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A comparison of the hormone levels in patients with Sjogren's syndrome and healthy controls

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Objective. The purpose of this study was to compare the level and relative ratio of estrogen, progesterone, and prolactin in patients with Sjögren's syndrome and healthy controls.
Study design. Serum samples were collected from 17 SS patients and 19 age-, sex- and race-matched controls. All subjects were postmenopausal females who were not currently on hormone replacement therapy. Prolactin levels were measured using ELISA and progesterone and estrogen were measured using EIA.
Results. Mann-Whitney U test revealed a significantly higher levels of prolactin among patients than controls (11.41 ng/ml vs. 6.74 ng/ml, $p = 0.003$) with significantly higher prolactin/ progesterone (18.88 vs. 8.14, $p = 0.02$) and estrogen/ progesterone (71.51 vs. 42.02, $p = 0.05$) ratios. No significant differences were observed in the levels of estrogen and progesterone between patients and controls.
Conclusion. Abnormal levels and relative ratios of hormones may play a role in the pathogenesis of Sjögren's syndrome.
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Sjögren's syndrome (SS) is an inflammatory disease that affects the exocrine glands, including the salivary and lacrimal glands, resulting in dry mouth and dry eyes.[1-5] Sjögren's syndrome primarily affects women over the age of 40 years, and it is the second most common autoimmune disease, with an estimated prevalence of 1-3% of the world's population. Due to the high prevalence of Sjögren's syndrome among women, an association between hormonal changes and the disease has been suggested.[6] Studies have suggested that changes in the relative ratio between estrogen and androgen or the ratio of their receptors can modulate the cellular immune response, thus providing a means for endocrine regulation of the immune system. Estrogen and progesterone are believed to play an immune-stimulatory role, while androgens are believed to inhibit the immune response.[6-8] Androgen level tends to decline during menopause; this may explain, in part, the high incidence of Sjögren's syndrome among menopausal women. Estrogen deficiency in a murine model for SS has been linked with the development of severe destructive autoimmune lesions in salivary and lacrimal glands in mice.[9]

The immune function of the hormones ensues via interaction with other immune modulators. For example, estrogen has been shown to modulate lymphocytes growth, differentiation, proliferation, antigen presentation, cytokine production, antibody production, cell survival, and apoptosis.[8] While prolactin, which is considered a proinflammatory hormone, has been shown to participate in T cell proliferation, induce IL-2 receptor expression, support IFN-gamma production, and stimulate antibody production.[8,10,11] Further, recent evidence suggests that estrogen and prolactin share a reciprocal relationship; estrogen exhibits a stimulatory effect on prolactin secretion, while high prolactin concentrations suppress estrogen secretion.[8] Also, there is evidence that suggests that estrogen and progesterone can modulate the immune system through polyclonal activation of B cells.[12] The relationship between estrogen and prolactin was examined in the NZB X NZW F1 mouse model for

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Table I. Age distribution of study population (years)

Diagnosis	*N*	*Mean ± SEM*	*Median*	*Range*
Sjögren's Syndrome	17	65.35 ± 2.98	62	47-87
Healthy control	19	57.47 ± 2.63	53	44-79

lupus. In this model, prolactin accelerated disease expression, and estrogen devoid of its prolactin stimulatory properties became immunosuppressive and inhibited IL-2 production.[8] Currently, there is no study that examines the levels and relative ratio of hormones in the same SS patients. Therefore, the purpose of this study was to compare the level and ratio of estrogen, prolactin, and progesterone in serum of post-menopausal women diagnosed with SS and age-, sex-, and race- matched healthy control (HC).

MATERIAL AND METHODS

Study population

Patients were selected at random from the Salivary Dysfunction Clinic at the Baylor College of Dentistry. In order to be eligible for the study, patients had to have Sjögren's syndrome, be postmenopausal female, and not be on hormone replacement therapy. Patients with primary liver cirrhosis, graft-versus-host disease sarcoidosis, amyloidosis, HIV infection, or history of radiation therapy were excluded from the study. Healthy controls were age-, sex-, and race-matched postmenopausal females who were not on hormone replacement therapy. The diagnosis of Sjögren's syndrome was based on the European Community Criteria[1] with modification. The modification requires that, in addition to the dryness symptoms and reduced salivary and/or tear secretions, patients must have at least one positive autoantibody (ANA, RF, SS-A, or SS-B) and/ or positive minor salivary gland biopsy in order to be diagnosed with Sjögren's syndrome. Each study participant signed an informed consent, which was approved by the Institutional Review Board at Baylor College of Dentistry. Patients and controls were then requested to fill out a questionnaire pertaining to oral and ocular dryness and donate approximately 5-8 ml of blood. The questionnaire included symptoms of dry/sore mouth, difficulty in swallowing, difficulty in chewing, and altered taste/smell. The ocular symptoms included dry eyes, discharge, itching, sandy feeling, excessive tear, foreign body sensation, tired eyes, and intolerance to air draft/light.[13] Positive responses were given a score of one while negative responses were given a score of zero. Total symptom score was calculated by adding all responses.

Serum collection

Blood samples were collected into Vacutainer® containing SST® gel and clot activator (Becton Dickinson, NJ). The samples were allowed to stand at room temperature for at least 30 minutes before separating the serum by centrifugation at 2,500 rpm, using a bench centrifuge, for 15 minutes. The samples were then stored with sodium azide (0.1%, Sigma, St Louis, Mo), at 4° C until used.

Measurement of hormone levels

Hormone levels were determined using either ELISA kit (for prolactin), or EIA kit (for estrogen and progesterone), which were purchased from Diagnostic Systems Laboratories (San Antonio, Tex). The assays were performed according to the manufacturer's instructions. Standards were assayed in duplicates and unknowns in triplicates. The results were expressed as ng/ml or pg/ml, and the relative ratio between various hormones was calculated for each participant separately.

Statistical analyses

Levels and relative ratios of each hormone (prolactin, progesterone, and estrogen) were compared between patients and controls using Mann-Whitney U test at $p < 0.05$. Spearman correlation analysis at $p < 0.05$ was used to evaluate the association between hormone levels with total oral and ocular symptom scores.

RESULTS

Demographics

Thirty-six postmenopausal females participated in the study; 17 had Sjögren's syndrome and 19 were age-, sex-, and race-matched healthy controls. Each of the study participants (patients and controls) was postmenopausal female; none of them was using any form of hormone replacement therapy. SS patients had dry mouth, dry eye, positive serology, and/or a positive salivary gland biopsy with a focus score of ≥ 1 (a focus score is defined as 50 lymphocyte per 4 mm^2). The mean age for patients was 65.35 ± 2.98, and the median was 62 years (range, 47-87 years). The mean age for controls was 57.47 ± 2.63, and the median was 53 years (range, 44-79 years) (Table I).

Prolactin

The mean serum level of prolactin for SS was 11.41 ng/ml ± 1.25 SEM, and the median was 11 ng/ml. The mean serum level for HC was 6.74 ng/ml ± 0.74 SEM, and the median was 6 ng/ml. Mann-Whitney U test revealed a significant difference in the prolactin level between SS patients and controls ($p = 0.003$) (Table II).

Estrogen

The mean serum level of estrogen for SS was 57.35 pg/ml ± 17.31 SEM, and the median was 35.00 pg/ml.

Table II. Comparison*of hormone levels in Sjögren's syndrome and healthy controls

	Mean ± SEM (Median)		
Hormone	*Sjögren's syndrome*	*Healthy control*	*p**
Prolactin (ng/ml)	11.41 ± 1.25 (11)	6.74 ± 0.74 (6.0)	0.003
Estrogen (pg/ml)	57.35 ± 17.31 (35)	35.84 ± 5.20 (27)	0.16
Progesterone (ng/ml)	1.43 ± 0.54 (0.70)	1.73 ± 0.36 (1.10)	0.22

*Mann-Whitney U test.

The mean serum level for HC was 35.84 pg/ml ± 5.20 SEM, and the median was 27 pg/ml. Mann-Whitney U test revealed no significant differences in the estrogen level between SS and HC ($p = 0.16$) (Table II).

Progesterone

The mean serum level of progesterone for SS was 1.43 ng/ml ± 0.54 SEM, and the median was 0.70 pg/m. The mean serum level for HC was 1.73 ng/ml ± 0.36 SEM, and the median was 1.10 ng/ml. Mann-Whitney U test revealed no significant differences in the progesterone level between SS and HC ($p = 0.22$) (Table II).

Relative ratio of prolactin to estrogen

The mean relative ratio of prolactin/estrogen for SS was 0.32 ± 0.06, and the median was 0.26. The mean ratio of prolactin/ progesterone for HC was 0.23 ± 0.03, and the median was 0.22. Mann-Whitney U test revealed no significant difference in the prolactin/estrogen level between SS and HC ($p = 0.25$) (Table III).

Relative ratio of prolactin to progesterone

The mean relative ratio of prolactin/progesterone for SS was 18.88 ± 4.27, and the median was 13.33. The mean ratio of prolactin/ progesterone for HC was 8.145 ± 1.85, and the median was 4.55. Mann-Whitney U test revealed a significant difference in the prolactin/progesterone ratio between SS and HC ($p = 0.02$) (Table III).

Relative ratio of estrogen to progesterone

The mean relative ratio of estrogen/progesterone for SS was 71.51.28 ± 14.51, and the median was 51.25. The mean ratio of estrogen/ progesterone for HC was 42.02 ± 8.94, and the median was 23.08. Mann-Whitney U test revealed a significant difference in the estrogen/progesterone level between SS and HC ($p = 0.05$) (Table III).

Total oral symptom scores

The relationship between hormone levels and total oral symptoms scores is shown in Table IV. Spearman correlation analyses revealed a significant negative correlation between estrogen level and oral symptoms ($p = 0.02$). Similarly, there was a significant negative correlation between progesterone level and oral symptoms ($p = 0.03$). However, no correlation was found between the level of prolactin and oral symptoms ($p = 0.45$).

Table III. Comparison of hormone relative ratios in Sjögren's syndrome and healthy controls

	Mean ± SEM (Median)		
Hormone	*Sjögren's syndrome*	*Healthy control*	*p**
Prolactin/ Estrogen	0.32 ± 0.06 (0.26)	0.23 ± 0.03 (0.22)	0.25
Prolactin/ Progesterone	18.88 ± 4.27 (13.33)	8.14 ± 1.85 (4.55)	0.02
Estrogen/ progesterone	71.51 ± 14.51 (51.25)	42.02 ± 8.94 (23.08)	0.05

*Mann-Whitney U test.

Total ocular symptom scores

Spearman correlation revealed no significant correlation between various hormones and ocular symptoms, although prolactin level approached a significant negative correlation ($p = 0.09$), Table IV.

DISCUSSION

The high incidence of autoimmune diseases among females suggests that gender may be a risk factor in the development of autoimmune disease. Females constitute 65-75% cases of rheumatoid arthritis, Addison's disease, and myasthenia gravis; 85% of Hashimoto's thyroiditis; and over 90% of systemic lupus and Sjögren's syndrome.[14] For this reason, sex hormones were implicated in modulating the immune response.[8] Hormonal modulation of autoimmunity was reported in animal models.[9,15] Studies have suggested that estrogen deficiency can lead to severe destructive autoimmune lesions in murine salivary and lacrimal glands.[9] Estrogen exhibits stimulatory effect on prolactin secretion, but a high level of prolactin suppresses estrogen production. In contrast, progesterone exhibits an inverse relationship with estrogen.[15] Although the role of hormones remains unclear, these findings suggest that the relative ratio of hormones may be as important as the individual level of each hormone.

Results of this study indicate that the level of prolactin for both patients and controls were well within the normal range for adult women (2.5-25.6 ng/ml), as determined by the ELISA kit that was used in this investigation. However, our results also indicated that the mean level of prolactin was significantly higher among Sjögren's patients than healthy controls (11.41 vs. 6.74 ng/ml, $p = 0.003$). Prolactin, which is secreted by the pituitary gland, is a hormone that has been shown to be elevated in patients with autoimmune disease(s).[17] Research evidence suggests that prolactin can act as a

Table IV. Relationship between hormone levels and total oral and ocular symptoms

	Total oral symptom score		*Total ocular symptom score*	
Hormone	*r*	*p*	*r*	*p*
Prolactin	−0.186	0.455	−0.413	0.09
Estrogen	−0.552	0.027	−0.103	0.67
Progesterone	−0.518	0.038	−0.293	0.24

*Spearman correlation

pro-inflammatory mediator and may modulate the immune response. Hyperprolactinemia was reported in 46% of patients with primary Sjögren's syndrome and in 59% of patients with scleroderma.[17] These findings are in agreement with other studies, which suggest that prolactin is a potent immune stimulator, modulates proliferation and differentiation of variety of cells in the immune system, stimulates macrophage, and plays a role in macrophages tissue depletion. Prolactin shares many properties with cytokines and could potentially play a role in the pathogenesis of autoimmune diseases.[15,16]

Unlike the results obtained from animal studies, which associate estrogen deficiency with autoimmune diseases,[9] our study showed higher levels of estrogen among SS patients than controls (57.35 vs. 35.84 pg/ml, respectively). These results are in agreement with other studies, which demonstrate higher level of estrogen among SS patients, and they also support the hypothesis, which suggests that higher level of prolactin is associated with elevated estrogen levels.[5,8,15] Despite its inhibitory effect on T-cell dependent immune function, estrogen is known to aggravate B-cell dependent response in diseases and leads to increased antibody production.[14] This finding is also consistent with the theory that proposes a biphasic effect for estrogen: at lower doses estrogen is believed to enhance the immune response, while at higher doses (such as during pregnancy) it may have an inhibitory effect.[18]

Finally, results of our studies did not show a significant difference in progesterone level between patients and controls (1.43 vs. 1.73 ng/ml, $p = 0.22$). These values were within the normal range (0.1-1.1 ng/ml) for postmenopausal women, as determined by the manufacturer of the EIA kit (Diagnostic Systems Laboratories) that was used in this study. The ratio of prolactin to progesterone, however, was significantly higher in patients than controls (18.8 vs. 8.1, $p = 0.02$). Similarly, the ratio of estrogen to progesterone was significantly higher in SS than HC (71.5 vs. 42.0, $p = 0.05$). It is possible that the relative ratio of the hormones is more critical for the integrity of the biological system than their absolute concentrations. Currently, there is no study that addresses the potential effect of changes in the relative ratios of sex hormones in Sjögren's syndrome.

As for the relationship between the hormones and dryness symptoms, there was significant negative correlation between estrogen and progesterone levels with oral symptoms ($p = 0.02$ and 0.03 respectively). But, except for prolactin, there was no correlation between hormone levels and ocular symptoms (Table IV). Previous studies have linked synergistic elevation of estrogen and progesterone with the severity of symptoms in Sjögren's syndrome.[6] However, a recent study did not support a correlation between estrogen and disease activity in Sjögren's syndrome, while a positive correlation was found between testosterone and various measures of disease activity in the same patient population.[19]

Due to financial constraints, the study population was relatively small, and only a limited number of hormones was examined. In order to reduce the possibility of an artificial variable on the results, none of the study participants was using hormone replacement therapy. The outcome of this study appears to support a role for sex hormones in Sjögren's syndrome. However, a meaningful understanding of the role of hormones action and interaction in Sjögren's syndrome requires studying a larger population and evaluation of all sex hormones. Such a study may allow better understanding of the relationship between the endocrine system and autoimmune diseases.

In conclusion, this is the first study that examines estrogen, prolactin, and progesterone in the same SS population and evaluates their effects on dryness symptoms. The overall results of this study suggest that despite the normal values of these hormones among the study population, there were noticeable differences in their levels between Sjögren's patients and healthy controls. These differences may contribute to the development and/or progression of Sjögren's syndrome and could potentially affect the severity of the symptoms. Evaluation of the concentration of hormones and their receptors within the salivary gland may shed a light on the mechanism of pathogenesis of Sjögren's syndrome. Our results also suggest that in order to understand the role(s) of sex hormones in autoimmune diseases, it may be important to examine their relative ratios in addition to their absolute values.

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