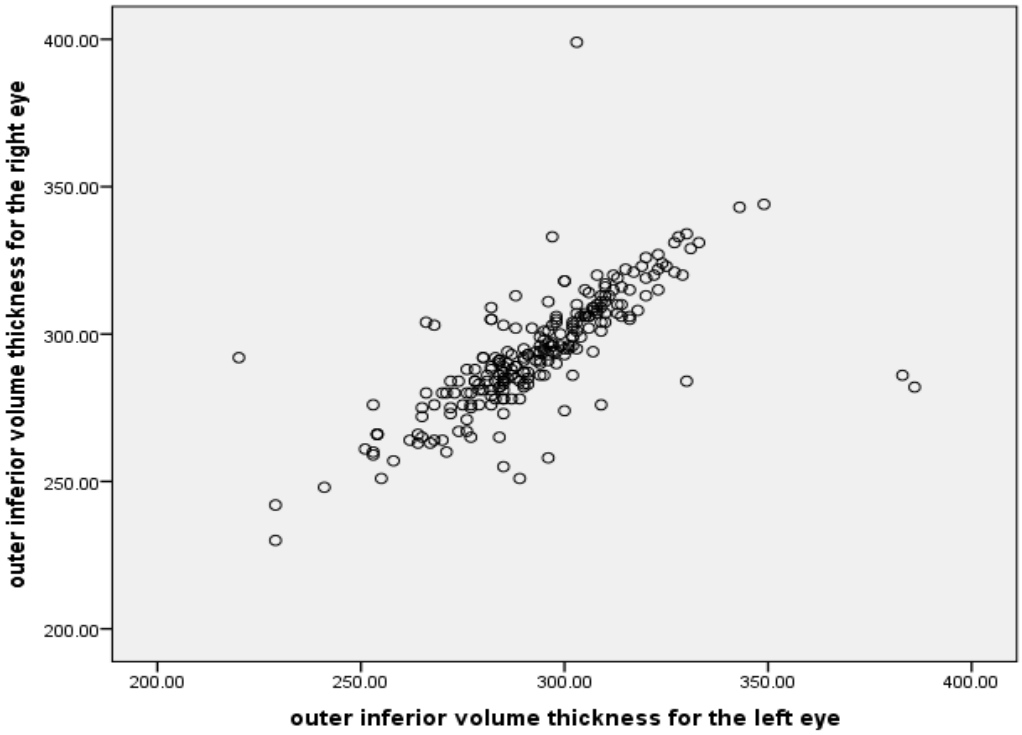

ring 3 amplitude for the right eye
20.00
15.00
10.00
5.00
.00
.00
5.00
10.00
15.00
20.00
ring 3 amplitude for the left eye

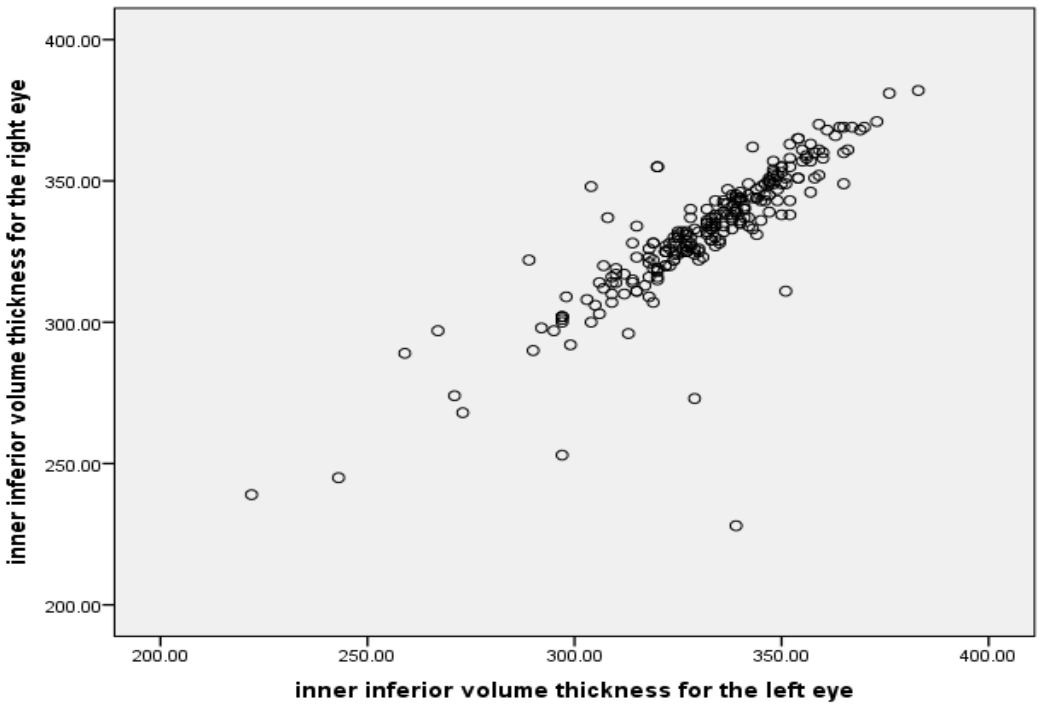

ring 1/2 ratio for the right eye
5.00
4.00
3.00
2.00
1.00
1.00
2.00
3.00
4.00
ring 1/2 ratio for the left eye

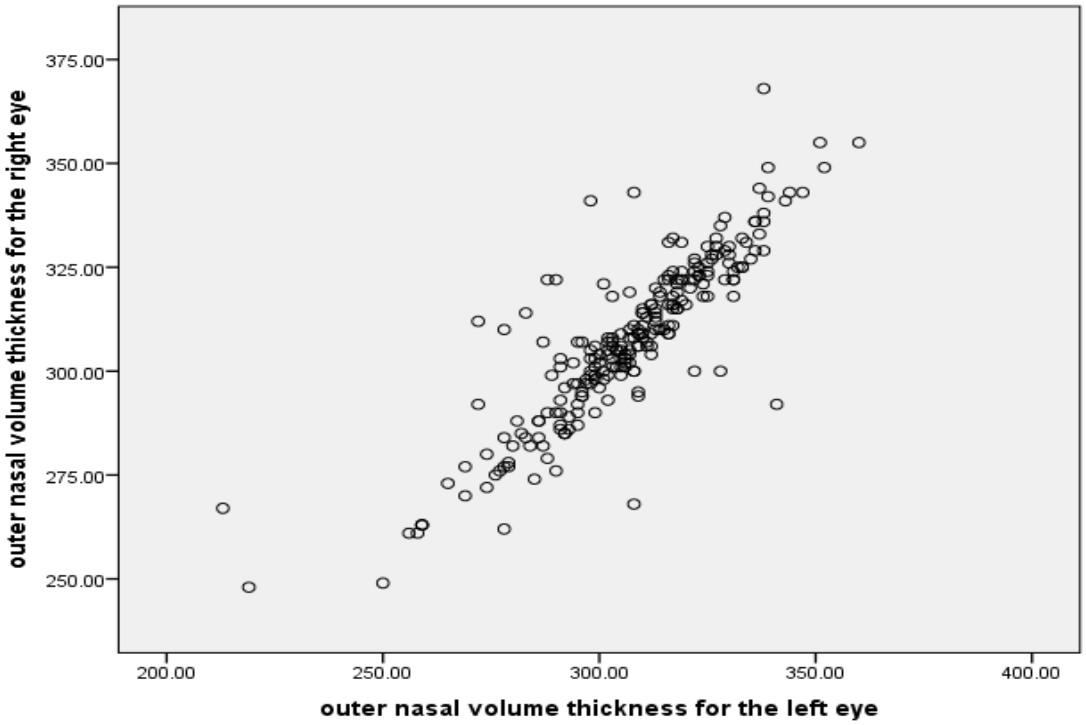

ring1/3 ratio for the right eye
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
ring 1/3 ratio for the left eye

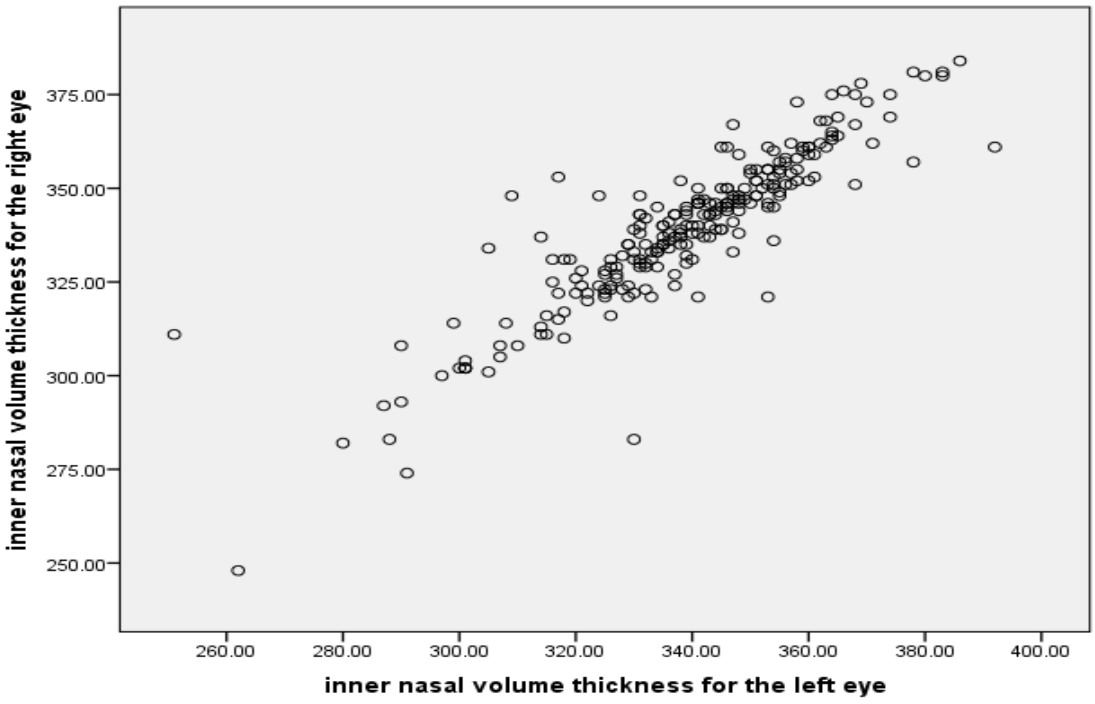

outer superior volume thickness for the right eye
350.00
325.00
300.00
275.00
250.00
225.00
150.00
200.00
250.00
300.00
350.00
outer superior volume thickness for the left eye

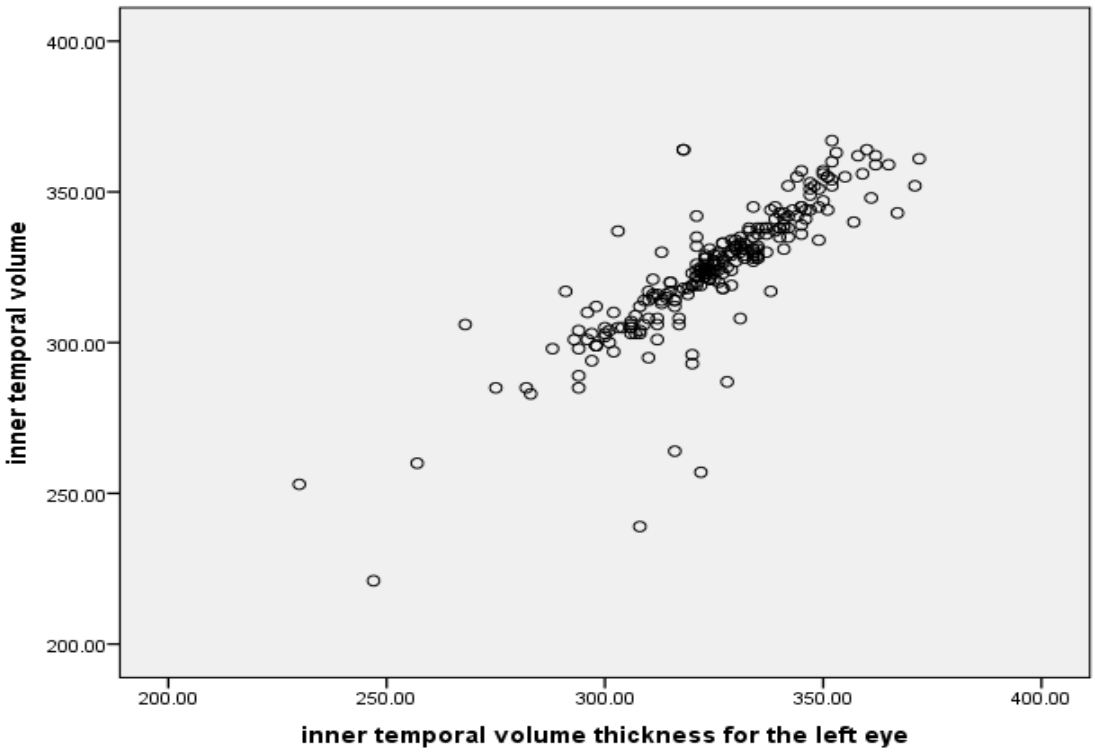

inner superior volume thickness for the right eye
400.00
350.00
300.00
250.00
200.00
200.00
250.00
300.00
350.00
400.00
inner superior volume thickness for the left eye

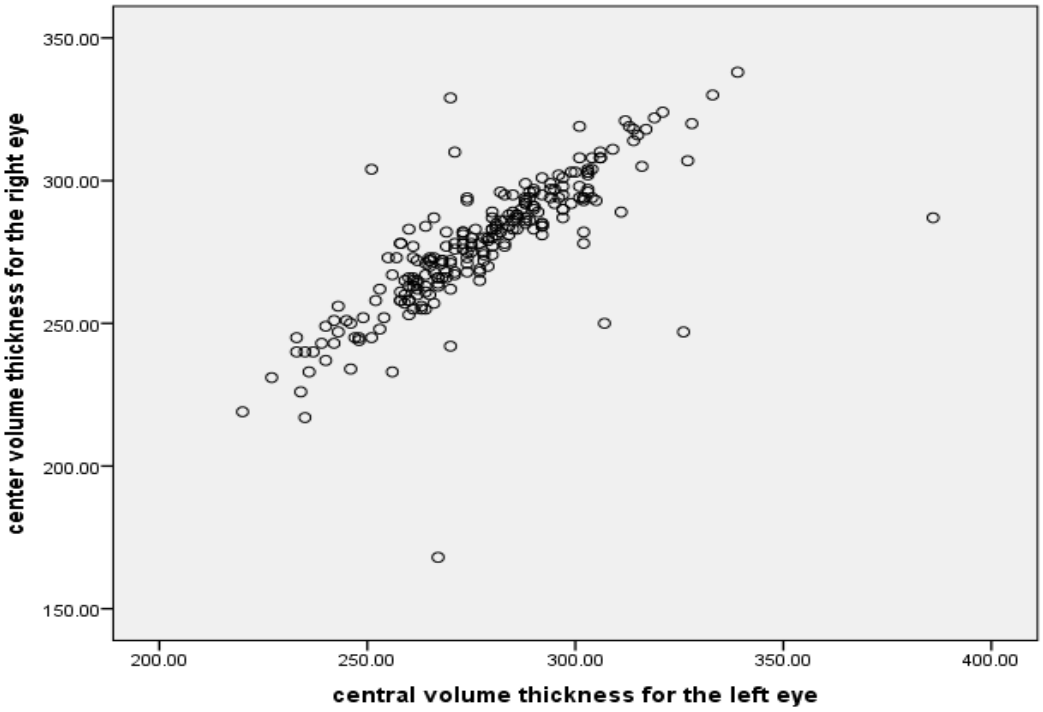

outer inferior volume thickness for the right eye
400.00
350.00
300.00
250.00
200.00
200.00
250.00
300.00
350.00
400.00
outer inferior volume thickness for the left eye

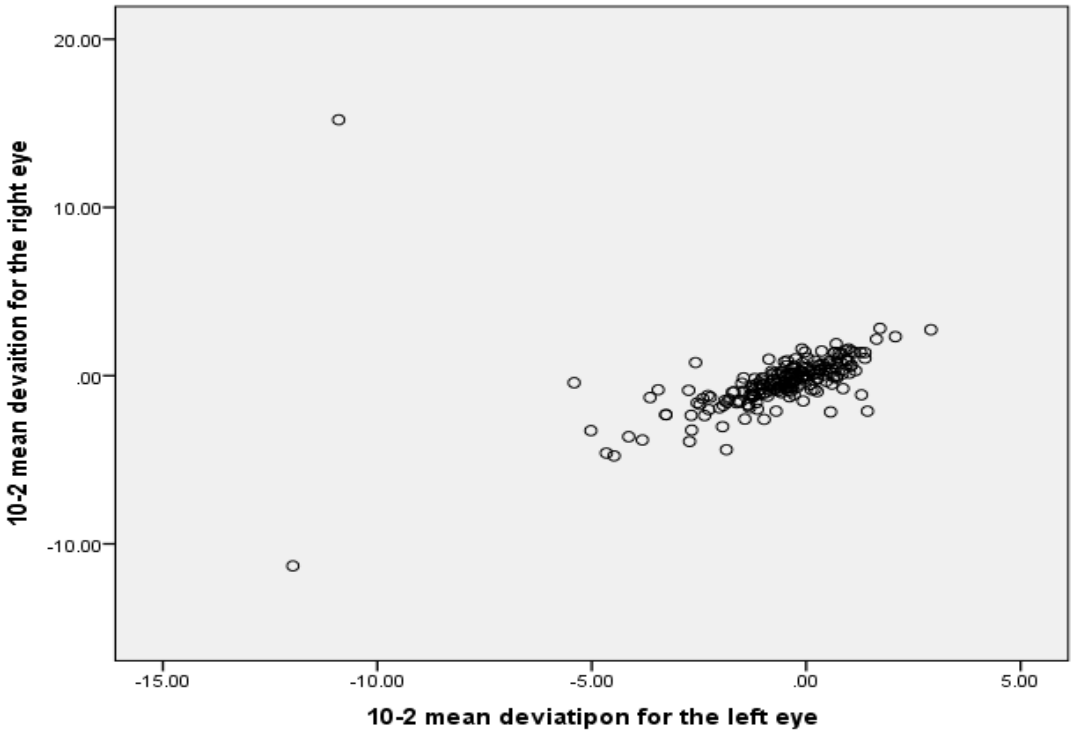

inner inferior volume thickness for the right eye
400.00
350.00
300.00
250.00
200.00
200.00
250.00
300.00
350.00
400.00
inner inferior volume thickness for the left eye

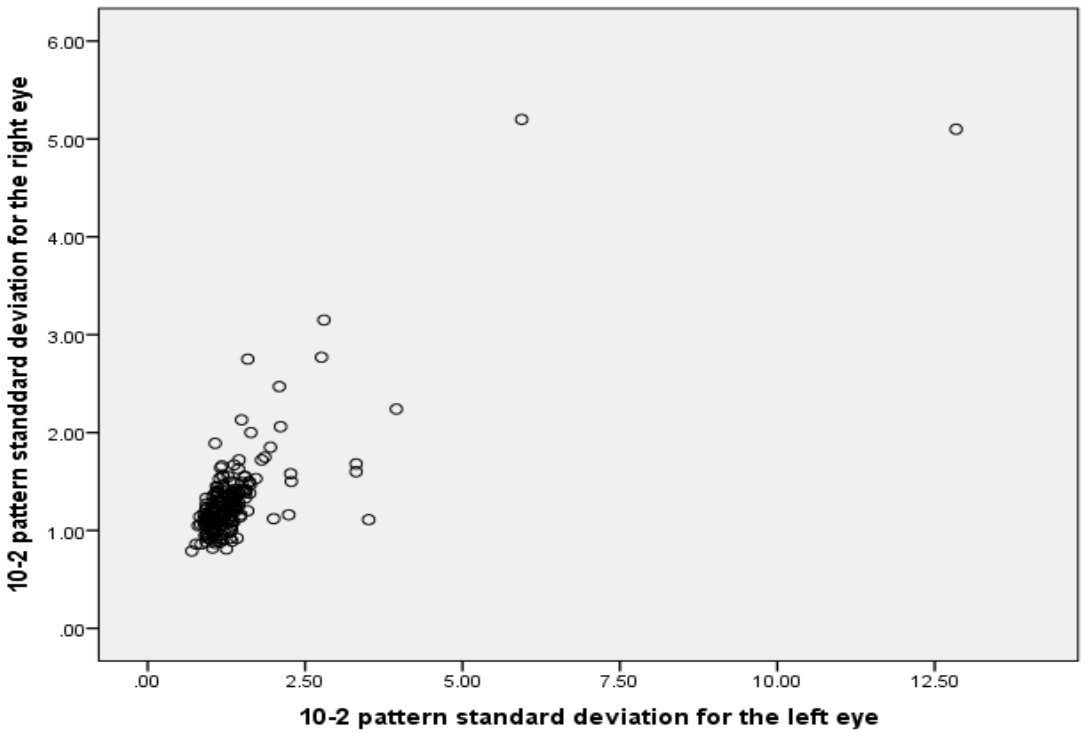

outer nasal volume thickness for the right eye
375.00
350.00
325.00
300.00
275.00
250.00
200.00
250.00
300.00
350.00
400.00
outer nasal volume thickness for the left eye


inner nasal volume thickness for the right eye
375.00
350.00
325.00
300.00
275.00
250.00
260.00
280.00
300.00
320.00
340.00
360.00
380.00
400.00
inner nasal volume thickness for the left eye


400.00
350.00
300.00
250.00
200.00
inner temporal volume
200.00
250.00
300.00
350.00
400.00
inner temporal volume thickness for the left eye


350.00
300.00
250.00
200.00
150.00
center volume thickness for the right eye
200.00
250.00
300.00
350.00
400.00
central volume thickness for the left eye


20.00
10.00
.00
-10.00
10-2 mean devaition for the right eye
-15.00
-10.00
-5.00
.00
5.00
10-2 mean deviatipon for the left eye


6.00
5.00
4.00
3.00
2.00
1.00
.00
10-2 pattern standdard deviation for the right eye
.00
2.50
5.00
7.50
10.00
12.50
10-2 pattern standard deviation for the left eye

Appendix 4 mfERG, SD-OCT and 10-2 VFT images of 14 patients who discontinued Plaquenil therapy

Patient	MfERG ring amplitude	T		DC1		DC2	
		OD	OS	OD	OS	OD	OS
P1	R1	10.9	13.7	15.5	17.1		
	R2	6.0	7.3	8.5	9.5		
	R3	3.7	4.1	5.0	5.9		
	1/2 ratio	1.82	1.88	1.82	1.80		
	1/3 ratio	2.95	3.34	3.10	2.90		
P2	R1	8.55	7.6	19.2	17.8		
	R2	5.15	2.3	11.4	10.7		
	R3	3.3	3.3	6.8	6.9		
	1/2 ratio	1.72	3.30	1.68	1.66		
	1/3 ratio	1.59	2.34	2.82	2.58		
P3	R1	10.2	13.9	20.9	20.0	16.3	27.1
	R2	4.8	4.8	12.2	11.6	11.5	15.9
	R3	3.1	3.5	7.3	7.3	8.0	9.4
	1/2 ratio	2.15	2.94	1.71	1.72	1.42	1.70
	1/3 ratio	3.34	4.04	2.86	2.74	2.04	2.88
P4	R1	14.2	16.8	20.2	17.7	23.0	21.6
	R2	5.0	6.6	11.3	9.8	12.9	11.6
	R3	2.7	2.7	6.0	5.4	6.9	6.8
	1/2 ratio	2.90	2.56	1.79	1.81	1.78	1.86
	1/3 ratio	5.26	6.20	3.37	3.28	3.33	3.18
P5	R1	17.7	16.4	30.4	33.1		
	R2	3.9	5.0	14.9	17.2		
	R3	4.0	4.4	10.7	12.0		
	1/2 ratio	4.54	3.30	2.04	1.92		
	1/3 ratio	4.48	3.72	2.84	2.76		
	R1	9.5	12.3	18.1	19.7		

	R2	5.2	5.7	11.5	11.5		
P6	R3	2.9	3.3	7.1	7.2		
	1/2 ratio	1.83	2.18	1.57	1.71		
	1/3 ratio	3.28	3.73	2.54	2.74		
	R1	9.0	10.8	14.8	13.7		
	R2	4.7	4.7	8.3	7.7		
P7	R3	2.8	2.7	5.2	5.1		
	1/2 ratio	1.9	2.3	1.78	1.78		
	1/3 ratio	3.20	4.00	2.85	2.69		
	R1	12.1	6.6	20.8	11.7	19.4	15.7
	R2	7.0	4.1	12.1	6.6	11.2	10.6
P8	R3	3.8	2.4	6.9	4.0	7.2	6.1
	1/2 ratio	1.73	1.61	1.70	1.77	1.73	1.48
	1/3 ratio	3.18	2.8	3.01	2.93	2.69	2.57
	R1	13.3	9.2	8.8	9.4	15.4	18.3
	R2	5.5	4.3	5.3	5.5	9.3	11.9
P9	R3	3.5	2.7	3.2	3.4	6.2	7.7
	1/2 ratio	2.444	2.15	1.66	1.7	1.66	1.54
	1/3 ratio	6.65	3.39	2.75	2.76	2.48	2.38
	R1	2.2	1.9	6.8	5.6	4.0	2.7
	R2	0.7	1.3	2.4	2.2	1.9	1.2
P10	R3	0.5	0.7	1.0	1.0	1.1	0.6
	1/2 ratio	3.14	1.46	2.83	2.55	2.11	2.25
	1/3 ratio	4.4	2.71	6.80	5.60	3.64	4.50
	R1	6.6	5.2	8.8	8.0	8.8	6.5
	R2	3.6	3.1	4.8	5.4	4.8	3.8
P11	R3	3.0	2.8	3.7	1.2	4.0	3.4
	1/2 ratio	1.83	1.48	1.83	1.48	1.83	1.71
	1/3 ratio	2.20	1.96	2.38	6.67	2.20	1.90
	R1	0.0	1.7	4.3	1.3	4.3	5.2
	R2	1.0	1.5	1.2	1.7	3.6	4.2

P12	R3	2.0	2.4	2.3	2.5	3.6	3.5
	1/2 ratio	0.00	1.13	3.58	0.76	1.19	1.24
	1/3 ratio	0.00	0.71	1.87	0.52	1.19	1.49
	R1	6.1	6.4	11.5	23.0	9.2	14.9
	R2	3.0	3.9	5.8	12.1	5.8	8.4
P13	R3	2.1	2.7	3.8	7.1	3.7	3.3
	1/2 ratio	2.03	1.64	1.98	1.90	1.60	1.77
	1/3 ratio	2.90	2.37	3.03	3.24	2.51	3.39
	R1	8.7	8.3	9.9	8.4	9.9	8.7
	R2	4.3	3.8	5.1	3.9	4.6	4.0
P14	R3	2.1	2.2	2.3	2.0	2.3	2.1
	1/2 ratio	2.02	2.18	1.94	2.15	1.15	2.18
	1/3 ratio	4.14	3.7	4.30	4.2	4.30	4.14

T= mfERG ring amplitudes and ratios at the point of toxicity

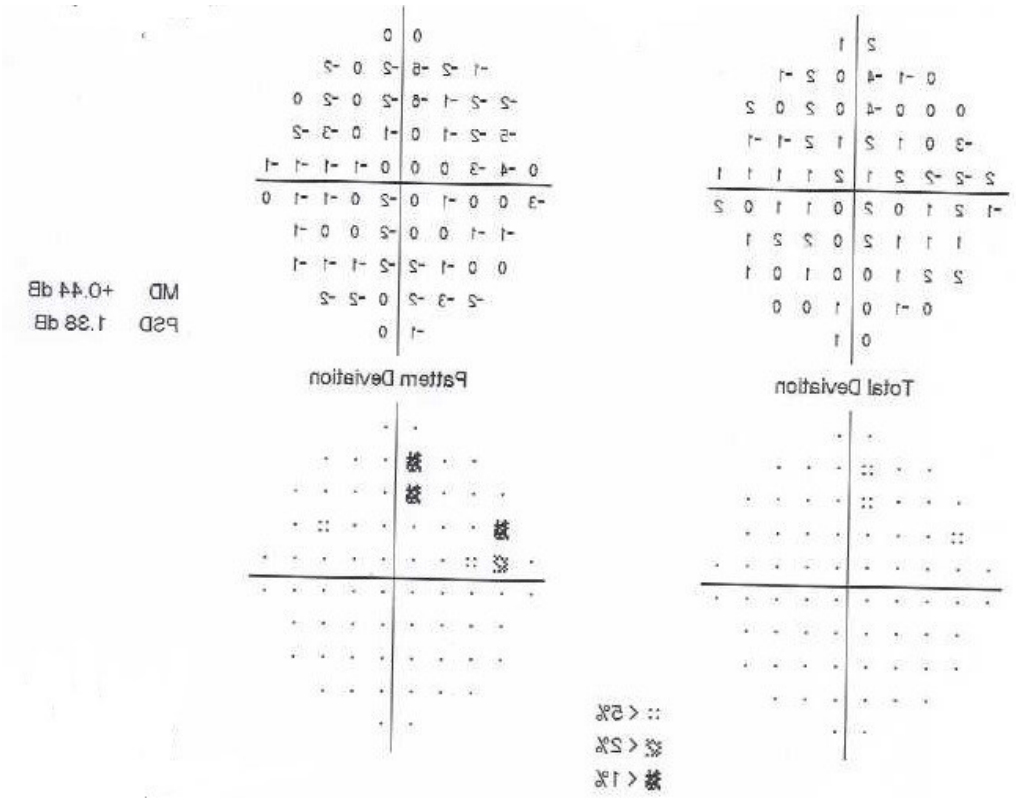
DC1= mfERG ring amplitudes and ratios at the 1st of examination after discontinuation of Plaquenil therapy (1-1.5 years after therapy cessation)

DC2= mfERG ring amplitudes at the 2nd time point of examination after Plaquenil therapy cessation (3.5-4 years)

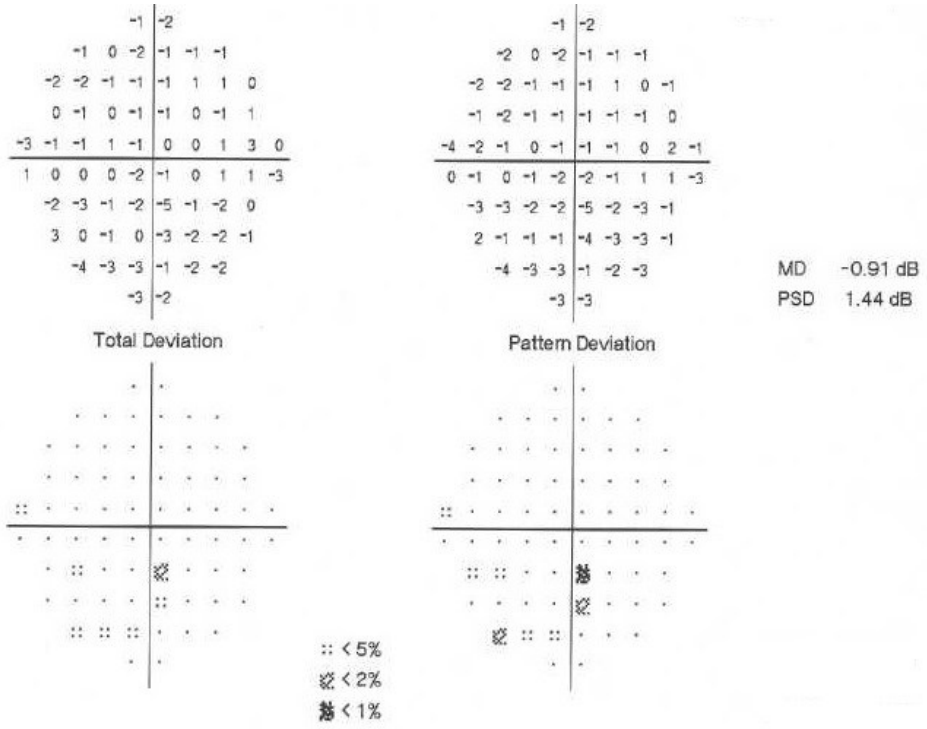
10-2 Humphrey VFT and SD-OCT for patients who discontinued therapy where data is available at the point of toxicity

P1

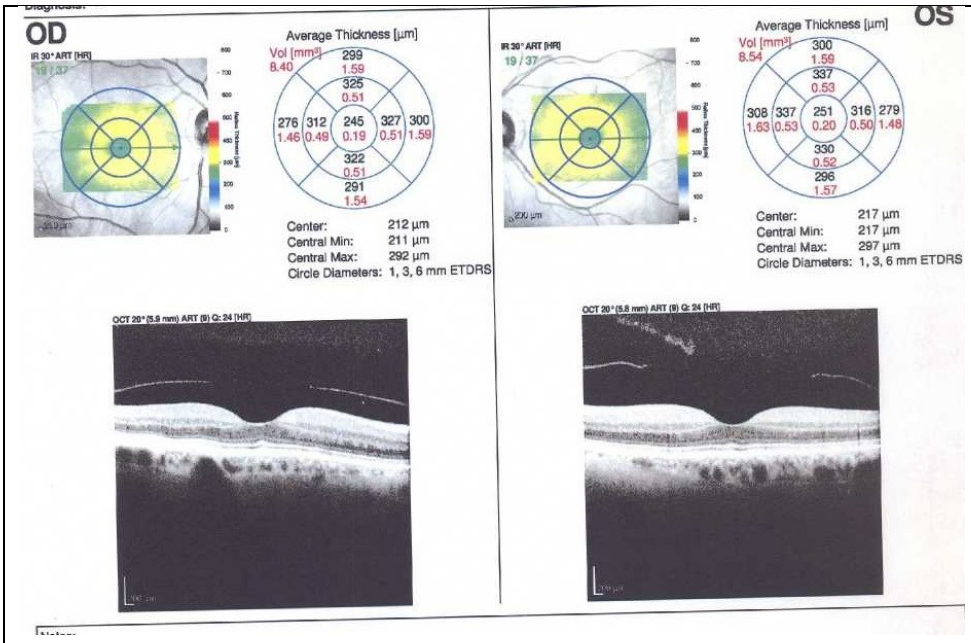
10-2 Humphrey VFT image



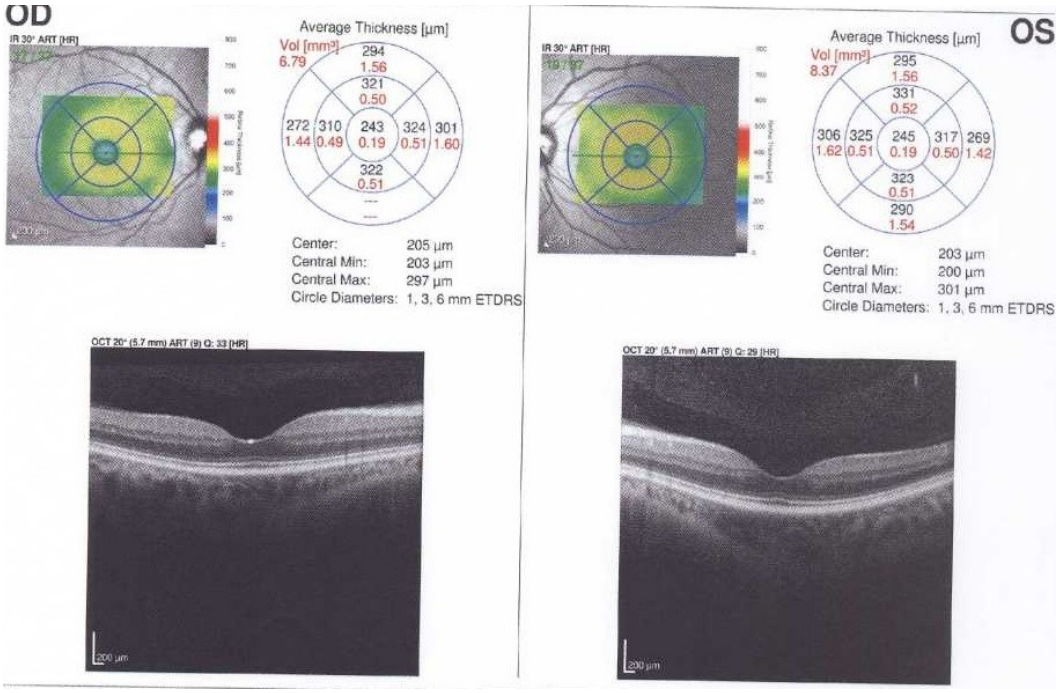
P3
10-2 Humphrey VFT image



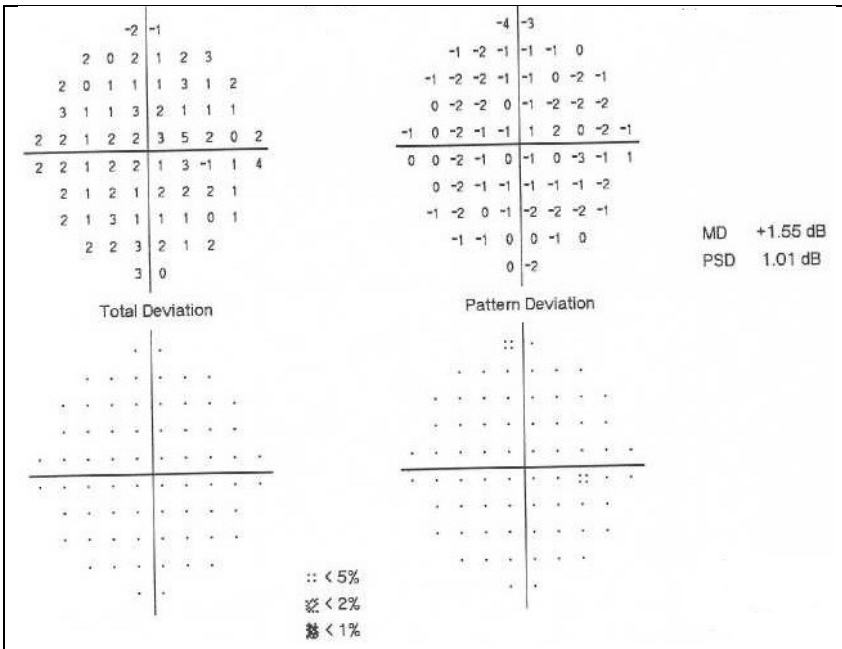
SD-OCT image



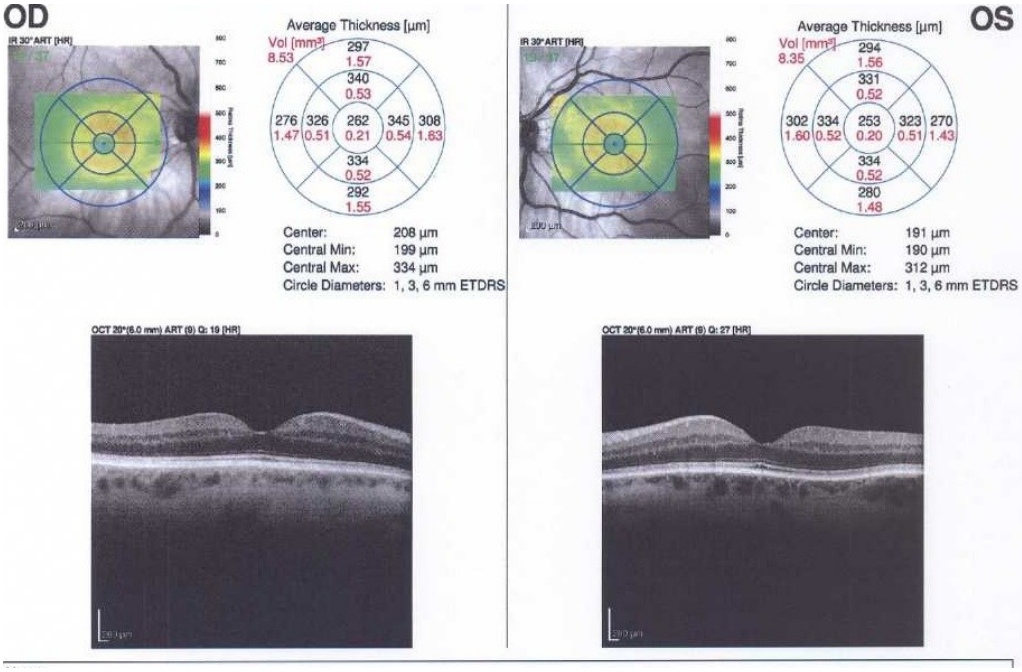
P4
SD-OCT image



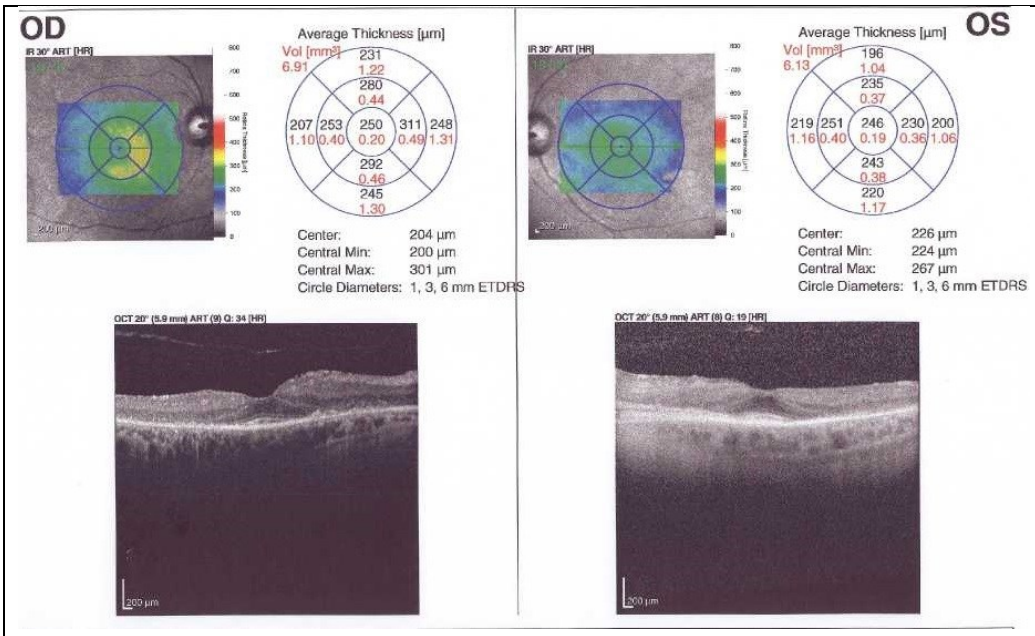
P8
10-2 VFT image



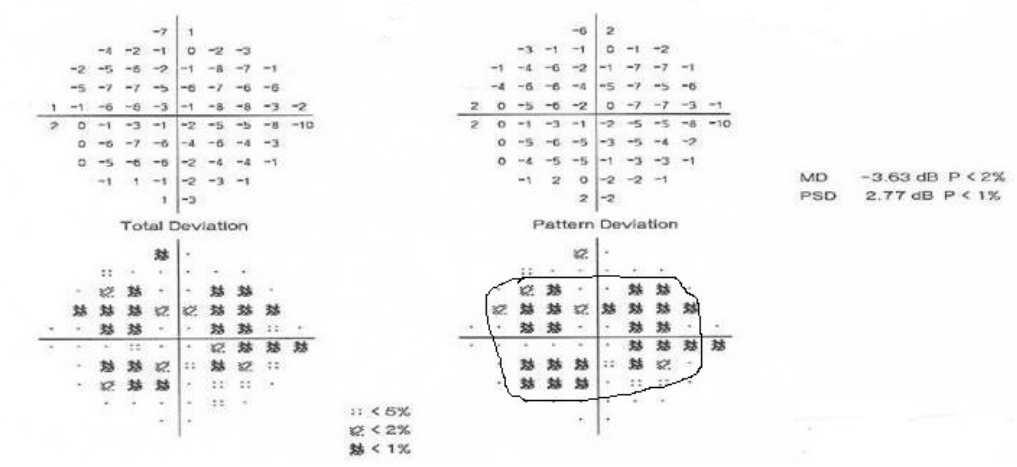
P9
SD-OCT image



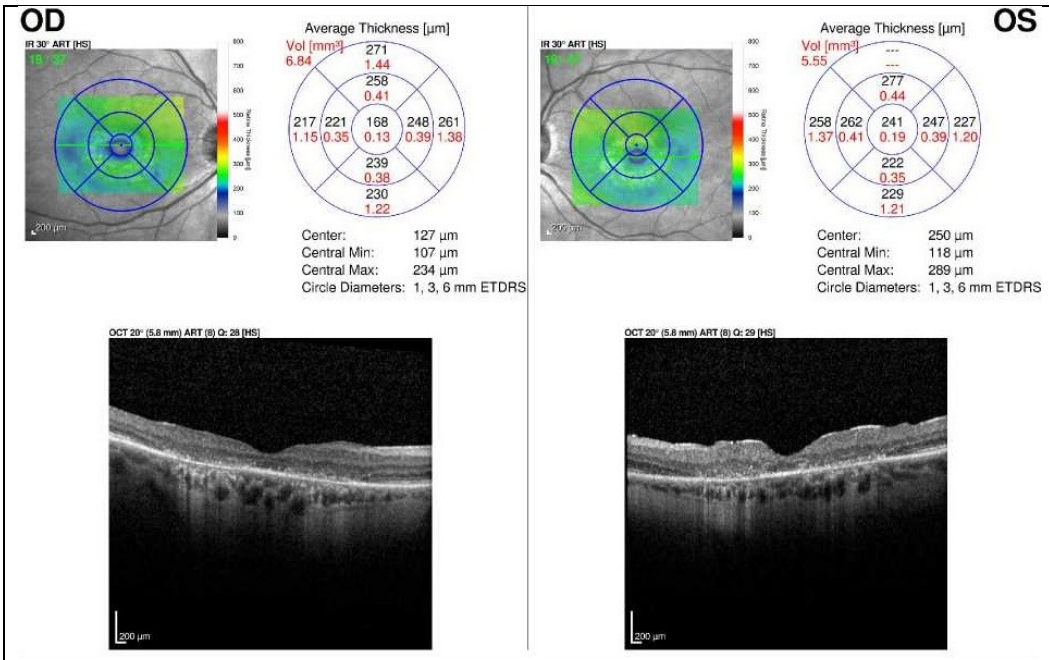
P10
SD-OCT image



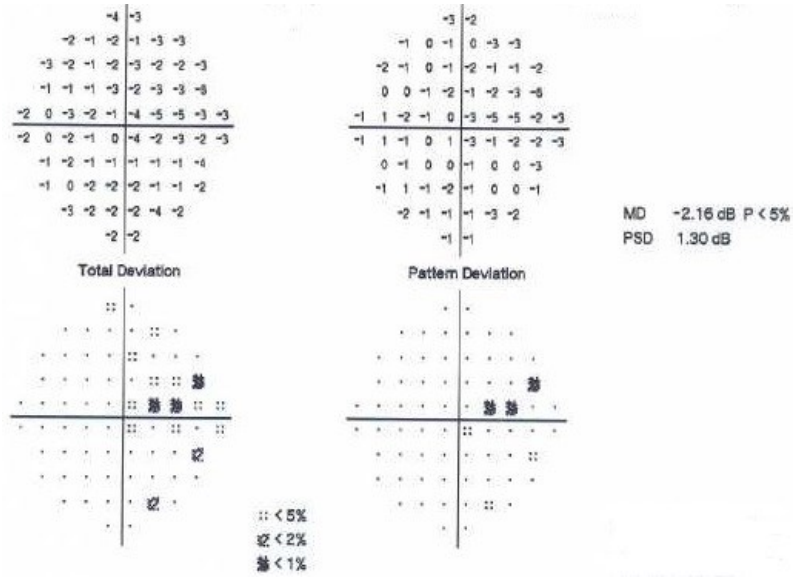
P12



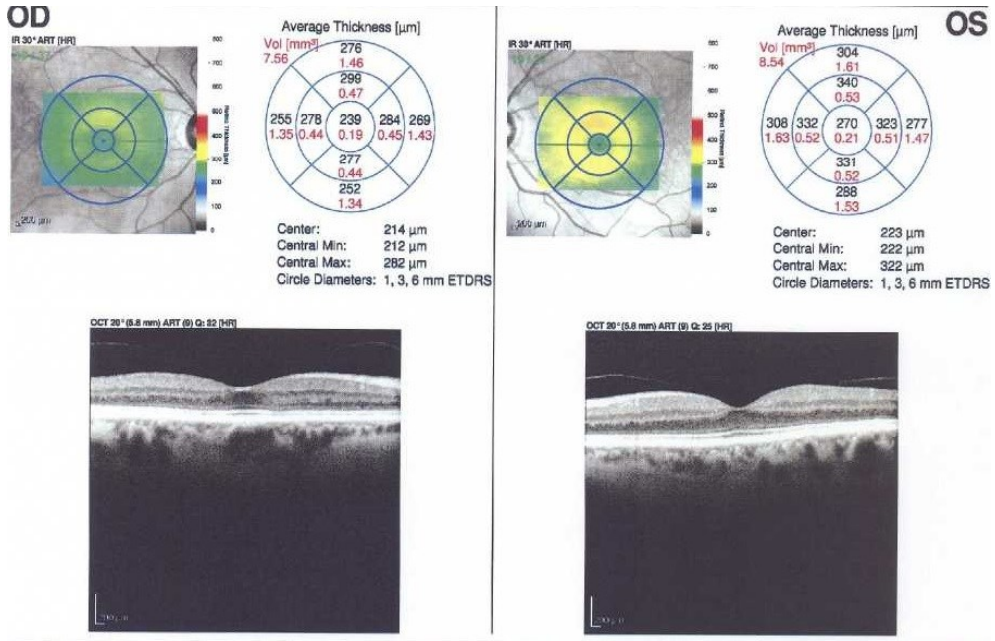
SD-OCT image



P13
10-2 Humphrey VFT image



SD-OCT image


OD
Average Thickness [µm]
Vol [mm³]
7.56
276
1.46
298
0.47
255
1.35
278
0.44
239
0.19
284
0.45
269
1.43
277
0.44
252
1.34
Center: 214 µm
Central Min: 212 µm
Central Max: 282 µm
Circle Diameters: 1, 3, 6 mm ETDRS
OS
Average Thickness [µm]
Vol [mm³]
8.54
304
1.61
340
0.53
308
1.63
332
0.52
270
0.21
323
0.51
277
1.47
331
0.52
288
1.53
Center: 223 µm
Central Min: 222 µm
Central Max: 322 µm
Circle Diameters: 1, 3, 6 mm ETDRS

Best corrected visual acuity (logMAR) changes after therapy discontinuation

Patient	BCVA at T		BCVA at DC1		BCVA at DC2	
	OD	OS	OD	OS	OD	OS
P1	0.00	0.00	0.00	0.00		
P2	0.30	0.60	0.10	0.10		
P3	0.10	0.00	0.00	0.10		
P4	0.00	0.00	0.00	0.00		
P5	0.00	0.10	0.00	0.00		
P6	0.18	0.10	0.10	0.00		
P7	0.10	0.00	0.30	0.30		
P8	0.00	0.30	0.10	0.00	0.10	0.30
P9	0.10	0.00	0.10	0.00		
P10	0.10	0.00	0.10	0.00	0.10	0.10
P11	0.00	0.00	0.00	0.00	0.00	0.00
P12	0.10	0.30	0.40	0.30	0.48	0.48
P13	0.18	0.10	0.10	0.00	0.18	0.00
P14	0.18	0.30	0.10	0.40	0.18	0.18

BCVA= Best corrected visual acuity (logMAR notation).

T= best corrected visual acuity at the point of toxicity

DC1= best corrected visual acuity at the 1st of examination after discontinuation of Plaquenil therapy (1- 1.5 years after therapy cessation)

DC2= best corrected visual acuity at the 2nd time point of examination after Plaquenil therapy cessation (3.5-4 years).