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Cortisol awakening response is blunted and pain perception is increased during menses in cyclic women

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ABSTRACT

Background and aims: The incidence of menstrual symptoms is reported to be as high as 90% in cyclic women. These symptoms, including anxiety and pain, might be associated with cortisol, as its receptors are widely distributed in the brain areas associated with behavior. Therefore, the current study aimed to assess the cortisol awakening response (CAR) throughout the menstrual cycle and correlate it with pain perception and trait anxiety.

Materials and methods: CAR was assessed by measuring salivary cortisol at 0, 15, 30, and 60 min following awakening in the same women (n = 59, age 22.2 ± 0.37 years) at various stages of the menstrual cycle (menses, midcycle, luteal and premenstrual phases). Progesterone and estradiol concentrations were also determined in saliva samples to assess cyclic changes. Self-reported pain, trait anxiety, and menstrual symptoms were assessed by visual analog scale (VAS), state-trait anxiety inventory (STAI-T), and the Daily Record of Severity of Problems (DRSP), respectively.

Results: Estradiol was significantly elevated during the midcycle period and remained high during the early luteal phase ($p < 0.05$). Progesterone was increased during the luteal phase ($p < 0.05$). Post-awakening cortisol values increased during midcycle, luteal phase, and premenstrual phase ($p < 0.05$, classical CAR), but not during the menses ($p > 0.05$, blunted or flat CAR). Positive and significant correlations were found between cortisol and estradiol ($R^2 = 0.322$; $p = 0.000$), cortisol and progesterone ($R^2 = 0.156$; $p = 0.000$), and estradiol and progesterone ($R^2 = 0.349$; $p = 0.001$). Premenstrual symptom scores were higher in the menses and premenstrual phases than in the midcycle and luteal phases ($p < 0.001$). Pain perception was the highest during the menses followed by the premenstrual phase ($p < 0.01$).

Conclusions: CAR was blunted during the menses, suggesting that cortisol might play a phase-specific role in the regulation of the cycle. Additionally, premenstrual symptoms, including pain, were more severe when ovarian steroid levels reduced (i.e., menses and the premenstrual phase).

1. Introduction

Cortisol is a steroid hormone secreted from the adrenal cortex in response to stressful events (Adam et al., 2014). It can easily pass the lipid barriers in its active (free, unconjugated) form (Kirschbaum and Hellhammer, 2000) and disperses into all body fluids, including saliva (Kaushik et al., 2014). It has a circadian rhythm and reaches a maximum level 30 min following awakening in the morning (Kudielka and Kirschbaum, 2003). This increase is described as the cortisol awakening response (CAR) (Pruessner et al., 1997), and it is measured in saliva as a noninvasive indicator of adrenocortical activity (Spath-Schwalbe et al., 1992; Doom and Gunnar, 2013). The precise role of CAR remains unknown (Law et al., 2015), but it has been demonstrated that CAR levels might be associated with degree of regional or generalized pain perception (Riva et al., 2012; Markert et al., 2016), stress and anxiety (Adam et al., 2014; Gallagher et al., 2016), and depressive symptomatology (Vargas et al., 2016).

Menstrual cycles occur throughout the active reproductive life of a woman and include cyclical changes in progesterone and estradiol secretions. Decreased ovarian steroids during the perimenstrual period are generally associated with a variety of psychological changes, including depression, anxiety, headache, and nervousness (Gerrish et al., 2010). However, these symptoms are not observed in every woman and with same severity (Tanaka et al., 2013). This suggests that other factors may affect the observation

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Table 1
General characteristics of participants (n = 59).

	Mean ± SEM	Min.	Max.
Age (years)	22.2 ± 0.37	18	30
Length of menstrual cycle (days)	28.5 ± 0.29	23	35
Age at menarche (years)	13.2 ± 0.14	11	17
Body mass index (BMI)	21.64 ± 0.36	17.1	29.3
Sleep duration (h)	7.4 ± 0.1	4.5	9.0
STAI-T score[a]	48.6 ± 0.8	38.0	68.0

[a] STAI-T score may vary between 20 (lowest anxiety) and 80 (highest anxiety).

of neuroendocrinological changes on behavior. One of the candidate factors might be cortisol, as its receptors, like progesterone and estradiol, are widely and densely expressed in many parts of the brain associated with behavior, including the amygdala, cerebellum, cortex, hippocampus, and hypothalamus (Toffoletto et al., 2014; Colciago et al., 2015). It is probable that increased cortisol release in the beginning of the day might interact with decreased sex steroids at certain times of the menstrual cycle. Therefore, the measurement of the CAR throughout the cycle and assessing its temporal relationship with progesterone, estradiol, and behavioral changes seems to be a feasible and necessary undertaking, considering the burden of these symptoms in women worldwide (Biggs and Demuth, 2011; Tanaka et al., 2013).

There are few studies on the relationship between the menstrual cycle and cortisol secretion. CAR levels did not differ between the follicular and luteal phases (Kudielka and Kirschbaum, 2003; Wolfram et al., 2011), but increased during the ovulation period (Wolfram et al., 2011). In another study, it was reported that there is a directly proportional increase in both salivary estradiol and cortisol in the 25–28 days of the menstrual cycle (Bao et al., 2004). Salivary cortisol response following the Trier Social Stress Test (TSST) was not different between the follicular and luteal phases (Duchesne and Pruessner, 2013). Therefore, rather than dividing the menstrual cycle into follicular and luteal phases, it may be more physiologically relevant to divide it into menses, midcycle, luteal phase, and premenstrual phase when menstrual symptoms are considered.

The aim of the current study was to measure cortisol (as CAR), estradiol, and progesterone in saliva at physiologically distinct stages of the menstrual cycle (i.e. menses, midcycle, luteal phase, premenstrual phase) in the same women throughout their complete cycle and correlate them with self-reported pain and anxiety perception.

2. Methods

2.1. Participants

The study protocol was approved by of the Malatya Clinical Research Ethics Committee (Protocol # 2015/45). A face-to-face meeting was conducted with apparently healthy female students (n = 82, second- and third-year students in the Faculty of Medicine of Inonu University in Turkey), and postgraduate research assistants (n = 6) were volunteered to enroll in the study. All participants signed an informed consent. Subjects were assessed by an anamnestic medical interview, and they were reported to be healthy as they did not have any health problems or chronic diseases. Of the 88 participants, 2 had longer cycles than >35 days (42 and 45 days), 5 were using analgesics (one of them was obese), 1 was using antidepressants, and 3 were smoking (1 was also using oral contraceptives). These participants were removed from the study. A final total of 59 participants completed the study protocol and were included in the analyses. The descriptive data of women are summarized in Table 1. Information about the length of the menstrual cycles of participants was noted by face-to-face meeting. The slight differences between the length of the expected and observed cycles were not significant enough to disrupt the timing of the phases. Some participants (n = 12) were unaware of their cycle length in the last 3 cycles, and therefore, they were not asked to provide midcycle samples. Each participant's own calendar was created based on their cycle lengths in the last three months. Expected timing of menses, midcycle, luteal phase, and premenstrual phase was calculated accordingly. Each participant's expected menstrual cycle phases were noted, and they were reminded with a notification message sent to their mobile phones in the evening on the day before CAR samples were collected.

2.2. Study design

Study design and saliva collection protocol are explained in Fig. 1. Expected cycle lengths were in accordance with observed cycle lengths (explained above), and therefore, for ease of understanding we standardized this to the mean cycle length observed in our study (i.e. 28 days). Cycles were divided into menses (1–3 days), midcycle, luteal (20–21 days), and premenstrual (24–27 days) phases (Houghton et al., 2002; Bao et al., 2004; Pamuk and Cakir, 2005; Wallace et al., 2010). The saliva samples for CAR were taken at 0, 15, 30, and 60 min following spontaneous awakening in the morning (Kudielka and Krischbaum, 2003; Wolfram et al., 2011). Saliva samples for estradiol and progesterone were taken at the beginning of the cycle (menses) and every other day from the middle of the cycle to the end. Saliva samples were collected at 09:00–12:00 a.m., but if this coincided with CAR sampling, the last sample of CAR (i.e. 60 min sample) was used to analyze ovarian steroids. It has been shown that estradiol and progesterone measured in whole saliva collected by the participants provide a non-invasive, feasible method for determining menstrual cycle profiles (Gandara et al., 2007).

In this study, the premenstrual syndrome scale (DRSP, the Daily Record of Severity of Problems) was filled in on the days when CAR was measured. The visual analog scale (VAS) was completed for pain assessment in the menses, early luteal period, and premenstrual phases. To measure the general anxiety levels of the individuals, the State-Trait Anxiety Inventory-Trait Anxiety (STAI-T) scale was used.

2.3. Saliva collection

Saliva samples were collected according to expert consensus guidelines reported by Stalder et al. (2016). Experimental procedure and study protocol was explained to the participants during the face-to-face meeting. The study protocol was also given to the participants in a printed version together with a mechanical ringing timer adjustable to 60 min with 1 min increments. This timer was used to ensure that the next saliva sampling was carried out on time. DRSP, VAS, and STAI-T questionnaires were given to each participant for filling in at the specified times. Polypropylene tubes (1.5 mL, ISOLAB, Germany) labeled with participant's ID, phase of the menstrual cycle, and time of the saliva sampling (e.g. 0, 15, 30, 60 min) were given to the participant. To accommodate easy handling of the samples, tubes for each phase were placed together in a polypropylene specimen container (60 mL) with screw caps labeled with participant's ID and the phase of the cycle.

The passive drool method was used to collect saliva samples for CAR determinations, and the protocol used was that described by Hanrahan et al. (2006) and Stalder et al. (2016). Accordingly, individuals were warned to avoid any kind of activities such as eating, drinking, and brushing teeth in the first half-hour after waking up. Water drinking was allowed, provided that it was ceased at least 10 min before the next saliva sampling. Tooth brushing, eating, and drinking, except milk and caffeinated drinks, was allowed during