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Evaluation of Hydroxychloroquine Effect on the Retinal Layers by Spectral-Domain Optical Coherence Tomography

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Abstract

Objective: Hydroxychloroquine is a commonly used immunosuppressive agent in the treatment of various autoimmune diseases, despite lesser systemic toxicity compared to other drugs; it can cause severe retinal dysfunction and loss of vision. The aim of this study is to determine the early OCT findings in preclinical Hydroxychloroquine (HCQ) retinopathy. Patients and methods: This cross sectional comparative study held place in Ophthalmology and Rheumatology Clinics, Benha university Hospitals; from March 2019 to April 2021, twenty eyes of rheumatic patients on HCQ for more than two years and other twenty eyes of normal gender matched individuals, all underwent full ophthalmological examination and imaging with Spectral Domain Optical Coherence Tomography (SD-OCT). The entire study group had normal fundus appearance. Results: Outer retinal thinning in the form of central foveal and para-foveal thinning was detected by SD-OCT in patients on chronic HCQ for more than 2 year use with still normal fundus. IS/OS junction was interrupted in only 2 rheumatic patients (10%). Color vision was affected only in three rheumatic patients (15%). Conclusion: Screening of rheumatic patients by SD-OCT can detect preclinical HCQ retinopathy and this could be used as a screening technique especially with the increased availability of SD-OCT machines in most eye centers.

Key words: Retinopathy, hydroxychloroquine, Spectral domain OCT.

1.Introduction

Hydroxychloroquine is a commonly used immunosuppressive agent in the treatment of various autoimmune diseases, despite lesser systemic toxicity compared to other drugs; it can cause severe retinal dysfunction and loss of vision**.** Retinal toxicity can occur as a side effect of long-term hydroxychloroquine therapy, the most important risk factors are high dose and long duration of use, dosage > 5.0 mg/kg dramatically increases both population risk and annual incremental risk, and extreme doses can be exceedingly dangerous, other major factors are concomitant renal disease or use of tamoxifen [1]**.**

The major pathogenic effect of Hydroxychloroquine is induction of lysosomal dysfunction in photoreceptors and RPE cells which leads to the accumulation of lipofuscin in RPE, melanin binding increases the concentration of the drug in retina and further contributes or prolongs the toxic effects. The macular localization of the disease suggests that light absorption or cone metabolism may play a role [2].

Discontinuing the drug in the early stages can prevent permanent damage; therefore, the screening of patients for the early detection of asymptomatic retinal structural changes is important [3]**.**

In 2016, the American Academy of Ophthalmology published recommendations on screening for chloroquine and hydroxychloroquine retinopathy, the primary screening tests are automated visual fields plus spectral-domain optical coherence tomography (SD-OCT), the multifocal electroretinogram (mfERG) can provide objective corroboration for visual fields, and fundus autofluorescence (FAF) can show damage topographically [1]**.**

Modern screening should detect retinopathy before it is visible in the fundus, of these tests, SD-OCT is the most commonly used device for the detection of retinal structural changes. Therefore, in this study we will investigate the early signs of retinal toxicity, we aim to detect early abnormalities of the photoreceptor inner segment (IS), outer segment (OS), and RPE-Bruch's membrane complex thicknesses using SD-OCT image segmentation algorithms [1]**.**

SD-OCT is a highly sensitive and reproducible imaging modality commonly used in clinical practice; it's capable of detecting characteristic macular changes of HCQ toxicity, these typical macular abnormalities include loss of the parafoveal ellipsoid zone (EZ), parafoveal thinning of the outer nuclear layer (ONL) and inner plexiform layer (IPL), the "flying saucer" sign, and peripapillary nerve fiber layer thinning [4]**.**

SD-OCT testing visualizes the retinal layers in as much detail as microscopic examination and in HCQ retinopathy a loss of the photoreceptor inner-outer segment junction (the IS/OS line, also called the ellipsoid zone or the photoreceptor integrity line (PIL) and a thinning of the outer nuclear layer of the retina are observed [5]**.**

This study aimed to evaluate the effect of hydroxychloroquine on Macular Retinal Layers using spectral-domain optical coherence tomography (SD-OCT)

2.Patients and methods

This prospective cohort study was done at Banha university hospitals from March 2019 to April 2021.

The protocol was approved by the scientific committee of ophthalmology department and the Ethical Committee: Faculty of Medicine, Banha University. Informed consents were obtained from all participants. Neither the researcher nor the supervisors are financially interested in commercial products mentioned in the thesis.

The study was performed on 40 eyes of 40 subjects recruited from Ophthalmology and Rheumatology Outpatient clinics Banha university hospitals.

2.1Subjects were divided into two groups

- **Hydroxyhloroquine group:** 20 eyes of 20 patients treated with hydroxychloroquine for more than 2 years with normal fundus examination.
- **Control group:** 20 eyes of 20 normal subjects with completely normal ophthalmological examination of age and sex matched participants.

2.2Inclusion criteria

- Patients on Hydroxychloroquine for rheumatic diseases for more than 2 years.
- Good candidates for OCT imaging (no media opacity)
- No posterior segment pathology
- Non significant refractive error.
- No chronic uveitis.
- No history of glaucoma, retinopathy, retinal therapy, or other intraocular surgical intervention
- Not on other medication affecting the Retina.

2.3Exclusion criteria

- People who refuse to give consent.
- Any posterior segment pathology.
- Significant refractive error.
- chronic uveitis.
- History of glaucoma, retinopathy, retinal therapy, or other intraocular surgical intervention

2.4All patients underwent examination through: History taking, and complete ophthalmologic examination was done, including:

- Pupillary reaction.
- Best corrected visual acuity (BCVA) using snellen's chart testing.
- Anterior segment assessment by slit lamp examination.
- Intraocular pressure measurement with an applanation tonometer.
- Fundus examination using +20 D lens (to evaluate the periphery of the retina) and +90 D lens (biomicroscopy for evaluating the posterior pole).
- Spectral-Domain Optical Coherence Tomography (Structural Assessment): Pupils dilated for OCT examination in all subjects with 1% cyclopentolate. OCT was done using 3D OCT 2OOO FA was used (TOPCON CORPORATION, Tokyo, Japan), 3D macula mode and 5 lines cross scan mode will be used to obtain high quality images.

2.5Statistical Analysis

Data were collected, coded, revised and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The data were presented as number and percentages for the qualitative data, mean, standard deviations and ranges for the quantitative data with parametric distribution and median with inter quartile range (IQR) for the quantitative data with non-parametric distribution. Chi-square test was used in the comparison between two groups with qualitative data and Fisher exact test was used instead of the Chi-square test when the expected count in any cell found less than 5. Independent t-test was used in the comparison between two groups with quantitative data and parametric distribution and Mann-Whitney test was used in the comparison between two groups with quantitative data and non-parametric distribution. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05: Non significant (NS), P < 0.05: Significant (S), P < 0.01: Highly significant (HS).

3.Results

In this study, 19 patients (95%) were females and only 1 patient was male, mean of age was 43.55 among HCQ group, So there was no statistically significant difference between studied groups among demographic data, table 1

Table 1: Demographic data among studied groups

		HCQ group (No.=20)		Control group (No.=20)		Chi square test/ Independent t test	
		No	%	No	%	x^2/t*	p value
Sex	Female	19	95.0%	19	95.0%	0.000	1.000
	Male	1	5.0%	1	5.0%		
Age	Mean ± SD	43.55	15.42	41.30	13.43	0.492*	0.626

Table 2 shows that slit lamp exam of all patients was Free, color vision of 3 patients (15%) was desaturated, of 17 patients (85%) was normal among HCQ group, IS\OS of 2 patients (10%) was interrupted, of 18 patients (90%) was Intact among HCQ group, so there was no statistically significant

deference between slit lamp exam, Color vision and IS/OS among control group in comparison to HCQ group

Table 2: slit lamp examination, Color vision and IS/OS among studied groups

		HCQ group (No.=20)		Control group (No.=20)		Chi square test	
		No	%	No	%	x^2	p value
SLE (slit lamp exam)	NAD	20	100.0%	20	100.0%	NA	NA
Color vision	Desaturation	3	15.0%	0	0.0%	3.243	0.198
	Good	17	85.0%	20	100.0%		
IS/OS	interrupted	2	10.0%	0	0.0%	2.105	0.146
	Intact	18	90.0%	20	100.0%		

Fig1 shows that mean of central foveal thickness was 199.50 **µm** among HCQ group. So there was statistically significant decrease CFT (central foveal thickness) in HCQ group in comparison to control group

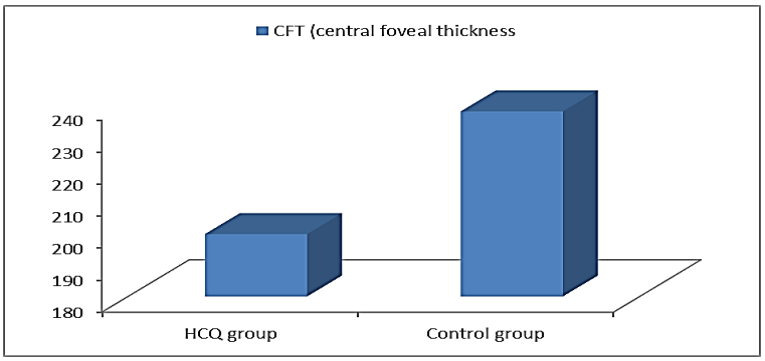

Fig 1: CFT among studied groups

Table 3 shows that mean of Superior parafoveal was 289.75 µm, mean of Inferior parafoveal was 280.35 µm, of Nasal parafoveal was 276.25 µm, of Temporal parafoveal was 269.20 µm among HCQ group. So there was statistically significant decrease Superior parafoveal, Inferior parafoveal, Nasal parafoveal and Temporal parafoveal in HCQ group in comparison to control group.

Table (3): Parafoveal thickness among studied groups

	HCQ group (No.=20)		Control group (No.=20)		Independent t test	
	Mean	SD	Mean	SD	T	p value
Superior parafoveal	289.75	15.81	301.75	12.06	2.699-	0.010
Inferior parafoveal	280.35	18.59	302.20	25.38	3.106-	0.004
Nasal parafoveal	276.25	30.44	302.00	10.73	3.569-	0.001
Temporal parafoveal	269.20	20.60	288.00	10.43	3.641-	0.001

Table 4 shows that mean of Superior perifoveal was 257.50 **µm**, mean of Inferior perifoveal was 260.95 **µm**, of Nasal perifoveal was 269.90 **µm**, of Temporal perifoveal was 245.35 **µm** among HCQ group.So there was no statistically significant difference between HCQ group and control group among Perifoveal region thickness.

Table 4 : Perifoveal thickness among studied groups

	HCQ group (No.=20)		Control group (No.=20)		Independent t test	
	Mean	SD	Mean	SD	T	p value
Superior perifoveal	257.50	11.01	259.15	10.71	0.480-	0.634
Inferior perifoveal	260.95	16.56	263.55	12.39	0.0562-	0.577
Nasal perifoveal	269.90	13.12	277.60	11.65	1.962-	0.057
Temporal perifoveal	245.35	15.72	247.55	12.76	0.486-	0.630

CFT showed negative correlation with cumulative dose of HCQ (r= -0.737), table 5

Table 5: Correlation between central foveal thickness and Cumulative dose in grams :

	Cumulative dose (grams	
	R	p value
CFT (central foveal thickness	-0.737	0.001

Table 6 shows that Cumulative dose (grams) has negative correlation with parafoveal quadrants thickness (r= 0.001) in HCQ group

Table (6): Correlation between parafoveal thickness and Cumulative dose in HCQ group :

	Cumulative dose (grams	
	R	**p value**
Superior parafoveal	-0.645	0.001
Inferior parafoveal	-0.691	0.001
Nasal parafoveal	-0.746	0.001
Temporal parafoveal	-0.668	0.001

Table 7 shows that treatment duration has negative correlation with CFT (r=0.003), superior parafoveal (r=0.020) and temporal parafoveal (r= 0.013) in HCQ group

Table 72: Correlation between CFT and parafoveal thickness with treatment duration in HCQ group.

	Treatment duration	
	R	P value
central foveal thickness	-0.631	0.003
superior parafoveal	-0.516	0.020
inferior parafoveal	-0.379	0.099
nasal parafoveal	-0.324	0.163
temporal parafoveal	-0.547	0.013

4.Discussion

Hydroxychloroquine (HCQ) is an antimalarial drug which is widely used nowadays in treatment of rheumatic diseases such as rheumatoid arthritis and systemic lupus erythematosus, its most common complication is retinopathy and cautious screening has been much easier after introduction of SD-OCT [6].

The exact mechanism of retinal toxicity is unknown; in our study, OCT scans showed early affection to outer retinal structures with high significant difference ($p < 0.001$ each) as photoreceptors where maximal affection was in the foveal and parafoveal regions

Chen study agrees with us which implicated damage to outer retinal structures such as photoreceptors and RPE cells [4]**.**

While Bulut study suggest retinal toxicity is due to damage to retinal ganglion cells (GCL), the inner plexiform layer (IPL), or the Retinal nerve fiber layer (RNFL) [7]**.**

At present, not all patients examined who presented with SD-OCT abnormalities had visual problems. It is interesting that even in the patient with the mildest SD-OCT changes; the ONL thickness was reduced in all scans of the 3D series. These changes were detectable in areas where ophthalmoscopy, Fundus Autoflurescence appeared normal, and mfERG responses were normal as well. So, prospective evaluation of ONL thickness during HCQ treatment might provide even earlier suspicion of the onset of retinal degeneration. [3].

Kellner study agreed with us which found alterations of the photoreceptor layers in patients with HCQ retinopathy detected with SD-OCT in the form of loss of photoreceptor IS indicated by the reduced ONL thickness and the interruption or absence of the photoreceptor IS/OS junction. These structural retinal alterations correspond to photoreceptor dysfunction demonstrated with the mfERG and an increase in the lipofuscin concentration and a decrease in the melanin concentration in the RPE [8]**.**

Statistical difference as regards CFT (1mm from the foveola) and parafoveal thickness (3mm from the foveola) was clearly detected with average thickness thickness 199.50 µm ± 29.22 and P value 0.001 for CFT. And for parafoveal quadrants, P value for superior quadrant was 0.010 with average thickness 289.75 µm ±15.81, p value for inferior quadrant was 0.004 with average thickness 280.35µm ± 18.59, p value for temporal quadrant was 0.001 with average thickness 269.20µm ± 20.60 and p value for Nasal quadrant was 0.001 with average thickness 276.25µm ± 30.44.

Jonathan study agreed with us which stated that SD-OCT can identify cases of early retinal HCQ toxicity by measuring retinal thickness. And that the retinal thinning and structural abnormalities that occur in cases of early toxicity can be more obviously identified if measured at a specific landmark: 1.0 mm from the foveola. Retinal thinning also appears to precede both the loss of IS/OS layer and RPE dysfunction [9]**.**

Moreover, the thinning in the parafoveal region caused by HCQ observed in our study matched what **Korah** and **Thomas** found. They noticed that parafoveal retinal thickness and volume measurements may be evidence of retinal toxicity, and OCT readings as a part of screening of HCQ retinal toxicity may be useful in early detection of HCQ maculopathy [10].

In our study three patients developed central foveal thinning only, five patients showed combined central foveal and parafoveal thinning where the 4 parafoveal quadrants are almost similarly affected with the temporal quadrant showing most frequent

affection. while the perifoveal quadrants although were not widely affected, the inferior quadrant showed maximal affection. This needs further future research with larger scale of population.

However, histology has shown that peri-foveal photoreceptor cells are the most to be affected and that it may be a secondary effect due to RPE metabolism disruption [11]**.**

HCQ is 4-aminoquinolones which is known to disrupt lysosomal function of the RPE with with ensuing degeneration of photoreceptors leading to increased lipofuscin accumulation and so, alteration in RPE metabolism may play a role in retinal toxicity [12].

Sisternes study agreed with us which described the SD–OCT findings as localized thinning of the retinal layers in the parafoveal region and confirmed early toxicity by loss of the IS/OS segment line [13]**.**

5.Conclusion

We assessed the examination results of rheumatic patients taking hydroxychloroquine for more than 2 years having normal fundus appearance as regards spectral domain optical coherence tomography compared to gender matched control group. Hydroxychloroquine group patients had statistically significant thinning of the central foveal and para-foveal regions compared to the control group. So, SD-OCT can be used by ophthalmologists as a screening tool of patients taking hydroxychloroquine using 3D macula mode and 5 lines cross scan mode 1 and 3mm and 5 mm from the foveola. In addition to its rapidity, specificity and availability in most eye centers, SD-OCT is an objective method of assessment that gives accurate measurements of the retinal thickness and layers. Based on our study results we recommend using SD-OCT as a screening tool before the start and during follow up of patients taking HCQ as a treatment for Rheumatic diseases.

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