

## Variation of Hydroxychloroquine Blood Levels in Moroccan Patients with Systemic Lupus Erythematosus; A Pilot Study

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### Abstract

**Aim of the study:** The aim was to determine the blood concentrations of hydroxychloroquine in Moroccan systemic lupus erythematosus patients and identify the factors associated with interindividual variation. **Methods:** A cross-sectional study enrolled patients with lupus erythematosus. We recorded demographic features of patients and main clinical and biological characteristics of the disease. We determined the blood concentrations of hydroxychloroquine for the entire patient enrolled in this study by liquid chromatography – mass spectrometry. Patients were divided according to their blood hydroxychloroquine level into three groups: low blood concentration (<500 ng/mL), therapeutic range (500-2000 ng/mL) and high blood HCQ concentration (>2000). **Results:** Eighty subjects were included; 77 were female and the mean age was 36.9±12, 2 years. The median concentration of hydroxychloroquine was 830 ng/mL (range: 35-3,200 ng/mL); 13 patients (16%) had low blood levels, 59 (74%) were in therapeutic range while 8 (10%) had high blood concentrations. Low body mass index and the use of corticosteroids were associated with high hydroxychloroquine concentrations (p=0.03 and p=0.02 respectively). **Conclusion:** We report for the first time the blood concentration of hydroxychloroquine in Moroccan lupus patients and we found significant variability between patients for the same dose. Various factors affected this concentration and further studies are needed to expand the sample.

**Keywords:** Systemic lupus erythematosus, Hydroxychloroquine, Whole blood, Interindividual variation, Liquid chromatography, Tandem mass spectrometry.

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### INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic inflammatory and autoimmune disease occurring preferentially in women (more than 90%) and frequently starting at childbearing age. SLE can affect almost any organ system but the most frequently symptoms are cutaneous, articular, haematological and visceral [1]. Several drugs can be used for the treatment of SLE like corticosteroids, immunosuppressive agents, biological agents; but, hydroxychloroquine (HCQ) is considered as an essential therapeutic element in SLE. Indeed, this non expensive drug can prevent flares, thrombotic events, diabetes mellitus and dyslipidemia [2-5].

The usual dose of HCQ is 400 mg daily in one or two divided doses. This drug appears to be relatively safe; common side effects include headaches, dizziness, gastrointestinal upset and rash. However, HCQ may have serious side effects such as retinopathy that

imposes regular ophthalmologic monitoring for patients on long term therapy [6].

Blood concentrations of HCQ vary widely among patients with SLE, but little is known about factors that influence this blood concentration variability. Monitoring of HCQ blood concentrations in chronically treated patients helps clinicians in identifying adherence to treatment. A pharmacokinetic/pharmacodynamic relationship has been found in different situations, and a very low blood concentration of HCQ is a simple marker of nonadherence to treatment. This monitoring has been shown to contribute to predicting HCQ treatment efficacy [7-10]. HCQ blood levels can be quantified by high performance liquid chromatography coupled to several detectors such as mass spectrometry which is the most specific and sensitive detection [11-13].

The purpose of our work is to determine for the first time in Morocco, the blood concentrations of HCQ in SLE patients and identify the factors associated with interindividual variation in blood HCQ concentrations. Liquid chromatography coupled to tandem mass spectrometry was used for this determination.

## METHODS

The study involved 80 Moroccan patients with SLE. All SLE patients were followed in the department of Internal Medicine B, Mohammed V Military Teaching Hospital of Rabat, Morocco, and fulfilled at least four of eleven criteria for SLE classification [14]. All patients were treated with HCQ prescribed at a stable oral dose of 400 mg/day (200 mg twice a day) for at least 6 months. The main exclusion criteria include: pregnancy, renal failure and any patient refusing to participate in the study. The samples from all patients were taken at the same time of the day. This study was conducted according to the principles of the declaration of Helsinki and was approved by the local Ethical Committee from the Faculty of Medicine and Pharmacy, Rabat, Morocco.

For HCQ assays, blood was collected in EDTA vacutainers tube. HCQ was quantified by Acquity ultraperformance liquid chromatography coupled to a Tandem mass spectrometer (ACQUITY UPLC-TQD, Waters) in multiple reactions monitoring mode.

In a 5 mL test glass tubes, 20  $\mu$ L of internal standard solution at 1 mg/L (Milnacipran; Merck) and 100  $\mu$ L of 5% ammonia solution were added to 200  $\mu$ L of blood, calibrator or control and then vortexed for 1 min. Organic extraction was carried out by adding 2 mL of Dichloromethane/Ether (4/6), with vortexing for 1 min, then centrifuging the mixture for 5 min at 4000 rpm. The organic layer was subsequently removed and placed in maximum recovery vials (Waters Corporation, Milford, MA, USA) and dried under a stream of nitrogen at 50 °C. The samples were then reconstituted in 200 mL of mobile phase, and 5  $\mu$ L was injected into an ACQUITY UPLC-TQD, giving an injection-to-injection time of 4 min. The mass spectrometer conditions were: capillary voltage, 1.1 kV; cone voltage, 40 V; desolvation temperature, 450 °C; desolvation gas, 800 L/h; source temperature, 120 °C; and collision gas flow, 5.1  $10^{-3}$  mbar. The instrument was operated in positive electrospray ionization mode using MassLynx™ 4.1 software, with auto data processing by TargetLynx™ Application Manager (Waters Corp). Chromatographic separations were achieved using a BEH C18 UPLC column (1.7  $\mu$ m, 2.1 - 50 mm; Waters Corp). Mobile phase A comprised acetonitrile, and phase B consisted of 2 mM ammonium formate with 0.1% formic acid in water; the flow rate in all steps was 0.6 mL/min in a gradient program. For

each analytical run, calibrators and quality control samples were freshly prepared.

For statistical analysis the patients were divided according to their blood level. Levels less than 500 ng/mL were considered to have a low blood HCQ concentration, levels between 500 and 2000 ng/mL were therapeutic to have a therapeutic range and greater than 2000 ng/mL were considered high blood HCQ concentration. Statistical analysis was performed with SPSS 13.0 for windows (SPSS, Inc., Chicago, IL, USA). Depending on their normal or skewed distribution, data are reported as mean  $\pm$  standard deviation (SD) or median and full range and qualitative data as absolute numbers and percentage. Comparison between variables was performed using ANOVA test or the chi square test. The level of significance was set as  $p < 0.05$  for all analyses.

## RESULTS AND DISCUSSION

The determination of blood HCQ levels was carried out by liquid chromatography tandem mass spectrometry (Figure 1). The method was validated and found to be linear across the wide range of 20-2000 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 5 ng/mL and 20 ng/mL, respectively.

The demographics data of the group are outlined in Table 1. The analysis included 80 patients, they were for the most part female (n=77, 96%). Their mean age was  $36.9 \pm 12.2$  years. The median concentration of HCQ was 830 ng/mL (range: 35-3,200 ng/mL). The distribution of these concentrations was as follows: 13 patients (16%) had low blood levels (<500 ng/mL), of these, 6 had levels of HCQ in their blood <200 ng/mL; 59 patients (74%) were in therapeutic range while 8 (10%) patients had high blood HCQ concentrations (>2000 ng/mL) (Table 2).

There was a statistically significant difference seen when the body mass index (BMI) was compared with respect to the proportion in each hydroxychloroquine group; low BMI seems to be associated to high HCQ blood levels ( $p=0.03$ ). Cutaneous disorder, mucosal ulcers, arthritis, serositis, neuropsychiatric disorder, anemia, lymphopenia, anti double stranded deoxyribose nucleic acid antibodies, anti-Smith antibodies and antinuclear antibodies did not distinguish any differences in hydroxychloroquine level. However, the use of corticosteroids appears to increase this blood concentration ( $p=0.02$ ) (Table 2).

HCQ is a cornerstone in medical management of SLE with its affordable price and relative safety. In chronically treated patients, monitoring of HCQ concentrations helps clinicians in identifying adherence and prevents side effects which contributes to the success of the treatment [15]. In our study we measured

HCQ blood levels for 80 SLE patients with liquid chromatography-tandem mass spectrometry.

The median concentration of HCQ was 830 ng/mL with extremes between 35 and 3200 ng/mL.

It is obviously difficult to compare our result with those of other studies because of the difference in sample size, ethnicities and the diversity of therapeutic protocols. For example, Jallouli *et al.* report a median concentration of 917 ng/mL (range 208–3,316 ng/mL) for 509 patients [16]. In another study, Miceli *et al.* found a median level of 722 ng/mL (range 0-2466 ng/mL) for 55 patients [17]. The optimal blood level of HCQ to achieve efficacy in SLE is still a subject of controversy. It has been reported that low HCQ blood levels are associated with higher SLE disease activity; while HCQ levels above 1000 ng/mL tend to improve the course of the disease [7]. However, this level is often difficult to maintain.

Among our patients, 6 had levels of blood HCQ below 200 ng/mL; these patients were clearly nonadherent even if they declared apply the treatment properly. The cut-off of 200 ng/mL was suggested by further studies to distinguish nonadherent patients [16]. Non-adherence to medication and/or low blood HCQ levels are associates with poor outcomes and disease flare [18, 19].

Several factors could influence the blood level of HCQ. In addition to adherence to treatment, gender and therapeutic protocol may be factors in varying this concentration. For the same daily dose, the blood concentration of HCQ may be higher in women than in men [16]. This result was not verified in our study because of the small proportion of men compared to women.

The treatment schedule may influence the blood levels of HCQ; even if the optimal daily dose of HCQ in SLE is a subject of controversy, it has been found that patients receiving 400 mg once a day have higher blood HCQ concentration than those receiving 200 mg twice a day [16]. In our study, all patients receive 200 mg twice a day. Taking 400 mg of HCQ once a day may be more convenient for patients and help with treatment success and improvement of adherence.

Low BMI was found to be associated to high HCQ concentration (p=0.03). This finding could be explained by the large volume of distribution of HCQ; therefore, therapeutic monitoring of HQC could be particularly useful in patients with low BMI. This result has been found in other studies [16, 20, 21] and low body weight was previously found to be a risk factor for HCQ toxicity particularly ophthalmic [20, 21].

Treatment with corticosteroids seems to increase the blood concentration of HCQ (p=0.02). This result has been reported by a previous study; this may be due to a potentiating effect as suggested by Jallouli *et al.* but it remains a theory [16]. The same study reports that low platelet and neutrophil were associated to low blood HCQ concentration [16]; this founding was not revealed by our results.

This study is, to the best of our knowledge, the first of its kind in Morocco; however, the relatively small sample size was one of our limitations in this study; we suggest that multicenter approaches may be necessary to attain larger sample size.

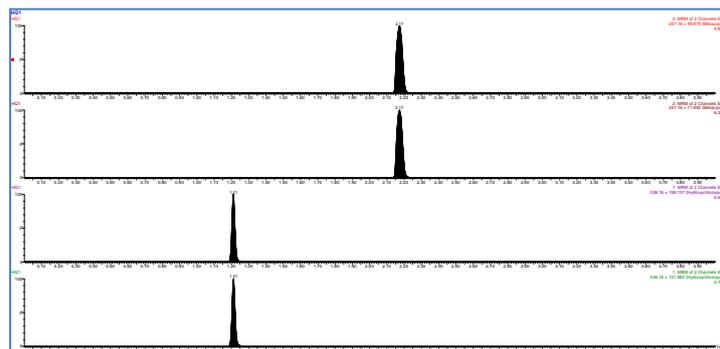


Fig-1: Chromatogram of Hydroxychloroquine (MRM transitions m/z 336>158, 336>102, retention time 1.21 min) and Milnacipran as an internal standard (MRM transitions m/z 247>72, 247>158, retention time 2.17 min)

Table-1: Characteristic and blood HCQ levels of SLE patients

	SLE
Number of subjects	80
Female/male	77/3
Age (years)	36.9±12.2
Hydroxychloroquine (ng/mL)	830 (35-3200)

**Table-2: Characteristics of patients with SLE, by blood HCQ concentrations**

	Low blood HCQ concentration (n=13, 16%)	Therapeutic range (n=59, 74%)	High blood HCQ concentration (n=8, 10%)	P
Age (years)	36.7±13.1	37.0±12.6	37.1±13.8	0.51
BMI (kg/m <sup>2</sup> )	24.9±3.2	23.7±1.9	23.1±2.3	0,03
Cutaneous disorder (68/80)	10 (15%)	51 (75%)	7 (10%)	0.43
Mucosal ulcers (10/80)	3 (30%)	6 (60%)	1 (10%)	0.35
Arthritis (77/80)	11 (14.3%)	60 (77.9%)	6 (7.8%)	0.54
Serositis (20/80)	6 (30%)	12 (60%)	2 (10%)	0.52
Neuropsychiatric disorder (22/80)	4 (18%)	16 (73%)	2 (9%)	0.16
Anaemia (24/80)	3 (12.5%)	20 (83.3%)	1 (4.2%)	0.16
Thrombocytopenia (20/80)	4 (20%)	10 (50%)	6 (30%)	0.06
Leukopenia (26/80)	4 (15.4%)	21 (80.8%)	1 (3.8%)	0.15
Lymphopenia (30/80)	5 (16.7%)	22 (66.6%)	5 (16.7%)	0.31
Anti-dsDNA (57/80)	9 (15.8%)	44 (77.2%)	4 (7%)	0.58
Anti-Sm (13/80)	2 (15.4%)	10 (76.9%)	1 (7.7%)	0.68
ANA presence (71/80)	12 (16.9%)	53 (74.7%)	6 (8.4%)	0.53
Use of corticosteroids (53/80)	3 (5.7%)	42 (79.2%)	8 (15.1%)	0,03

ANA: Antinuclear antibodies; dsDNA: double stranded deoxyribose nucleic acid; Sm: Smith

## CONCLUSION

We reported for the first time a determination of HCQ blood levels in SLE Moroccan patients. Monitoring HCQ blood levels is considered as an important tool to improve medication adherence in patients with SLE and prevent side effects. Unfortunately, this assay cannot be generalized in the Moroccan laboratory for lack of the necessary equipment, in particular the liquid chromatography tandem mass spectrometry.

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