Acta Dermatovenerol Croat 2022;30(3):146-150 ORIGINAL SCIENTIFIC ARTICLE

A Preliminary Study on the Therapeutic Effects of Hydroxychloroquine on Generalized Vitiligo

Soudabeh Sazgarnia[1], Pouran Layegh[2], Emadodin Darchini-Maragheh[2], Vahid Sepehr[1], Mojtaba Meshkat[3], Sedigheh Fathi-Najafi[2], Sara Rahsepar[2]

[1]Mashhad Azad University of Medical Sciences, Mashhad, Iran; [2]Cutaneous Leishmaniasis Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [3]Department of Library, Mashhad Azad University of Medical Sciences, Mashhad, Iran;

Corresponding author:
Emadodin Darchini-Maragheh, MD
Mashhad University of Medical Sciences
Mashhad, Iran
darchinime971@mums.ac.ir

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ABSTRACT Vitiligo is a recalcitrant depigmentary autoimmune skin disorder. Hydroxychloroquine (HCQ) is an effective immunomodulatory drug which is widely used in treatment of autoimmune disorders. HCQ-induced pigmentation has been previously found in patients taking HCQ due to other autoimmune diseases. The present study aimed to determine whether HCQ improves re-pigmentation of generalized vitiligo. HCQ was orally administered 400 mg daily (6.5 mg/Kg of body weight) by 15 patients with generalized vitiligo (more than 10% involvement of body surface area) for three months. Patients were evaluated monthly and skin re-pigmentation was assessed using the Vitiligo Area Scoring Index (VASI). Laboratory data were obtained and repeated monthly. Fifteen patients (12 women and 3 men) with a mean age of 30.13±12.75 years were studied. After 3 months, the extent of re-pigmentation on all the body regions, including the upper extremities, hands, trunk, lower extremities, feet, and head and neck was significantly higher than the baseline (*P* value <0.001, 0.016, 0.029, <0.001, 0.006, 0.006, respectively). Patients with concomitant autoimmune diseases had significantly more re-pigmentation compared with others (*P*=0.020). No irregular laboratory data were observed during the study. HCQ could be an effective treatment for generalized vitiligo. The benefits are likely to be more evident in case of concomitant autoimmune disease. The authors recommend additional large-scale controlled studies to draw further conclusions.

KEY WORDS: hydroxychloroquine; vitiligo; skin; pigmentation

INTRODUCTION

Vitiligo is an acquired polygenic autoimmune disorder characterized by loss of pigmentation due to melanocyte destruction. White macules appear in the skin in response to melanocyte destruction. Variable types of vitiligo are distinguished according to the distribution patterns of the lesions. Generalized vitiligo is the predominant type and is characterized by multiple scattered symmetrical lesions and is often progressive. Localized vitiligo presents as one or more focal or segmental unilateral macules, which is more common in pediatric patients (1).

The three major hypotheses explaining the pathogenesis of vitiligo are autoimmune injury, oxidative stress, and the neural hypothesis. Mainly, vitiligo is considered to be a T-cell and autoantibody-induced immune disorder. The current standard therapy for vitiligo is UV light and topical agents (corticosteroids and calcineurin inhibitors). However, some patients

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do not achieve satisfactory results despite UV light therapy and thus need systemic treatment (2).

Hydroxychloroquine (HCQ) is an antimalarial agent and potent immunosuppressant which is widely prescribed in rheumatology and dermatology. Although adverse effects are rare, its use can be complicated by mainly gastrointestinal and cutaneous side-effects. In a review of 689 dermatologic adverse effects, drug eruption (358 cases) and cutaneous pigmentation (116 cases) were the most common cutaneous adverse effects (3). In a dermatologic survey of 41 patients who had been treated with HCQ for over 6 months due to internal diseases, HCQ-induced pigmentation was the most common dermatologic manifestation which presented in 29% of patients (4). Furthermore, special skin sample staining revealed higher concentrations of melanin and iron in the dermis among patients who administered HCQ (5). Possible therapeutic effects of HCQ in skin pigmentation are also hypothesized to be immune mediated. Li *et al.* (2016) obtained antimelanocyte antibodies from the serum of 32 patients with generalized vitiligo and observed the effects of HCQ in preventing the autoantibody-induced disruption of melanocytes. Cell-based ELISA, indirect immunofluorescence, and western blotting were used to analyze the autoantibody content, and MTT assay was applied to detect cytotoxicity of HCQ on the autoantibody-melanocyte complex. Serum concentrations of autoantibodies against melanocytes were higher in patients with vitiligo compared with controls. HCQ significantly reduced both antibody-mediated and complement-mediated cytotoxicity that were mediated by vitiligo IgG. The study provided evidence that HCQ can have protective effect against autoantibodyinduced immune injury in generalized vitiligo (6).

Previously, HCQ has been anecdotally shown to be effective in vitiligo re-pigmentation. Joo *et al.* (2015) reported the case of a 39 years old woman who received HCQ due to rheumatoid arthritis. Concomitant acro-facial vitiligo improved 4 months after starting HCQ, worsened during the period when HCQ was discontinued, and continued to improve after restarting the treatment (7).

The aim of the present study was to assess induced re-pigmentation activity of HCQ in patients with generalized vitiligo. The hypothesis was presumed improvement in lesion depigmentation among patients 3 months after HCQ administration.

PATIENTS AND METHODS

Study design and population

This was an open label before-after study, conducted during 2019-2020 at two major dermatology

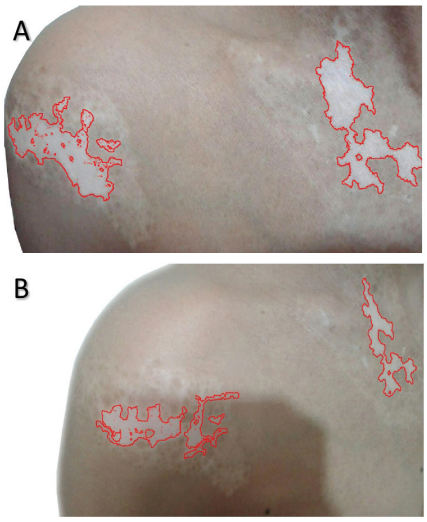

Figure 1. Vitiligo before (A) and after (B) treatment with hydroxychloroquine.

Table 1. Demographic data and clinical characteristics of 15 patients with generalized vitiligo registered in the study

Demographic Characteristics	Values	Clinical Characteristic	Values
Male Female	3 (20%) 12 (80%)	Disease duration (years)	7.20±6.60
Body Mass Index (kg/m^2)	24.80±4.21	Koebner's phenomenon	3 (20%)
Age (years old) Less than 30 More than 30	30.13±12.75 6 (40%) 9 (60%)	Halo nevi	0 (0%)
Skin types 1 2 3 4	 0 (0%) 8 (53.33%) 7 (46.66%) 0 (0%)	Associated autoimmune disease Thyroid disease Lupus erythematous Diabetes Mellitus 1 Rheumatoid Arthritis	 2 (13.33%) 0 (0%) 1 (6.66%) 1 (6.66%)

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Table 2. Vitiligo Area Scoring Index (VASI) scores in 15 patients with generalized vitiligo treated with hydroxychloroquine

Body Regions	Treatment Period	VASI Score (mean ± S.D.)	*P* value
Total	Baseline	9.08±6.37	0.037
	After 3 months	8.63±6.06	
Upper extremities	Baseline	1.45±1.35	<0.001
	After 3 months	1.38±1.27	
Hands	Baseline	0.57±0.59	0.016
	After 3 months	0.54±0.58	
Trunk	Baseline	2.51±3.01	0.029
	After 3 months	2.44±2.96	
Lower extremities	Baseline	3.00±2.81	<0.001
	After 3 months	2.84±2.57	
Feet	Baseline	1.02±1.29	0.006
	After 3 months	0.96±1.25	
Head and neck	Baseline	0.53±0.63	0.006
	After 3 months	0.47±0.56	

clinics in eastern Iran (Emam Reza Hospital and Arya Hospital Mashhad, Iran) on 15 patients with generalized vitiligo.

Initially, patients who met the inclusion criteria were included in the study according to a non-probability convenience sampling method. All patients had generalized vitiligo with a body surface area involvement of more than 10% and no prior systemic treatment or phototherapy during the past 6 months. Written informed consent has been obtained from all the patients. Those who were under 16 years of age or had systemic underlying disease including hepatic or kidney diseases, were pregnant/breastfeeding mothers, or declined to participate were excluded from the study.

As the first step, all the patients were examined by an ophthalmologist due to possible ophthalmologic evidence of retinopathy. Laboratory data including blood cell count (CBC), clinical chemistry (creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and Glucose-6-phosphate dehydrogenase (G6PD) were obtained prior to the study.

Treatment period

Oral hydroxychloroquine (AminPharma®) was prescribed 400 mg daily (6.5 mg/Kg of body weight) to each patient for 3 months. Each month, patients were evaluated by means of the Vitiligo Area Scoring Index (VASI) score and laboratory data, and possible adverse effects were recorded. Patients were examined by an independent dermatologist, and photographs were taken. At each visit, re-pigmentation in each topographical area was estimated by a standard protocol of digital photography (Canon Power Shot G9 camera) and graded using VASI score (8).

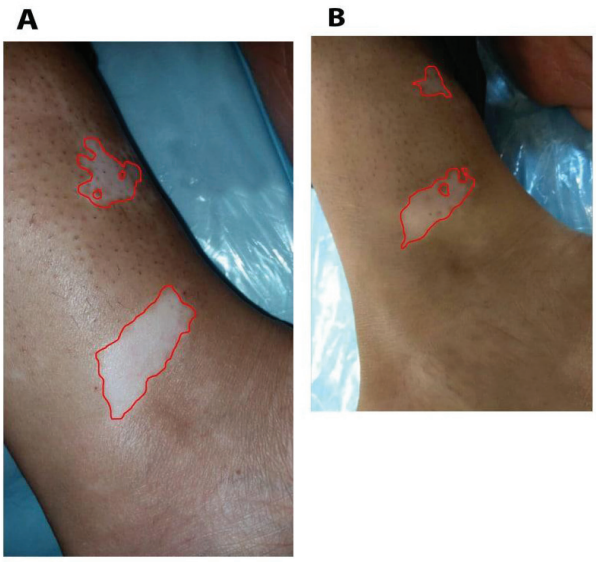

Figure 2. Vitiligo before (A) and after (B) treatment with hydroxychloroquine.

VASI score was determined by calculating body part involved and depigmentation severity. VASI was calculated by means of a formula that includes all body regions: VASI = ∑ Hand Units of each body region × Residual Depigmentation. One hand unit is approximately 1% of the total body surface area and encompasses the palm plus the volar surface of all the digits. It is used as a guide to estimate the baseline percentage of vitiligo involvement in each body region. The body is divided in to six regions: upper extremities (excluding hands), hands, trunk, lower extremities (excluding feet), feet, and head and neck. VASI score was calculated separately for each body region according to the formula. The final body VASI score was the sum of the scores for each region.

Residual depigmentation was expressed by the following percentages: 0, 10%, 25%, 50%, 75%, 90%, and 100%. At 100% depigmentation, no pigment was present; at 90%. specks of pigment were present; at 75%, the depigmented area exceeded the pigmented area; at 50%, the depigmented and pigmented areas were equal; at 25%, the pigmented area exceeded the depigmented area; at 10%, only specks of depigmentation were present.

This study was approved by the institutional ethics board of Mashhad Islamic Azad University of Medical Sciences, Mashhad, Iran and was in accord with the Helsinki Declaration. The protocol was also registered on Iranian Registry of Clinical Trials (IRCT20201212049690N1).

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Statistical analysis

Quantitative variables are reported as mean ± SD. Qualitative variables are indicated as frequency and percentages. The Kolmogorov-Smirnov test was used for evaluation of normality of the scores. Paired-samples t-test and Wilcoxon's test were used to compare the means within groups. Statistical analysis was performed using SPSS version 17 software (Statistical Package for Social Science, version 17) and Prism version 3.02 (Graph Pad Software, San Diego, CA, USA) for the Wilcoxon's test. For all analyses, $P<0.05$ was considered statistically significant.

RESULTS

Subjects

The study compromised 12 women and 3 men. Mean age of the patients was 30.13±12.75 (range 16-55) years old. Mean disease duration was 7.20±6.60 years. Demographic characteristics and clinical history are summarized in Table 1.

VASI score

At the beginning of the study, the mean VASI score was 9.08±6.37, which was significantly reduced to 8.63±6.06 after three months ($P=0.037$). In all the six body regions, including the upper extremities, hands, trunk, lower extremities, feet, and head and neck, VASI score was significantly reduced after three months of therapy (P value <0.001, 0.016, 0.029, <0.001, 0.006, 0.006, respectively). VASI score evaluation at each visit is shown in Table 2. Figure 1 and Figure 2 indicate vitiligo re-pigmentation after the treatment period in the upper and lower extremities, respectively.

VASI score changes did not show significant correlation with patients' age. Those who had other autoimmune diseases had significantly more improvement in the lesion after 3 months of therapy ($P=0.020$).

Adverse effects and drop-outs

Patients underwent clinical examination and laboratory exams at each visit. No laboratory disturbances or patient dissatisfaction was observed during the study period.

DISCUSSION

HCQ was previously utilized to treat malaria and is widely used today for autoimmune diseases due to its immunosuppressive and anti-inflammatory action. Despite its substantial efficacy, it is complicated by side-effects, including HCQ-induced pigmentation. Cutaneous hyperpigmentation occurs in approximately 10% to 30% of patients (9).

The exact mechanism of hyperpigmentation is unknown. HCQ inhibits acidic proteases action involved in many cellular functions, such as toll-like receptor (TLR) signaling, antigen presentation, and production of anti-inflammatory and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNFα). Van den Borne *et al.* (1997) demonstrated that HCQ inhibits phytohemagglutinin-induced TNF-α and interferon-γ production and lipopolysaccharide-induced TNF-α and interleukin-6 production. Possible immune-pathogenetic factors of vitiligo are reactivation of CD8+ T cells, melanocytotoxicity of CD4+ T-cells, reduced regulatory T-cells, and excess inflammatory cytokines (10).

Recently, Laddha *et al.* (2013) reported that TNF-α promotor polymorphisms may be genetic risk factors for susceptibility and progression of vitiligo (11). Furthermore, although cellular immunity was the main subject of investigation in recent years, humoral immunity has also been demonstrated to participate in destruction of melanocytes in vitiligo (6).

Since HCQ inhibits TNF-α production (7, 11) and dissociates antibody-antigen complexes against melanocytes (6), it could be involved in improvement of vitiligo lesions through both cellular and humoral immunity pathways. However, the exact underlying mechanism of HCQ in improving vitiligo lesions remains to be elucidated.

HCQ-induced pigmentation was reported to begin after a few months or years of treatment; however, no significant association with duration nor with cumulative dose of HCQ has been reported in the literature (12,13). Previously, acro-facial vitiligo lesions have been reported to improve 4 months after starting HCQ and worsen during drug discontinuation (13). In contrast, Bahloul *et* al. (2017) reported pigmentation appeared after a median duration of 32 months with a median cumulative dose of 361 g (4). We observed significant HCQ-induced pigmentation on vitiligo lesions 3 months after drug initiation; however, our patients will continue to visit the dermatologist each month and further results will be subsequently reported.

Possible predisposing factors of HQ-induced pigmentation have been previously reported in the literature (9,13). An association between pigmentation and the use of oral anticoagulants and antiplatelet drugs has been found. A case-control study in 24 patients with systemic lupus erythematosus and HCQ-associated skin pigmentation found that 23 (96%) of those with pigmentation had conditions that

predisposed to bruising; 22 (92%) also experienced local bruising before the appearance of pigmentation. The mechanism by which HCQ either encourages bruising or prevents proper healing and resorption of pigment is unclear (13). Although our patients did not have the abovementioned predisposing conditions, those with concomitant autoimmune diseases showed significantly more improvement compared with other patients.

In the only published report on the impact of HCQ on vitiligo re-pigmentation, Joo *et al.* (2015) observed considerable improvement of skin lesions in a patient with concomitant rheumatoid arthritis and acro-facial vitiligo. The author suggested that rheumatoid arthritis therapy with HCQ could have added benefits in re-pigmentation of vitiligo (7).

To our knowledge, this is the first clinical trial that evaluates vitiligo improvement by HCQ treatment. The current study was an open label non-controlled case series. The results of this study should be evaluated in the light of some limitations. Major limitations were mainly due to the study's small sample size and lack of a control group, which limits the strength of its conclusions. Further large and prospective studies with long follow-ups are needed to precisely determine the effect of HCQ in re-pigmentation of vitiligo lesions. Furthermore, persistency of HCQ-induced pigmentation despite cessation of HCQ therapy makes establishing of imputability more difficult. Additional studies are also required to further elucidate the molecular mechanisms underlying these observations.

CONCLUSION

The present study presents preliminary evidence that oral HCQ could be an effective treatment for generalized vitiligo. The benefits are likely to be more evident in case of concomitant autoimmune diseases. The authors recommend additional large-scale controlled clinical trials to draw further conclusions.

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