Diagnostic accuracy of salivary cortisol as a marker of premenstrual syndrome degrees in adolescents

M.F.G. SIREGAR

Summary: Diagnostic accuracy of salivary cortisol as a marker of premenstrual syndrome degrees in adolescents.

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Aim. Psychoneuroendocrine may play a role in the pathogenesis of premenstrual syndrome. There is an increase in cortisol level which resulted from psychosocial stressors, especially in adolescent. The aim of this study was to determine if salivary cortisol levels in adolescent can be used to determine the degree of premenstrual syndrome severity.

Method. This is a cross-sectional study and diagnostic tests performed in academy of midwifery in Medan since July 2011 to February 2012. Samples were 77 adolescent girls aged 17-21 years at the academy of midwifery who have experience premenstrual syndrome and has fulfilled the inclusion and exclusion criteria. L-MM-PI (Lie-Minnesota Multiphasic Personality Inventory) Scale questionnaire were used to assess the honesty of subjects. Adrenal stress questionnaires were filled in 7 days prior to the next cycle after which saliva cortisol were measured using ELISA test. Severity degrees were assessed using the PSST (Premenstrual Symptoms Screening Tool) as a reference standard.

Result. Salivary cortisol was significantly associated with PMS degrees (P<.022). Using a cut off value of 0.116 ug/dl, moderate-severe and mild cases of PMS had sensitivity, specificity, positive and negative predictive values of 95.35, 91.17, 93.18, and 93.94, and 8.86, 4.65, 6.82, and 6.06%, respectively.

Conclusion. Salivary cortisol is significantly associated with psychosocial stressors and premenstrual syndrome degrees and is an accurate diagnostic tool for cases of moderate-severe PMS.

Key words: Premenstrual syndrome - Cortisol - Adolescent.

"What is new in this paper?" The most used tool to assess premenstrual syndrome severity is Premenstrual Symptoms Screening Tool (PSST). Since PSST is subjective, an objective biomarker is needed for this disease. Salivary cortisol may be used as a feasible and non-invasive alternative method to replace PSST.

Introduction

Approximately 80-95% of reproductive aged women experience life interfering symptoms, that usually are predictable and occur regularly on approximately 2 weeks prior to menstruation (1, 2). Even though several menstural hormonal changes are thought to cause PMS, the main etiology remains unclear (3). Siregar (4) in 2010 stated that PMS events were significantly associated with magnesium serum. Productivity rates are affected, which Borenstein et al. (5) described to decline due to increased work absent rates, resulting in salary issues. Furthermore, Lutan and Pujiasuti (2) reported that PMS symptoms highly affected daily activities of PMS subjects.

Epidemiology studies showed that 20% of adolescent aged women experience moderate to severe menstrual periods (6). One study in Yogyakarta (7), Indonesia, reported severe and mild PMS symptoms in 39.2 and 60.8% female students, respectively. Results from Anggraini (8) showed lack of academic motivation reported in midwifery students.

Previously, diagnosing PMS was somewhat problematic. However, based on the Montreal Consensus, the Premenstrual Symptoms Screening Tool (PSST) was, as of October 2010, used as the official diagnostic tool (9).

Stress induced cortisol secretion has been postulated to
produce a cascade of physiological changes that trigger PMS (10-12). Consequently, it is only natural that cortisol testing is currently proposed to assess severity degrees. As it represents free circulating cortisol (12, 13) salivary cortisol should serve as a significant PMS degree indicator. A definitive cut off value is therefore mandatory, to determine the effective treatment for this syndrome. This study was conducted to determine a salivary cortisol cut off value that could be used as a significant marker of PMS degrees in adolescent women.

**Methods**

Seventy seven female attending students of the Medan midwifery academy participated in this cross sectional diagnostic study from July 2011 until February 2012, on approval by the Health Research Ethical Committee, Medical Faculty, University Sumatera Utara (Registration Number: 202/KOMET/FK USU/ 2011). As this study was designed to test a proposed null hypothesis and diagnostic accuracy of salivary cortisol testing, two formulas to determine sample size that met both purposes were applied, two way hypothesis and AUC outcome, respectively. The largest sample size of 77 subjects, obtained from the latter formula, was used. Written informed consent were obtained from 17-21-year-old volunteer students with regular menstrual cycles; with no history of brain tumor, Cushing syndrome, Addison’s disease, altered liver function tests, diabetes, thyroid disease, hyperprolactinemia, history of alcohol use and not undergoing hormonal therapy or corticosteroid drugs. Subsequently, recruited students underwent the Lie-Minnesota Multiphasic Personality Inventory (L-MMPI) test; subjects with raw L-MMPI scores ≤ 5 followed a two menstrual cycle observational period for PMS in the next two consecutive months (PMS diagnosis using American College of Obstetrics and Gynecology criteria) (9).

As it designated dishonesty and would potentially tamper further assessments, students with raw L-MMPI scores > 5 were denied further participation in this study, and were subsequently replaced. Subjects experiencing menstrual disorders during the observational period, defined as a menstrual cycle under 24 days or exceeding 35 days, were also excluded. Candidates were continuously recruited until the minimal sample size (n=77) was met. Last menstrual dates were self recorded by eligible subjects. Seven days prior to the subsequent menstrual date, subjects were asked to fill the adrenal stress questionnaire (see appendix 7). This tool consists of 20 questions, assigned to be either untrue (value=0), somewhat true (value=3), or very true (value=5), on which subjects were allocated into one of the following groups: good health, under some stress, candidate for adrenal burn out, in adrenal burn out, and in several adrenal burn outs, as designated by overall scores of 0 – 30, >30 – 40, >40 – 50, >50 – 60, and >60, respectively (14).

Saliva samples were then collected on the same day. Subjects were previously asked to fast for more than an hour (in cases of alcohol consumption, sampling was limited to 12 hours post intake) and on 10 minutes of oral rinsing, instructed to bend down with an opened mouth towards a polypropylene tube, in which approximately 1.5 cc saliva was left to self run into. Three cup samples comprised one specimen, with each identified and assigned to a specific test. Bacterial growth was prevented by temporarily storing samples in a 2°C – 8°C refrigerator. Samples were then directly sent to the Prodia Laboratory, Medan, and stored at -20°C. Salivary cortisol, defined as cortisol concentration in saliva, was examined by Salimetrics kit in ug/dL units and measured using the Enzyme Linked Immunoassay (ELISA). As a note, in this study, salivary cortisol testing was limited from 08.00 am-12.00 pm.

Still on the same day, severity assessment for PMS was then performed using PSST, a reference standard based on the Montreal Consensus (2010) that also met criteria from the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and International Classification of Diseases (ICD X). Premenstrual screening tool comprises 14 items on PMS symptoms (1-14) and 5 daily activity inquiries (A-E). Subjects were allocated into one of the following categories: mild PMS, moderate-severe PMS, and Premenstrual Dysphoric Disorders (PMDD) (9). Subjects were diagnosed having PMDD if at least one of items 1-4 and one inquiry of A-E as severe with an additional 4 of items 1-14 as moderate to severe. Whereas subjects with at least one of 1-4 with an additional four of 1-14 and at least one of A-E assigned as moderate-severe were diagnosed with moderate-severe PMS. All subjects were blindly measured and assessed by professional personnel.

Descriptive data were analyzed using univariate analysis. Bivariate analysis focused on activity patterns in the midwifery academy by assessing psychosocial stressor effects on adrenal stress. One way anova test was employed to perform statistical analysis followed by a comparative analysis between adrenal stress (categorical variable) and saliva cortisol levels (numerical variable) (15, 16). Multivariate analysis was conducted using the Spearman’s and Pearson’s Correlation Test. Linear regression correlation analysis adjusted confounding factors that affect saliva cortisol levels, including: age, body mass index (BMI), menstrual length, and menarche age. Premenstrual Syndrome degrees were considered mediator variables, determined based by PSST assessment. Cut off, sensitivity, specificity, positive and negative predictive values, accu-
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...racy, and area under the curve (AUC) of saliva cortisol (numeric variable), set here as a marker of PMS degrees (categorical and dependant variable), were determined by diagnostic tests. Statistical analysis was performed using the SPSS v 18.0 (Statistical Package for Social Sciences).

Results

Seventy seven subjects were collected since July, 28th 2011, with mild and moderate-severe premenstrual syndrome subjects of 34 and 43, respectively, all of which participated throughout the entire study. Univariate analysis conducted on the following characteristics: Body mass index (BMI), menstrual period length, and menarch, described that 52 subjects (67.5%) were normoweight, 75 subjects (97.4%) experienced a 3-7 day menstrual period length, and 54 subjects (70.1%) were aged 13-16 years old on their first menstrual period.

Students with normal BMI, menstrual period of 3 to 7 days, and attending first academic year were dominant in adrenal stress group > 30-40 (19, 26, and 18 students respectively; $P=0.936$, $P=0.597$, and $P=0.137$, respectively). However, this study failed to test the association between adrenal stress and interpersonal relationship, environment, and finance, a fact attributed to the constant condition all subjects had concerning these three stressors.

Compared to other groups, adrenal stress group > 50-60 had the widest salivary cortisol interval and apparently were averagely aged older during menarch (0.1583-0.3470 ug/dL; CI=95% and 13.5 years old, SD=1.03 years, $P=0.623$, respectively). Table 1 showed that average salivary cortisol significantly differ among all adrenal stress groups ($P=0.022$), by which the null hypothesis was rejected.

Table 2 displays the association between salivary cortisol and age, menarche age and BMI was determined using Pearson’s correlation test where as Spearman’s correlation test determined associations between salivary cortisol and menstrual duration (nominal variable) and stress levels (ordinal variables). On assessing Table 3, average salivary cortisol in each PMS subjects significantly differed between the two groups. Mild PMS subjects had average salivary cortisol of 0.0807 ug/dL (CI=0.0627-0.0987), whereas subjects with moderate-severe premenstrual syndrome had average salivary cortisol of 0.2650 (ug/dl) (CI range: 0.2202-0.3098).

**Table 1 - Average Levels of Salivary Cortisol Saliva based on Stress Degrees (Adrenal Stress) on Women with Premenstrual Syndrome.**

<table>
<thead>
<tr>
<th>(Adrenal Stress) Women with Premenstrual Syndrome</th>
<th>Salivary Cortisol Level (µg/dl)</th>
<th>Mean</th>
<th>SD</th>
<th>CI 95 %</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal Stress Score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0-30</td>
<td></td>
<td>0.1192</td>
<td>0.11322</td>
<td>0.0677</td>
<td>0.1708</td>
</tr>
<tr>
<td>- &gt;30-40</td>
<td></td>
<td>0.1696</td>
<td>0.12910</td>
<td>0.1185</td>
<td>0.2206</td>
</tr>
<tr>
<td>- &gt;40-50</td>
<td></td>
<td>0.2320</td>
<td>0.14837</td>
<td>0.1423</td>
<td>0.3217</td>
</tr>
<tr>
<td>- &gt;50-60</td>
<td></td>
<td>0.2526</td>
<td>0.17705</td>
<td>0.1583</td>
<td>0.3470</td>
</tr>
</tbody>
</table>

*One Way Anova Test

**Table 2 - The Association between Age, Menarche, BMI, Menstrual Length, and Adrenal Stress and Salivary Cortisol.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient towards salivary cortisol*</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Age</td>
<td>R=0.105</td>
</tr>
<tr>
<td>- Menarche</td>
<td>R=-0.006</td>
</tr>
<tr>
<td>- Body Mass Index (BMI)</td>
<td>R=-0.039</td>
</tr>
<tr>
<td>- Menstrual length</td>
<td>R=0.118</td>
</tr>
<tr>
<td>- Adrenal stress</td>
<td>R=0.380</td>
</tr>
</tbody>
</table>

* Spearman’s correlation test

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TABLE 3 - AVERAGE SALIVARY CORTISOL BASED ON PREMENSTRUAL SYNDROME DEGREES (PMS).

<table>
<thead>
<tr>
<th>Premenstrual syndrome</th>
<th>Salivary cortisol (µg/dl)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Mild PMS</td>
<td>0.0807</td>
<td>0.0516</td>
</tr>
<tr>
<td>- Moderate-severe PMS</td>
<td>0.2650</td>
<td>0.1456</td>
</tr>
</tbody>
</table>

* One way Anova test

The salivary cortisol cut off value of 0.116 µg/dL was set on coordinate 30. Sensitivity, specificity, positive predictive and negative predictive values of mild and moderate-severe PMS set on coordinate 30 were 8.86, 4.65, 6.82, and 6.06, and 95.35, 91.17, of 93.18, and 93.94%, respectively.

Discussion

Salivary cortisol test is a feasible and non-invasive method (17, 18). Here, salivary cortisol was significantly associated with PMS degrees (P< 0.022). However, this contradicts findings by Oda in Japan, who reported lower levels in PMS subjects compared to the control group (19). Weak correlation coefficients of 0.105 and 0.118 between salivary cortisol and mild and moderate-severe PMS, respectively, were presumably due to the narrow sample age range and low diversified menstrual length categories applied in this study. Although somehow predictable, naturally one would assume these findings as disadvantages. But this study did in fact deliberately target female samples aged 18 to 22 years old due to moderately high rates of premenstrual syndrome in adolescents and complaints experienced by adolescent aged women (6). Furthermore, the primary aim was to determine the association between salivary cortisol and PMS, which was successfully achieved by a significant positive correlation coefficient of 0.381. Consequently, this value indicated that the strength of this study was not undermined by the weak association established between salivary cortisol and the remaining variables (menstrual length, age, menarche, age, and BMI; 0.118, 0.105, - 0.006 and -0.039, respectively).

Bertone-Johnson et al. indicated appropriate BMI maintenance to be essential in preventing PMS (20). However, here, several discrepancies to previous studies were revealed, a fact attributed to the non-diversified nature of samples enrolled in this study.

In 1986, Crammer reported elevated plasma cortisol in PMS subjects, that normalized on estradiol/ testosterone medication, followed by a reduction of PMS symptoms (21). Similar results obtained by Odber et al. (22) showed significantly elevated menstrual cortisol secretion, compared to post menstrual, in subjects with mild premenstrual physical and emotional changes.

Salivary cortisol significantly differs between moderate-severe and mild PMS groups. A systematic PUBMED online search (inquiry: salivary cortisol AND premenstrual syndrome AND degree), revealed that, until this study was completed, no previous studies have reported on salivary cortisol assessed based on PMS degrees.

Statistical analysis on moderate-severe PMS ROC curve concluded salivary cortisol cut off value of 0.116 µg/dl may be used as a marker of PMS degrees in adolescent women. This combined with results from Table 3 imply that salivary cortisol is accurate in diagnosing moderate-severe PMS, but is inadequate in detecting mild PMS, as mild PMS seldomly show any significant clinical manifestation. This study has thus offered a specific salivary cortisol level cut off value that could be apparently used as a marker of premenstrual syndrome, a diagnostic indicator that has not been described in previous studies (Systematic PUBMED online search, inquiry: diagnostic accuracy AND salivary cortisol AND Premenstrual Syndrome).

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Details of ethics approval

This study was approved by the Health Research Ethical Committee, Medical Faculty, University Sumatera Utara (Registration Number: 202/KOMET/FK USU/2011).

Disclosure of Interests: this study has no conflict of interest.

Contribution to Authorship: Muhammad Fidel Ganis Siregar is the sole Author of this study.

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Appendix

ADRENAL STRESS QUESTIONNAIRE

Next to each question assign a number between 0 and 5. You should assign values as follows:

0 = Not true  3 = Somewhat true  5 = Very true

Once you have completed the questionnaire calculate your total and locate the range you fall under at the bottom of the page.

1. I experience problems falling asleep.
2. I experience problems staying asleep.
3. I frequently experience a second wind (high energy) late at night.
4. I have energy highs and lows throughout the day.
5. I feel tired all the time.
6. I need caffeine (coffee, tea, cola, etc.) to get going in the morning.
7. I usually go to bed after 10 pm.
8. I frequently get less than 8 hours of sleep per night.
9. I am easily irritated.
10. Things I used to enjoy seem like a chore lately.
11. My sex drive is lower than it used to be.
12. I suffer from depression, or have recently been experiencing feelings of depression such as sadness, or loss of motivation.
13. If I skip meals I feel low energy or foggy and disoriented.
14. My ability to handle stress has decreased.
15. I dislike that I am easily irritated or upset.
16. I have had one or more stressful major life events. (e.g. divorce, death of a loved one, job loss, new baby, new job)
17. I tend to overwork with little time for play or relaxation for extended periods of time.
18. I crave sweets.
19. I frequently skip meals or eat sporadically.
20. I am experiencing increased physical symptoms such as muscle aches, headaches, and/or frequent illnesses.

0 - 30 You are in good health.
30 - 40 You are under some stress.
40 - 50 You are a candidate for adrenal burnout.
50 - 60 You are in adrenal burnout.
60 + You are in severe adrenal burnout.

References