Running head: CORTISOL LEVELS AND AUTOIMMUNE DISEASES

Title: Analyses of hair and salivary cortisol for evaluating hypothalamic-pituitary-adrenal axis activation in patients with autoimmune disease

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Abstract

Although many studies have shown that patients with autoimmune disease present a hypoactive hypothalamic-pituitary-adrenal axis (HPA), controversial results have been described. Our objective was to study HPA axis activity in women with autoimmune disease compared to healthy women. Therefore, we analyzed salivary cortisol over the course of a day, and hair cortisol concentrations from the three preceding months, from 65 women divided into two groups: healthy women (n = 30), with a mean age of 44.70 ± 11.65 years; and women with autoimmune disease (n = 35), with a mean age of 48.26 ± 9.04 years. The latter group comprises women with systemic lupus erythematosus (SLE), Sjögren’s syndrome (SS), and systemic sclerosis (SSc). Perceived stress and psychopathological symptomatology...
were also evaluated. Autoimmune disease group scored higher on the somatization subscale SCL-90-R and lower on the anxiety subscale than the control group. Regarding HPA axis activation, the area under curve for cortisol levels during the day was higher for the autoimmune disease group. In addition, higher cortisol levels in hair were found in the group with autoimmune disease. Our findings show greater short and long-term HPA axis activity in women with autoimmune disease than in healthy women.

**Keywords:** stress, cortisol, HPA axis, systemic lupus erythematosus, Sjögren’s syndrome, systemic sclerosis
Introduction

The main systemic autoimmune diseases are characterized largely by affecting multiple organs and systems in a single patient, by their unknown etiology, and by periods of disease aggravation alternating with periods of disease inactivity (Jiménez-Alonso et al., 2011). One of the changes seen in these patients is altered activity of the HPA axis (Tzioufas et al., 2008). The hypothalamic-pituitary-adrenal axis (HPA) axis plays an important role in modulating autoimmune activity by release of glucocorticoids (Charmandari et al., 2005; Heesen et al., 2007), hormones secreted in response to stress (McEwen, 2000). Psychological stress is considered one of the main causes for the onset and aggravation of symptoms of various autoimmune diseases (McCray and Agrawal, 2001; Johnson et al., 2006; Peralta-Ramírez, et al., 2004; Stojanovich and Marisavljevich, 2008).

Cortisol, the main hormone produced in response to stress, has been widely used as an HPA axis activity biomarker, and elevated levels of cortisol have important physical and psychological consequences. Traditionally, saliva, blood, and urine analyses are conducted to establish the amount of cortisol secreted at a precise time within the past 24 hours. However, a new procedure has recently been developed using the patient’s hair to provide information regarding stress exposure from days up to months (Meyer and Novak, 2012). Hair cortisol is considered by some to be an objective and retrospective measure of chronic stress (Gow et al., 2010). Furthermore, the use of hair avoids the variability of cortisol levels in fluids associated to individual and environmental characteristics, study procedures (Wolfram et al., 2013), the time of day (Adam et al., 2006), and food consumption (Gibson et al., 1999).

No consensus has been reached regarding HPA regulation in autoimmune diseases. Some studies show that the psychological stress that patients with autoimmune disease experience causes hypoactivity of the HPA axis (Delevaux et al., 2013; Shalimar et al., 2006;
Silverman and Sternberg, 2012; Van der Goes et al., 2011). However, Finan and Zautra (2013) did not observe that daily stressors or worries influence the cortisol levels of patients with rheumatoid arthritis (RA).

Jung et al. (2015) were the first to evaluate the relationship of salivary cortisol levels with depression, stress, and disease activity in patients with systemic lupus erythematosus (SLE) compared to healthy controls. Their results showed similar levels in perceived stress and salivary cortisol in both groups, but higher levels of alpha-amylase and depression in patients with SLE. Significant correlations between depression and 1) erythrocyte sedimentation rate (ESR) and 2) disease activity index were found. These results indicated autonomous nervous system (ANS) and HPA axis dysregulation in patients with SLE.

Harle et al. (2006) measured serum neuropeptide Y and cortisol or plasma ACTH levels to assess sympathetic nervous system (SNS) and HPA activities in patients with SLE or RA, and healthy controls. Their results showed that patients with SLE or RA had greater SNS activation and lesser HPA axis activation than the healthy controls. Thus, they concluded that an altered activity of the SNS and HPA axis is present in patients with autoimmune disease.

On the other hand, various studies have analyzed HPA axis regulation in patients with Sjögren’s syndrome (SS) by measuring salivary cortisol levels. Van der Goes et al. (2011) compared salivary cortisol levels during the day and ESR in patients with SLE, patients with (SS), and healthy persons. Their results showed that SLE and SS patients with high ESR had lower cortisol awakening response (CAR) than patients with low ESR. However, the authors concluded that HPA axis dysfunction in the patients with SLE and SS was not clear, because the cortisol levels were no different than those of the healthy persons. In a later study, Miller et al. (2012) evaluated salivary cortisol between 10 and 12 am in patients with SS and healthy persons, and found no differences between the two groups.
Consequently, no consensus has been reached regarding HPA axis response in patients with autoimmune disease, because it has not been studied in many specific diseases, and because the findings from those studies show different directionality regarding regulation. Furthermore, the methods used for analyzing salivary cortisol, urine, and plasma show values for isolated measures of HPA axis activity. We believe that in order to gain a deeper understanding of the relation between psychological stress and autoimmune-disease aggravation, and to know better in which direction HPA regulation is being produced, it is essential to research HPA axis activity, by studying cortisol levels retrospectively as well as in the context of laboratory testing. Consequently, the objective of our investigation has been to study HPA axis activity between women with autoimmune disease compared to healthy women by analyzing salivary cortisol during the day as a current measure, and hair cortisol as a retrospective measure of chronic stress. For the latter, we analyze hair cortisol concentrations from the three preceding months and psychological stress through self-report measures.

Material and methods

Participants

Sixty-five women participated in this study. Thirty were healthy and had a mean age of 44.70 years (SD = 11.65). Thirty-five had an autoimmune disease and a mean age of 48.26 years (SD = 9.04). Within this second group 11 women had SLE, 12 SS, and 12 systemic sclerosis (SSc).

The healthy women were recruited through posters in public institutions, newspapers, and local radio. The inclusion criteria were age 18–65, literate, and not presenting a physical or mental disease at the time of the study. Because of their potentially negative effect on cortisol levels (Williams et al., 2004), the exclusion criteria were: hypertension, heart disease,
obesity, clinical diagnosis of depression or anxiety, personality disorders, and substance use (i.e., amphetamines, methadone, barbiturates, or muscle relaxants). This information was obtained through a semi-structured interview conducted when the women called to participate in the study.

The women with autoimmune disease were recruited by medical staff from the Systemic Autoimmune Disease Units (Internal Medicine Service) at the Virgen de las Nieves University Hospital and the San Cecilio Clinical Hospital, both located in Granada, Spain. They were recruited during an appointment with their doctor to confirm they were not experiencing symptom flare up at the time of study. The inclusion criteria were age 18–65, literate, diagnosed according to medical diagnostic criteria with systemic lupus erythematosus, Sjögren’s syndrome, or systemic sclerosis; no corticosteroid treatment for a minimum of one year previous to the study; and no severe psychiatric disorders. The exclusion criteria were: obesity, clinical diagnosis of depression or anxiety, personality disorders, and substance use (i.e., amphetamines, methadone, barbiturates, or muscle relaxants). The study is focused in these diseases for they are by far more prevalent in females in both hospitals. In addition, these are systemic and autoimmune diseases with a common genetics. There considerable literature explaining common manifestations of the autoimmune diseases and possible therapeutic targets in adults (Kochi, 2016) and children (Li et al., 2015).

**Procedure**

A brief interview was conducted by telephone with each woman interested in participating in the study to ensure they met the inclusion criteria for either the healthy group or the autoimmune disease group. Once accepted, the participants were provided information regarding the study. They then read and signed the informed consent form, which had been approved by both hospitals’ ethics committees and was in accordance with the
recommendations of the Helsinki Declaration. Subsequently, the women were interviewed, and then they completed the perceived stress and psychopathological symptomatology questionnaires.

All the participants were tested in the lab between Mondays to Thursdays, and they collected the saliva samples the next day. In this way, the salivary cortisol samples were collected on weekdays, not on weekends.

The procedure for collecting salivary cortisol samples during the day was explained to the participants. They were then given a kit containing 5 Salivette® tubes, a detailed instruction-sheet explaining sample collection and a record sheet for indicating the day and the time the sample was collected. We instructed them to avoid eating (neither chewing gum nor candy) or drinking except water and were not allowed to smoke during the half hour preceding each sample collection. For sample collection, they were told to introduce and soak the cotton swab during one minute. For example, they self-collected one salivary sample in the lab after the explanation, to assure they could collect the sample correctly and that the saliva did not have red blood cells or another contaminants element. Finally, a lock of head hair was cut, which upon analysis is thought to provide cortisol levels from the previous 3 months. Two days later the participants were to deliver the salivary cortisol samples and record sheet to the laboratory. For assure the adherence we paid them 20 € delivered the samples.

The salivary cortisol samples of two patients with SS could not be measured because there was not enough saliva to analyze the cortisol levels. There were three outliers values from hair samples in the healthy group and two outliers from hair samples in the autoimmune group that were included for the analysis.

**Instruments**
The questionnaires below provide meaningful covariates that were used in the statistical analyses of psychological measures and cortisol levels.

*Semi-structured interview:* The subjects provided information on socio-demographic factors, daily life and sleep habits, medication, and history of psychiatric or psychological treatment. This information was completed with the medical history from patients with autoimmune diseases.

*Perceived Stress Scale (PSS)* (Cohen, Kamarak, and Mermeistein, 1983; Spanish adaptation by Remor and Carrobles, 2001): The PSS is a self-report scale used to evaluate perceived stress levels and the degree to which people find their lives unpredictable, uncontrollable, or overwhelming (aspects that contribute to stress) over the past seven days, including the day the scale is administered. The PSS consists of 14 items with five response alternatives. The highest score corresponds to the highest perceived stress level. The Spanish version of the PSS (14 items) has adequate reliability (internal consistency=0.81 and test-retest=0.73), concurrent validity, and sensitivity (Remor, 2006). Here, we considered scores over 22 (i.e., the mean score for the Spanish population; Remor and Carrobles, 2001) as reflecting high levels of perceived stress.

*SCL-90-R Symptoms Inventory (SCL-90-R):* The SCL-90-R (Derogatis, 1994; Spanish adaptation by Gonzalez de Rivera et al., 1988) is a self-report questionnaire that was developed to assess symptoms of psychopathology. It comprises 90 items with five response alternatives (0-4) on a Likert scale. Subjects respond according to how they have felt within the past seven days, including the day the inventory is administered. The inventory is scored and interpreted according to nine main dimensions (somatization, obsessive-compulsive symptoms, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism) and three global indices of psychological distress (Global
Severity Index [GSI], Positive Symptom Total [PST], and Positive Symptom Distress Index [PSDI]). This instrument demonstrates satisfactory levels of reliability and validity (De las Cuevas et al., 1991).

**Cortisol measures**

*Measurement of cortisol during the day:* Salivary cortisol sample-collection was performed using a Salivette® Cortisol kit (Sarstedt, Numbrecht, Germany, Ref.51.1534) that consists of two small tubes, one of which contains a small piece of cotton. Participants chewed the cotton for approximately 60 seconds and then placed it back into the same tube for analysis. Samples were analyzed at the San Cecilio Clinical Hospital using the electrochemiluminescence immunoassay “ECLIA” method. This method was designed for use in Roche Elecsys 1010/2010 automated analyzers with the Elecsys MODULAR ANALYTICS E170 module.

Each participant collected five samples of salivary cortisol during one day. The first sample (Cortisol 1) was collected 30 minutes after waking up (while still fasting), the second (Cortisol 2) at +4 hours, the third (Cortisol 3) at +8 hours, the fourth (Cortisol 4) at +12 hours, and the fifth (Cortisol 5) at +16 hours. We asked them to write down in the record sheet the times of each sample collection, and set an alarm on the subjects’ phone for to ensure they collected the saliva samples at the appropriate time.

*Measurement of hair cortisol:* Hair samples were cut with scissors from the posterior vertex of the head as closely to the scalp as possible by a researcher experienced with hair collection. Each sample consisted of approximately 150 strands of hair. The samples were then wrapped, labeling the scalp end in a piece of aluminum foil to protect the hair from light and humidity and stored in an envelope at room temperature. Later, they were sent to the Faculty of Pharmacy at the University of Granada where the 3 cm segments of hair most proximal to the scalp were analyzed (assuming an average growth rate of 1 cm/month (Wennig, 2000), a 3 cm
segment contains cortisol that has been deposited over approximately the last 3 months). The sample was washed twice with 1 ml of high performance liquid chromatography (HPLC)-grade isopropanol, by repeated inversion for 3 minutes, using a rotator. Subsequently, the sample was weighed and ground to a fine powder to break up the hair’s protein matrix and increase the surface area for extraction using a ball mill (Bullet Blender Storm, Swedesboro NJ, USA). Cortisol from the interior of the hair shaft was extracted into HPLC-grade methanol by incubation of the sample for 72 hours at room temperature in the dark, with constant inversion using a rotator. After incubation, the supernatant was evaporated until completely dry using a vacuum evaporator (Centrivac, Heraeus, Hanau, Germany) and the extract was reconstituted in 150 uL of phosphate buffered saline (PBS) at pH 8.0. The reconstituted sample was immediately frozen at -20ºC for later analysis (Sauvé, 2007; Chen et al., 2013; Meyer et al., 2014).

The hair cortisol sample was measured using the Salivary ELISA Cortisol kit© (Alpco Diagnostics®, Windham, NH) as per the manufacturer’s directions with the reagent provided, conscious that the results from the ELISA method would be higher than those obtained through mass spectrometry (Slominski et al., 2015). The cross reactivity as reported by the manufacturer is as follows: Prednisolone 13.6%, Corticosterone 7.6%, Deoxycorticosterone 7.2%, Progesterone 7.2%, Cortisone 6.2%, Deoxycortisol 5.6%, Prednisone 5.6% and Dexamethasone 1.6%. No cross-reaction was detected with DHEAS and Tetrahydrocortisone.

The intra- and inter-assay variations were analyzed on internal quality controls used for routine saliva cortisol measurement, analyzed in duplicate on ten consecutive assays. The intra-assay coefficients of variance were 4.4% at 11.1ng/ml and 2.6% at 54.4 ng/ml. The inter-assay coefficients of variance were 12.7 and 10.2%, respectively. In addition, it is important to note that the ELISA kit used by our group is the same one used by Russell et al.
In a study carried out by four leading laboratories in hair testing. In this study, four immunoassay methods and two liquids chromatography–mass spectrometry (LC–MS/MS) methods were compared by analyzing the same hair samples representing the low, intermediate and, high ranges of hair cortisol concentrations (HCC). The results of this study showed that HCC determined by the four immunoassay methods were highly and positively inter-correlated ($r^2$ between 0.92 and 0.97; all $P < 0.0001$) in all comparisons of individual laboratories. Additionally, each laboratory’s HCC immunoassay had significant positive correlations ($r^2$ between 0.88 and 0.97; all $P < 0.0001$) with each of the two LC–MS/MS methods, which produced practically identical results.

The hair cortisol levels were expressed in pg/mg values as in the studies of Sauvé et al. (2007) and Henley et al. (2013). Sauvé et al. (2007) used Alpco ELISA kit and they established one reference range of 1.7 - 153.2 pg/mg (log-transformed) in people without obesity. As well, Henley et al. (2013) used the same kit and the results showed a median hair cortisol level (range) of 116 (26 - 204) pg/mg for healthy persons and 177 (93 - 273) pg/mg for individuals reporting stress.

**Statistical analyses**

Student’s $t$-tests were conducted to control for differences between both groups (the autoimmune disease group and the healthy group) with regard to socio-demographic variables (age and education level) and psychological variables (SLC-90-R and PSS).

A mixed $2 \times 5$ analysis of covariance (ANCOVA) was then conducted to check for statistically significant differences between both groups regarding cortisol levels during the day. The first factor includes two levels (healthy group and autoimmune disease group) between the independent groups. The second was a repeated-measures within-subject factor at five time points: (Cortisol 1) collected 30 minutes after waking up (while still fasting),
(Cortisol 2) at +4 hours, (Cortisol 3) at +8 hours, (Cortisol 4) at +12 hours, and (Cortisol 5) at +16 hours. The Greenhouse-Geisser Correction was applied in the repeated-measures analyses. The anxiety and somatization subscales of the SLC-90-R were included as covariates. Student’s t-tests were conducted to determine whether there were differences in saliva cortisol samples between both groups.

Further, the AUCg was measured in all participants, which provided information regarding the changes occurring during observation of the dependent variable (salivary cortisol levels). The AUCg was calculated using the trapezoid formula from Pruessner et al. (2003). An ANCOVA was then run to check for significant differences in AUCg between the healthy group and the autoimmune disease group. The anxiety and somatization subscales of the SLC-90-R were included as covariates.

Hair cortisol concentrations were natural log (ln, base e) transformed to normalize values. An ANCOVA was then run to check for significant differences in hair cortisol between the healthy group and the autoimmune disease group. The anxiety and somatization subscales of the SLC-90-R were included as covariates.

Pearson correlation analyses were used to examine cross-sectional associations among hair cortisol concentrations and the total amount of salivary cortisol produced (AUCg) and hair cortisol concentrations and the five time points of salivary cortisol during the day.

All data were analyzed with SPSS version 21.

Results

Sample description

Table 1 presents the participants’ socio-demographic and psychological variables. There were no group differences in socio-demographic, perceived stress, and
psychopathological symptomatology variables, with the exception of the anxiety and somatization subscales of the SCL-90-R. The autoimmune disease group shows higher scores than the healthy group on the somatization subscale and lower scores on the anxiety subscale. There were no significant differences between years of illness and the three diseases: SLE (10.34 ± 9.24 years), SS (5.04 ± 4.85 years), and SSc (9.17 ± 5.95 years). The average years of disease in the autoimmune group was 8.12 ± 7.03.

**Insert Table 1**

**Cortisol during the day**

The statistical analyses show that there was no significant time × group interaction effect, however, there were statistically significant differences in AUCg cortisol levels (F (1,59) = 4,209.031; \( p \leq 0.001 \)): the autoimmune disease group exhibited higher levels (M = 21,578.92 ± 7,936.25 nmol/L) than the healthy group (M = 15.18 ± 7.90 nmol/L). Figure 1 shows the AUCg salivary cortisol values.

There were significant differences at the Cortisol 2 (t = -2.949; p = 0.005), Cortisol 3 (t = -2.395; p = 0.020), and Cortisol 5 (t = -3.081; p = 0.003) collections, where the autoimmune disease group showed higher levels than the healthy group. Data cortisol levels at different times during the day are shown in Table 2.

**Insert Figure 1**

**Insert Table 2**

**Hair cortisol**
The results revealed statistically significant differences between groups with regard to hair cortisol concentrations ($F(1,63) = 5.542; \ p = 0.022$), with the autoimmune disease group exhibited higher concentrations than the healthy group (Figure 2).

**INSERT FIGURE 2**

There were three outliers values from hair samples in the healthy group with concentrations of 499.72 pg/mg, 365.43 pm/mg, and 27.59 pg/mg. The two outliers from hair samples in the autoimmune group were of 24.05 pg/mg and 30.10 pg/mg.

There was a positive correlation between the hair cortisol levels and AUCg levels ($r = 0.402; \ p = 0.001$) and a positive correlation between the hair cortisol levels and the Cortisol 5 samples ($r = 0.268; \ p = 0.034$).

**Discussion**

The objective of our research was to study HPA axis activity among women with autoimmune disease compared to healthy women on two levels: by analyzing the salivary cortisol levels during one day as a measure of current stress, and hair cortisol as a retrospective measure of chronic stress. For the latter, we analyzed hair cortisol measures containing concentrations from hair samples approximating the three preceding months, and psychological stress levels through self-report measures.

All socio-demographic and psychological variables were similar for both groups, with the exception of the somatization subscale, in which the autoimmune disease group showed higher scores, foreseeable when comparing healthy persons to others with disease. Differences on the anxiety subscale were also found, reflecting higher scores for the healthy group. Nevertheless, even though both somatization and anxiety affect AUCg levels and the
healthy group was experiencing more anxiety, AUCg levels were still significantly higher in the autoimmune disease group.

However, tests of HPA axis activity indicated significant differences in AUCg, with higher levels observed in the autoimmune disease group than in the healthy group. These results indicate HPA axis hyper-activation in patients with autoimmune disease that could be caused by the alteration of the immune system. Thus, we cannot confirm that autoimmune patients will have lower levels of cortisol than the healthy population. One example of this is a study by Straub et al. (2010), in which patients with RA showed HPA axis activation similar to that of healthy persons. According to Straub, this led to chronic activation of the sympathetic nervous system in the autoimmune disease patients, which acted as a compensation mechanism between the two axes: because these patients were experiencing inflammation, HPA axis activation could not resemble that of healthy persons. This produces a desynchronization between both axes.

Similarly, Jung et al. (2015) found that patients with SLE had higher levels of alpha-amylase than healthy controls. Nevertheless, differences in salivary cortisol levels were not found between patients with SLE and healthy controls. However, unlike our study, the patients with SLE who participated in that study were undergoing corticosteroid treatment, and that could affect the results and how HPA axis activation was interpreted in those SLE patients. The differences in results between both studies could be explained by the fact that our study included patients with other autoimmune diseases, in addition to patients with SLE, and none of our patients had received corticosteroid treatment for a minimum of one year preceding the study, so as to guarantee elimination of corticosteroids in the patients’ systems and the hyper atrophy of the adrenal glands that results from corticosteroid use (Ruiz-Arruza et al., 2014).
Van der Goes et al. (2011) also evaluated salivary cortisol in patients with primary SS and patients with SLE. The study found that the ESR predicted HPA axis activation in those patients, thus patients with elevated ESR had lower CAR than patients with low ESR. Since our patients did not suffer from intense inflammation (as shown by the absence of corticoid treatment), and although we did not evaluate these variables, we think that they could explain the differences we found in cortisol levels during the day between the autoimmune disease and the healthy group.

On the other hand, the results of higher saliva cortisol levels in the Cortisol 2, Cortisol 3, and Cortisol 5 samples of the day collected in autoimmune diseases group seem like a lack of circadian rhythm in cortisol levels across the day. Chung, Son, and Kim (2011) revised studies where patients with abnormal HPA axis activity showed circadian rhythm related symptoms. They concluded that this had implications and risks factors in the health and disease.

Currently, all studies of this axis have focused on cortisol in blood, saliva or urine, measures that provide scores from minutes, hours or days. To date, however, HPA axis activity has not been studied retrospectively through hair cortisol in persons with SLE, SS and SSc versus healthy people. This is a technique that provides stable cortisol readings unaffected by individual and environmental characteristics, study procedures (Wolfram et al., 2013), the time of day (Adam et al., 2006), and food consumption (Gibson et al., 1999) maintained during months.

Regarding hair cortisol levels accumulated during the preceding three months, the results revealed the same as those for salivary cortisol levels AUCg; namely, that women with autoimmune disease showed higher cortisol levels than healthy women. The significant correlation between both measures confirms these results.
Our results concerning salivary and hair cortisol indicate that the autoimmune disease group presents higher HPA axis activity. The HPA axis behaves similarly in both the long and short-term, as women with autoimmune disease have greater HPA axis activity than healthy women. This fact could also be due to the time that they have been suffering the illness and elevated blood interleukin-6 (IL-6) levels. Mastorakos et al. (2013) associated elevated IL-6 levels with elevated HPA axis activity of patients with sarcoidosis. Furthermore, Fujio et al. (2016) investigated the influence of interleukin-6 levels on the HPA axis after onset of corticoids treatment in patients with autoimmune diseases and they found positive correlation between levels of IL-6 and basal cortisol.

Our findings are similar to those from studies included in the review by Meyer and Novak (2012), in which long-term HPA axis activity measured by hair cortisol levels and short-term HPA axis activity measured by salivary cortisol are related under certain conditions. In this regard, ours is the first study to include salivary and hair cortisol measures for evaluating and comparing HPA axis activity in women with autoimmune disease who are not undergoing corticosteroid treatment with healthy women.

There are some limitations to our study. It would be interesting to evaluate a larger sample of patients with autoimmune disease with a broader scope of autoimmune pathologies, as well as other hormones, such as alpha-amylase, that are directly involved in regulating the HPA axis and sympathetic adrenomedullary system, because both are key components in the response to stress and carry out an important modulator role in the immune system. Additionally, it is recommended that saliva samples should be collected during the day at approximately the same time for all participants (difficult due to differences in awakening hours and bedtime). It is further recommended to collect saliva samples over two consecutive days and calculate the means at each sampling time.
Conclusions

Both higher cortisol hair concentrations and hormonal production indicate that women with autoimmune disease have increased long and short-term HPA axis activity than healthy women. Anxiety and somatization levels could be predictor variables of a greater response to daily stress. This could be caused by dysregulation of HPA axis in patients with autoimmune disease. These findings provide novel information on HPA axis behavior in persons with autoimmune disease.

Lay summary

This study examines the HPA axis activity in healthy women and women with autoimmune diseases (systemic lupus erythematosus, Sjögren’s syndrome and systemic sclerosis) by analyzing salivary cortisol, hair cortisol levels, and self-report measures of stress. We found that autoimmune disease group scored higher on the somatization subscale SCL-90-R, the area under curve for salivary cortisol levels during the day, and hair cortisol concentrations. This could suggest greater short and long-term HPA axis activity in women with autoimmune disease than in healthy women.

Declaration of interest

None-declared
References


Table 1. Means and standard deviations (SD) of socio-demographic and psychological variables for the participants in both groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy Group (n = 30)</th>
<th>Autoimmune Group (n = 35)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.70 ± 11.65</td>
<td>48.26 ± 9.04</td>
<td>1.92</td>
<td>0.17</td>
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<tr>
<td>Education (years)</td>
<td>15.33 ± 3.57</td>
<td>14.14 ± 3.79</td>
<td>1.68</td>
<td>0.20</td>
</tr>
<tr>
<td>Perceived Stress Scale</td>
<td>25.47 ± 5.93</td>
<td>26.74 ± 9.93</td>
<td>0.38</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Symptom Checklist SCL-90-R**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatization</td>
<td>51.73 ± 27.48</td>
<td>74.70 ± 22.67</td>
<td>13.18</td>
<td>0.001**</td>
</tr>
<tr>
<td>Obsessions and compulsions</td>
<td>78.43 ± 21.97</td>
<td>78.70 ± 24.84</td>
<td>0.002</td>
<td>0.96</td>
</tr>
<tr>
<td>Interpersonal sensitivity</td>
<td>71.33 ± 28.21</td>
<td>67.06 ± 33.65</td>
<td>0.29</td>
<td>0.59</td>
</tr>
<tr>
<td>Depression</td>
<td>66.77 ± 28.46</td>
<td>63.61 ± 32.39</td>
<td>0.17</td>
<td>0.68</td>
</tr>
<tr>
<td>Anxiety</td>
<td>71.07 ± 20.75</td>
<td>63.21 ± 31.93</td>
<td>4.08</td>
<td>0.048*</td>
</tr>
<tr>
<td>Hostility</td>
<td>65.97 ± 22.85</td>
<td>56.91 ± 33.64</td>
<td>1.53</td>
<td>0.22</td>
</tr>
<tr>
<td>Phobic Anxiety</td>
<td>48.37 ± 38.77</td>
<td>48.88 ± 38.31</td>
<td>0.003</td>
<td>0.96</td>
</tr>
<tr>
<td>Paranoia</td>
<td>65.27 ± 32.75</td>
<td>54.70 ± 36.67</td>
<td>1.44</td>
<td>0.23</td>
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<tr>
<td>Psychoticism</td>
<td>69.43 ± 32.74</td>
<td>59.97 ± 32.38</td>
<td>1.33</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*t*: t-student value; *p ≤ 0.05. **p ≤ 0.01
Table 2. Means and standard deviations (SD) of salivary cortisol during the day

<table>
<thead>
<tr>
<th>Cortisol samples (nmol/L)</th>
<th>Healthy Group (n = 30)</th>
<th>Autoimmune Group (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± SD</td>
<td>M ± SD</td>
</tr>
<tr>
<td>Cortisol 1 (30’ after waking)</td>
<td>15.56 ± 7.89</td>
<td>18.15 ± 8.74</td>
</tr>
<tr>
<td>Cortisol 2 (at +4 hours)</td>
<td>6.10 ± 3.21</td>
<td>9.03 ± 4.60</td>
</tr>
<tr>
<td>Cortisol 3 (at +8 hours)</td>
<td>5.49 ± 3.33</td>
<td>7.61 ± 3.70</td>
</tr>
<tr>
<td>Cortisol 4 (at +12 hours)</td>
<td>5.27 ± 4.67</td>
<td>6.47 ± 4.44</td>
</tr>
<tr>
<td>Cortisol 5 (at +16 hours)</td>
<td>3.89 ± 3.77</td>
<td>6.75 ± 3.55</td>
</tr>
</tbody>
</table>
Figure 1. AUCg levels from the salivary cortisol during the day for the healthy group and the autoimmune disease group.
Figure 2. Hair cortisol levels from the previous three months for the healthy group and the autoimmune disease group.