Paper

Risk factors for early severe preeclampsia in obstetric antiphospholipid syndrome with conventional treatment. The impact of hydroxychloroquine

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Abstract

Objective: The first aim was to retrospectively identify risk factors for the development of early severe preeclampsia (sPE) in patients with obstetric antiphospholipid syndrome (OAPS) who received conventional treatment (CT). The second aim was to evaluate the impact of hydroxychloroquine (HCQ) in preventing early sPE among a subgroup of patients considered at high risk.

Methods: A total of 102 women diagnosed with OAPS and treated with CT since the diagnosis of pregnancy were selected. At the end of pregnancy, we identified risk factors associated with early sPE. According to these risk factors, we collected a new cohort of 42 patients who presented high-risk factors for developing early sPE and split them into two groups according to the treatment received: group A, CT (30 patients); and group B, CT+HCQ (12 patients). We evaluated and compared pregnancy outcomes in both groups.

Results: According to the multivariate analysis, risk factors associated with early sPE and CT were triple positivity for antiphospholipid antibodies (aPL) (OR = 24.70, [4.27–142.92], $p < 0.001$) and a history of early sPE (OR = 7.11, [1.13–44.64], $p = 0.036$). A low-risk aPL profile was associated with a good response to CT in preventing early sPE (OR = 0.073, [0.014–0.382], $p = 0.002$). High-risk patients treated with CT+HCQ had a significantly lower early sPE rate than those treated with CT only (8.3% vs 40.0%; $p = 0.03$).

Conclusion: Triple positivity for aPL and a history of early sPE are potential strong risk factors for the development of early sPE. HCQ might be an interesting therapeutic option for patients with high-risk factors for early sPE.

Keywords

Pregnancy, pregnancy loss, antiphospholipid antibodies, low-molecular-weight heparin, hypertensive disorders

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Introduction

Currently, preeclampsia (PE) is a chief cause of maternal and neonatal morbidity/mortality worldwide, affecting 2 to 5% of pregnant women. Reducing the risk of PE during pregnancy remains a major challenge for scientists.[1–4]

According to a meta-analysis of 25.356.688 pregnancies, women with antiphospholipid syndrome (APS) had the highest pooled rate of PE (17.3%, 95% CI [6.8%-31.4%]).[5] In fact, in pregnant women with APS, PE usually occurs with a severe phenotype and at early stages, even before fetal viability; hence, early severe PE (sPE) is associated with higher

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perinatal mortality.[6,7] Despite the controversy over the considerable heterogeneity and variability among the results of studies showing an association between the presence of antiphospholipid antibodies (aPL) and PE,[6,8] a recent prospective case-control study with a rigorous design and strict selection criteria provided strong evidence for an association between early sPE and aPL (11.5% in the aPL group vs 1.4% in the control group, OR = 8.9 [95% CI 1.9-41.4]).[4]

Conventional treatment (CT) based on low-molecular-weight heparin (LMWH) and aspirin is only partially effective in preventing early sPE in patients with obstetric APS (OAPS).[9] In fact, different study groups have proposed new therapeutic alternatives for the prevention of early sPE in pregnant women with APS, such as hydroxychloroquine (HCQ) and statins.[1,6,10–12] Therefore, our study aimed to extensively evaluate and classify patients with OAPS according to their risk of developing early sPE and then to evaluate the effects of treatment with HCQ on that risk.

Materials and methods

Study population

The first aim of our study was to retrospectively identify risk factors for the development of early sPE in patients with OAPS who received CT. We carefully selected 102 women diagnosed with OAPS with complete data who attended our centers between April 2010 and July 2017.

All pregnant women fulfilled the criteria for OAPS.[13] These patients were treated for OAPS with CT only, including low-dose aspirin (LDA, 100 mg/day) beginning in the preconception period (at least one month before attempting conception) and a prophylactic dose of LMWH (40 mg/day) since the diagnosis of pregnancy.

Only 6 pregnant women who had a history of deep venous thrombosis received therapeutic doses of LMWH + LDA. All systemic lupus erythematosus (SLE) patients were in remission and not receiving any SLE-specific treatment.

The strict exclusion criteria were as follows: patients who initiated conventional therapy for APS after the 7th week of gestation; patients with other thrombophilia, metabolic or endocrine alterations (Cushing's disease, diabetes, thyroid disease, etc.); patients with clinical conditions that might negatively affect pregnancy outcomes (cardiovascular disease, chronic renal failure, severe respiratory disease, among others); or patients who received additional treatment for rheumatic diseases.

At the end of their following pregnancy, we evaluated the association of OAPS and consequent treatment with CT with the risk of developing early sPE, taking into account the following risk factors: obesity, metabolic syndrome, chronic hypertension, hypertriglyceridemia, diabetes, maternal age, smoking history, history of previous PE, presence of lupus anticoagulant, aPL positivity (triple, double, or single), SLE, late pregnancy loss, history of intrauterine growth restriction and high titer of aPL.

Once risk factors for sPE were established in OAPS patients on CT, we collected a new cohort of patients with these risk factors and evaluated the effect of adding HCQ to CT. To properly analyze the relationship between high-risk variables and treatment, we analyzed two groups of patients: group A: patients treated with CT (30 patients); and group B: patients treated with CT + HCQ (12 patients). We evaluated and compared pregnancy outcomes in both groups.

Definitions

Pregnancy loss was defined as the loss of the product of conception during pregnancy, including fetal loss and miscarriage. Miscarriage was defined as pregnancy loss prior to 20 weeks of gestation; early and late miscarriage were defined as pregnancy loss prior to and after 10 weeks, respectively; and fetal loss was defined as pregnancy loss after 20 weeks of gestation. Pregnancy complications included PE, fetal growth restriction (FGR) and prematurity. PE was defined as diastolic blood pressure of 90 mmHg or higher or systolic blood pressure of 140 mmHg or higher on 2 occasions at least 4 hours apart after 20 weeks of gestation with significant proteinuria (0.3 grams in a 24-hour urine sample). PE was classified as early if the onset was before 34 weeks. PE was classified as severe if it included one of the following factors: severe hypertension (diastolic blood pressure >110 mmHg or systolic blood pressure >160 mm Hg), eclampsia (seizures), pulmonary edema, symptoms of hypertensive disease (defined as severe headache, right upper quadrant pain, visual changes, and/or epigastric pain), renal insufficiency as revealed by abnormal creatinine levels (creatinine >1.1 mg/dL), platelet count <100.000/μL, and abnormally high liver enzyme levels (aspartate aminotransferase or alanine aminotransferase >80 IU/L). FGR was defined as fetal weight below the 10th percentile. Prematurity was defined as birth prior to 36 completed weeks of gestation.

Laboratory tests

All patients were diagnosed with OAPS if their laboratory criteria were positive on two or more occasions at

least 12 weeks after their first positive result. The following aPL tests were used: lupus anticoagulant (LA), anti-β2 glycoprotein I (aβ2GPI) antibody IgG and/or IgM, and anticardiolipin antibodies (aCLs) IgG and/or IgM.

Blood samples. Blood was obtained by clean venipuncture (after an 8-hour fast) and collected into plastic tubes containing sodium citrate (ratio, 9:1). After two centrifugation steps at 2500 $\times g$ for 15 minutes, the platelet-poor plasma was immediately assayed for LA and then stored at -40°C. For serum preparation, the blood was collected into tubes, allowed to clot at 37 °C and then centrifuged at 1500 $\times g$. The serum was stored at -40°C until use.

Serum. aCLs were measured using standardized enzyme immunoassays of IgG and IgM isotypes that were developed in-house as previously reported.[14] aβ_2GPI was measured by a commercial enzyme immunoassay kit for IgG and IgM isotypes (BioSystems S. A., Barcelona, Spain). Medium or high titers of serum aCL or aβ_2GPI were considered a positive result. A medium titer was defined as >99th percentile (greater than 40 GPL or MPL or greater than 40 UG or UM according to the international criteria). A high titer was arbitrarily defined as 80 GPL or MPL or as 80 UG or UM (according to previous publications).[15,16]

Plasma citrate. The plasma samples were evaluated for the presence of LA activity using two tests: the dilute Russell viper venom time (TriniCLOT Lupus Screen and/or Confirm, Tcoag, Co. Wicklow, Ireland) and silica clotting time (HemosIL, Instrumentation Laboratory, Bedford, MA, USA). To identify the inhibitor, these clotting tests were conducted with a 1:1 mixture of patient plasma and normal plasma. As a confirmatory test, we used a phospholipid neutralization procedure. LA was diagnosed when at least one of the screening and one of the confirmatory procedures were positive according to the international criteria.[13]

aPL profile. We classified the patients according to aPL positivity in the laboratory tests: triple-positive for aPL (LA+, aCL+ and aβ2GPI+), double-positive for aPL (LA+ and aCL+, LA+ and aβ2GPI+, or aCL+ and aβ2GPI+) and single-positive for aPL (LA+, aCL+, or aβ2GPI+).

We classified the patients into the low-, medium- and high-titer groups according to the serum levels of aCL IgG and IgM or aβ2GPI IgG and IgM. A low titer was defined as less than 39 GPL or MPL or less than 39 UG or UM, a medium titer was defined as 40 to 79 GPL or MPL or as 40 to 79 UG or UM, and a high titer was arbitrarily defined as greater than or equal to 80 GPL or MPL or greater than or equal to 80 UG or UM (according to previous publications).[15,16]

Ethics

This study was approved by the ethics committee of the respective medical center and was performed according to the principles of the Declaration of Helsinki and current national laws. Informed consent was obtained from all the participants.

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (version 23.0 for Windows, SPSS, Chicago, IL, USA). Because the data were not normally distributed, the results are presented as medians and percentiles (25 and 75) or percentages. Nonparametric tests (Mann-Whitney U) were used to compare quantitative data, and the chi-square test or Fisher's exact test was used to compare proportions, with McNemar's chi-square test for paired data. A simple binary logistic regression and multivariable logistic regression analyses were performed to evaluate the association between early sPE and the studied variables. The odds ratio (OR) and corresponding 95% confidence interval (CI) were estimated to assess the strength of the association between early sPE and the studied variables. $P < 0.05$ was considered to indicate statistical significance.

Results

This study included 102 pregnant women diagnosed with OAPS (median age: 31 years; interquartile range: 28-36 years) who were treated at our clinical centers between 2010 and 2017. Demographic, clinical and laboratory characteristics are described in Table 1.

After CT, 18 patients (17.6%) suffered pregnancy loss, of which 8 (7.8%) were fetal losses and 10 (9.8%) were miscarriages. Meanwhile, live births were achieved by 84 patients (82.3%). Overall, 9 patients (8.8%) developed early sPE, and 11 (10.8%) presented with FGR. From the subgroup of patients with only miscarriage, no one presented early sPE.

We also analyzed the potential effect of CT on preventing early sPE in OAPS patients according to their aPL profile. In the first analysis, we observed a significantly decreased early sPE rate in patients with single or double positivity for aPL after CT treatment [17.2% (15/87) before CT vs 3.5% (3/87) after CT; $p = 0.0015$]. Meanwhile, in patients with triple positivity for aPL, the rate of early sPE increased after CT, although the difference was not statistically significant (20.0% (3/15) before CT vs 40.0% (6/15) after CT; $p = 0.248$).

We analyzed which variables are associated with the risk of developing early sPE among OAPS patients on CT (Table 2); and then a univariable binary logistic regression was performed. We found that the presence of triple positivity for aPL [OR = 18.667 (95% CI: 3.974-87.686), $p < 0.001$], SLE [OR = 9.829 (95% CI: 2.141-45.112), $p = 0.003$] and a history of previous early sPE [OR = 4.514 (95% CI: 1.078-18.909), $p = 0.039$] were associated with a higher chance of developing sPE despite CT. Moreover, the presence of single or double positivity for aPL (low risk) was associated with significantly decreased odds of suffering early sPE on CT [OR = 0.073 (95% CI: 0.014–0.382), $p = 0.002$], which reflects the possible efficacy of this treatment in this subgroup of OAPS patients.

Other risk factors associated with early sPE, such as double positivity for aPL, a history of fetal loss, high aPL titers, the presence of LA, a history of chronic hypertension, diabetes, obesity, smoking habit and other cardiovascular risk factors, were not associated with the development of early sPE in our cohort.

We then performed a multivariate analysis to confirm which variables are associated with a higher risk of developing early sPE. The results of this analysis showed that triple positivity for aPL [OR = 24.70 (95% CI: 4.27-142.92); $p < 0.001$] and a history of early sPE [OR = 7.11 (95% CI: 1.13-44.64); $p = 0.036$] were the only strong risk factors (high risk) for developing new-onset early sPE in OAPS patients being treated with CT.

The second part of our study aimed to evaluate the impact of HCQ in preventing early sPE. We identified a new cohort of 42 patients with OAPS and at least one high-risk factor (triple positivity for aPL and/or a history of previous early sPE) and grouped them according to the treatment received and pregnancy outcome. Group A (n = 30) included patients treated with CT only, while group B (n = 12) included patients treated with CT+ HCQ. The demographic and clinical characteristics of this cohort of patients are described in Table 3. The results of this analysis showed that OAPS patients who received CT+HCQ had a significantly lower early sPE rate than those treated with CT only ($p = 0.03$) (Table 4).

Table 1. Demographic, clinical and laboratory characteristics of patients with OAPS.

Number of patients	All (n = 102)	%
Age	31 (28–36)	
History of early sPE	18	17.6
History of FGR	17	16.7
Systemic lupus erythematosus	11	10.8
History of thrombosis	6	5.9
Early miscarriage (<10 weeks)	71	69.6
Late miscarriage (10 to 20 weeks)	34	33.3
Only miscarriage (<20 weeks)	19	18.6
Fetal loss (>20 weeks)	46	45.1
Lupus anticoagulant	63	61.8
aCL titer (GPL/MPL)		
>40	46	45.1
>80	12	11.8
aβ2GPI titer (UG/UM)		
>40	33	32.4
>80	12	11.8
High aβ2GPI and aCL titers	20	19.6
aPL profile		
Single positivity	76	74.5
Double positivity	11	10.8
Triple positivity	15	14.7

aPL: antiphospholipid antibodies; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2 glycoprotein I; FGR: fetal growth restriction; sPE: severe preeclampsia; OAPS: obstetric antiphospholipid syndrome.

Table 2. Variables associated with risk of developing early sPE among OAPS patients on CT.

Variables	No Early sPE % (n)	Early sPE % (n)	p
History of early sPE	15.1 (14)	44.4 (4)	0.049
History of FGR	16.1 (15)	22.2 (2)	0.643
Systemic lupus erythematosus	7.5 (7)	44.4 (4)	0.007
History of thrombosis	5.4 (5)	11.1 (1)	0.434
Fetal loss (>20 weeks)	43.0 (40)	66.7 (6)	0.293
Lupus anticoagulant	58.1 (54)	100 (9)	0.012
High aCL titer (>80 GPL/MPL)	41.9 (39)	77.8 (7)	0.075
High aβ2GPI titer (>80 UG/UM)	29.0 (27)	66.7 (6)	0.055
High aβ2GPI and aCL titers	17.2 (16)	44.4 (4)	0.071
Triple positivity of aPL	9.7 (9)	66.7 (6)	<0.0001

aPL: antiphospholipid antibodies; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2 glycoprotein I; FGR: fetal growth restriction. sPE: severe preeclampsia; OAPS: obstetric antiphospholipid syndrome; CT: conventional treatment.

Table 3. Demographic and clinical characteristics of patients in Groups A with conventional treatment and B with conventional treatment + hydroxychloroquine.

Number of patients	All (n = 42)	Group A (n = 30) CT	Group B (n = 12) CT+HCQ
History of early severe preeclampsia	54.7% (23/42)	60.0% (18/30)	41.7% (5/12)
History of intrauterine growth restriction	23.8% (10/42)	16.7% (5/30)	41.7% (5/12)
Systemic lupus erythematosus	16.7% (7/42)	16.7% (5/30)	16.7% (2/12)
Triple positivity for aPL	54.7% (23/42)	50.0% (15/30)	66.6% (8/12)
Double positivity for aPL	2.4% (1/42)	0.0% (0/30)	8.3% (1/12)
Single positivity for aPL	42.8% (18/42)	50.0% (15/30)	25.0% (3/12)
Lupus anticoagulant	80.9% (34/42)	80.0% (24/30)	83.3% (10/12)
High aCL titer (>80 GPL/MPL)	35.7% (15/42)	26.7% (8/30)	58.3% (7/12)
High aβ2GPI titer (>80 UG/UM)	38.1% (16/42)	36.7% (11/30)	41.7% (5/12)

aPL: antiphospholipid antibodies; aCL: anticardiolipin antibodies; aβ2GPI: anti-β2 glycoprotein I; CT: conventional treatment; HCQ: hydroxychloroquine.

Table 4. Early severe preeclampsia rates in groups A and B after treatment.

	Early severe preeclampsia		
	NO	YES	
Group A = CT	18 (60.0%)	12 (40.0%)	$P = 0.03$
Group B = CT + HCQ	11 (91.7%)	1 (8.3%)	

CT: conventional treatment; HCQ: hydroxychloroquine.

Discussion

In the last few years, LMWH and LDA have dramatically improved pregnancy outcomes in patients with OAPS, and according to the most recent EUROAPS survey, maternal and fetal outcomes were excellent when the recommended therapy was used appropriately.[17] Despite this advance, CT still fails in approximately 20–30% of pregnant women with OAPS.[6,15,18] According to different studies, these CT failures might be associated with certain risk factors, among which serological factors are certainly relevant.[15,19–23]

The NOH-APS study compared the pregnancy outcomes of 513 patients with OAPS who received CT to those of 791 women with a history of unexplained pregnancy loss who tested negative for aPL (control group, no treatment) and concluded that CT failed to reduce the incidence of PE. They alleged that CT may be only partially effective at preventing sPE in OAPS patients.[9] The FRUIT-RCT studied 32 pregnant women with aPL and recurrent hypertensive disorders before 34 weeks of pregnancy who were separated into two groups according to treatment: 16 patients treated with deltaparin and aspirin and 16 patients treated with aspirin alone. This randomized trial did not find a significant difference in PE prevention between the two groups. Notably, the overall PE recurrence was just 3.1%. This finding could be because most of the patients had a low-risk aPL profile.[24] Based on this result, we could presume that both treatments were acceptable at preventing PE in this cohort of patients, since the prevalence of PE was similar to that in the general population. Other previous randomized controlled trials involved APS women with pregnancy loss, such as those by Farquharson RG et al.[25] and Kutteh WH et al.,[26] showed similar results, with no superiority of heparin + aspirin at preventing PE. All this evidence suggests that CT has limited efficacy in the prevention of PE.

When we evaluated the rates of early sPE in our full cohort of patients, we found a high rate of this pathology (8.8%) compared to the general population, but this rate was similar to that reported by other groups.[4,5,7,27]

According to the results obtained from our cohort of 102 women with OAPS, triple positivity for aPL and a history of early sPE were identified as strong risk factors for the development of early sPE despite the therapeutic use of CT. However, other previously described risk factors for early sPE, such as cardiovascular disease, LA, a history of fetal loss, obesity, smoking habit, maternal age, aPL titer and other autoimmune diseases, were not found to be associated with this pregnancy complication in our study.

Considering that the results of several studies from the last ten years highlight the relevance of triple positivity for aPL as a risk factor for pregnancy morbidity and mortality, it is reasonable that this factor is associated with the risk of developing early sPE, as we have found.[15,19–23,28] Indeed, our results are in agreement with those reported in two major studies on APS by Saccone et al.[29] and Alijota-Reig et al.,[17] in which the PE rate was higher in OAPS patients with double or triple positivity for aPL. Moreover, we also found that OAPS patients with single and double positivity for

aPL who were treated with CT had a low rate of sPE (3.45%), similar to what was found in the FRUIT study, where most patients had low-risk aPL profiles.[24] This result might reflect the possible efficacy of this treatment in this subgroup of low-risk patients. Based on these results, we agree with other groups that CT is partially effective at preventing sPE.

Additionally, the identification of an association between a history of previous sPE and the onset of early sPE in OAPS patients on CT was not surprising, considering that a history of sPE is one of the most significant and recognized risk factors for recurrence.[30,31] In addition, it is important to mention that the evidence indicates that the number of risk factors exponentially increases the risk of PE.[31]

The second part of our study aimed to evaluate the impact of HCQ at preventing early sPE among a selected group of OAPS patients with high-risk factors for this obstetric pathology. We found that high-risk patients treated with CT+HCQ had a significantly lower early sPE rate than those treated with CT only. Considering the different pathways through which the antimalarial drug HCQ exerts its effects, it might help prevent early sPE by reducing oxidative stress in both the placenta and endothelium and by preventing local inflammatory processes.[32] Moreover, Seo et al. found that patients with SLE who were treated with HCQ during pregnancy showed an 89.4% reduction in the PE rate.[33] Sciascia et al. also demonstrated a reduction in aPL-related pregnancy morbidity in patients with APS+SLE who received HCQ treatment, and a recent study conducted by Do S. et al. also found that HCQ might have a protective effect against PE in pregnant women with SLE.[10,34]

In our opinion, one of the strengths of our study arises from our methodology of patient selection, since we conducted a thorough analysis to identify the risk factors for early sPE and according to these factors selected a group of high-risk patients to evaluate the impact of HCQ in preventing early sPE. Few studies on patients with OAPS have focused on early sPE while considering topics such as risk factors and alternative treatments such as HCQ. However, we acknowledge that a weakness of our study was the limited number of patients in the second cohort; although our study had a multicenter design, patient numbers were limited, as is common in other similar cohorts.

In conclusion, our findings suggest that CT is partially effective at preventing early sPE in OAPS patients. Triple positivity for aPL and a history of early sPE were strong risk factors for the development of early sPE. CT was only effective in patients at low risk according to their aPL profile, while CT in combination with HCQ was an interesting option for preventing sPE in patients with high-risk factors, such as triple positivity for aPL and/or a history of early sPE.

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Contributorship

All the authors were involved in drafting the article. SU and JOL had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All the authors were involved in the study conception and design. Data analysis and interpretation were performed by FA, SU and GDL.

Conflict of Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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