Hydroxychloroquine Levels throughout Pregnancies Complicated by Rheumatic Disease: Implications for Maternal and Neonatal Outcomes

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ABSTRACT. ***Objective.*** Pregnancies in women with active rheumatic disease often result in poor neonatal outcomes. Hydroxychloroquine (HCQ) reduces disease activity and flares; however, pregnancy causes significant physiologic changes that may alter HCQ levels and lead to therapeutic failure. Therefore, our objective was to evaluate HCQ concentrations during pregnancy and relate levels to outcomes.

Methods. We performed an observational study of pregnant patients with rheumatic disease who were taking HCQ from a single center during 2013–2016. Serum samples were analyzed using high-performance liquid chromatography/mass spectrometry. Primary HCQ exposure was categorized as nontherapeutic (≤ 100 ng/ml) or therapeutic (> 100 ng/ml). Categorical outcomes were analyzed using Fisher's exact test and continuous outcomes using linear regression models, Wilcoxon signed-rank test, Kruskal-Wallis test, t test, and ANOVA.

Results. We analyzed 145 samples from 50 patients with rheumatic disease, 56% of whom had systemic lupus erythematosus (SLE). HCQ concentration varied widely among individuals at each trimester. Mean physician's global assessment scores in patients with SLE were significantly higher in those with average drug levels ≤ 100 ng/ml compared to > 100 ng/ml (0.93 vs 0.32, p = 0.01). Of patients with SLE, 83% with average drug levels ≤ 100 ng/ml delivered prematurely (n = 6), compared to only 21% with average levels > 100 ng/ml (n = 19; p = 0.01). HCQ levels were not associated with prematurity or disease activity in non-SLE patients.

Conclusion. With both high and low HCQ levels associated with preterm birth and disease activity in SLE, further study is necessary to understand HCQ disposition throughout pregnancy and to clarify the relationship between drug levels and outcomes. (J Rheumatol First Release October 1 2018; doi:10.3899/jrheum.180158)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS HYDROXYCHLOROQUINE PREGNANCY

In pregnant women with rheumatic disease, neonatal complications are common and include preterm birth and low birth weight[1,2]. Poor neonatal outcomes occur in pregnancies complicated by many rheumatic diseases, including systemic lupus erythematosus (SLE) and rheumatoid arthritis, among others[3]. Increased maternal disease activity during pregnancy further increases the risk for pregnancy and neonatal complications[1,3,4,5]. To control disease activity and optimize outcomes, many pregnant women with SLE are treated with hydroxychloroquine (HCQ) during pregnancy, because it can reduce disease flare rates and disease activity, allow for lower doses of corticosteroids, and reduce prematurity[3,6,7,8].

In nonpregnant populations, low systemic levels of HCQ are associated with increased disease activity, increased risk of disease flares, and therapeutic failure[9,10,11,12,13]. In clinically inactive patients with SLE, whole blood HCQ levels were the only independent predictors of disease flare, with an increase in drug levels of about 500 ng/ml predicting a 60% decreased risk of subsequent flare[9]. In addition, SLE patients with very low whole blood HCQ levels (e.g., < 100 ng/ml) are at a high risk for active disease and flares[11,14]. While whole blood is often the preferred matrix for testing HCQ concentration, serum matrices have also been studied in SLE[10]. For example, SLE patients with inactive disease trended toward lower

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disease activity scores and reduced incidence of flares when serum HCQ levels were > 500 ng/ml[10].

Despite the importance of maintaining adequate HCQ exposure, drug levels during pregnancy have not been systematically studied. Similar doses of HCQ in nonpregnant patients result in differing levels owing to high inter-individual variability[9,15]. The variability may be due to several features such as elevated body mass index, medication nonadherence, and increased renal clearance, all of which have been associated with low HCQ levels in nonpregnant SLE populations[16]. Physiologic changes in pregnancy, including dramatic increases in the volume of distribution[17], may cause additional changes in drug disposition. However, it is currently unknown whether low HCQ drug levels occur during pregnancy and whether lower drug levels correlate with outcomes. To close this knowledge gap, we performed an observational analysis of a registry of pregnant women with rheumatic diseases to identify HCQ levels throughout pregnancy and to study the effect of low drug levels on maternal disease activity and neonatal outcomes.

MATERIALS AND METHODS

Study design. This was an analysis of data collected through the Duke Autoimmunity in Pregnancy (DAP) registry, which prospectively collects biospecimens and clinical data. Serum samples from study participants were sent to a laboratory for measurement of HCQ level using validated high-performance liquid chromatography/tandem mass spectrometry. The study was conducted in compliance with the Declaration of Helsinki. The Duke institutional review board approved the study protocol (Pro00000775/Pro00000756).

Participants. From November 2013 to December 2016, patients with rheumatic disease taking HCQ prior to pregnancy were identified from the DAP registry, and serum samples were analyzed if patients continued HCQ longitudinally throughout pregnancy and provided at least 1 blood sample. Women with multiple births were excluded owing to the confounding risk of preterm birth and pregnancy complications. All included patients consented to having research blood collected.

Data collection. Registry study visits occur, on average, 2–3 times during pregnancy and once after delivery. For SLE patients, disease activity was measured by 1 rheumatologist (MEC) using the physician's global assessment (PGA) and the Systemic Lupus Erythematosus Pregnancy Disease Activity Index (SLEPDAI)[18]. The PGA for SLE is a visual analog scale ranging from 0 cm (no disease activity) to 3 cm (severe disease activity), with extensive use as a reliable outcome measure in SLE pregnancies[19,20,21,22]. In patients without SLE, disease activity was measured by the same rheumatologist (MEC) using the PGA, adjusted to represent a visual analog scale ranging from 0 mm (no disease activity) to 100 mm (highest activity). Exploratory measures of SLE disease activity included complement levels (C3/C4) and anti-dsDNA antibody levels. For analysis of neonatal outcomes, neonatal gestational age at birth was the primary outcome[21], with preterm birth (< 37 weeks) and birth weight as secondary outcomes.

HCQ definitions. Based on previous literature, our primary analyses categorized the HCQ level as nontherapeutic (≤ 100 ng/ml)[9,11] and therapeutic (> 100 ng/ml). In exploratory analyses, the therapeutic category was further categorized into suboptimal (101–500 ng/ml), or optimal (> 500 ng/ml)[10,23].

Patient perspective of HCQ adherence during pregnancy. Five patient representatives from the DAP Registry Patient Advisors and Collaborators (DAPPAC) were asked what percentage of women with SLE in pregnancy they estimated did not take prescribed HCQ reliably.

Statistical analysis. For HCQ during pregnancy, mean and median HCQ levels by trimester were measured by ANOVA and Kruskal-Wallis test, respectively, with undetectable drug levels (< 10 ng/ml) imputed as 5 ng/ml. Mean drug levels by year were measured by ANOVA. Differences in use of concomitant medications across categorical HCQ levels were measured using Fisher's exact test.

For neonatal outcomes, the difference in median gestational age at birth and birth weight by average categorical HCQ level during pregnancy was determined using the Wilcoxon signed-rank test (2 groups) or the Kruskal-Wallis test (3 groups). Differences in the prevalence of preterm birth by average HCQ level category were determined using Fisher's exact test. Nonlive births were excluded from neonatal outcome analyses. The association of pregnancy average maternal PGA and neonatal gestational age was estimated by unadjusted linear regression models.

For maternal disease activity, the effect of average HCQ levels on disease activity was analyzed using ANOVA, t test, and unadjusted linear regression models. The effect of HCQ level on C3, C4, and dsDNA was analyzed using unadjusted linear regression models per 100 ng/ml increases in HCQ level. For patients with SLE, disease activity was characterized as low (PGA ≤ 1) or high (PGA > 1). For patients without SLE, disease activity was characterized as low (PGA ≤ 25 mm) or high (PGA > 25 mm). All analyses were performed in SAS 9.4.

RESULTS

Demographics and samples. Fifty patients with 145 serum samples were included in the study (Table 1). The majority of patients had SLE (56%). Six samples (4%) were below the limit of quantitation (< 10 ng/ml). At some point during pregnancy, 28% of patients took concomitant prednisone, with an average dose recorded at each visit of 16.7 mg, while 18% took concomitant azathioprine (AZA). The most common

Table 1. Clinical characteristics (n = 50).

Characteristics	n (%) or Mean ± SD
Age, yrs	31 ± 5.2
Body mass index, kg/m^2	28.2 ± 5.6
Diagnosis	
SLE	28 (56)
RA or JIA	7 (14)
Undifferentiated CTD	5 (10)
Other*	10 (20)
Race	
White	32 (64)
Black/African American	16 (32)
Other	2 (4)
Corticosteroids	
Use, concomitant	14 (28)
Average dose, mg	16.7 ± 11
Maximum dose, mg	21.3 ± 19
AZA use, concomitant	9 (18)
Disease activity	
SLE PGA (0–3 cm)	0.5 ± 0.6
Non-SLE PGA (0–100 mm)	11.9 ± 12.7

Categorical variables listed as n (%); continuous variables listed as mean ± SD. * Other diagnoses included antiphospholipid antibody syndrome (n = 1), autoimmune hepatitis (n = 1), cutaneous SLE (n = 2), psoriatic arthritis (n = 1), SSc (n = 1), Sjögren syndrome (n = 3), and positive ANA/Ro (n = 1). SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; JIA: juvenile idiopathic arthritis; CTD: connective tissue disease; AZA: azathioprine; PGA: physician's global assessment; ANA: antinuclear antibody; SSc: systemic sclerosis.

total daily HCQ dose was 400 mg, while the median weight-based dose was 5.1 mg/kg (IQR 4.3–5.5). Because of weight gain, 1 patient had HCQ dosage increased during pregnancy.

Of subjects with SLE and available testing, 18 (64.3%) were Ro-positive, 7 (25%) were La-positive, and 12 (44.4%) were RNP-positive. No subjects had antiphospholipid antibody syndrome or triple antiphospholipid antibody positivity; 1 subject (3.8%) had a positive β2-glycoprotein IgM or IgG, 1 subject (3.7%) was positive for anticardiolipin IgM or IgG, and 1 subject (3.8%) was positive for lupus anticoagulant. Seven subjects (25%) had a history of confirmed or suspected lupus nephritis (LN); 3 of whom (10.7%) had active nephritis during pregnancy. The highest observed creatinine for any subject during pregnancy was 1 mg/dl.

HCQ matrix stability. No sample degradation occurred over time. For the most common dosing (400 mg), the mean HCQ level (± SD) was 420 ng/ml in 2013 (n = 1), 412 ng/ml (286) in 2014 (n = 44), 377 ng/ml (198) in 2015 (n = 42), and 355 ng/ml (229) in 2016 (n = 40, p = 0.7).

HCQ levels during pregnancy. There was significant variability in serum HCQ concentrations among individuals, resulting in a wide range of concentrations at all doses and at each trimester (Table 2). The median HCQ level for patients taking the same dose (400 mg) during pregnancy did not differ between those with SLE (355 ng/ml) and without it (345 ng/ml, p = 0.8). Twelve of 50 patients (24%) had at least 1 HCQ level ≤ 100 ng/ml during pregnancy, suggesting HCQ nonadherence. Seven of these women had average HCQ levels ≤ 100 ng/ml throughout pregnancy.

Patient perspective of HCQ adherence during pregnancy. The women in the DAPPAC predicted poor adherence in the study population, estimating that 20% of women with SLE would not be taking HCQ, 30% would be taking it inconsistently, and only 50% would be taking it reliably.

Neonatal outcomes. There were 2 neonatal losses (1 mother with autoimmune hepatitis and 1 with SLE) and 2 pregnancies with unknown neonatal outcomes (both mothers with SLE); resulting in 46 total live births with known outcomes (25 SLE and 21 non-SLE). Among the pregnancies in women with SLE, those with average drug levels ≤ 100 ng/ml had a higher frequency of preterm delivery (p = 0.01) and lower gestational ages at birth than those with levels > 100 ng/ml (p = 0.03; Table 3). Neonatal outcomes in women without SLE did not significantly differ based on HCQ level.

When drug levels were further stratified, the median neonatal gestational age in patients with SLE was highest in subjects with average HCQ levels 101–500 ng/ml (Table 3). Among 5 women with SLE and HCQ > 500 ng/ml, 4 delivered preterm. There was no linear association between gestational age and average HCQ level as a continuous variable.

For patients with SLE, neonatal gestational age was significantly lower in mothers with high disease activity compared to those with low disease activity, median (IQR) 29.5 (27–30) weeks versus 38 (37–39) weeks, respectively (p = 0.002). When PGA was analyzed as a continuous variable, neonatal gestational age decreased by 4.9 weeks for every 1 unit increase in average PGA ($p < 0.0001$, $R^2 = 0.5$). However, in non-SLE patients, disease activity was not associated with neonatal gestational age.

Of the mothers with active LN during pregnancy (n = 3), all infants were born ≤ 30 weeks. Two of these pregnancies had an average HCQ level ≤ 100 ng/ml while 1 had an average HCQ level > 500 ng/ml. Of the mothers with a history of proven or suspected LN that was not active during pregnancy (n = 4), one delivered at 25 weeks while the remainder delivered at ≥ 36 weeks.

Maternal disease activity by HCQ level. SLE patients with average HCQ ≤ 100 ng/ml had higher disease activity compared to those with average HCQ > 100 ng/ml, with an

Table 2. HCQ levels throughout pregnancy.

Trimester	Total Daily Dose, mg	n (samples)	HCQ Level, ng/ml Mean (SD)	HCQ Level, ng/ml Range	HCQ Level, ng/ml Median (IQR)
1st	200	2	158 (116)	76–240	158 (76–240)
	300	5	219 (131.6)	< 10–350	250 (200–290)
	400	17	440.6 (281.3)	< 10–1000	430 (260–500)
	Overall	24	370.9 (266.8)	< 10–1000	320 (220–485)
2nd	200	2	290 (113.1)	210–370	290 (210–370)
	300	5	350 (160.3)	190–520	300 (220–520)
	400	53	373.7 (238.6)	< 10–1100	370 (220–500)
	Overall	60	369 (228.9)	< 10–1100	365 (215–505)
3rd	300	3	160 (120)	40–280	160 (40–280)
	400	31	319 (194.6)	< 10–900	310 (230–380)
	Overall	34	304.9 (193.4)	< 10–900	300 (230–370)
Postpartum	400	26	438.1 (255)	< 10–1100	385 (310–600)
	457*	1	48	48	48
	Overall	27	423.6 (261.1)	< 10–1100	360 (310–600)

* Participant reported taking 400 mg 5 days/week and 600 mg 2 days/week. HCQ: hydroxychloroquine; IQR: interquartile range.

Table 3. Neonatal outcomes* by average HCQ level.

	HCQ, ≤ 100 ng/ml	HCQ, 101–500 ng/ml	HCQ, > 500 ng/ml	p**	p***
All patients	n = 7	n = 32	n = 7		
GA, weeks	36 (30–37)	39 (38–39)	36 (29–37)	**< 0.01**	**< 0.0001**
Preterm birth	5 (71)	2 (6)	4 (57)	**< 0.01**	**< 0.0001**
Birth weight, g	2451 (1530–2580)	3118 (2835–3629)	2175 (915–2750)	0.07	**0.003**
SLE patients	n = 6	n = 14	n = 5		
GA, weeks	35.5 (30–36)	38.5 (38–39)	35 (29–36)	**0.03**	**0.0003**
Preterm birth	5 (83)	0 (0)	4 (80)	**0.01**	**< 0.0001**
Birth weight, g	2347 (1530–2555)	3147 (2665–3685)	1885 (915–2505)	0.3	**0.02**
Non-SLE patients	n = 1	n = 18	n = 2		
GA, weeks	37	39 (37–39)	37.5 (37–38)	0.4	0.4
Preterm birth	0 (0)	2 (11)	0 (0)	1	1
Birth weight, g	2580	3118 (2977–3345)	2877.5 (2175–3580)	0.2	0.4

Categorical variables listed as n (%); continuous variables listed as median (IQR). * N = 46; 2 pregnancies ended in nonlive births and 2 pregnancies had unknown neonatal outcomes. ** Comparing ≤ 100 ng/ml vs > 100 ng/ml. *** Comparing ≤ 100 ng/ml vs 101–500 ng/ml vs > 500 ng/l. Values in bold face are statistically significant. HCQ: hydroxychloroquine; GA: gestational age at birth; SLE: systemic lupus erythematosus; IQR: interquartile range.

average PGA of 0.93 versus 0.32, respectively (p = 0.01; Figure 1). There was no significant difference between disease activity and HCQ level in non-SLE patients.

When drug levels were further stratified, the average PGA was lowest in SLE patients with average drug levels 101–500 ng/ml (0.23 ± SD 0.35, n = 16) compared to those with > 500 ng/ml (0.56 ± SD 0.65, n = 6) and ≤ 100 ng/ml (0.93 ± SD 0.65, n = 6; p = 0.02). When drug levels were analyzed as a continuous variable, the average SLE PGA decreased by 0.01 for every 100 ng/ml increase in HCQ (p < 0.0001), however, the correlation was weak (R^2 = 0.07; Appendix 1).

There was no association between HCQ level and SLEPDAI, or between HCQ level and C3, C4, and dsDNA antibody.

Concomitant medications. Concomitant use of AZA was higher in SLE subjects with average HCQ > 500 ng/ml (n = 5, 83.3%) compared to those with HCQ 101–500 ng/ml (n = 2, 12.5%) and HCQ ≤ 100 ng/ml (n = 1, 16.7%; p = 0.006). Otherwise, prednisone use was not significantly different among SLE groups. Among non-SLE subjects, there was no significant difference in AZA or prednisone use by categorical HCQ levels.

Pregnancy complications. Eight SLE mothers with preterm deliveries were induced because of pregnancy complications; 4 of these mothers had average HCQ levels ≤ 100 ng/ml and 4 had average HCQ > 500 ng/ml. The most common pregnancy complications leading to induction in SLE were maternal hypertension or preeclampsia (n = 7), infant not

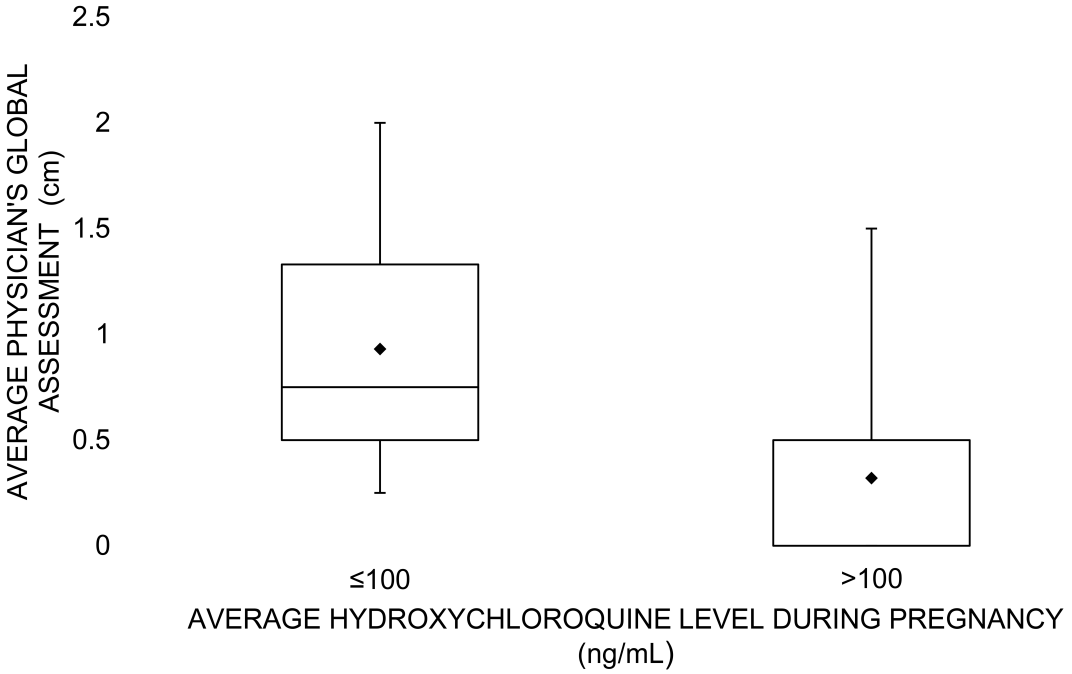

Figure 1. Systemic lupus erythematosus disease activity by hydroxychloroquine level.

being well (n = 4), and maternal disease activity (n = 3). Other rare complications included cholestasis (n = 1) and pericardial effusion (n = 1). No mothers without SLE required a preterm induction of labor.

DISCUSSION

To our knowledge, we present the first longitudinal study of HCQ drug levels during pregnancy. Very low HCQ levels consistent with nonadherence (≤ 100 ng/ml) were found in 24% of pregnancies, with 14% having average levels persistently low. This finding is highly consistent with estimates of nonadherence (about 1 in 5) observed using HCQ blood levels in a prospective international study[24], as well as our patient estimates of adherence. We observed a high frequency of preterm birth and lower neonatal gestational ages in women with SLE and either HCQ ≤ 100 ng/ml or > 500 ng/ml. Otherwise, in women without SLE, the HCQ level did not clearly correlate with neonatal outcomes. In addition, our study confirmed the significant relationship between high maternal disease activity in SLE and lower neonatal gestational age. All the severely preterm births (≤ 30 weeks) occurred in SLE patients with high disease activity.

Mothers with SLE have a higher risk for poor neonatal outcomes compared to other rheumatic diseases; therefore, we analyzed neonatal outcomes separately in SLE and non-SLE patients. We found that SLE patients with very low HCQ levels (≤ 100 ng/ml) had more preterm births and babies born at a lower gestational age than those with HCQ levels > 100 ng/ml. Because we observed the highest neonatal gestational age in SLE groups with the lowest disease activity and fewest pregnancy complications, the higher preterm delivery in patients with SLE may be related to disease activity. For example, 2 out of the 3 mothers with active LN had average drug levels ≤ 100 ng/ml and severe preterm births. Alternatively, low drug levels may be associated with other factors (e.g., poor medication and healthcare adherence)[11] that could also contribute to preterm birth. Although using different assays, most studies define nonadherence as whole blood HCQ levels < 100–200 ng/ml[14,24,25,26]. Because low HCQ levels likely identify severe nonadherence[24], identifying medication nonadherence becomes critical to patient management by affording the opportunity to improve adherence through counseling. For example, in the Hopkins Lupus Cohort, only 56% of patients were completely adherent with HCQ at baseline, and through routine check of medication levels and counseling, adherence increased to 80%[23]. Poor medication adherence in subjects with SLE has also been associated with depression and emergency room visits[27,28]. Therefore, with additional data, there may be a future role for serum HCQ drug monitoring during pregnancy to identify SLE mothers nonadherent with HCQ.

We also observed a high rate of preterm birth among women with SLE and HCQ > 500 ng/ml, likely because of concurrent medical complications during pregnancy. AZA use was also more common in SLE subjects with average HCQ > 500 ng/ml, which may be a marker for baseline disease severity. The pregnancy with the highest HCQ level delivered at 25 weeks because of active SLE and LN in the setting of placental abruption and severe HELLP syndrome (hemolysis, elevated liver enzymes, low platelets). Two additional pregnancies with average HCQ > 500 ng/ml and LN delivered at 29 weeks (nephritis active during pregnancy) and 36 weeks (nephritis not active during pregnancy). Conversely, the other 2 SLE patients with average HCQ levels > 500 ng/ml and no history of nephritis delivered at 35 weeks and 37 weeks. This underscores that preterm birth in SLE is multifactorial. Because sample sizes were low within subgroups (e.g., HCQ levels ≤ 100 ng/ml and > 500 ng/ml), an outlier effect is also possible. While we considered the possibility of a direct negative effect of HCQ on birth outcomes at high drug levels, we reassuringly did not observe a higher incidence of preterm birth in non-SLE patients with drug levels > 500 ng/ml. Overall, HCQ levels were not associated with neonatal outcomes in non-SLE patients. The lack of association in non-SLE patients is likely because of heterogeneity in underlying disease in this subgroup, whereby HCQ may not have contributed equally to disease control.

Defining an optimal "therapeutic window" for HCQ levels remains challenging. First, the use of different matrices (e.g., whole blood vs serum) limits comparisons of drug levels between studies. Second, HCQ has several pharmacodynamic effects, including altering antigen presentation and MHC class II expression, reduced cytokine production and lymphocyte proliferation, and control of toll-like receptors, among others[15]. It is likely that these effects are mediated at different HCQ concentrations, and further, differences in disease phenotypes and severity may also dictate response to HCQ at a given level. Despite these limitations, studies confirm an exposure-response relationship between HCQ levels and rheumatic disease activity. For example, 1 study found that in SLE patients without active disease at baseline, those who had subsequent disease flares had lower mean whole blood HCQ levels at baseline (703 ± 534 ng/ml) compared to those who did not have flares (1128 ± 507 ng/ml)[9,11]. This study defined an optimal whole blood HCQ level as ≥ 1000 ng/ml; however, a subsequent clinical trial targeting HCQ levels ≥ 1000 ng/ml did not reduce flare rates except in a subset without early flares who maintained target levels throughout followup[29]. Other studies further support a relationship between whole blood HCQ levels, cutaneous SLE activity[30], and SLE Disease Activity Index[25]. In our study of pregnant women, we found significant associations between drug levels and disease activity in SLE patients, with the highest disease activity observed in those with drug levels ≤ 100 ng/ml and the lowest disease activity with drug levels between 101–500 ng/ml. However, because of lower sample sizes within subgroups and possible outliers, further investigation is necessary to characterize the exposure-response

relationship of HCQ in SLE pregnancies. We also did not observe an association between HCQ levels and either SLEPDAI or biomarkers of SLE disease activity; the latter observation may be due to heterogeneity in SLE phenotypes, whereby not all subjects manifested active disease by a change in biomarkers.

There are several potential limitations with our study, including the sample matrix. Although our assay was validated only for frozen serum samples ≤ 12 months of age, our data suggest no significant sample degradation occurred. Further, while whole blood testing for HCQ has historically been considered more precise[11], whole blood was not available in the DAP registry. However, generalization is possible with other cohorts that have used serum HCQ assays[10]. In addition, red blood cells, platelets, and leukocytes sequester HCQ[16,25,31]. As a result, whole blood measurements quantify HCQ concentration in these cells as well as the bound and unbound (biologically active) drug in the vascular compartment. Therefore, cytopenias from SLE may reduce the effective "compartment size" for HCQ and potentially lower whole blood concentrations of HCQ. Serum measurements, which quantify only the bound and unbound drug in the vascular space, may be less prone to confounding from cytopenias in active rheumatic diseases, or from the physiologic anemia of pregnancy. As an example, tacrolimus, which also partitions into red blood cells, can become supratherapeutic when whole blood drug levels instead of plasma (or unbound drug) levels are adjusted to maintain target concentrations during pregnancy[32].

Several assumptions were necessary for our analysis. We enrolled individuals taking HCQ prior to pregnancy, assuming they were at steady-state concentration. On review of available medical records, we confirmed that over half were taking HCQ for > 1 year, with only 4 participants taking HCQ < 3 months. It is possible some participants were not reliably taking the prescribed dose before or during pregnancy and therefore were not at steady state. While subjects were routinely asked about medication use during physician interview, we did not use a validated tool to directly measure adherence. Further, administration times were not available in the registry, precluding the ability to account for time effects since last dose in the drug levels. Despite this potential limitation, most studies suggest the within-day and inter-day variability of HCQ levels are small owing to the drug's long half-life[9,13]. Although a recent report found HCQ levels were associated with administration time, the magnitude of the effect is unclear[25]. Lastly, outlier drug levels and suspected medication nonadherence increased variability in drug concentrations.

Observational studies have inherent design limitations, including confounding. We observed that maternal disease activity was strongly associated with gestational age at delivery in SLE, but because of the low sample sizes within subgroups, we could not stratify drug levels and preterm birth/gestational age at birth by disease activity. Further, it is possible that observed associations between categorical drug exposure and outcomes could represent artifact from the cutoffs chosen for each group. Therefore, future studies should use a larger sample size, stratify by disease activity, and consider using modeling to clarify the relationship between HCQ drug levels, disease activity, and neonatal outcomes.

In this cohort of patients, we observed very low drug levels consistent with nonadherence in almost one-quarter of participants. HCQ levels in women with SLE were associated with the PGA of disease activity, but no other measures of SLE or non-SLE activity. In addition, disease activity and pregnancy complications were significantly associated with preterm birth and low neonatal gestational age in patients with SLE, underscoring the importance of controlling active disease during pregnancy. While drug levels did not correlate with preterm birth in non-SLE patients, both higher and lower HCQ levels were associated with preterm birth and lower neonatal gestational age in women with SLE. The association between HCQ levels and both disease activity and pregnancy outcomes in SLE underscores the importance of further work to understand the pharmacokinetics, pharmacodynamics, and optimal exposure of HCQ during pregnancy.

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APPENDIX 1. Disease activity in systemic lupus erythematosus per 100 ng/ml hydroxychloroquine increase.