

# Prolactin and Estradiol Profile in a Cohort of Colombian Women with Systemic Lupus Erythematosus

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**ABSTRACT:** **Background:** Systemic lupus erythematosus (SLE) is an autoimmune disease with multiorgan involvement and wide variability in presentation and course. Although it can appear at any age, women of childbearing age are primarily affected. This has led to the proposal of a hormonal role in the development of SLE. Among the main hormones shown to have immunomodulatory effects are estradiol, progesterone and prolactin.

**Objectives:** To report the levels of estradiol and prolactin in SLE patients and establish the relationship between these levels and disease activity, and to determine whether the phases of the menstrual cycle influence the activity of SLE and its relationship to hormone levels.

**Methods:** In this cross-sectional study, we examined 60 women with SLE. We measured disease activity using SLEDAI and BILAG. We obtained peripheral blood samples to determine the levels of estradiol, progesterone and prolactin.

**Results:** Patients' age ranged between 16 and 65 years and the mean disease duration was 5.5 years (0–20). SLE was active (SLEDAI > 6) in 13 patients and inactive in 47. Thirty patients were in a pre-ovulatory menstrual cycle phase, 13 in a post-ovulatory cycle, and 17 were menopausal. We found a significant association between C4 levels and disease activity ( $P = 0.01$ ) and between estradiol levels and disease activity in the kidney ( $P = 0.04$ ). We did not find hyperprolactinemia in any patient.

**Conclusions:** In this population, we found an association between estradiol levels and organ-specific activity in the kidney. One may speculate as to whether our population might benefit from the implementation of anti-estrogen therapy for control of disease activity, particularly in the kidney.

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**KEY WORDS:** systemic lupus erythematosus (SLE), prolactin, estradiol, menstrual cycle

50 years is approximately 9:1, but 2:1 when it occurs during childhood or after menopause [1]. This prevalence observed in female patients and the observation of a rise in disease activity during pregnancy [2] led to the proposal of a hormonal role in the development of SLE. Among the main hormones that have displayed immunomodulatory effects are estradiol, progesterone and prolactin [3].

The role of estradiol is characterized mainly in murine models. For instance, estradiol breaks tolerance of high DNA affinity B lymphocytes, it facilitates the maturation of “naïve” autoreactive B cells, and hinders the development of protective B cells [4]. It also accelerates the disease in both sexes, decreases survival, causes increased autoantibodies in non-autoimmune mice, and increases immune complexes in glomerulonephritis [5].

The immunomodulatory role of prolactin has also been described. It is produced by B and T cells, their receptors are part of the cytokine superfamily, and it has an influence on intracellular processes such as gene transcription and signaling [6,7]. Several authors noted that a high proportion of SLE patients have hyperprolactinemia (up to 40%) [8–12] and in some studies this correlated with increased disease activity [8–10].

There is very little information regarding the menstrual cycle and what we know is mostly derived from observational studies. Interestingly, although there is great interest in the role of sex hormones in the pathophysiology of SLE, only a few studies have investigated the impact of the menstrual cycle on SLE activity [13]. Most studies focus on the opposite, i.e., determining the impact of SLE on the menstrual cycle.

In order to clarify the contradictions regarding the role of hormones in SLE, we performed the current study whose aims were: (i) to describe the levels of estradiol and prolactin in SLE patients, (ii) to establish if there is an association between these levels and disease activity, and (iii) to determine whether the phases of the menstrual cycle affect the activity of SLE and its relationship to hormone levels.

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**A**lthough systemic lupus erythematosus (SLE) can appear at any age, women of childbearing age are mainly affected. The female:male ratio of disease onset between age 15 and

## PATIENTS AND METHODS

In this cross-sectional study, we examined 60 women with SLE who presented to Hospital Universitario de la Samaritana,

Bogotá, Colombia from January to December 2011. They met the American College of Rheumatology (ACR) criteria for SLE classification and were > 16 years old. We defined disease activity by using SLEDAI and BILAG scores and obtained peripheral blood samples to determine hormonal levels (estradiol, progesterone and prolactin).

To measure prolactin levels we took three blood samples at 30 minute intervals. Exclusion criteria were:

- patients on hormone replacement therapy
- patients with a diagnosis of prolactinoma
- patients of childbearing age who had undergone a total hysterectomy (including oophorectomy)
- pregnant or lactating women
- patients with a concomitant diagnosis of other rheumatic disease, chronic renal disease and/or on hemodialysis
- patients with decompensated hypothyroidism
- patients treated with anxiolytics and antipsychotics such as haloperidol, olanzapine, risperidone, domperidone, monoaminooxidase inhibitors, fluoxetine; antihypertensive such as labetalol, reserpine,  $\alpha$ -methyl dopa and verapamil; gastric protectors such as ranitidine, metoclopramide and cimetidine
- patients who did not wish to participate and did not sign the informed consent.

In addition, patients had to meet the following conditions before blood samples were taken: they had not engaged in sexual intercourse the night before, had not consumed alcoholic beverages, had fasted for more than 8 hours, and rested for at least 30 minutes prior to sampling. The samples were taken before 9 a.m. All patients delivered 24 hour collected urine and an isolated urine sample.

We separated serum samples and stored them at  $-20^{\circ}\text{C}$ . We processed the samples by chemiluminescence (Siemens Healthcare Diagnostics Kits, Germany). We measured estradiol and progesterone from the first sample obtained. Hyperprolactinemia was defined as serum prolactin levels > 20 ng/ml. Estradiol was defined as follicular phase 18.9–246.7 pg/ml, midpoint of the cycle 35.5–570.8 pg/ml, luteal phase 22.4–256 pg/ml, and postmenopausal 0–44.5 pg/ml. Progesterone was defined as follicular phase not detectable level of 1.40 ng/ml, and luteal phase 3.34–35.56 ng/ml. We only used progesterone to determine the menstrual cycle phase.

#### STATISTICAL ANALYSIS

We performed statistical analysis using Stata 10.0. The following descriptive statistics were performed: measures of central tendency and dispersion for quantitative variables and frequency measures for qualitative variables. The following bivariate analysis was also performed: mean difference of estradiol and prolactin levels between patients with active and inactive SLE using Student's *t*-test. The analyses

of quantitative variables are presented as median and interquartile ranges. The calculated *P* value was performed using a non-parametric Mann-Whitney test. Stratified analysis by menstrual phase, consistent with the levels of progesterone cycle, was performed.

## RESULTS

The study population comprised 60 women, whose mean age was  $37 \pm 14$  years (range 16–65) and median disease duration of 5.5 years (range 0–20 years). Forty-three patients (72%) were women of childbearing age and 17 (28%) were in menopause. Among women of childbearing age, 30 (70%) were in the pre-ovulatory phase of the menstrual cycle and 13 (30%) in the post-ovulatory phase.

The immunological profile of the population showed a median titer of 1/10 anti-dsDNA antibodies (range 0–2560) and C3 and C4 complement levels 98 and 18 mg/dl, respectively. In the hormonal profile, the prolactin median value was 7.07 ng/ml and a peak of 20.2 ng/ml was the highest level recorded (only one patient). The median estradiol level was 51.3 pg/ml (range 0–339 pg/ml). The pharmacological profile showed that 60% of the patients were taking more than 10 mg prednisolone, 80% were using some sort of immunomodulatory/immunosuppressive drugs, and less than 3% were on biological therapy.

Table 1 presents a comparison of the immunological and hormonal profiles in the total population between the active and inactive SLE groups. There was no statistically significant association between groups except for the C4 complement level, which was more decreased in the active group ( $P = 0.01$ ). As shown in Table 2 we sought to determine whether there is an association of prolactin and estradiol with SLE activity in different organs and systems. No association was found with the hormonal levels studied except in the renal system where a statistically significant association ( $P = 0.04$ ) with estradiol levels was found.

Although no statistically significant differences were found, a higher percentage of women of childbearing age had disease activity than menopausal women. Also, the percentage of women without disease activity was greater in the pre-ovulatory

**Table 1.** Immunological and hormone profile of the cohort

60 patients	Active SLE n=13 (22%)	Non-active SLE n= 47 (78%)	<i>P</i>
Anti-DNA	40 (0–40)	10 (0–80)	0.72
C3 (mg/dl)	78.1 (68–98.8)	101.9 (79.6–118)	0.07
C4 (mg/dl)	12 (10–14.1)	19.8 (14–24.9)	0.01
Prolactin (ng/ml)	9.7 (5–11.25)	7.06 (5.1–10.2)	0.7
Estradiol (pg/ml)	104.2 (52.2–147.6)	36.7 (10.9–114)	0.07

Mean and interquartile range values

phase. In Table 3 we analyze the phases of the menstrual cycle (and the menopause subgroup), disease activity and levels of prolactin and estradiol to determine whether there was any hormonal effect on the other variables in our study population. No statistically significant association was found.

## DISCUSSION

In view of the ongoing controversy regarding the hormonal role in triggering autoimmune disease and its activity, we proposed this study, trying to exert maximum control over disruptive hormonal agents. We also determined hormonal levels at the same time that we performed the laboratory tests so that the activity measurements correspond to the hormonal levels. To the best of our knowledge, this is the first study with these particular features.

Most of the sampled women were in a non-active phase of the disease, largely because patients with very active disease were likely to require hospitalization and medication or invasive measurements. These correspond with the exclusion criteria – as all these parameters can result in increased prolactin levels – disqualifying these women from the study. Within the active disease group, the most affected systems were renal, cardiorespiratory and hematological. A significant association of complement C4 consumption in active SLE was found, particularly in relation to renal activity [14].

Only a few patients use biological treatment, likely due to the low economic circumstances of the population attending our hospital and the lack of state or health insurance for this type of therapy.

Our study found that estradiol levels were higher in the active group; however, there was no statistically significant difference ( $P = 0.07$ ). A significant association was found with activity in the kidney ( $P = 0.04$ ), where the median estradiol level in the active group nearly tripled the no activity group. Other systems also showed higher levels, but the difference was not statistically significant, likely due to sample size.

McMurray and May [15] found significantly higher estradiol levels in women with SLE compared to controls; the same was not found in studies of men with SLE. Others, like Folomeev et al. [16] showed that patients with SLE have increased activity of the aromatic hydroxylase enzyme, leading to the aromatization of testosterone in women. Wang et al. [17] demonstrated polymorphisms in  $\alpha$ -estradiol receptor genes associated with SLE, showing increased risk for this disease in women.

Mainly derived from studies in murine models, there is strong evidence that estradiol generates autoimmunity in B and T cells. In B cells, its influence over genes such as CD22, SHP-1, bcl-2, and VCAM 1 blocks the induction of tolerance of naive B cell resistance to apoptosis and expands the population of marginal B cells, breaking tolerance of high affinity B cells to DNA [3,4], and increases the levels of BAFF, which in turn increases

**Table 2.** Disease activity by organs/systems (SLEDAI) and hormone levels

System	Prolactin (ng/ml)		P	Estradiol (pg/ml)		P
	Active	Non-active		Active	Non-active	
CNS	7.9 (3.9–11.5)	7.07 (5.12–10.3)	0.8	88.8 (61.2–147)	40.9 (14.5–120)	0.2
Vascular	NA	NA		NA	NA	
Skeletal/muscle	9.3 (3.8–11.2)	7.06 (5.2–10.3)	0.9	123 (15–164.2)	41.9 (18.7–104)	0.3
Renal	9.8 (6.7–11.5)	7.02 (5–10.2)	0.16	111.5 (101–186)	38.3 (14–107)	0.04
Dermatological	7.5 (3.6–10.3)	7.07 (5.2–10.6)	0.59	67.3 (23–145)	39.3 (11.7–116)	0.4
Serositis	9.7 (3–30.2)	7.06 (5–0.3)	0.6	18.9 (15–251.7)	52.2 (18.7–118)	0.9
Immunological	8.9 (5.5–11.2)	7.02 (5–10.2)	0.6	85.4 (5–148)	46 (18.9–108)	0.9
Constitutional	NA	NA		NA	NA	
Hematological	3.8 (3.8–3.8)	7.09 (5–10.4)	0.2	142 (142–142)	50 (15–118)	0.3

Mean and interquartile range values  
NA = not applicable

**Table 3.** Menstrual cycle phase, menopause subgroup, disease activity and relationship with hormone levels

	Prolactin (ng/ml)			Estradiol (pg/ml)		
	Active	Non-active	P	Active	Non-active	P
Pre-ovulatory	11.27 (5.02–13.9)	9.7 (6.04–10.2)	0.96	95.7 (48.9–144.7)	93.92 (35.3–123)	0.41
Post-ovulatory	8.3 (6.2–10.5)	8.08 (6.7–10.4)	0.87	147.92 (76.7–219.1)	144.42 (61.8–192.8)	0.75
Menopause	3.06 (3.06–3.06)	6.93 (3.56–9.81)	0.27	15.03 (15.03–15.03)	10.19 (4.8–16.3)	0.74

Mean and interquartile range values

the survival of autoreactive B cells and thus the generation of antibodies [4]. On T lymphocytes, it inhibits apoptosis, allowing the persistence of autoreactive T cells.

With regard to the relationship of elevated estradiol levels with disease activity specifically in the kidney, we found no description of human models; the only models to date were murine. In mice, the induction of nephritis by estradiol would be mediated by  $\alpha$ -estrogen receptor (ER $\alpha$ ) [18]. Irsik and co-workers [19] determined that different variants of these receptors are present in the kidney. This is corroborated by studies from Wu et al. [20] demonstrating the effectiveness of tamoxifen in the murine autoimmune model NZB/WF1, where the group receiving the drug showed lower renal injury. Abdou and team [21] showed that the use of fulvestrant in women with SLE decreases the activity of the disease and patients require less use of medication to control it. Some other studies support the empirical use of tamoxifen in SLE.

In this study we did not find hyperprolactinemia in any patient with SLE. There was no difference in prolactin levels between the group with active SLE and the group without disease activity. These findings contrast with several studies of the past 20 years which found at least mild to moderate

hyperprolactinemia in SLE patients, between 2 and 40% [8-12]. SLE activity has been explored in several studies that found a relationship with the levels of prolactin [8-10], rather than those that did not [11].

Haghighi and team [10] identified that in SLE patients with hyperprolactinemia the most affected systems were the kidney and central nervous system, and the presence of vasculitis. The immunomodulatory role of prolactin has been described: it is produced by B and T lymphocytes [6], whose receptors are part of the cytokine superfamily [6], and it influences intracellular processes such as gene transcription and signaling [7]. It is also known that the production of prolactin by lymphocytes is regulated by a gene promoter different from pituitary prolactin.

In murine models, prolactin has a clear deleterious role. It significantly increases mortality and induces autoimmunity not only in populations likely to have SLE (NZB/WF1), but also in others (non-SLE populations) [7]. We know that prolactin interferes in the three mechanisms known to induce tolerance in B lymphocytes: B cell receptor-mediated suppression, anergy, and receptor editing. This results in increased survival of autoreactive B lymphocytes and anti-DNA and IgG deposition in the kidney [7].

One feature of note in the current study is that patients were strictly selected and we tried hard to control any agent that could alter the prolactin levels. Furthermore, we used a method whereby the hormonal levels are determined in three different samples taken consecutively with intervals of 30 minutes, rendering results that are more accurate. One might suspect that in other studies the control of agents that distort prolactin levels was not very strict since it is not described in the literature. An alternative explanation is that our population definitely behaves differently from those in other parts of the world; this is the first study of its kind in South America. With these findings, we could speculate that our population does not benefit from the use of antagonists of prolactin, contrary to the results established by some studies [22]. Also, we do not think that prolactin can be used as a biomarker of the disease.

One of the objectives of our study was to determine whether the ovulatory cycle influenced the activity of SLE in some way. We did not find any significant association, nor did we find a relationship between the hormonal effect, the phase of the cycle, or the activity of the disease.

Despite the considerable interest in the role of sex hormones in the pathophysiology of SLE, there are few studies on the impact of the menstrual cycle on disease activity [13]. We found only one study that attempted to document this, reporting that patients experienced worsening of symptoms during the luteal phase [23]. This is noteworthy since it is precisely when a decrease in estradiol levels would be expected.

Although not statistically significant, it was striking that estradiol levels in our study were higher in the post-ovulatory phase. Most studies focus on determining the impact of SLE on

the menstrual cycle and not vice versa, as was our purpose. They conclude that a subgroup of patients have menstrual abnormalities such as oligomenorrhea or amenorrhea, and in some cases these were more frequent when the disease was more active [9,24]. Colangelo et. al. [25], in a descriptive study, attempted to determine the phase of the cycle that would have more SLE activity, concluding that it is higher before the menstrual period. However, that study lacks true objectivity due to data collection methods (e.g., patient self-reports and questionnaires). It is unclear whether patients may have confused the symptoms of SLE with premenstrual syndrome.

## CONCLUSIONS

- Our population showed a trend toward higher levels of estradiol in patients with active SLE when compared to those without activity, although statistical significance was not obtained
- We determined a statistically significant association between estradiol levels and organ-specific activity in the kidney, which had previously been described in murine models only
- In our population we found neither hyperprolactinemia nor prolactin elevation associated with SLE activity, which is in contrast to most studies published to date. This could be attributed to the fact that our population behaves differently from other studied populations, or to the strict control of agents that may modify the hormone levels
- Our study found no relationship between the phase of the menstrual cycle and SLE activity
- It is noteworthy that high estradiol levels were maintained in the post-ovulatory phase when physiologically it is normal for these to drop. This might be considered a feature of women with SLE, but more studies are needed to confirm this
- One could also speculate that our population might benefit from the implementation of antiestrogen therapy for control of activity, particularly in the kidney, but further studies are needed.

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