Prolactin, estradiol and anticardiolipin antibodies in premenopausal women with systemic lupus erythematosus: a pilot study

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease, with higher prevalence in women. An incidence peak occurs during the reproductive years, suggesting that estradiol may play a role in the clinical presentation of SLE. Anticardiolipin antibodies (ACA) are associated with antiphospholipid antibody syndrome (APLS), but can be found in patients with SLE without APLS, and relate to cardiovascular risk and nephrite. **Objective:** This study aimed at assessing whether the presence of ACA is associated with hormonal changes in a sample of women with SLE. **Methods:** Forty-seven women diagnosed with SLE according to the American College of Rheumatology criteria, aged 30.8 ± 8.12 years, were evaluated. None was on hormonal contraception, and their SLE activity was estimated using the SLE Disease Activity Index (SLEDAI). Patients were stratified, according to the presence or absence of ACA, and estradiol and prolactin levels were measured. **Results:** Nine (19.1%) of 47 patients were positive for ACA. No differences were found between groups concerning age, duration of disease, and SLEDAI. In contrast, the median estradiol level was lower in the ACA-positive group [46.8 (21.0-72.1) pg/mL] than in the ACA-negative group [122.3 (64.8-172.7) pg/mL, P = 0.004]. **Conclusion:** These results suggest, for the first time, an inverse association between ACA and estradiol levels in premenopausal SLE patients. Considering that both lower endogenous estradiol levels and ACA positivity are related to atherosclerosis, our finding may be clinically relevant in predicting cardiovascular risk and/or APLS development in SLE.

Keywords: systemic lupus erythematosus, antibodies, anticardiolipin, estradiol, premenopause, prolactin.

INTRODUCTION

The observed difference in prevalence of autoimmune diseases between men and women has intrigued many investigators and stimulated studies on the role of sexual hormones in immunity. Considering systemic lupus erythematosus (SLE), its prevalence in women during their reproductive years compared to men is 9:1. In addition, its clinical manifestations also seem to differ between genders: men have more severe renal disease, as well as greater neurological and cardiorespiratory impairment than women.[1,2]

Sexual hormones, such as testosterone, estradiol and prolactin, have been shown to influence the mechanisms of the immune system in animals and human beings, affecting several immune functions, including lymphocytic maturation and activation, and synthesis of autoantibodies and cytokines.[3] The observed results are conflicting regarding the role played by estrogen, perhaps due to different effects of this hormone on

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different cell lines. However, estrogen is considered to promote an increase in cell proliferation and humoral immune response.[4]

Recently, the hypothesis that sexual hormones may be associated with manifestations of antiphospholipid antibody syndrome (APLS) has been formulated.[5,6] This syndrome is defined by the presence of antiphospholipid antibodies in patients with a history of fetal loss and/or recurring venous and arterial thromboembolism.

The antiphospholipid antibodies comprise the lupus anticoagulant, anticardiolipin (ACA) and anti-β2-glycoprotein I antibodies, and can prolong phospholipid-dependent coagulometric tests, such as activated partial thromboplastin time. Despite the laboratory findings, patients with antiphospholipid antibodies have a higher risk for thromboembolic than hemorrhagic events.[5] The ACAs are detected by ELISA (enzyme-linked immunosorbent assay) and comprise IgG, IgM and IgA isotypes. IgG is strongly associated with thrombosis.[7,8]

The present study aimed at assessing whether there is a relationship between the presence of ACA (IgG and IgM) and estradiol and prolactin levels in a sample of women at reproductive age diagnosed with SLE.

PATIENTS AND METHODS

This is a non-controlled cross-sectional study. It included 47 premenopausal patients followed-up at the Rheumatology Service of Hospital de Clínicas de Porto Alegre (HCPA), who were not on hormonal contraception and met at least four American College of Rheumatology diagnostic criteria for SLE.[9]

Patients with changes in liver function tests (GOT, GPT or LDH), renal failure (creatinine levels over 1.5), changes in thyroid function, concomitant presence of another autoimmune disease, or users of drugs that alter the circulating levels of prolactin were excluded from the study.

The SLE activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).[10,11] The disease was considered active when SLEDAI ≥ 4, and inactive when SLEDAI < 4.

Estradiol and prolactin serum levels were measured in all patients by electrochemiluminescence (Elecsys, Roche Diagnostics, Mannheim, Germany), with analytical sensitivity of 5.0 pg/mL and 0.047 ng/mL, respectively. The reference values for estradiol were between 10 and 520 pg/mL and between 6.0 and 29.9 ng/mL for prolactin. Blood collection for such measurements was performed simultaneously with those for the tests used for determining SLEDAI.

Positivity for ACA (IgG and IgM) was assessed by Hemagen® anticardiolipin kit (Hemagen Diagnostics, Columbia, USA). Values below 10 U GPL for IgG and 10 U MPL for IgM are considered negative. Test relative sensitivity is 95% and specificity 100% (with 98% agreement when compared with the reference kit provided by the Antiphospholipid Standardization Laboratory). Based on these analyses, the patients were stratified into two groups: serum ACA-positive and serum ACA-negative (IgG or IgM).

On the same day of blood collection, the patients were interviewed regarding previous pathological history and gynecological and obstetrical findings. In addition, their medical records were reviewed to assess the presence of a previous diagnosis of APLS and history of target-organ impairment.

This study was approved by the Health and Research Ethics Committee of HCPA (GPPG 04-215). All patients provided written informed consent.

Statistical analysis

The categorical variables were presented as absolute and percent relative frequencies. The quantitative variables were presented as mean ± standard deviation when their distribution was symmetrical or as median and interquartile interval when their distribution was asymmetrical. For comparing variables with symmetrical distribution in the categories of dichotomous variables, the Student *t* test was used, while for those with asymmetrical distribution, Mann-Whitney test was used. The significance level adopted was 95% ($P\alpha \leq 0.05$). Data were analyzed using the SPSS software, version 14.0 (Chicago, IL, USA).

RESULTS

The study sample comprised 47 premenopausal women diagnosed with SLE, whose mean age was 30.8 ± 8.12 years.

Of the 47 study participants, nine (19.1%) were positive for ACA (IgG and/or IgM) in the serum. Most patients had SLE in remission or with mild activity, as shown by the SLEDAI scores (Table 1).

Table 1 shows data of the study patients stratified according to ACA (IgG and/or IgM) positivity or negativity. Factors that could influence estrogen levels were assessed. The groups were similar regarding age, age at menarche, and duration of disease. In addition, no difference between groups was observed regarding lupus activity estimated by use of SLEDAI or presence of anti-DNA. The prolactin levels were also similar between groups ($P = 0.43$).

Table 1
Characteristics of the patients with SLE stratified according to ACA (IgG or IgM) positivity or negativity

	ACA-positive (n = 9)	ACA-negative (n = 38)	P
Age (years)	29.75 (3.30)	32.36 (7.56)	0.51[a]
Duration of disease (years)	7.0 (8.52)	7.21 (4.70)	0.10[a]
Menarche (years)	11.75 (2.06)	12.82 (1.58)	0.79[a]
Anti-DNA	1/40 (0-1/200)	0 (0-0)	0.49[b]
SLEDAI	4 (2-9)	2 (0-7)	0.193[b]
Prolactin (ng/mL)	10.68 (8.43-14.04)	13.38 (9.13-20.27)	0.43[b]

(a) Mean ± SD; Student´s *t* test.

(b) Median (IQ interval); Mann-Whitney test.

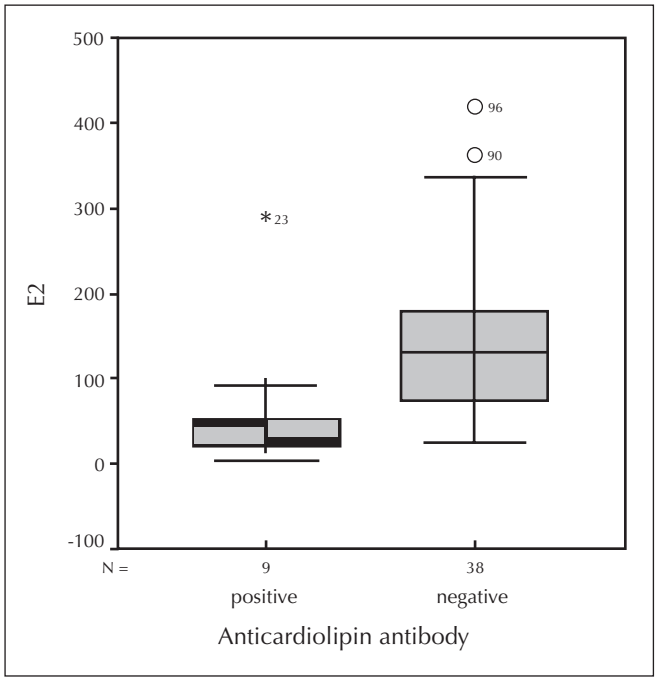

Figure 1
Serum estradiol in premenopausal ACA positive or negative women.

Figure 1 shows that ACA-positive patients had significantly lower estradiol levels than ACA-negative ones: 46.8 (21.0-72.1) versus 122.3 (64.8-172.7) pg/mL, respectively (P = 0.004).

No significant difference was observed in estradiol levels between patients undergoing blood collection during their early follicular phase of the menstrual cycle (up to the eighth day of the cycle) and those undergoing blood collection in other phases of the menstrual cycle (P = 0.737).

None of the patients had a previous or current history of acute myocardial infarction or stroke. No association was found between estradiol levels and the presence of vasculitis, nephritis or other vascular alterations associated with APLS.

DISCUSSION

This study assessed whether ACA positivity, frequently found in patients with SLE and associated with thrombotic events and APLS, is correlated with the estradiol and prolactin levels in a group of women with SLE at reproductive age.

Our results showed that, in the sample studied, the presence of ACA in the serum was associated with lower estradiol levels. This result partially disagrees with the notion that higher estrogen levels usually associate with the production of autoantibodies.[12] This observation is relevant, considering that both estrogen deficiency and ACA are related to the risk for atherosclerotic disease. So far, the association between hypoestrogenism and the presence of ACA in premenopausal women with SLE has not been described.

Low-density lipoprotein (LDL) oxidation is widely recognized to play an important role in atherogenesis.[13] Recent studies have suggested that the production of ACA is associated with exposure to antigens expressed in endothelial cells during apoptosis, as well as exposure to oxidized LDL.[14] A study published by Tuominen et al.[15] has shown that LDL oxidation also induces immune response to epitopes of oxidized cardiolipin. Some authors have suggested that atherosclerosis and autoimmunity are intrinsically correlated events.[14]

Some studies have indicated that endogenous estrogen, unlike the exogenous one, can reduce apoptosis of endothelial cells, leading to a protective cardiovascular effect.[12,16] A study

published in 2008[17] investigated the in vitro effect of estrogen on apoptosis of endothelial cells in culture, demonstrating that estrogen inhibits, although incompletely, apoptosis induced by TNF-α and oxidized LDL. Thus, a possible explanation for the association of low estradiol levels and the presence of ACA would be the increased activation of endothelial apoptosis caused by the low endogenous estrogen levels, leading to increased exposure to endothelial and even subendothelial antigens, such as oxidized LDL.

Although it is an original observation, the literature review includes some indirect evidence on the association between estrogens, APLS and cardiovascular risk. In 2005, Jara et al.[18] published one study comparing, for the first time, the clinical differences between men and women with APLS at baseline and during follow-up. No difference between genders was observed regarding arterial and venous thrombosis or ACA levels. However, the incidence of stroke was greater in women than in men (31.5% and 10%, respectively). Although the incidence of atherosclerotic disease in women increases after menopause as compared to that of menacme,[19] in this study the patients were young but had no protection as compared to men regarding the appearance of stroke – according to these authors, probably because of a possible APLS-associated hypoestrogenism.[18]

The effect of the exogenous administration of estrogen has been reported. In a study published in 2004, Todorova et al.[20] investigated the presence of ACA (IgG and IgM) during the use of hormone therapy in a group of healthy postmenopausal women, with no clinical history of previous thrombotic events. The patients were divided into two groups: a control group and a group receiving hormone therapy containing 2 mg of 17-β estradiol and 1 mg of norethisterone acetate for six months. No significant change in positive ACA prevalence was observed in the control group for six months of follow-up. The group receiving hormone therapy showed an increase in the IgM ACA levels during the course of hormone therapy, and this change was significant after the third month of treatment; a reduction in the ACA levels was observed in the sixth month of treatment, but with no return to baseline levels.[20]

Similarly, a study in animals using C57BL/6 rats without autoimmune disease[21] has shown that the animals treated with estrogen had an increased expression of IgG and IgM ACA.

However, some review articles[22] have reported that, in postmenopausal Chinese women treated with estradiol derivatives, the IgG ACA levels have decreased and those of high-density lipoprotein (HDL) increased. Thus, one can speculate that the effects of estrogens on the expression of antiphospholipid antibodies may depend on their concentration, ethnicity, origin (endogenous vs. exogenous) and the endothelial activation status.[23]

It is worth emphasizing that, in the present study, the difference in estradiol levels between the groups with and without ACA positivity did not depend on serum concentrations of prolactin. Hyperprolactinemia is a relatively common finding in patients with SLE and can cause a lower ovarian estradiol secretion through common central mechanisms of regulation (through hypothalamic dopamine and gonadotropin-releasing hormone). The lack of hyperprolactinemia in our patients may be due to low lupus activity at the moment of their inclusion in the study.[24,25] However, more importantly, it shows that the association observed between lower concentrations of estradiol and higher frequency of ACA positivity has not been contaminated by prolactin-related changes.

CONCLUSION

In our study, the presence of antiphospholipid antibodies in premenopausal patients with SLE was associated with lower circulating estradiol levels, but not with prolactin levels. To explain the inverse association between estradiol levels and the presence of ACA, one can speculate that, with the reduction in estrogen levels, there is an increase in apoptosis with consequent exposure to antigens that increase ACA expression. This observation, if confirmed by other studies, raises interesting questions about the interrelation between estrogens, endothelium and autoimmunity, with a potential impact on the risk of thrombosis and cardiovascular disease. Thus, longitudinal studies are required to assess whether reduced levels of estradiol in women with SLE during menacme can be considered risk markers for atherosclerosis and stroke, independently of APLS.

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