Characteristics of Perceived Stress and Salivary Levels of Secretory Immunoglobulin A and Cortisol in Japanese Women With Premenstrual Syndrome

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1. Background

More than 85 to 90% of all women report premenstrual syndrome (PMS), which includes psychosomatic changes, including irritation, depression, food cravings, and breast pain before and during menstruation (1, 2). Approximately 24 - 40% of reproductive-aged women experience PMS (3, 4). This syndrome has been reported to account for 3 - 16% of the economic loss from workforce degradation (5). Thus, PMS is not only a personal issue, but also a social concern. Multiple physiological and psychosocial factors such as ovarian hormones, abnormalities in neurotransmitter physiology, lifestyle habits and stress are related to PMS (6, 7). Relieving the syndrome through self-care is currently one of the therapeutic approaches available for PMS because its exact pathology is unknown, and no effective therapy has been discovered.

Some studies have shown that stress is associated with PMS (3, 4, 8). Most measurements of the stress response in women with PMS have used perceived stress, and no studies quantifying stress based on biochemical parameters have been conducted.

2. Patients and Methods

A longitudinal observational study was conducted in 2010 in the Kansai region of Japan. Thirteen women with premenstrual syndrome and 11 controls, all with regular menstrual cycles, participated in this study. Salivary secretory immunoglobulin A (S-IgA) and cortisol levels were measured as biochemical parameters, and scores on the Stress Check List KM (SCL-KM) (Cronbach’s α in this study ranged from 0.76 to 0.84) were used to indicate perceived stress through two complete menstrual cycles. Before stress measurements were taken, premenstrual, menstrual and postmenstrual phases were confirmed based on records of basal body temperature across two menstrual cycles. Data analysis was performed using the Student’s t-test, analysis of variance with repeated measures, and Pearson’s correlation coefficient, as appropriate.

3. Results

Both the postmenstrual S-IgA concentration and secretion rate were significantly lower in the group with premenstrual syndrome than in controls (P < 0.05). Premenstrual S-IgA concentrations were significantly higher than postmenstrual levels in the group with premenstrual syndrome (P < 0.05). No significant differences in cortisol levels were seen in either group during any phase. Premenstrual and postmenstrual phase SCL-KM scores were significantly higher in the group with premenstrual syndrome than in controls (P < 0.05). No significant changes in the SCL-KM scores were observed among menstrual cycle phases in either group. Postmenstrual S-IgA levels were negatively correlated with the SCL-KM score (P < 0.05).

4. Conclusions

The stress due to psychosomatic changes in the menstrual cycle is associated with premenstrual syndrome. Measures of S-IgA, rather than cortisol or subjective responses to stress, may be most closely associated with PMS.

5. Keywords: Immunoglobulin A; Menstruation; Premenstrual Syndrome; Stress; Psychological; Hydrocortisone

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and cortisol levels has become available (11, 12).

2. Objectives

The aim of this study was to analyze the association between PMS and stress across two menstrual cycles using biochemical parameters and questionnaire-based reports.

3. Patients and Methods

3.1. Subjects

A longitudinal observational study was performed in 2010 in the Kansai region of Japan. Sampling was performed via the convenience sampling method. The sample size was calculated based on power analysis by G*Power (13, 14), with power of 0.80 and an effect size of 0.40 at P < 0.05 with analysis of variance with repeated measures. A sample size of 12 was estimated for each group. The subjects were recruited using the following process:

The subjects were required to be residents of the Kansai region in Japan, so that the researchers could meet with them to explain the research methods. The general method of recruitment was through the investigators' personal networks, including social groups, a junior high school parent-teacher association, and a university nursing school. Subjects were included only if they were not pregnant or breast-feeding, had no chronic health conditions or psychiatric conditions, and were not currently using oral contraceptives. At this point, there were 31 subjects.

In the second step, the following subjects withdrew from participation during the study: two subjects who were unable to have their stress levels evaluated on saliva testing due to irregular menstrual cycles; one subject who discovered she was pregnant; and four subjects who were unable to continue taking basal body temperature measurements. The final study set comprised 24 subjects (age range of 21 to 38 years).

These subjects had regular self-reported menstrual cycle lengths of 25 - 38 days. Among the subjects, 13 reported having premenstrual syndrome (PMS group), and 11 reported not having premenstrual syndrome (N-PMS group). Based on published diagnostic criteria for PMS (1), the subjects were classified in the two mentioned groups based on both their basal body temperature during three menstrual cycles and subjective records kept in a menstrual symptom journal, the PMS Memory Diary (15).

3.2. Measures

The time schedule of measurements is shown in Figure 1. The PMS Memory Diary recorded 17 listed symptoms and the respondents’ perceived symptoms at four different levels (blank or 0, no symptoms; 1) moderate symptoms that did not affect everyday activities; 2) symptoms that affected everyday activities; and 3) severe symptoms). Cumulative scores were calculated based on the severity and frequency of symptoms in each phase. Day 1 was defined as the day of the onset of menses, which was recorded as the start of the menstrual cycle. Cycle phases were defined as follows: premenstrual phase (one to seven days before menstruation); menstrual phase (days one to seven after the start of menstruation); and postmenstrual phase (days eight to fourteen after the start of menstruation). The premenstrual phase was confirmed at the start of menstruation.

Perceived stress and biochemical parameters were measured twice over two complete menstrual cycles, with two measurements for each item, to ensure reproducibility. Before stress measurements were taken, premenstrual, menstrual and postmenstrual phases were confirmed based on records of basal body temperature taken across two menstrual cycles.

Salivary S-IgA concentrations (μg/mL) and secretion rates (μg/min), as well as cortisol concentrations (μg/dL), were measured as biochemical parameters. Cortisol concentrations are high in the morning and decrease in the evening, indicating circadian variations typical of humans (16). Given this circadian pattern, collection early in the morning was avoided, and the effects of subjects’ everyday activities were taken into account. Saliva samples were collected using a Salimetrics Oral Swab (Salimetrics LLC, State College, PA) by a medical technologist. To standardize collection conditions, samples were collected between 5:00 p.m. and 8:00 p.m. In addition, the difference in collection time between menstrual phases was strictly kept to less than 30 minutes. Subjects were asked to avoid a big meal within 60 minutes before sample collection. The oral cavity of each subject was rinsed with water 10 minutes before sample collection. The oral swab was placed under the tongue for two minutes and then transferred to a Swab Storage Tube (Salimetrics LLC). The volume of saliva collected through the oral swab was determined by weighing the device along with the storage tube before and after collection. Collected samples were frozen immediately at -20°C and then thawed completely, vortexed, and centrifuged at 1500 g for 15 minutes. The supernatants were stored at 80°C until analysis (within six months). The cortisol levels and both the S-IgA concentrations and secretion rates were determined using enzyme-linked immunoassay kits (Salimetrics LLC). Sampling methods and laboratory procedures were followed according to the Japanese version of the Stress Checklist KM (SCL-KM) (19). The SCL-KM uses a binary-choice format for 30 items of psychosomatic complaints that can develop to a stress response. Each item is a two-point response scale (0=’not at all’ or 1=’yes’). One point is given to any item reflecting high evaluation of stress; thus, the possible point range is from 0 to 30. The SCL-KM has high reliability and validity (19). Cronbach’s α in this study ranged from 0.76 to 0.84.

3.3. Statistical Analysis

Watanabe K et al.
The SPSS software (version 20.0, IBM, Tokyo, Japan) was used for data analysis. The Mann-Whitney nonparametric U test was used to examine any differences in the present age and age at menarche between the PMS and N-PMS groups. Chi-square tests were used to examine differences in marital status, parity, smoking status, and alcohol use between the groups. Outcome data were not normally distributed and were transformed to log 10 values prior to statistical analyses. A two-way repeated measures analysis of variance was used to test among the menstrual cycle phases in each group. Post hoc comparisons of the mean values were performed using the Bonferroni test. After confirmation of the reasonably normal distribution of the two-paired samples, Student’s t-test was used to evaluate differences between groups. Correlation testing between perceived stress and biochemical parameters was conducted using Pearson’s correlation coefficient for both groups combined. Significance was set at P < 0.05.

3.4. Ethical Considerations

The objectives of the study were explained, and informed consent was obtained from each subject included in the study. The Kobe University Graduate School of Medicine, Medical Ethics Committee approved the study (No. 799). All procedures were conducted in accordance with the Declaration of Helsinki.

4. Results

4.1. Subjects’ Characteristics

The PMS and N-PMS groups did not differ significantly regarding age, marital status or parity. All subjects were nonsmokers, and their alcohol drinking frequency was less than one drink a week for most subjects (Table 1).

![Figure 1. Timetable of the Stress Checklist KM, Salivary Secretory IgA Level, Cortisol Level, and Premenstrual Syndrome Assessment During the Menstrual Cycle Phase](image-url)

| Menstrual cycle phase | Baseline ±2 | Cycle 1 | Postmenstrual | Cycle 2 | Postmenstrual |
|----------------------|-------------|---------|---------------|---------|---------------|
| Menstrual cycle days | [7 → 1]     | [7 → 1] | [8 → 14]      | [7 → 1] | [8 → 14]      |
| BBT                  |             |         |               |         |               |
| Symptoms             |             |         |               |         |               |
| Instrument           |             |         |               |         |               |
| SCL-KM               | ○           | ○       | ○             | ○       | ○             |
| S-IgA                | ○           | ○       | ○             | ○       | ○             |
| Cortisol             | ○           | ○       | ○             | ○       | ○             |

Abbreviations: BBT, Basal Body Temperature; SCL-KM, Stress Checklist KM; S-IgA, Secretory Immunoglobulin A. Day 1, onset of menses and start of the menstrual cycle. The preceding menstrual phase was confirmed at the start of menstruation.

| Variables          | PMS (n = 13) | N-PMS (n = 11) | P Value |
|--------------------|--------------|----------------|---------|
| Age, y             | 26.30 ± 6.20 | 29.0 ± 6.67    | 0.317   |
| Age at menarche, y | 11.77 ± 1.42 | 11.73 ± 1.1    | 0.937   |
| Marital status     |              |                | 0.215   |
| Single             | 9 (69)       | 10 (91)        |         |
| Married            | 4 (31)       | 1 (9)          |         |
| Parity             |              |                | 0.067   |
| Nulliparous        | 9 (69)       | 11 (100)       |         |
| Parous             | 4 (31)       | 0              |         |
| Cigarette smoking  |              |                | 0.99    |
| Nonsmoker          | 13 (100)     | 11 (100)       |         |
| Smoker             | 0            | 0              |         |
| Alcohol use        |              |                | 0.556   |
| None               | 3 (23)       | 3 (27)         |         |
| Light (≤1 drinks per week) | 7 (54) | 7 (64) |         |
| Moderate (1−3 drinks per week) | 2 (15) | 0 |         |
| Heavy (4+ drinks per week) | 1 (8) | 1 (9) |         |

Data are presented as mean ± SD or No. (%). Abbreviations: PMS, having premenstrual syndrome; N-PMS, not having premenstrual syndrome. Mann-Whitney U test. Chi-squared test.

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4.2. Premenstrual Syndrome and Biochemical Parameters: Salivary Secretory IgA (Concentration and Secretion Rate) and Cortisol

Two-way analysis of variance showed no interaction between groups and menstrual cycle phases. No significant differences were found in the S-IgA concentration and secretion rate and the cortisol level between the groups (Figures 2 and 3). No significant differences were observed in the S-IgA concentration and secretion rate among menstrual cycle phases in the N-PMS group. The S-IgA concentrations in the first \( F(2, 36) = 9.330, P < 0.001 \) and second \( F(2, 36) = 6.009, P < 0.01 \) measurements were significantly higher in the premenstrual phase than in the postmenstrual phase in the PMS group. The S-IgA concentrations in the premenstrual phase \( F(2, 36) = 9.330, P < 0.001 \) and second \( F(2, 36) = 6.009, P < 0.01 \) measurements were significantly higher than in the postmenstrual phase in the PMS group. Moreover, the S-IgA concentration in the first measurement was significantly higher in the menstrual phase than in the postmenstrual phase in the PMS group. The S-IgA secretion rate in the second measurement was significantly higher in the menstrual phase than in the postmenstrual phase in the PMS group. The S-IgA concentration in the first measurement \( F(2, 36) = 2.090, P < 0.05 \) was significantly higher in the premenstrual phase than in the postmenstrual phase in the PMS group. Moreover, the S-IgA concentration in the first measurement was significantly higher in the menstrual phase than in the postmenstrual phase in the PMS group. The S-IgA concentration in the first measurement \( F(2, 36) = 2.129, P < 0.05 \) was significantly higher in the menstrual phase than in the postmenstrual phase in the PMS group. The S-IgA secretion rate in the second measurement \( F(2, 36) = 5.178, P < 0.05 \) was significantly higher in the menstrual phase than in the postmenstrual phase in the PMS group. No significant differences in the cortisol level were observed in any phase (Figure 3).

4.3. Premenstrual Syndrome and Perceived Stress

No significant changes in the SCL-KM score were observed among menstrual cycle phases in either group. Premenstrual \( t(22) = 3.034, P < 0.01, 95\% \text{ CI } [1.3, 6.9] \) and postmenstrual phase \( t(22) = 2.098, P < 0.05, 95\% \text{ CI } [1.7, 7.1] \) SCL-KM scores were significantly higher in the PMS group than in the N-PMS group (for both measurements). Menstrual phase SCL-KM scores in the second measurement were significantly higher in the PMS group than in the N-PMS group \( t(22) = 3.034, P < 0.01, 95\% \text{ CI } [1.3, 6.9] \) (Figure 4).

4.4. Correlations Between Salivary Biochemical Parameters and Perceived Stress Scores

A significant negative correlation was observed between S-IgA concentration \( r = –0.491, P < 0.05 \) and secretion rate \( r = –0.543, P < 0.01 \) and SCL-KM scores at the second measurement in the postmenstrual phase. Furthermore, no significant correlations were found between the cortisol level and SCL-KM scores.

**Figure 2.** Changes in Salivary Secretory Immunoglobulin A (S-IgA) Concentrations (µg/mL) and Secretion Rates (µg/min) in the PMS Group \( (n = 13) \) and N-PMS Group \( (n = 11) \) During the Menstrual Cycle and Differences Between the Two Measurements

**Figure 3.** Changes in Cortisol Concentrations in the PMS Group \( (n = 13) \) and N-PMS Group \( (n = 11) \) During the Menstrual Cycle and Differences Between the Two Measurements

**Figure 4.** Changes in the Stress Checklist KM Scores in the PMS Group \( (n = 13) \) and N-PMS Group \( (n = 11) \) During the Menstrual Cycle and Differences Between the Two Measurements
5. Discussion

This study examined changes in biochemical parameters of stress, and measured perceived stress during the menstrual cycle in women with PMS. The findings of this study indicated that the postmenstrual S-IgA concentration and secretion rate were significantly lower at the second measurement in the PMS group than in the N-PMS group. The S-IgA is a protein that plays a major role in the local immune system of the mucosal epithelium. It is secreted in saliva, airway mucus and breast milk. Evidence indicates that its secretion sensitively reflects changes in the immune system (10). Secretory IgA has been reported to decrease in response to chronic stress and general life events, and it has been suggested to be a chronic stress marker (20, 21). The present study results suggest that lower S-IgA levels indicate chronic stress in the postmenstrual phase, even without the psychosomatic burden caused by PMS. Thus, this study showed that chronic stress is present in women with PMS.

Conversely, the findings of this study indicated that S-IgA concentration was significantly higher during the premenstrual or menstrual phase than the postmenstrual phase in the PMS group. Significantly higher levels of S-IgA were reported during the menstrual phase in women with dysmenorrhea compared with women without dysmenorrhea; this was likely to be a result of pain (17). In addition, elevated S-IgA levels have been shown to indicate a transient acute stress response (22, 23). This suggests that, accompanied by chronic stress conditions, psychosomatic changes associated with PMS increase stress responses in women with premenstrual syndrome.

No correlation was observed between cortisol level and PMS. Cortisol has been reported to be secreted in response to stronger stress loads than S-IgA (24, 25). Stresses from everyday activities have a greater effect on PMS than unusual severe stress (26). In the present study, changes in cortisol levels were not observed, presumably because the subjects in this study did not have a severe stress load. The SCL-KM score was significantly higher in the PMS group than in the N-PMS group in both the premenstrual and menstrual phases. In addition, perceived stress scores were similar among menstrual cycle phases in both groups, with or without PMS. This suggests a high perceived stress condition in women with PMS, supporting the results of previous studies (27). Higher levels of nervous tension, anxiety, depression, and anger have been reported to be caused by either PMS (28) or perceived stress (29), primarily in the premenstrual phase. However, other studies reported no differences in stress responses among menstrual cycle phases (30). Since these studies used different scales and found no differences among menstrual phases in small studies with 21-25 subjects, further studies are necessary to verify the methods of measuring perceived stress. Furthermore, it has been reported that 27% of the perceived stress response varies depending on the subject’s status at the time of measurement (27); changing stress levels across the two menstrual cycles were reflected in salivary biochemical parameters and perceived stress scores. These might have affected the agreement between the results of the first and second measurements.

There were negative correlations in the postmenstrual phase between the S-IgA concentration and secretion rate and the SCL-KM score. These results indicate that S-IgA can be used for the quantitative evaluation of chronic stress in women with PMS. Conversely, no significant correlations were observed between cortisol levels and the SCL-KM scores. This result is compatible with a previous study that used daily urine samples, in which no correlation was observed between cortisol and perceived stress (31). In conclusion, whether cortisol would respond to the perceived stress analyzed in the present study remains to be clarified. The absence of significant correlations between cortisol levels and perceived stress scores might imply a fundamental difference between these two types of stress.

The present study had several methodological limitations. First, the subjects were from a limited area in Japan and were recruited through the researchers’ personal networks. Therefore, the study was not randomized, which limits the generalizability of the results. Second, the number of subjects was small, which limits the power of the analysis of the results of S-IgA concentration and secretion rate from different menstrual cycle phases. Third, the difference in sample size between the two groups increased type 1 error, which could potentially account for differences in marital status and parity between the groups, as well as for cortisol and perceived stress not being detected as statistically significant. For future research, further analyses of the associations of salivary S-IgA and cortisol with PMS are needed with more subjects and experimental stress loads to test the usefulness of these biochemical parameters for quantitative stress measurements.

The results of the present study suggest that measurements of S-IgA, rather than cortisol or subjective responses to stress, may be most closely associated with PMS. Salivary S-IgA can be used as a highly sensitive method for stress evaluation in women with PMS in the general population. In addition, it can be a very helpful tool for evaluating the progress and success of stress management and reduction of PMS symptoms.

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Authors’ Contributions

Kaori Watanabe developed the original idea and the protocol, abstracted and analyzed data, wrote the manu-
script, and was the guarantor. Taku Shirakawa contributed to the development of the protocol, and analysis and interpretation of data.

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