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Original Article

Linking Premenstrual Syndrome With Salivary Alpha-Amylase Levels and Stress Levels: A Cross-Sectional Study

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Abstract

Objective: This study aims to analyze the correlation between salivary alpha-amylase (sAA) and stress in adolescents with premenstrual syndrome (PMS), and to assess the predictive ability of sAA levels and stress for PMS.

Materials and methods: Sixty-two adolescents with and without PMS (31 each) were grouped based on their PMS status, measured using the Shortened Premenstrual Assessment Form (SPAF). Stress was measured using the Kessler Psychological Distress Scale (K10). The levels of sAA were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Data were analyzed using the Pearson correlation test, Receiver Operating Characteristic (ROC) curve analysis, and multiple linear regression analysis.

Results: Stress and PMS expressed a significant positive correlation ($p=0.001$; $r=0.66$). sAA levels and PMS showed a significant positive correlation ($p<0.05$; $r=0.42$). The level of sAA in adolescents with PMS was 23.28±12.02 ng/mL, almost twice higher than in adolescents without PMS (12.10±7.5 ng/mL). The cut-off value of sAA level on PMS was ≥15.02 ng/mL with a sensitivity and specificity of 67.7%. The level of sAA and stress were significantly able to predict PMS ($p<0.001$). The correlation value of SAA levels and stress with PMS is positive ($r=0.705$), suggesting they can predict PMS by 49.7% ($R^2=0.497$).

Conclusion: There is a positive relationship between sAA levels and stress in adolescents with PMS. SAA levels and stress were able to predict PMS in adolescents with an accuracy of 49.7%.

Keywords: Salivary Alpha-Amylase; Premenstrual Syndrome; Stress

Introduction

Physiologically, women of reproductive age experience the circumlunar cycle of menstruation (1). Menstruation affects biopsychosocial in all socioeconomic and cultural levels and is an important marker in reproductive health (1, 2). Menstrual cycle levels, symptoms, and premenstrual disturbances are important in assessing menstrual health (2).

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Premenstrual disorders include premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD), and other clinical symptoms aggravating pre-menstruation and disrupting psychosocial functions (3).

PMS is a common problem in the reproductive age with varied intensity of symptoms (3-5). The American College of Obstetricians and Gynecologists (ACOG) describes PMS as physical and emotional symptoms occurring in a cycle from five days before menstruation (luteal phase) to the fourth day of menstruation, followed by symptoms-free period until the thirteenth day (follicular phase) and is not related to organic diseases (6-9). At least 3 out of 10 women with PMS have trouble with their daily activities (3, 10-12).

A recent study found a prevalence of 76.35% of students with PMS, where stress is the primary risk factor for PMS in adolescents (13). PMS and stress have a complex and interrelated relationship (13). Stress affects PMS pathophysiology, severity, and occurrence (9, 13, 14). The sympathetic nervous system (SNS) and hypothalamic pituitary adrenal (HPA) axis are the primary systems responsive to acute stress (psychosocial) (15-18). Within the last year, salivary alpha-amylase (sAA) has been used as a valid non-invasive stress biomarker sensitive to SNS activities due to stress (15, 19, 20).

The high prevalence of PMS in adolescents requires special attention considering stress highly affects academic performance. Many studies investigate the correlation between stress and PMS. However, no study determined the correlation between sAA levels and PMS in adolescents. Thus, this study aims to determine the correlation between sAA levels and stress in adolescents with PMS, as well as to assess the predictive ability of sAA levels and stress for PMS.

Materials and methods

Research subjects: Since February 2024, researchers conducted a study in 5 public high schools in Makassar City and the Research Laboratory of Hasanuddin University Hospital until April 2024. The selection of schools was based on regional diversity and accessibility. The researchers employed a cross-sectional study aimed at examining the occurrence of PMS and stress among adolescents, measuring sAA levels, and comparing sAA levels between those with PMS and those without, as well as between those experiencing stress and those not. Screening of 745 female students from these 5 schools identified 62 adolescents (31 with PMS and 31 without PMS) who met the inclusion and exclusion criteria.

Inclusion criteria: Participants had normal menstrual cycles ranging from 21 to 35 days, normal menstrual duration between 5-7 days, normal body mass index, and were enrolled in classes X and XI.

Exclusion criteria: Adolescents with a history of mental disorders, ovary removal, hormonal therapy, or marriage were excluded.

Research tools

General information questionnaire: A general information questionnaire was designed by the investigators, which includes demographic information such as age, school, class, place of residence, history of ovariectomy, first day of the last menstrual period, menstrual cycle, and menstrual duration.

The Shortened Premenstrual Assessment Form (SPAF): SPAF, developed by Allen in 1991, has become a valid tool for measuring the severity of PMS (Premenstrual Syndrome) (21). SPAF consists of 10 questions about changes experienced during the week before menstruation: 4 psychological changes and 6 physical changes. Each question is subjectively rated by the respondent on a 6-point scale: 1 = no change at all or no change from usual, 2 = minimal change, 3 = mild change, 4 = moderate change, 5 = severe change, and 6 = extreme change. The total sum of points assigned is the SPAF score, which ranges from 10 to 60. Scores of 10-19 fall into the non-PMS category, while scores of 20-60 indicate PMS.

Kessler psychological distress scale (K10): The Kessler psychological distress scale (K10) is a psychological screening tool designed by Kessler *et al* in 2002 to measure psychological distress levels (18). There are 10 questionnaire items that reflect the psychological pressure experienced over the last 4 weeks. Each item is scored from 1 (none of the time) to 5 (all of the time). The scores from these 10 items are then summed, resulting in a score ranging from a minimum of 10 to a maximum of 50. The total scores can be categorized into four stress levels: well (10-19), mild (20-24), moderate (25-29), and severe (30-50).

Salivary alpha amylase measurements: Two milliliters of saliva samples were taken at the end of the luteal phase, in the morning after waking up, between 09:00 a.m. GMT+8 and 12:00 p.m. GMT+8. Saliva can be stored for up to two hours at room temperature, 24 hours in cold temperature, and three months in the freezer. The level of sAA was measured using an Enzyme-Linked Immunosorbent

Assay (ELISA) kit from Elabscience.

Data collection method: The eligible students were given instructions and counseling regarding this study. Data were first collected by screening to determine participants with and without PMS using the SPAF questionnaire. The participants were given an instruction sheet, informed consent, and a stress level questionnaire (K10) at the end of the luteal phase of their menstruation cycle. They were given an explanation of the procedures for filling out the questionnaire and the requirements. Participants were also guided using standard instructions. The completed questionnaires were collected and then verified.

Statistical methods: Univariate, bivariate and multivariate analyses were conducted with a computerization system using the SPSS software version 25. Univariate analysis was used to describe the incidence of PMS in the students of public senior high schools in Makassar and the characteristics of each variable. The results were presented as mean, standard deviation (SD), frequency distribution, and percentage. Meanwhile, bivariate analysis was used to identify the correlation between independent and dependent variables. Pearson correlation test was used to determine the correlation between two variables with a significance value of 0.05 (p-value 0.05), an independent T-test was used to assess the level of sAA in each variable and the magnitude of risk factor impact on an effect, and multiple linear regression analysis was also used to analyze the multivariate data.

Results

Table 1 describes the demographic characteristics of adolescent with PMS. Table 2 reveals the comparison of salivary alpha-amylase (sAA) levels between adolescents with PMS and those without PMS. Figure 1 displays the analysis of receiver operating characteristic (ROC) curve to determine the predictive ability of sAA on PMS. Table 3 shows the t-test between adolescents with PMS and those without PMS based on the Shortened Premenstrual Assessment Form (SPAF). Table 4 shows Pearson correlation between variables. Figure 2 illustrates the pearson correlation between stress score andPMS score.

Most participants were class XI (53.2%) and more than half of them were 16 years old. The results of the stress level analysis revealed that the majority of participants experienced non-stress levels (40.32%), followed by mild stress (20.97%). Moderate and severe stress levels were equally distributed at 19.34% each. Based on the results of Levene's Test, the variations in age, class, and stress levels among adolescent groups with PMS and those without PMS are homogeneous, indicating no significant differences in variances between the groups ($p > 0.05$).

Table 1. Demographic characteristics of adolescents with PMS (n=31) and without PMS (n=31)

Characteristics	With PMS n (%)	Without PMS n (%)	p-Value
Age			
15 years	13 (41.9)	6 (19.4)	0.058[a]
16 years	14 (45.2)	19 (61.3)	
17 years	4 (12.9)	6 (19.4)	
Class			
X	17 (54.8)	12 (38.7)	0.346[a]
XI	14 (45.2)	19 (61.3)	
Stress Levels			
Well	4 (12.9)	21 (67.7)	0.099[a]
Mild	7 (22.6)	6 (19.4)	
Moderate	9 (29.0)	3 (9.7)	
Severe	11 (35.5)	1 (3.2)	
sAA Levels (ng/mL)			
≥ 15.02	21 (68)	10 (32)	> 0.05[b]
< 15.02	10 (32)	21 (68)	

Data are expressed as the n (%).
PMS, Premenstrual Syndrome; sAA, Salivary Alpha Amylase.
[a]Levene's test, [b]Kolmogorov-Smirnov test.

Previously, table 2 shows the comparison of salivary alpha-amylase (sAA) levels between adolescents with PMS and those without PMS.

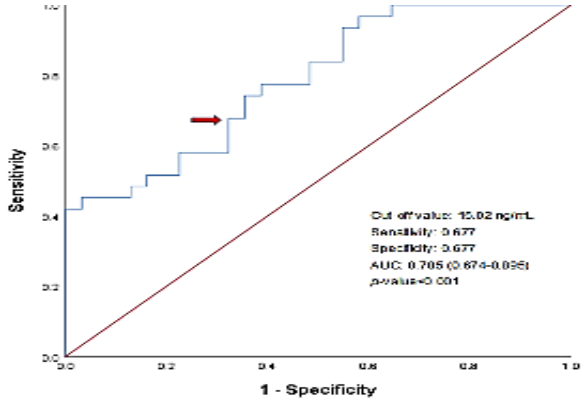

Figure 1. Analysis of receiver operating characteristic (ROC) curve to determine the predictive ability of sAA on PMS. The curve is presented based on sensitivity and 1-specificity for sAA level on PMS. AUC, the area under ROC curve

Table 5 presents the multiple linear regression analysis of sAA levels and stress in adolescents with PMS.

Table 2. Comparison of Salivary Alpha-Amylase (sAA) Levels in Adolescents with and without PMS

Variable	With PMS		Without PMS		*p*-Value
	Range	Mean ± SD	Range	Mean ± SD	
sAA Levels (ng/mL)	8.06 – 49.77	23.28 ± 12.02	2.57 – 26.20	12.10 ± 7.05	0.001[a]

PMS, Premenstrual Syndrome; sAA, Salivary Alpha Amylase.
[a]Statistical significant

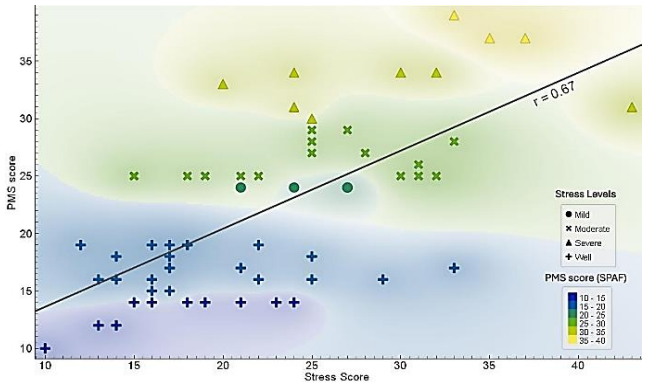

Figure 2. Pearson correlation depicting a positive linear correlation (r=0.67) between stress score and PMS score (p=0.001). Stress and PMS score describe symptom severity. PMS, premenstrual syndrome; SPAF, the shortened premenstrual assessment form

The mean sAA level for adolescents with PMS is 23.28 ± 12.02 µg/mL, which is nearly twice as high and significantly different (p = 0.001) compared to the sAA level of adolescents without PMS, which is 12.10 ± 7.05 µg/mL.

The receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic potential of sAA levels in adolescents with PMS.

The ROC curve determined a cut-off point of 15.02 ng/mL for sAA levels, with an Area Under the Curve (AUC) of 0.785 (95% CI: 0.674–0.895, p < 0.001), and a sensitivity and specificity of 67.7% (Figure 1).

Meanwhile (Table 1), the presentation of the analysis of sAA levels among participants is based on the cut-off value of the ROC curve, with levels of ≥ 15.02 ng/mL and < 15.02 ng/mL having the same frequency (50%). According to the results of the Kolmogorov-Smirnov test, the sAA level data for adolescents with PMS and those without PMS are normally distributed (p > 0.05).

The three most common PMS symptoms experienced by participants were abdominal pain, back/joint/muscle pain, and irritable outbursts, while edema, puffiness, or fluid retention, weight gain, and painful breast were the least frequently reported PMS symptoms (Table 3). The affective subscale was the most dominant, with a total score of 626 (45.58%) compared to the total scores (1371) obtained from all 62 participants.

Eight out of ten PMS symptoms assessed using the SPAF showed differences in scores between adolescents with PMS and those without PMS. Adolescents with PMS exhibited higher symptom scores compared to adolescents without PMS.

Table 3. The adolescent t-test shows the differences in premenstrual symptoms between adolescents with PMS and those without PMS based on the Shortened Premenstrual Assessment Form (SPAF), which is grouped into effect, pain, and fluid retention subscale

Subscale	SPAF Question	Total Score (n = 1373)	With PMS (mean ± SD)	Without PMS (mean ± SD)	p-Value
Affect	Irritable Outbursts	**194 (14.13)**	4.10 ± 1.25	2.16 ± 1.13	< 0.001[a]
	Overwhelmed	131 (9.54)	2.84 ± 1.29	1.39 ± 0.72	< 0.001[a]
	Feel Under Stress	151 (10.99)	3.39 ± 1.36	1.48 ± 0.63	< 0.001[a]
	Feel Sad or Blue	150 (10.92)	3.48 ± 1.61	1.35 ± 0.71	< 0.001[a]
Pain	Painful Breasts	94 (6.85)	1.81 ± 1.25	1.23 ± 0.56	< 0.05[a]
	Back/Joint/Muscle Pain	**172 (12.53)**	3.55 ± 1.55	2.00 ± 1.21	< 0.001[a]
	Abdominal Pain	**206 (15.00)**	4.10 ± 1.22	2.55 ± 1.34	< 0.001[a]
Fluid Retention	Weight Gain	90 (6.55)	1.61 ± 0.95	1.29 ± 0.53	> 0.05
	Edema, Puffiness, or Fluid Retention	72 (5.24)	1.10 ± 0.39	1.23 ± 0.72	> 0.05
	Feel Bloated	113 (8.23)	2.39 ± 1.48	1.26 ± 0.51	< 0.001[a]

Data total score are expressed as the n (%).
The numbers in bold show the 3 symptoms with the most dominant scores
SD, Standard Deviation; PMS, Premenstrual Syndrome; SPAF, the Shortened Premenstrual Assessment Form.
[a]Statistical significant

Table 4. Pearson correlation showing the levels of linear association between variables

	Coefficient of correlation (r)	*p*-Value
sAA levels and PMS	0.42	0.001[a]
sAA levels and stress	0.30	0.017[a]
Stress and PMS	0.67	<0.001[a]

PMS, Premenstrual Syndrome; sAA, Salivary Alpha Amylase.
[a]Statistical significant

However, the symptoms of weight gain and edema, puffiness, or fluid retention did not show significant differences between PMS and non-PMS adolescents (Table 3).

A positive correlation between sAA and PMS was found from the Pearson test ($p<0.05$; $r=0.42$) (Table 4). Similarly, pearson correlation test revealed a positive correlation between sAA level and stress ($p<0.05$; $r=0.30$) (Table 4). The average sAA levels were 6.13 ng/mL higher ($p=0.006$) in adolescents experiencing stress (20.16±12.62 ng/mL) compared to adolescents without stress (14.03±7.81 ng/mL).

Table 5. Multiple linear regression analysis of sAA levels and stress in adolescents with PMS

Variable	β	SE	β[1]	t	p-Value
Constant	5.751	2.265	-	2.539	0.014
sAA levels	0.157	0.062	0.246	2.538	0.014
Stress	0.604	0.99	0.591	6.110	< 0.001

R^2=0.497, adjusted R^2=0.480, F=29.206, p<0.001.
sAA, Salivary Alpha Amylase.

PMS and stress showed a positive correlation ($p=0.001$; $r=0.67$) (Table 4). Figure 2 showed that a higher PMS score means a higher stress score. In other words, the greater the stress level, the more severe the PMS symptoms will be. There was a significant difference ($p < 0.001$) in the mean stress scores between adolescents with PMS and those without PMS. The majority of adolescents were at a mild stress level (Figure 2). Specifically, adolescents without PMS were at a good stress level, while adolescents with PMS were at a moderate stress level. Thus, it appears that adolescents with PMS have higher levels of stress compared to adolescents without PMS.

In this multiple linear regression analysis, we used PMS (SPAF score) as the dependent variable, identified as significant in the single factor analysis. Stress scores and sAA levels were the independent variables. Based on Table 5, the correlation between sAA levels, stress, and PMS is positive ($r = 0.705$), with an ability to predict PMS at 49.7% ($R^2 = 0.497$, $F = 29.206$). The effective contribution of sAA levels is 10.4%, and the effective contribution of stress is 39.3%, making the combined effective contribution 49.7%.

Discussion

PMS showed a significant correlation to stress. This study was in line with Lustyk et al. who suggested a significant correlation between the PMS scale and stress score. Thus, more severe PMS symptoms are related to higher stress (22). Cheng et al. also reported a correlation between the two variables and believed that the incidence and severity of PMS are caused by stress (23) There is a relationship between the severity of PMS and the stress experienced (24).

Stress affects both physical and psychological well-being (25). PMS and stress have a complex and interrelated relationship (13). Stress plays a role in the pathophysiology of PMS and influences the severity and recurrence of PMS symptoms (9, 13, 14). When stressed, cortisol is released, disrupting hormonal stability (estrogen, progesterone, and serotonin), neurotransmitters, and overall physiological function, leading to more severe and difficult-to-manage PMS symptoms (9, 13, 26).

The prevalence of PMS among adolescents is 76.35%, with stress being the main risk factor for the high incidence of PMS in adolescents. PMS can affect adolescents' quality of life, mental status, and academic performance, leading to issues such as loss of concentration and motivation to study, poor interaction and academic participation, low grades, and absenteeism (1, 6, 10, 13). Stress in PMS causes mood changes, anxiety, and increased sensitivity (13). However, PMS symptoms can improve with proper stress management (27, 28).

However, other studies show test results that do not indicate a correlation between stress and PMS (29). This study did not measure the potential risk of PMS in adolescents based on stress levels due to the unequal and small sample sizes for each stress level. However, Purnawati *et al* (30) explained in his research that adolescents are 10 times more likely to experience PMS if they experience severe stress. Fernandez *et al* (31) found that adolescents have a 2.5 times higher risk of experiencing PMS if they experience moderate stress and a 5 times higher risk if they experience severe stress.

The participants in this study were senior high school students aged 15-18 years old enrolling in class X and XI. The mean value showed that most

participants had mild stress. Specifically, adolescents without PMS were in the non-PMS category. Meanwhile, adolescents with PMS were in the moderate stress category. This shows that higher stress levels are associated with a more severe occurrence of PMS symptoms in adolescents.

The sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis are the main systems responsive to acute (psychosocial) stress (15–18). The HPA axis is activated by the hypothalamus, which stimulates the release of glucocorticoids, such as cortisol, from the adrenal cortex (16-18). Meanwhile, the SNS stimulates the release of catecholamines (adrenaline and noradrenaline) from the adrenal medulla (16, 18). Catecholamines, in turn, stimulate the secretion of the alpha-amylase enzyme in saliva, serving as an indicator of SNS activity due to stress (15–18). Nearly half of the total salivary protein consists of the alpha-amylase enzyme, most of which is secreted by the parotid gland (19).

In recent 10 years, salivary alpha-amylase (sAA) has been recognized as a valid and sensitive non-invasive biomarker of stress-induced SNS activity (15, 19). SAA is better than cortisol at showing stress reactivity, autonomic activity, and noradrenergic actions (32, 33). This aligns with the results of our research, which show a relationship between sAA levels and stress events.

The mean value of sAA level in adolescents with stress (6.13 ng/mL) was compared to higher than in adolescents without stress. This was in line with Hoferichter et al. who found that adolescents with more parental support have lower sAA levels (34). However, Alsalman et al. proved the contrary, where there was a significant correlation between the perceived stress scale (PSS) score of men with tinnitus and baseline measured sAA (higher stress means lower sAA level) (35).

The sAA level analysis followed the established guideline and the mean value of two samples was determined to obtain a more reliable estimation (36). However, measurement was limited to one-time sample collection within a day. Therefore, we could not assess the fluctuation of sAA levels in adolescents during the luteal phase.

The analysis results of sAA level and PMS showed a statistically significant correlation (p=0.001). Adolescents with PMS tend to have sAA levels twice as high compared to adolescents without PMS. This may be caused by stress because correlation analysis on stress and PMS showed a strong value.

Another study by Espin et al. shows the autonomic response (sAA levels) to psychosocial stress based on gender and menstrual cycle phase. The results indicated that during the luteal phase (premenstruation), the response of sAA levels to stress was sharper compared to the follicular phase (37). sAA levels tend to decrease during the menstrual phase (38). This suggests that hormonal fluctuations increase the sensitivity of the central nervous system during the luteal phase, manifesting as premenstrual syndrome. Symptoms appear cyclically during the luteal phase and diminish as the follicular phase begins. Dysregulation of progesterone metabolites, such as allopregnanolone, along with a combination of serotonergic and GABAergic compounds, may be the mechanisms involved (39).

This study is limited by the sampling method, as it was conducted only once during the luteal phase, which may not fully capture fluctuations in sAA levels. To better understand these variations, we recommend that future research employ a longitudinal design to assess sAA fluctuations more comprehensively. Furthermore, this study lacks in-depth interviews that explore other potential factors contributing to stress in adolescents. We recommend that future research incorporate qualitative methods to examine additional stress triggers, enabling a more comprehensive understanding.

Moreover, since most sample collection occurred during school hours, this may not fully capture fluctuations in daily stress levels. We recommend that future studies incorporate sampling at multiple points throughout the day to obtain a more comprehensive assessment.

Other than that, Multivariable analysis using multiple linear regression with sAA and stress (K10) as independent variables yielded an R^2 value of only 0.497, indicating that these factors account for just 49.7% of the variance in PMS (SPAF). This indicates that the remaining 50.3% may be influenced by other unconsidered factors that could impact PMS. Several additional factors can contribute to the onset of PMS, potentially leading to increased sAA levels in adolescents' saliva. These factors encompass dietary habits characterized by insufficient essential nutrients, inadequate sleep patterns and quality, a lack of physical activity, exposure to environmental stressors such as familial conflicts, genetic predispositions, coexisting health conditions such as anxiety, and excessive caffeine consumption. Researchers find it

particularly challenging to exert control over these factors. We recommend that future research take into account these and other relevant influences to provide a more comprehensive understanding of the factors affecting PMS in adolescents.

Additionally, ROC analysis demonstrated that the sensitivity and specificity of sAA levels in assessing the potential diagnosis of adolescents with PMS, at 67.7%, are suboptimal for clinical application. In light of this, we emphasize that while sAA levels may not currently be adequate for clinical diagnosis, they can still provide valuable insights into stress levels and their correlation with PMS. We suggest that future research should focus on improving the diagnostic accuracy of sAA by considering additional biomarkers or integrating sAA measurements with other clinical assessments.

Conclusion

There is a positive relationship between sAA levels and stress in adolescents with PMS. SAA levels and stress were able to predict PMS in adolescents with an accuracy of 49.7%.

Conflict of Interests

Authors declare no conflict of interests.

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