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Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine

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Abstract

Background—Based on the potential involvement of Toll-like receptor (TLR) signalling in the pathogenesis of neonatal lupus (NL), it was hypothesised that fetal exposure to hydroxychloroquine (HCQ), a TLR inhibitor, might reduce the risk of anti-SSA/Ro/SSB/La antibody-associated cardiac manifestations of NL (cardiac-NL).

Methods—Cardiac-NL children (N=50) and controls (N=151) were drawn from the following overlapping pregnancy studies: Research Registry for NL; PR Interval and Dexamethasone Evaluation in Cardiac-NL; and Predictors of Pregnancy Outcomes: Biomarkers in Antiphospholipid Syndrome and Systemic Lupus Erythematosus (SLE). Pregnancies met the following inclusion criteria: documentation of maternal anti-SSA/Ro/SSB/La antibodies at pregnancy, confirmation of medication use and child's outcome, a diagnosis of SLE before pregnancy and birth by 31 December 2007.

Results—Seven (14%) of the cardiac-NL children were exposed to HCQ compared with 56 (37%) of the controls (p=0.002; OR 0.28; 95% CI 0.12 to 0.63). Cases and controls were similar with respect to demographic and antibody status. Multivariable analysis adjusting for birth year, maternal race/ethnicity, antibody status, non-fluorinated steroid use and prior cardiac-NL risk yielded an OR associated with HCQ use of 0.46 (95% CI 0.18 to 1.18; p=0.10).

Conclusion—This case–control study suggests that, in mothers with SLE with anti-SSA/Ro/SSB/La antibodies, exposure to HCQ during pregnancy may decrease the risk of fetal development of cardiac-NL. Prospective studies are needed for confirmation.

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INTRODUCTION

Neonatal lupus (NL) represents a pathological manifestation of passively acquired autoimmunity. Maternal autoantibodies, regardless of health status, reactive with the ribonuculeoproteins SSA/Ro and/or SSB/La are almost universally present in cases of isolated fetal heart block.[1] The cardiac manifestations of NL (cardiac-NL) are well characterised and include conduction disease and life-threatening cardiomyopathy.[12] Cardiac-NL is associated with significant morbidity and mortality. [13] Prospective studies of anti-SSA/Ro positive women without previously affected pregnancies have shown an estimated 2% risk of cardiac-NL.[4–6] The recurrence rates in subsequent pregnancies are 10-fold higher.[17] Despite monitoring at-risk fetuses and immediate treatment of conduction abnormalities, complete block has never been reversed.

This irreversibility supports the need for prevention, best formulated based on the pathophysiology of disease. While the mechanism of antibody-mediated cardiac damage is not fully delineated, it has been posited that Toll-like receptor (TLR) activation may promote cardiac inflammation and scarring.[8] This suggests that hydroxychloroquine (HCQ), which inhibits endosomal acidification required for optimal TLR signalling[9] and is a medication used by patients with systemic lupus erythematosus (SLE) even during pregnancy, may prevent cardiac injury. Accordingly, this study addressed the hypothesis that mothers continuing HCQ treatment throughout pregnancy have a decreased risk of having a child with cardiac-NL by mining three of the largest well-characterised studies of pregnant anti-SSA/Ro-SSB/La antibody positive women with SLE.

METHODS

Study population

Pregnancies resulting in cases (cardiac-NL) and controls (non-cardiac-NL) were identified from three overlapping sources: Research Registry for Neonatal Lupus (RRNL)[1]; PR Interval and Dexamethasone Evaluation (PRIDE) in cardiac-NL[410]; and Predictors of Pregnancy Outcomes: Biomarkers in Antiphospholipid Syndrome and Systemic Lupus Erythematosus (PROMISSE). Each database has IRB approval for evaluation of de-identified information. All pregnancies present in two of the studies were identified and counted once.

Since patients with SLE were more likely to be prescribed HCQ than other anti-SSA/Ro-SSB/La positive patients, the analysis was limited to mothers who had a diagnosis of SLE at the time of pregnancy to minimise potential biases due to confounding by indication.

Inclusion/exclusion criteria

Pregnancies were included if all the following criteria were met: (1) documentation of maternal antibodies reactive with SSA/Ro and/or SSB/La at the time of or prior to pregnancy from either NYU or another CLIA approved laboratory (see Appendix to Methods in online supplement); (2) confirmation of the child's outcome based on medical records; (3) information on medications used and health status during pregnancy based on questionnaires (regarding signs and symptoms of SLE) and medical records; (4) birth of child by 31 December 2007; (5) a rheumatologist's diagnosis of SLE reported in the medical records prior to conception (see Appendix to Methods in online supplement).

Study design, outcome measure and data collection

This was a case–control study to determine whether exposure to HCQ reduced the risk of cardiac-NL. The primary outcomes (cardiac-NL and non-cardiac-NL) have been previously

defined.[7] A pregnancy was considered exposed to HCQ if the mother took ≥200 mg/day throughout pregnancy. A pregnancy was considered unexposed if HCQ was never taken or was discontinued at the knowledge of pregnancy.

The prior risk of developing cardiac-NL was estimated based on previous pregnancy outcomes and categorised as 2% if no prior affected child,[4–6] 13–18% if a prior child had a cutaneous manifestation of NL,[11] 18–20% if a prior child had cardiac-NL[17] and 50% if two prior children had cardiac-NL.[7] Additional data collected included maternal anti-SSA/Ro-SSB/La antibody status and treatments including plasmapheresis, azathioprine, intravenous immunoglobulin (IVIG) and steroids (fluorinated or nonfluorinated) given either before the diagnosis of cardiac-NL or, if non-cardiac-NL, before 30 weeks of gestation (given the unlikelihood of cardiac-NL being first detected after this time).[1]

Statistical analysis

Generalised linear models were fit to the data using the statistical software program SAS V. 9.1 (SAS Institute, Cary, North Carolina, USA) to evaluate the effects of HCQ use on the risk of cardiac-NL and to compare demographic and clinical characteristics between cardiac-NL cases and controls. Because multiple children from the same family were included in the analysis, generalised estimating equations (GEE) were used to account for within-family correlation in the data.[12] Two-sided p values <0.05 were considered statistically significant.

RESULTS

Patient demographics, maternal autoantibody status and medication use

Two hundred and one children met the inclusion criteria, 50 cardiac-NL (cases) and 151 non-cardiac-NL (controls) (table 1). Of the 50 cases, 46 had heart block (43 second or third degree, 3 first degree) and four had an isolated cardiomyopathy. Of the three cases with first degree heart block, two reversed following dexamethasone (one had an accompanying cardiomyopathy) and one remained in first degree at 3 years. Of the controls, 123 were unaffected, 25 had cutaneous-NL and 3 had isolated hepatic/haematological manifestations.

The demographic characteristics, antibody status, risk of NL and immunosuppressive medications are shown in table 2 and, except for year of birth and use of non-fluorinated steroids, were not significantly different between cases and controls. Specifically, cardiac-NL children were more likely to have been born before 2000 than non-cardiac-NL children ($p<0.0001$) and their mothers were less likely to have been exposed to non-fluorinated steroids before 30 weeks ($p=0.05$). There were no differences in the use of plasmapheresis, azathioprine or IVIG between cases and controls (data not shown).

Evaluation of overall HCQ exposure

Sixty-three of the 201 children were exposed to HCQ, 7 (14%) of the cardiac-NL cases and 56 (37%) of the controls, corresponding to an estimated OR for cardiac-NL associated with HCQ use of 0.28 (95% CI 0.12 to 0.63; $p=0.002$). Most cardiac-NL cases came from the RRNL cohort where HCQ use was infrequent (15.7%). In pregnancies from the PRIDE and PROMISSE cohorts, HCQ use was greater (44.7% and 59.6%, respectively). In addition to pregnancies in which the mother was not exposed to HCQ at any time during pregnancy, the following five pregnancies were considered unexposed: four mothers discontinued HCQ upon conception with two subsequently developing cardiac-NL and one mother who began HCQ after 11 weeks of pregnancy and had a non-cardiac-NL child. There was no difference in the dosage of HCQ between cases and controls (table 2).

Analysis of HCQ effects by birth year and non-fluorinated steroid use

Additional analyses were performed to assess whether the observed protective effect of HCQ may have been confounded by other factors. For example, fewer cardiac-NL cases were born after 2000: 20 (40%) compared with controls 106 (70.2%), p<0.0001. Birth year was highly correlated with HCQ use: 4 (5.3%) of 75 pregnancies prior to 2000 were exposed to HCQ compared with 59 (46.8%) of 126 pregnancies occurring during or after 2000 (p<0.0001).

In analyses stratified by birth year, HCQ remained associated with a reduced risk of cardiac-NL. However, the magnitude of the protective effect was reduced and the results did not attain statistical significance. Among births during or after 2000, 6 (30%) of 20 cardiac-NL cases were exposed to HCQ compared with 53 (50%) of 106 in the non-cardiac-NL group (OR 0.43; 95% CI 0.16 to 1.14; p=0.09). The estimated OR was similar in the subset of births prior to 2000 (OR 0.48; 95% CI 0.09 to 2.6; p=0.39). The overall OR adjusted for birth year was 0.44 (95% CI 0.19 to 1.03; p=0.06).

Maternal exposure to non-fluorinated steroids before 30 weeks was less likely among cases than in controls (28% vs 44%; p=0.05) and was positively correlated with HCQ use (p=0.005). In analyses stratified by exposure to non-fluorinated steroids, HCQ remained associated with a lower risk of cardiac-NL regardless of exposure, with an overall OR adjusted for non-fluorinated steroid use of 0.31 (95% CI 0.13 to 0.73; p=0.007).

Multivariable analyses

In a multivariable GEE model fit to the combined data which adjusted for birth period (<2000 vs ≥2000), race/ethnicity, antibody status, non-fluorinated steroid use and prior cardiac-NL risk, the estimated OR for cardiac-NL associated with HCQ was 0.46 (95% CI 0.18 to 1.18; p=0.10) (table 3). Although HCQ use was no longer a statistically significant predictor of cardiac-NL status, the estimate of the OR remained in the direction of a protective effect, consistent with the results from the overall unadjusted analysis and the above subgroup analyses.

Evaluation of pregnancies subsequent to a cardiac-NL case

The recurrence rate of cardiac-NL in this study was 24.2%. None of the 8 subsequent pregnancies that developed cardiac-NL were exposed to HCQ. Of the 25 subsequent pregnancies that did not develop cardiac-NL, 3 were exposed to HCQ, limiting analysis of the influence of HCQ on recurrence rate.

DISCUSSION

Initial exploration into the potential protective effect of HCQ considered all mothers with anti-SSA/Ro antibodies regardless of health status.[13] Although the major risk factor for developing cardiac-NL is the presence of specific autoantibodies, the use of HCQ is influenced by maternal diagnosis. The study was therefore limited to mothers with SLE, which reduced the number of cardiac-NL children. The OR in mothers taking HCQ showed a 70% risk reduction. However, non-cardiac-NL children were more likely to be born during or after the year 2000 and their mothers were more likely to be taking non-fluorinated steroids. Using a multivariable GEE model to account for these and other potential confounders, the estimated risk remained decreased by nearly 50% but was no longer statistically significant.

Antimalarial drugs are frequently prescribed to patients with SLE and have been shown to prevent flares during pregnancy.[14,15] Despite accumulating evidence supporting HCQ use

during pregnancy, physicians and patients may remain reluctant to prescribe or take HCQ given isolated case reports of toxicity from chloroquine and quinine.[16,17] Supporting this concern, four patients discontinued HCQ once pregnant, and it remains unclear how many may have done so while planning a pregnancy. The current FDA designation of HCQ is pregnancy category risk C. However, recent literature supports HCQ safety during pregnancy.[18–20] Since the current study was retrospective, evaluation of adverse fetal effects is limited.

A third of the patients in this study were taking HCQ. Although cardiac-NL occurred in each database, most were from the RRNL cohort where HCQ use was infrequent compared with the other two sources. This observation raises the possibility of fundamental differences between groups not accounted for by adjustment for birth year and other patient characteristics, which could explain the finding that HCQ may be protective. While the diagnosis of SLE was based on the rheumatologist's judgement which, coupled with the patient questionnaire, resulted in four criteria; the absence of all criteria specifically listed in the medical records was a potential limitation of the study.

In conclusion, this case–control study suggests that HCQ use in mothers with SLE and anti-SSA/Ro-SSB/La antibodies may reduce the risk of fetal cardiac-NL. Confirmation with larger prospective studies is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Outcome of children

Cardiac-NL (N=50)	**N (%)**
First degree heart block	3 (6%)
Second/third degree heart block	43 (86%)
Isolated cardiomyopathy	4 (8%)
Non-cardiac-NL (N=151)	
Unaffected	123 (81.4%)
Isolated hepatic/haematological NL	3 (2.0%)
Cutaneous NL	25 (16.6%)

NL, neonatal lupus.

Table 2

Clinical and demographic characteristics of cardiac-NL cases and non-cardiac-NL controls

	Cardiac-NL (N=50)	Non-cardiac NL (N=151)	p Value
Age of mother at time of birth (years)	31.4±4.8	31.5±4.8	0.96
Race/ethnicity			0.30
White	33 (66.0%)	87 (57.6%)	
Black	9 (18.0%)	21 (13.9%)	
Hispanic	6 (12.0%)	17 (11.3%)	
Asian	2 (4.0%)	18 (11.9%)	
Other/NA	0 (0%)	8 (5.3%)	
Antibody status			0.49
Anti-Ro+/La+	30 (60.0%)	83 (55.0%)	
Anti-Ro+/La−	19 (38.0%)	66 (43.7%)	
Anti-Ro−/La+	1 (2.0%)	2 (1.3%)	
Sex of child			0.31
Male	25 (50.0%)	82 (54.3%)	
Female	24 (48.0%)	61 (40.4%)	
NA	1 (2.0%)	8 (5.3%)	
Medication			
HCQ use during entire pregnancy	7 (14.0%)	56 (37.1%)	0.002
HCQ dosage (per day)	342.9±97.6	336.5±90.7	0.86
Fluorinated steroids			
Total patients taking	26 (52.0%)	5 (3.3%)	<0.0001
Patients taking prior to cardiac-NL or 30 weeks gestation	0 (0%)	5 (3.3%)	0.33
Non-fluorinated steroids			
Total patients taking	22 (44.0%)	66 (43.7%)	0.97
Patients taking prior to cardiac-NL or 30 weeks gestation	14 (28.0%)	66 (43.7%)	0.05
Pregnancies with no prior affected child	39 (78.0%)	110 (72.9%)	0.45
Number of children born ≥year 2000	20 (40.0%)	106 (70.2%)	<0.0001

Age and hydroxychloroquine dosage are presented as mean±SD. All other data are reported as N (%).

HCQ, hydroxychloroquine; NL, neonatal lupus.

Table 3

Results of multivariable generalised estimating equations (GEE) analysis

Predictor variable	Adjusted OR (95% CI)
HCQ use	
No	1
Yes	0.46 (0.18 to 1.18)
Birth period	
≥2000	1
<2000	3.28 (1.45 to 7.45)
Non-fluorinated steroid use	
No	1
Yes	0.55 (0.25 to 1.19)
Race	
Caucasian	1
Non-Caucasian	1.03 (0.50 to 2.14)
Antibody status	
Anti-Ro+/La+ or Anti-Ro−/La+	1
Anti-Ro+/La−	1.16 (0.59 to 2.32)
Prior child with NL	
No	1
Yes	0.49 (0.20 to 1.17)

Estimates of adjusted OR and 95% CIs from a multivariable GEE model including hydroxychloroquine use, birth period, non-fluorinated steroid use, race, antibody status and prior child with NL as predictor variables.

HCQ, hydroxychloroquine; NL, neonatal lupus.