ORIGINAL RESEARCH

Safety and Efficacy of Oral Hydroxychloroquine in the Treatment of Ophthalmic Disease Associated with Sjögren's Syndrome

Wang Fang, MS; Zhou Qingqing, BSN; Luo Qihui, BSN; Zhong Bing, MS; Huang Xinyue, MD; Xiong Jie, MS

ABSTRACT

Background • Dry eye disease is common among patients with primary Sjögren's syndrome (pSS). Hydroxychloroquine (HCQ), known for its immunomodulatory effects and minimal adverse effects, has emerged as a pivotal treatment option for pSS. Nonetheless, conflicting evidence exists regarding the therapeutic efficacy of HCQ in managing dry eye disease associated with pSS.

Objectives • To evaluate the safety and efficacy of oral hydroxychloroquine in treating dry eye disease associated with pSS.

Methods • A prospective randomized controlled study was conducted, enrolling pSS patients with moderate to severe dry eye disease. Participants were randomly assigned to an oral HCQ group and an observation group. Various scales (ESSDAI, ESSPRI, OSDI, and SPEED questionnaire score), dry eye-related tests (OSS score, TBUT, and Schirmer test I), ophthalmology-specific tests (BCVA, SD-OCT RT, field of view, latency and amplitudes for multifocal ERG ring 1 and ring 2), whole body protein levels (serum IgA, IgG, and IgM), and blood glucose were assessed before and after 12 months of treatment.

Results • Pairwise comparison of the observed indicator baseline revealed no statistical significance ($P > .05$). After 12 months, the HCQ group exhibited notable improvements in ESSPRI, serum IgA, and Schirmer test I results compared to the control group ($P < .05$). Both groups demonstrated significant improvements in BCVA, OSDI, SPEED scores, and dry eye-associated examinations compared to baseline ($P < .05$). Serum IgG and IgM levels decreased in the HCQ group after 12 months of treatment, but without statistical significance ($P > .05$). None cases of HCQ retinopathy were reported during follow-up.

Conclusions • Oral HCQ was demonstrated safety and efficacy in managing pSS-related dry eye disease. Treatment with Oral HCQ markedly reduced the ESSPRI score, improve patients' systemic dryness symptoms, and greatly decreased blood IgA levels. Combined with topical cyclosporin, HCQ improved Schirmer test I scores and alleviated ocular surface inflammation and dry eye signs and symptoms. (*Altern Ther Health Med.* 2023;29(8):656-662).

Wang Fang, MS; **Zhou Qingqing**, BSN; **Luo Qihui**, BSN; **Huang Xinyue**, MD; **Xiong Jie**, MS, Department of Ophthalmology, The First Hospital Affiliated of the Army Medical University, Chongqing, China. **Zhong Bing**, MS, Department of Rheumatology and Immunology, The First Hospital Affiliated of the Army Medical University, Chongqing, China.

Corresponding author: Xiong Jie, MS
E-mail: xiongjie0419@outlook.com

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a slowly progressed systemic disease associated with the autoimmune system, and it mainly affects exocrine glands such as the lacrimal and salivary glands, leading to severe dryness in the ocular and oral mucosal surface.[1] The estimated global incidence of this disorder is between 0.061% and 0.4%.[2,3] Nearly 95% of pSS patients have reported dry eye and dry mouth symptoms, which can seriously affect the health and quality of life of pSS patients, posing a heavy burden on families and society.[4,5] Treatment regimens for pSS in recent decades have established dryness alleviation, prevention of complications, and extensive immunosuppression.[6] Dry eye disease is the most common eye disease in pSS patients, and its recommended treatment mostly includes long-term topical use of artificial tears and anti-inflammatory drugs. Muscarinic agonists can be used to treat pSS-related dry eye disease, but they only improve subjective dryness with many side effects.[7] Hydroxychloroquine (HCQ) plays an important role in the treatment of pSS due to its unique immune-modulating effect and fewer side effects.[8] HCQ is confirmed to greatly improve the systemic immunological indicators of pSS patients.[9] Due to the small sample size of HCQ in the treatment of pSS-related dry eye disease, limited observation

indicators, and different inclusion criteria, conclusions about the treatment effect of HCQ on pSS-related dry eye disease are different.[10-16] Therefore, the conclusive impact of HCQ on pSS-related dry eye is yet to be determined. This study intended to observe the safety and efficacy of HCQ in the treatment of pSS-related dry eye disease by designing a prospective randomized controlled trial, which is expected to provide some guidance for the clinical application of HCQ in the treatment of this disease.

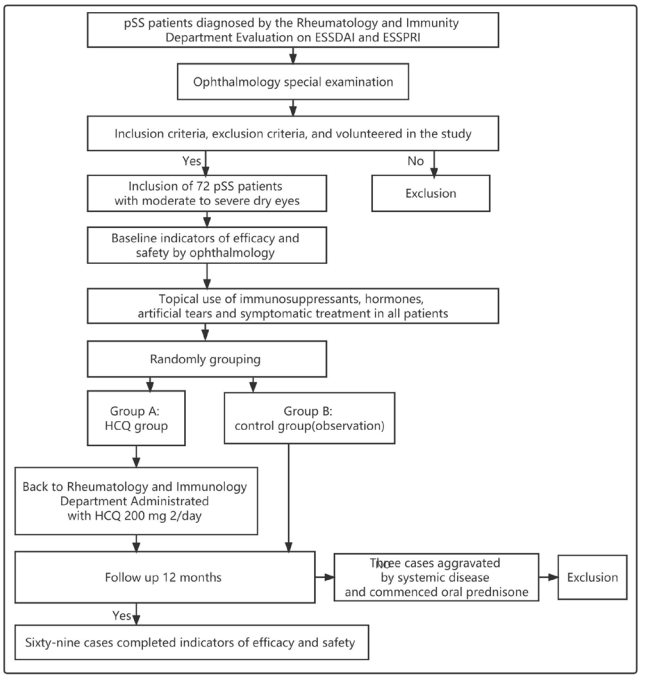
MATERIALS AND METHODS

Patient recruitment and study design

This prospective randomized controlled study was approved by the Ethics Committee of The First Hospital Affiliated of the Army Medical University (Ethics number: KY2020063). This study considered patients diagnosed with primary Sjögren's syndrome from the Department of Rheumatology and Immunology of the First Affiliated Hospital of the Military Medical University of the Army between October 2020 and October 2022 according to the diagnostic criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) in 2016. The ocular surface of the patients was evaluated in the Ophthalmology Department, and pSS patients with moderate and severe dry eye disease and 12 months of follow-up were included for observational analysis. Inclusion criteria: (i) age > 18 years; (ii) conform to pSS free from organ damage other than arthritis; (iii) combined with moderate or severe dry eye disease (containing 2 or more of the following conditions: Ocular Surface Disease Index (OSDI) questionnaire score > 23 points or/and Standard Patient Evaluation of Eye Dryness (SPEED) questionnaire score > 9 points, Ocular Surface Disease Severity (OSS) score ≥ 2 points, tear secretion experiment < 5 mm, tear meniscus height ≤ 0.10 mm, and tear-film rupture time < 5 s); (iv) be available for implementing treatment regimens and follow-up as required; (v) no additional serious diseases of the whole body. Exclusion criteria: (i) secondary Sjögren's syndrome (SS) patients; (ii) primary SS with severe viscera damage such as the heart, kidneys, or lungs; (iii) patients with glaucoma, fundus oculi disease, eye trauma, or other active ocular surface diseases; (iv) disease conditions of patients requiring oral glucocorticoids or immunosuppressants; (v) unavailable to implement treatment and follow-up as required; (vi) the presence of additional serious diseases of the whole body; (vii) pregnant or lactating women.

All included patients received 0.05% cyclosporine eye drops at the dose of 4 times/day and the dose could be decreased to twice/day after symptom remission, and this treatment continued. Prednisolone Acetate Ophthalmic Suspension was commenced 4 times/day for 2 to 4 consecutive weeks; Sodium Hyaluronate Eye Drops 4 times/day; Deproteinised Calf Serum Eye Gel 3 times/day. Patients with meibomian gland dysfunction (MGD) were introduced to apply Tobramycin Dexamethasone Eye Ointment on the palpebral margin, once/day for 2 consecutive weeks. The patients were instructed to pay attention to physical treatments such as palpebral margin cleaning and heat application. A pair of moisture chamber glasses and meibomian gland massage were necessary according to the conditions.

Figure 1. Patient Screening Flowchart



All patients signed the informed consent and were randomly assigned to two treatment groups, the oral HCQ group, and the control group. The dose of HCQ was 400 mg per day, one tablet per time, twice per day, and 200 mg for each tablet. Vitamin C tablets were given as a placebo in the control group (Figure 1).

Clinical evaluation

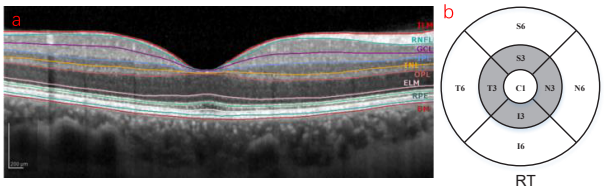
Efficacy indicators were ESSDAI, ESSPRI, OSDI, speed questionnaire score, serum IgG, serum IgA, serum IgM, blood glucose, BCVA, corneal OSS score, Schirmer test I, and TBUT. Safety indicators were intraocular pressure, macular OCT, visual field, and mfERG. Pre-treatment baseline conditions and 12-month post-treatment measures were collected, respectively.

Examinations of safety indicators

Apparatus and reagents: All patients underwent comprehensive eye examinations, including Best Corrected Visual Acuity (BCVA), slit lamp examination, automatic intraocular pressure gauge (KT-500 automatic intraocular pressure gauge; Kowa), automatic refraction (KW-1500; Kowa), and fundus examination. If both eyes meet the inclusion criteria, the right eye is used for the study. BCVA is converted to LogMAR vision. Humphrey Field View Analyzer II or III (Carl Zeiss Meditec Inc.) was used to perform a standard automated field of view examination based on the Swedish Interactive Threshold Algorithm 24-2 strategy. Only

reliable images with gaze loss < 20%, false positive rate < 15%, and false negative rate < 15% were included in the analysis. High-contrast and high-quality images were acquired via a fundus camera, without interference from severe refractive media turbidity (such as severe cataracts and vitreous opacities), and Visual Field Index (VFI) values and visual sensitivity were collected from both eyes. The Heidelberg Spectral Domain Optical Coherence Tomography (SD-OCT) scan was performed on a 6 × 6 mm grid centered on the fovea centralis of the macula, and retinal thickness (RT) was measured from the internal limiting membrane to the retinal pigment epithelium (RPE) (as shown in Figure 2a). RT was analyzed using the Early Treatment Diabetic Retinopathy Study (ETDRS) grid (Figure 2b), and the RT of the specific region was automatically generated via the SD-OCT software. The ETDRS grid divided the retina into three concentric rings: the central, inner, and outer rings, with diameters of 1, 3, and 6 mm, respectively. The inner ring (the circular area between the central ring and the outer ring) and the outer ring (the circular area between the inner ring and the outer ring) were uniformly divided into four quadrants, corresponding to temporal, superior, nasal, and inferior. In the macula area, the area inside the central ring is called the fovea centralis, the area of the central circle is called the parafovea, and the area inside the outer ring is called the perifovea. The average RT of the 4 quadrants of the central ring was obtained (as shown in the shaded region of Figure 2b). Multifocal Electroretinogram (mfERG) examination was performed using the Veris Science™ 4.2 multi-focus electrophysiology system (EDI, USA) following the International Society of Clinical Visual Electrophysiology (ISCEV) standard. Refractive error was corrected before the examination and amplitudes (P1 amplitude) and latencies for rings 1 and 2 in both eyes were collected.

Figure 2. Annotation of retinal layer segmentation and ETDRS grid analysis in optical coherence tomography images. **(a)** Represents an automatic segmentation line of the retina in OCT software, and the retinal thickness includes the thickness from the inner boundary membrane to the retinal pigment epithelium. **(b)** Indicates the macula ETDRS grid, and the research areas are marked in gray shadows in the Figure.



Examinations of effective indicators

pSS patients' four scale questionnaire score: Four major scales include the EULAR Sjögren's syndrome activity index (ESSDAI),[17] the EULAR Sjögren's syndrome patient reported index (ESSPRI),[18] the OSDI questionnaire score,[16] and the SPEED questionnaire score.[19] Final ESSDAI scores were calculated based on the activity level of the eight items including systemic symptoms, lymph nodes, glands, joints, skin, lungs, kidneys, and muscle lesions multiplied by the corresponding weights and made a sum, which was applied to evaluate the severity of disease activity in pSS patients. The ESSPRI score is the mean score of the three symptoms of dryness, limb pain, and fatigue, which can effectively quantify the symptoms of pSS patients and evaluate the efficacy of treatment. The OSDI questionnaire consists of 12 questions assessing the severity of dry eye from three aspects: eye symptoms, visual function, and environmental irritation triggers. Each question is given 0 points (never), 1 point (occasionally), 2 points (often), 3 points (mostly), and 4 points (always) by frequency. OSDI scores = [sum of answered questions × 100]/[number of answered questions × 25], from 0 to 100 points. Symptoms of the SPEED questionnaire included dry gravel or itching, pain or irritation, burning or dampness, and visual fatigue. The involved frequencies included never, sometimes, often, and persistent with scores of 0, 1, 2, and 3, respectively. The severity of the symptoms included no problems, tolerable, uncomfortable, bored, and intolerable, with scores of 0, 1, 2, 3, and 4, respectively. Both OSDI and SPEED scores could quantitatively evaluate the symptoms and severity of dry eye.

Evaluation of the severity of dry eyes: The OSS score, TBUT, and Schirmer test I of all patients tested each time were evaluated by the same experienced ophthalmologist. OSS[20] was determined following the Oxford scheme (0–5).[21] Corneal punctate epithelial erosion was counted and scored after the instillation of a preservative-free 1% fluorescein solution. We instill one drop of preservative-free 1% fluorescein solution into the superotemporal bulbar conjunctiva. We permitted patients to blink several times, then the patients were commanded to keep their eyes open, without blinking. We measured the duration between the last complete blink and the first appearance of any disturbance. The average value of three TBUT measurements was used for analysis. We placed standardized Schirmer's strips (Eagle Vision, Memphis, TN, USA) in the lateral one-third of the lower eyelid under topical anesthesia. After 5 min, we measured the length of the wet portion of each strip.

Evaluation of the serological indicators

Serological indicators included serum levels of IgG, IgM, IgA, and blood glucose testing.

Statistical analysis

Data were analyzed with IBM SPSS Statistics software and expressed as mean ± standard deviation. The pre-treatment baseline comparison was performed using two independent sample ranks in a non-parametric statistical method, the Mann-Whitney rank test. Two-factor repeated measurement analysis of variance was used for comparison between the two groups before and after the treatment, and LSD pairwise comparison was performed when a significant difference was revealed. $P < .05$ indicates a significant difference between the two groups.

Table 1. Details of the Baseline and the Indicators After 12 Months of Treatment in the Two Groups

	HCQ Group					Control group					*P* value	
	Baseline		After 12 m			Baseline		After 12 m				
Items	n	Mean (SD)	n	Mean (SD)	*P* value	n	Mean (SD)	n	Mean (SD)	*P* value	Baseline comparison between groups	Efficacy comparison before and after treatment between groups
Gender	n = 33 Male = 0 female = 33		n = 33 Male = 0 female = 33			n = 36 Male = 1 female = 35		n = 36 Male = 1 female = 35				
Age	33	46.82 (10.60)	33	46.82 (10.60)		36	50.42 (11.00)	36	50.42 (11.00)		.279	

Figure 3. Comparative analysis of baseline indicators in two groups for effectiveness and safety assessment in ocular study. Comparison of baseline between the two groups indicates that indicators of effectiveness (ESSDAI, ESSPRI, OSDI, SPEED questionnaire score, serum IgG, serum IgA, serum IgM, blood glucose, BCVA, corneal OSS score, Schirmer test I, and TBUT) and safety (intraocular pressure, SD-OCT RT, VFI, visual sensitivity, mfERG rings 1 and 2 latencies and amplitudes) are not statistically significant ($P > .05$).

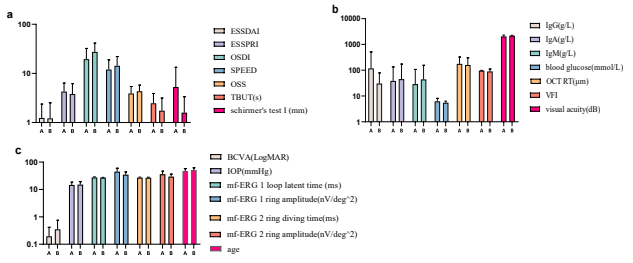
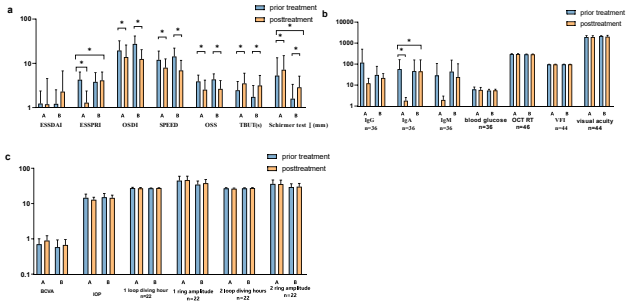


Figure 4. Comparative analysis of effective and safety indicators between prior treatment and posttreatment group. **(a)** Represents comparative analysis of effective indicators. **(b)** and **(c)**, represents a comparative analysis of safety indicators.



*$P < .05$.

RESULTS

Patients general information

A total of 72 patients were included in this study. To date, 3 patients have been excluded due to systemic conditions requiring oral glucocorticoids or immunosuppressants, and 69 of them were followed up to 12 months. The right eyes of all patients were selected for study; 33 cases (33 females, 0 male) aged between 22 and 70 years old (46.82 ± 10.6) were included in the oral hydroxychloroquine group; 36 patients in the observation group (35 females and 1 male) aged between 30 and 76 years old (50.42 ± 11) as shown in Table 1.

Baseline comparison of the two groups before treatment

The pre-treatment efficacy indicators (ESSDAI, ESSPRI, OSDI, SPEED questionnaire score, serum IgG, serum IgA, serum IgM, blood glucose, BCVA, corneal OSS score, Schirmer test I, and TBUT) and safety indicators (ocular pressure, macular OCT, visual field, and mfERG) are shown in Figure 3 and Table 2, and there exists no statistically significant difference between the two groups ($P > .05$).

Efficacy analysis

The four questionnaires (ESSDAI, ESSPRI, OSDI questionnaire score, and SPEED questionnaire score), serological indicators (IgA, IgG, IgM, and blood glucose), and dry eye-related tests (OSS score, TBUT, and Schirmer test I) in the two groups before and after treatment are shown in Figure 4 and Table 2.

As shown in Figure 4a, the difference in the HCQ group in ESSPRI before and after treatment were statistically significant ($P < .05$). A comparison of dry eye-related symptom scales (OSDI and SPEED) in the two groups before and after treatment revealed a statistically significant difference ($P < .05$). The ESSDAI comparison between the two groups before and after treatment revealed no significant difference ($P > .05$). The differences in ESSPRI among groups were statistically significant ($P < .05$). No statistically significant difference was revealed in ESSDAI, OSDI, and SPEED scores among groups ($P > .05$).

Dry eye-related examination indicators as shown in Figure 4a, the differences in OSS score, TBUT, and Schirmer test I of the two groups before and after the treatment were statistically significant ($P < .05$). The differences among groups in the Schirmer test I were statistically significant ($P < .05$), and there was a significant improvement in the Schirmer test I group 12 months after HCQ treatment compared with the control group. There was no statistical difference among each group ($P > .05$).

Serological indicators (n = 36) as in Figure 4b, the differences in serum IgA before and after HCQ treatment were statistically significant ($P < .05$). Compared with the observation group with oral HCQ treatment after 12 months, serum IgA decreased markedly. There was no statistically significant difference in serum IgG, serum IgM, and blood glucose in the two groups before and after treatment ($P > .05$), and no statistically significant difference exists in the serum IgA in the control group before and after treatment ($P > .05$). There was a significant difference in serum IgA among the groups ($P < .05$). No significant difference in serum IgG, serum IgM, and blood glucose was revealed between the two groups ($P > .05$).

BCVA (LogMAR) as shown in Figure 4c, BCVA (LogMAR) had no significant difference between the two groups ($P > .05$). There was no statistical difference in BCVA (LogMAR) among the groups ($P > .05$).

Table 2. Details of the Baseline Indicators in the Two Groups

		HCQ Group					Control group					*P* value	
		Baseline		After 12 m			Baseline		After 12 m				
Items		n	Mean (SD)	n	Mean (SD)	*P* value	n	Mean (SD)	n	Mean (SD)	*P* value	Baseline comparison between groups	Efficacy comparison before and after treatment between groups
Systemic Symptom-Related Scale	ESSDAI	33	1.24 (1.14)	33	1.05 (1.12)	.467	36	1.22 (1.31)	36	1.26 (1.14)	.587	.778	.466
	ESSPRI	33	4.27 (2.10)	33	3.63 (1.71)	.003[a]	36	3.82 (2.36)	36	4.01 (2.26)	.424	.370	.042[a]
Dry Eye-Related Scale	OSDI	33	19.5 (12.85)	33	10.58 (6.71)	.010[a]	36	27.44 (13.97)	36	12.56 (7.79)	.001[a]	.146	.139
	SPEED	33	12 (6.95)	33	8 (4.59)	.032[a]	36	14.31 (7.73)	36	6.94 (4.73)	.001[a]	.377	.728
Dry Eye Related Exams	OSS	33	3.92 (1.50)	33	2.54 (1.64)	.001[a]	36	4.34 (1.45)	36	2.66 (1.43)	.000[a]	.302	.451
	TBUT	33	2.47 (1.44)	33	3.53 (2.46)	.048[a]	36	1.75 (1.41)	36	3.17 (2.17)	.000[a]	.051	.129
	Schirmer test	33	5.29 (8.09)	33	7.17 (7.74)	.020[a]	36	1.59 (1.76)	36	2.88 (2.27)	.000[a]	.090	.001[a]
Serological Indicators	IgA	8	38.89 (95.47)	5	1.79 (0.65)	.017[a]	14	45.03 (127.89)	4	3.54 (1.11)	.176	.905	.045[a]
	IgG	18	118.15 (395.80)	6	19.47 (4.45)	.075	18	30.44 (48.25)	3	30.83 (21.65)	.285	.905	.976
	IgM	8	29.06 (78.77)	5	1.37 (0.38)	.173	14	44.19 (110.93)	4	1.23 (0.97)	.109	.413	.572
	Blood glucose	10	6.22 (1.90)	5	5.86 (2.14)	.310	13	5.52 (0.83)	4	5.46 (0.99)	.686	.730	.722
BCVA (LogMAR)		33	0.20 (0.22)	33	0.10(0.22)	.001[a]	36	0.35(0.4)	36	0.20(0.20)	.001[a]	.231	.150
The above table presents indicators of effectiveness, and the following table presents indicators of safety													
Intraocular pressure		33	14.65 (3.91)	33	12.9 (2.23)	.209	36	15.12 (4.19)	36	14.57 (2.77)	.700	.686	.086
OCT	RT	33	299.55 (16.03)	20	300.40 (13.05)	.180	36	292.14 (11.33)	26	291.77 (11.25)	.345	.096	.510
View field	VFI	33	96.75 (2.47)	28	96.71 (2.59)	.986	36	97.00 (2.56)	16	97.13 (1.50)	.562	.869	.911
	Visual acuity	33	1953.50 (322.62)	28	1931.43 (302.39)	.725	36	2171.94 (95.78)	16	2077.31 (342.40)	.624	.388	.097
mf-ERG	Ring 1 latency	33	27.06 (1.30)	14	27.02 (1.49)	.441	36	27.66 (1.19)	8	27.71 (0.39)	.083	.616	.493
	Ring 1 amplitude	33	41.70 (13.51)	14	46.07 (13.64)	.782	36	37.19 (10.38)	8	37.83 (10.11)	.505	.188	.055
	Ring 2 latency	33	26.92 (1.05)	14	26.55 (1.03)	.279	36	27.36 (1.19)	8	27.50 (0.89)	.382	.920	.173
	Ring 2 amplitude	33	32.46 (9.40)	14	35.63 (10.62)	.872	36	30.34 (8.07)	8	30.28 (6.91)	.798	.161	.073

[a]$P < .05$

Safety analysis

According to the diagnostic criteria for hydroxychloroquine retinopathy,[22] no adverse reactions such as hydroxychloroquine retinopathy and diarrhea were complained of in group A with oral HCQ administration. Two cases (6.06%) in group A and 3 cases (8.33%) in group B suffered from transient ocular hypertension, which decreased to normal after discontinuation of hormonal eye drops combined with antihypertensive therapy.

There was no statistically significant difference ($P > .05$) between the safety indicators (ocular pressure, SD-OCT RT (n = 46), VFI (n = 42), visual sensitivity (n = 42), and mfERG ring 1 latency (n = 22) and amplitude (n = 22), and ring 2 latency (n = 22), and amplitude (n = 22)), as shown in Figures 4b and 4c.

DISCUSSION

Many works of literature report that HCQ performs excellently in immunomodulation and is widely used in autoimmune-related diseases, such as Systemic Lupus Erythematosus (SLE),[23-25] rheumatoid arthritis,[26] anti-phospholipid syndrome,[27] Sjögren's syndrome,[8] or other inflammatory rheumatic diseases. In ophthalmology, HCQ can treat Graves eye disease by inhibiting the proliferation of fibroblasts, lipogenesis, and hyaluronic acid production.[28] In animal experiments, HCQ may alleviate experimental autoimmune uveitis (EAU) by modulating effector T cells/regulatory T cells (Teff/Treg) balance and improving retinal vascular endothelial cell (RVEC) dysfunction via the LOX-1/NF-kB axis.[29] However, the mechanism of HCQ for the treatment of different diseases remains unclear, and it is unknown whether its treatment effect in the clinic is the same mechanism or different mechanisms. HCQ is reported to inhibit immune activation by directly or indirectly reducing Toll-like receptor signaling, cytokine production, as well as CD154 expression in T cells.[30-32] pSS is a systemic autoimmune disease characterized by the infiltration of lymphocytes in the salivary gland and lacrimal gland. CD4+ helper T cells are common lymphocytes in these gland tissues in an early stage of the disease. The accumulation of such cells and secreted IFN-γ alters the tight connection of glandular epithelial cells and induces apoptosis of glandular epithelial cells, resulting in decreased glandular secretion.[33] Theoretically, HCQ is effective in tear gland inflammation due to Sjögren's Syndrome and improves the symptoms of this disease. The results of this study found no significant improvement in the ESSDAI, OSDI, and SPEED scores after 12 months of treatment in the oral HCQ group compared with the control group, which is consistent with those reported in the literature.[7,9] It may be that the patients included developed no other organ damage except arthritis, with low systemic involvement. Therefore, the improvement in disease activity indicators was difficult to observe due to a low baseline of the ESSDAI score. The use of HCQ in pSS patients is reported to be a protective factor against hospitalization.[34,35] In our study, changes in symptoms (ESSPRI score) of patients in the HCQ group were significantly improved compared with the control group, and this mainly manifested in the symptom improvement of patients' systemic dryness. Dry eye symptom-related scores (OSDI and SPEED) and relevant tests (OSS, TBUT, and Schirmer test I) marked improvement before and after treatment, which was closely related to the regular and topical application of cyclosporin eye drops in both groups, and this was consistent with Wan, Chen, and Young.[36]

Bodewes, et al.[9] uncovered reduced expression of pSS systemic interferon-stimulating genes and elevated ESR, IgG, and IgM levels of pSS 24 weeks after treatment with HCQ, but IgA was not included in their study. Serum IgA was markedly decreased after treatment with HCQ compared to the control group, and serum IgG and IgM in the HCQ group had a declining trend from the baseline, but there was no statistically significant difference. This might be explained by the small number of patients who completed serology in this

study. Whether there is a statistically significant change in serum IgG and IgM before and after HCQ treatment with a larger sample size requires further observation.

It was revealed that a significant improvement in Schirmer test I in both groups after 12 months of treatment compared with the baseline, and this was the main reason that 0.05% of cyclosporine eye drops were topically used in both groups. Cyclosporine eye drops can release a series of neurotransmitters by stimulating the peripheral nerves, especially the release of P substances, which can further activate muscarinic receptors in the body, thereby increasing the secretion of tears. Mo, et al. reported that 0.05% of cyclosporine eye drops improve Schirmer test I,[37] and the difference in the group before and after treatment may be related to the topical use of cyclosporine eye drops. Schirmer test I in the oral HCQ group was markedly improved at 12 months after treatment compared with the control group and tear secretion was significantly improved in the oral HCQ group. It is shown that oral HCQ can further increase the secretion of tears combined with the topical use of cyclosporine eye drops. Presently, little is known about the pathophysiological characteristics of the lacrimal gland. Bannier-Hélaouët, et al.[38] have established a lacrimal-gland-like tissue organoid derived from adult stem cells, which provides an experimental platform for studying the pathophysiology and pharmacology of the lacrimal gland. Several articles and/or reviews[39,40] have found that the pathogenesis of pSS is related to intestinal microecological disorders, and some studies[41,42] also have confirmed the role of traditional Chinese medicine in improving the gut microbiota and the symptoms of pSS patients. However, no study investigates the role of HCQ in the gut microbiome and lacrimal-gland-like tissue organoids of pSS patients, which will be our prime concern in the subsequent research.

In our study, HCQ improved serum IgA levels and it may have roles in the improvement of the secretory function of the lacrimal glands. pSS patients with HCQ treatment over 3 years can benefit from its significant protective effect[43] on new diabetes mellitus (DM) and it is also a protective factor for hospitalization. HCQ can improve the subjective and objective manifestations of pSS dry eye.[13] pSS is recognized as a chronic autoimmune disease involving multiple organs and tissues of the body, with a long course of treatment, and it is uncertain whether the long-term HCQ treatment can do some harm to the patients or not. Hydroxychloroquine retinopathy has long been recognized,[44] and its incidence has been considered to be very low and given insufficient attention. But lately, with the availability of increasing articles with long duration and large samples, its incidence is estimated to be about 5% in patients who have been using this drug for up to 5 years and about 10% in patients who have been using it for 10 years. It can be as high as 31% in patients with over 20 years of application, and hydroxychloroquine retinopathy was markedly associated with the duration and cumulative dose of hydroxychloroquine use.[45,46] However, this damage is irreversible, and it may continue to develop after discontinuation of the drug use. It is incurable at present,[47] and can lead to permanent vision loss at the late stage.[22] Early identification of this disease is of vital significance. In this study, hydroxychloroquine retinopathy was also used as an essential observational indicator, and the guidelines recommended baseline examination for patients within 1 year after the start of HCQ treatment,[22] mainly to improve the automatic field of view, macular SD-OCT examination, and fundus spontaneous fluorescence and mfERG examination if necessary. At baseline, the patients performed examinations for the field of view, macular SD-OCT, and mfERG, and the changes in the retinal layers at 1-3 mm from the fovea centralis on OCT preceded the occurrence of HCQ retinopathy. Tsang, et al.[48] also pointed out that the damage to the parafovea was more serious. Although it was previously thought that HCQ retinopathy occurred mainly in the outer retina, recent studies have found that retrogressive changes in the inner and middle layers of the retina are associated with HCQ retinopathy.[49,50] Therefore, our article mainly calculated the average thickness of the whole retina in the region where the center ring was located, and the results showed that the average thickness of the center ring retina at 12 months after HCQ treatment was not statistically significant compared with the control group, which was consistent with the results at 24 months after HCQ by Ueda-Consolvo, et al.[45] Except for SD-OCT, there was no statistically significant difference in view field VFI and visual acuity in patients using HCQ as compared with the control. The sensitivity and specificity of P1-N1 amplitudes for mfERG ring 1 are 76% and 96%, respectively, and those of P1-N1 amplitudes for ring 2 are 90% and 78%.[51] Meanwhile, the study of Tsang et al.[48] also reported that the sensitivity and specificity of mfERG in the diagnosis of HCQ retinopathy are 100% and 78%, respectively, of which the P1 amplitude of the ring 2 performs the best.

In summary, the latency P1 and amplitudes of mfERG ring 1 and ring 2 were statistically analyzed. The differences between the HCQ group and the control group were not statistically significant, which might be explained by the short observation time of HCQ treatment, and at present all patients did not develop HCQ retinopathy. Abnormal blood flow in the retinal capillary plexus and thinning of the choroid are reportedly observed with SS-OCTA in patients who received HCQ over 5 years, even though the optimal corrected vision, spontaneous fluorescence, mfERG, automatic field of view, and OCT examination findings are all normal.[52] The conclusion demonstrated that early identification of abnormalities in the fundus after HCQ treatment warranted further attention to changes in blood flow in the retinal capillary plexus and choroid. Because the specific mechanism of HCQ retinopathy is unclear, the mainstream view is that the accumulation of HCQ on RPE causes degeneration of outer retinal and photoreceptor cells,[53] factors affecting serum HCQ levels (including HCQ dose and systemic status)[54] will affect the occurrence of HCQ retinopathy. All patients are chronic and have a long treatment course, personalized guidance is necessary for patients who require long-term treatment considering both the safety and effectiveness of the treatment.

This study found that oral HCQ has significant therapeutic effects in improving patients' ESSPRI scores, IgA levels, and Schirmer test I results, making it a safe treatment method for dry eye disease associated with pSS. However, due to the small sample size, included pSS patients generally exhibited lower organ involvement, and short follow-up period, this study has limitations. Future research should explore HCQ as an independent treatment method, conduct larger-scale and longer-duration studies, and include objective measures for comprehensive evaluation. Furthermore, investigating the impact of HCQ on the gut microbiota of patients with pSS as well as its effects on lacrimal gland-like organs will be a key focus of our upcoming research.

DATA AVAILABILITY

The experiment data used to support the findings of this study is available from the corresponding author upon reasonable request.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest regarding the publication of this paper.

AUTHOR CONTRIBUTIONS

Wang Fang and Zhou Qingqing are co-first authors and have contributed equally to this study.

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