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Low serum zinc and total antioxidant capacity levels in individuals with premenstrual syndrome

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ABSTRACT

Aims: The aim of this study was to evaluate serum zinc (Zn) level, total antioxidant capacity (TAC) level, and nutritional status in premenstrual syndrome (PMS).

Methods: This cross-sectional, single-center study and case-control study enrolled university students 18 to 28 years old. Serum TAC analysis was performed via the enzyme-linked immunosorbent assay. Serum Zn level was measured using atomic absorption spectrometry. Nutritional status was assessed using the 24-hour dietary recall.

Results: The study population consisted of 110 individuals (mean±Sstandart deviation age: 20.3±1.9 years), 48 subjects with PMS, and 62 subjects without PMS. Individuals with PMS had lower serum Zn levels (28.04±31.40 µmol/L vs. 11.67±6.42 µmol/L, respectively), TAC levels (1.60±0.42 mmol/L vs. 1.47±0.10 mmol/L), and higher body mass index (22.30±02.71 kg/m^2 vs. 23.73±3.44 kg/m^2) than individuals without PMS. Individuals with PMS had lower fiber, polyunsaturated fatty acid (PUFA), vitamins E, vitamin B1, vitamin B2, vitamin B6, vitamin C, folate, and magnesium, and higher carbohydrate and sodium intake than those without PMS but the differences did not reach statistical significance. Serum TAC level, dietary antioxidant, and PUFA intake level were positively correlated ($p<0.05$), whereas serum TAC level was negatively correlated with dietary intake of protein, lipid, and carbohydrate intake ($p<0.05$).

Conclusions: This study showed that serum Zn and TAC levels were lower in PMS, and serum TAC levels were correlated with dietary antioxidant intake.

Introduction

Premenstrual syndrome (PMS) is a common condition that causes physical, emotional, and behavioral symptoms in women of reproductive age (1,2). It starts in the late luteal phase of the menstrual cycle, and the symptoms subside shortly after the onset of menstruation (3). The quality of life and reproductive health are significantly affected in affected individuals (2,4).

The Royal College of Obstetricians and Gynecologists has reported that 40.0% of women have premenstrual symptoms (5). The lowest incidence was reported in France (12.0%). The highest incidence was reported in Iran (98.0%) (6). In Turkey, the incidence has been reported by 66.0 to 91.8%, more commonly in young individuals (7,8).

The symptoms of PMS have been explained through reduced levels of progesterone and the progesterone metabolite

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allopregnanolone, which act as agonists to the gamma-aminobutyric acid-A (GABA-A) receptor in the luteal phase (9). Progesterone and allopregnanolone exert antioxidant and sedative actions via the GABA-A receptor and do not cause mood changes in healthy individuals (10). However, they may cause hormonal imbalance, increased pro-oxidant activity, oxidative stress, and the disrupted GABAergic system, leading to PMS symptoms (11).

The oxidant/antioxidant balance is disrupted in PMS, associated with the symptom occurrence (12). Psychiatric problems, usually major depression and anxiety, are among the symptoms that frequently occur in individuals with PMS (13), and increased levels of serum superoxide dismutase and malondialdehyde, as well as decreased levels of ascorbic acid, levels have been noted in major depression (14). Additionally, impaired immune functions and decreased cytokine levels have been reported in individuals with anxiety, associated with increased cortisol secretion and increased oxidative stress (15).

Sleep disturbance and poor sleep quality are also frequently observed in individuals with PMS, particularly at the end of the luteal phase (16). Additionally, PMS has been associated with high levels of malondialdehyde, which is considered an indicator of oxidative stress, and low glutathione and glutathione peroxidase levels (11). However, it has been reported that oxidative stress markers are not altered in healthy individuals with sleep disorders (17).

It has been emphasized that zinc (Zn) metabolism should be taken into account in PMS because of its antioxidant properties and roles in progesterone binding, prolactin secretion, opiate effect, gonadal secretion, and menstrual cycle regulation (18). Long-term insufficient Zn intake decreases Zn levels in the body and results in impaired glucocorticoid production. This is often associated with neurological symptoms such as irritability, emotional instability, and depression, common in PMS (11).

PMS or premenstrual symptoms have been associated with the inflammatory response (19). In women of childbearing age, the levels of inflammatory mediators increase after ovulation and reach the highest levels during menstruation (20). Elevated C-reactive protein levels lead to more severe PMS symptoms, particularly mood and pain symptoms (21). Zn is also defined as an anti-inflammatory agent, and it has been suggested to relieve PMS symptoms by regulating the levels of high-sensitivity C-reactive protein (22). It has also been reported to exhibit antidepressant activity by upregulating the gene expression of neurotrophic factors (23). In a previous study, 30 mg/day Zn supplementation for 12 weeks had favorable effects on physical and psychological symptoms and total antioxidant capacity (TAC) in individuals with PMS (22).

The relationship between Zn levels/deficiency and TAC levels has not been evaluated sufficiently in individuals with PMS. Only one study reported favorable effects of 30 mg/day Zn supplementation for 12 weeks on symptoms and TAC in PMS (22). Therefore, more research on serum Zn and TAC in PMS are necessary to find relationships between these and symptoms.

This study aimed to determine the serum TAC levels, Zn levels, and their relationship with nutritional intake in PMS.

Methods

This case-control study was conducted between May and July 2019 on university students aged between 18 and 28 years. The exclusion criteria included the use of antidepressants, receiving hormone support therapy, and oral contraceptives, receiving nutritional support, and a diagnosis of menstrual irregularity and polycystic ovary syndrome.

The study was approved by the Zekai Tahir Burak Clinical Research Ethics Committee (38/2019 - 14/05/2019). All procedures were performed in compliance with the Helsinki Declaration. Additionally, all patients were informed about the study, and written consent was obtained.

Data collection

Survey form

Menstrual cycle characteristics (e.g., first menstrual age, length of the menstrual cycle, and menstrual pattern), eating habits (number of meals, increased/decreased food consumption) related to the menstrual period, and preference of any food/drink for relaxation of the symptoms were evaluated by face-to-face interviews using a survey form.

Anthropometric measurements

The body weight was measured on an empty stomach, without shoes, and in light clothes. Height was measured using a stadiometer with the individual standing feet closed, head in the Frankfurt plane (eye triangle and top of auricle aligned, parallel to the ground). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (meter). Individuals with a BMI of 18.5 to 24.9 kg/m² were classified as normal weight, 25.0 to 29.9 kg/m² as overweight, 30.00-34.99 kg/m^2 as type 1 obese, and 35.00-39.99 kg/m^2 as type 2 obese (24).

Premenstrual syndrome scale

The PMS status was evaluated using a PMS scale developed and validated by Gençdoğan (25) in Turkey according to The Diagnostic and Statistical Manual of Mental Disorders-III (DSM) and DSM-IV-R criteria. It is a five-point Likert-type scale that consisted of 44 items. Items are scored 1 for a response of “not any/never’’, 2 for “very little”, 3 for “sometimes”, 4 for “frequently” and 5 for “most times’’. The highest score is 220 and scores of 111 or higher indicate PMS.

International physical activity questionnaire (short form)

The international physical activity questionnaire-short form (IPAQ-SF) is a validated tool to evaluate physical activity levels in our study (26).

IPAQ-SF quantifies physical activity during the last 7 days in four categories: Vigorous intensity, moderate intensity, walking, and sitting. In addition to intensity, frequency and duration of physical activity are assessed. The sum of duration (minutes) and frequency (days) of walking, moderate-intensity, and vigorous activity is used to calculate the total score. A standard metabolic equivalent (MET) value is calculated and the required energy is calculated using the MET-minute scores. The following MET values were considered in the current study: Walking=3.3 METs, moderate physical activity=4.0 METs, vigorous physical activity=8.0 METs, and sitting=1.5 METs. Daily and weekly physical activity levels are evaluated using these values.

Biochemical analyses

Blood samples were taken on the 21st day of the menstrual cycle. Serum Zn level was measured using a commercial test kit (Relassay, Turkey) with atomic absorption spectrometry. The Zn in the sample changes color from the red-orange color of 5-Br-PAPS to light pink under alkaline conditions. The change in absorbance at 548 nm is proportional to the total Zn level in the sample. The calibration was performed with Zn sulfate dissolved in deionized water. A serum Zn level <10.7 µmol/L was considered Zn deficiency (27).

Serum TAC level was measured using a commercial kit (Relassay, Turkey). The antioxidants in the sample convert the dark blue-green ABTS radical solution to the colorless ABTS form. Thus, the change in absorbance at 660 nm is proportional to the total number of antioxidants. The kit was calibrated using a stable antioxidant standard called Trolox equivalent, similar to vitamin E. The results were expressed in mmol/L Trolox equivalents (28).

Evaluation of nutritional status

Twenty-four-hour dietary recall was used to determine the daily nutrient intake. Food consumption records were evaluated using the Nutritional Information System Package Software (Bebispro for Windows, Stuttgart, Germany; Turkish version, 2010), and the mean daily dietary intake of energy and macro- and micronutrients were determined (29).

Determination of total dietary antioxidant capacity

A food consumption frequency form was used to determine the amounts of dietary antioxidant intake calculated using the antioxidant database of foods created by the ferric reducing antioxidant power (FRAP) assay method by Carlsen et al. (30). The FRAP method is simple, fast, and inexpensive. Moreover, it ensures the optimization of the extracts in the determination of both lipophilic and hydrophilic antioxidants (30).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Statistics for Mac, version 22.0 (IBM Corp., Armonk, NY) was used for the statistical analysis. The normality distribution was evaluated using the Kolmogorov-Smirnov test. Descriptive statistics were presented as number (n), percentage, and mean±standard deviation (SD). Student's t-test was used to evaluate differences between age, BMI, menarche age, duration/length of menstruation, serum TAC and Zn levels, and physical activity MET values of individuals with and without PMS. Pearson's chi-square test was used to compare the difference in the incidence of Zn deficiency between the two groups. The effect of nutrient intake on serum TAC and Zn levels was evaluated using multiple linear regression analysis. The level of significance was determined to be $p<0.05$.

Results

The study population consisted of 110 individuals (mean±SD age: 20.3±1.9 years), 48 subjects with PMS, and 62 subjects without PMS. Mean BMI was 21.8±3.1 kg/m^2, age at menarche was 13.3±1.1 years and the duration of menstruation was 5.7±1.4 days in the whole sample. Of the study sample, 8.4% were underweight, 74.8% were normal weight, 15.0% were overweight, and 1.8% were obese. Mean serum TAC and Zn levels were 1.5±0.3 mmol/L vs. 20.9±25.2 µmol/L, respectively.

Compared with the group without PMS, individuals with PMS had higher BMI (23.7±2.7 vs. 21.3±3.4 kg/m^2, $p<0.05$) and lower serum TAC level (1.4±0.1 vs. 1.6±0.4 mmol/L, $p<0.05$), Zn level (11.6±6.4 vs. 28.0±31.4 µmol/L, $p<0.05$) and physical activity MET values (1051.5±931.3 vs. 1315.5±825.1 min, $p<0.05$). There was no difference between the two groups in terms of age, age at menarche, and duration of menstruation (Table 1).

Most individuals in both groups (92.3% with PMS vs. 91.8% without PMS) consumed two meals and two snacks a day. However, when changes in eating habits (food consumption increased or decreased) during the menstrual period were evaluated, 42.7% of them stated a change in eating habits, and it was more common in individuals with PMS than without PMS (54.2% vs. 33.9%, $p<0.05$) (Table 2).

As was also shown in Table 2, 52.1% of the individuals with PMS and 33.8% of those without PMS preferred consuming a particular food during their menstrual period for symptom relief, and 67.4% of them reported chocolate intake. Also, 25.0% of the individuals with PMS and 17.7% of the individuals without PMS consumed a particular drink during the menstrual period for symptom relief, and 56.5% of them preferred herbal tea. Additionally, the percentage of individuals with PMS taking a nutritional supplement during the menstrual period was higher than without PMS (40.3% vs. 29.2%, $p<0.05$) (Table 2).

Individuals with PMS had a lower intake of energy, protein, fat, fiber, polyunsaturated fatty acids (PUFA), vitamin E, vitaminB_1,

vitamin B_2, vitamin B_6 and vitamin C, folate, magnesium (Mg), and higher intake of carbohydrate (CHO) and sodium than individuals without PMS, but the differences were not significant (Table 3).

In individuals with PMS, serum TAC level was correlated with dietary antioxidant and PUFA intakes and negatively correlated with the percentages of dietary energy from CHO, protein, and fat (Table 4).

No significant relationship was found between serum TAC level and nutrient intake in individuals without PMS.

Zn deficiency was observed in 34.5% of the individuals, which was higher in individuals with PMS than without PMS (47.9% vs. 24.2%, respectively, $p<0.05$). Additionally, serum Zn levels were negatively correlated with the percentages of dietary energy from CHO, protein, and fat in individuals with PMS (Table 5).

Discussion

The exact pathophysiology of PMS remains unknown. It has been suggested that micronutrients improve PMS symptoms through neurotransmitters and hormones, but only a few studies have been performed in this field (31,32). Therefore, we hypothesized that individuals with PMS could have lower serum Zn and TAC.

Conditions such as sleep disorders, increased anxiety, and depression that are commonly reported in obese individuals, are among the symptoms observed in individuals with PMS, and that obesity is a potential risk factor for PMS (33). Furthermore, studies have shown that the prevalence of PMS in obese individuals is twice as high as in non-obese and the importance of appropriate body weight for treating PMS has been demonstrated (33-35). In this study, a significant difference in BMI was observed based on the PMS status and the BMI values of individuals with PMS were higher, which is consistent with the literature.

Table 1. General characteristics of the individuals based on PMS

Variables		n	$\bar{X}$	Levene's F-test	Levene's P-test	p value (two-tailed)
Age (years)	PMS (+)	48	20.6441	6.835	0.010	0.245
	PMS (-)	62	20.1823			
Body mass index (kg/m^2)	PMS (+)	48	23.7317	0.207	0.650	**0.036**
	PMS (-)	62	21.3073			
Age at menarche (years)	PMS (+)	48	13.4029	1.989	0.161	0.345
	PMS (-)	62	13.2378			
Duration of menstruation (days)	PMS (+)	48	5.6678	4.413	0.038	0.701
	PMS (-)	62	5.7574			
Serum TAC (mmol/L)	PMS (+)	48	1.4671	7.469	0.007	**0.019**
	PMS (-)	62	1.6021			
Serum Zn level (µmol/L)	PMS (+)	48	11.6792	14.766	<0.001	**<0.001**
	PMS (-)	62	28.0435			
Physical activity MET value (min)	PMS (+)	48	1051.51	1.450	<0.001	**0.020**
	PMS (-)	62	1315.56			

MET: Metabolic equivalent, TAC: Total antioxidant capacity, Zn: Zinc, PMS: Premenstrual syndrome

Table 2. Eating habits of individuals

Variables	PMS (+)				PMS (-)				p
	Yes		No		Yes		No		
	n	%	n	%	n	%	n	%	
Do your eating habits change during your menstrual period?	26	54.2	22	45.8	21	33.9	41	66.1	**0.033**
Is there a particular food you prefer to consume during your menstrual period for relaxing symptoms?	25	52.1	23	47.9	21	33.8	41	66.2	0.453
Is there a particular drink you prefer to consume during your menstrual period for relaxing symptoms?	12	25.0	36	75.0	11	17.7	51	82.3	0.444
Is there any nutritional support you take during your menstrual period?	25	40.3	37	59.7	14	29.2	34	70.8	**0.017**

PMS: Premenstrual syndrome

We observed that changes in eating habits during the menstrual period were more common in individuals with PMS than those without PMS. In the PMS group, 80.1% indicated that their food intake increased during the premenstrual period. Our finding that CHO consumption was higher in this group also confirms this data. Accordingly, increased BMI was previously proposed as a risk factor for PMS (36), and our findings showed that food intake might also increase in individuals with PMS, which may also contribute to this risk. Most individuals (86.0%) stated that chocolate was a particular food they preferred during the menstrual period. The increased desire to consume chocolate may be due to the increased need for substances such as Mg and serotonin due to the physiological changes during this period or its effect like creating a sense of pleasure through the endocannabinoid system (37). It has also been suggested that food intake increases depending on the change in serum steroid level and basal metabolic rate during this period (38). In PMS, nutritional consultation seems to be a crucial component of maintaining appropriate body weight and improvement of symptoms.

One of the most critical findings of individuals with PMS is the change in oxidative balance. Many hypotheses have been proposed regarding the underlying mechanism. Although there are contradictory studies, it has been confirmed that estrogen, which has pro-oxidant properties, causes serum TAC levels to decrease and oxidant levels to increase in individuals with PMS (39-41). In a previous study, the levels of oxidant F2-isoprostane were higher in PMS which were linked to symptom severity (42).

We observed lower serum TAC levels in individuals with PMS, and serum TAC levels were correlated with dietary antioxidants and PUFA intake. Individuals with PMS should pay attention

Table 3. Mean nutrient intake of individuals based on PMS

Variable		n	$\bar{X}$	SD	p value
Energy (kcal/day)	PMS (+)	48	949.4	434.01	0.752
	PMS (-)	62	972.9	311.61	
Protein (g/day)	PMS (+)	48	43.8	15.31	0.928
	PMS (-)	62	44.0	9.78	
CHO (g/day)	PMS (+)	48	118.5	42.64	0.421
	PMS (-)	62	112.5	34.87	
Fat (g/day)	PMS (+)	48	47.5	14.98	0.067
	PMS (-)	62	58.5	38.94	
Fibre (g/day)	PMS (+)	48	14.4	6.26	0.316
	PMS (-)	62	24.1	66.65	
PUFA (g/day)	PMS (+)	48	13.0	20.01	0.466
	PMS (-)	62	18.4	48.27	
Vitamin E (mg/day)	PMS (+)	48	6.6	2.85	0.076
	PMS (-)	62	8.0	4.89	
Vitamin B_1 (mg/day)	PMS (+)	48	1.5	1.26	0.331
	PMS (-)	62	4.3	20.03	
Vitamin B_2 (mg/day)	PMS (+)	48	0.8	0.48	0.606
	PMS (-)	62	0.9	1.01	
Vitamin B_6 (mg/day)	PMS (+)	48	17.1	21.29	0.321
	PMS (-)	62	57.9	282.58	
Vitamin C (mg/day)	PMS (+)	48	97.1	185.93	0.616
	PMS (-)	62	118.6	245.44	
Folate (mcg/day)	PMS (+)	48	183.3	81.28	0.517
	PMS (-)	62	205.6	226.60	
Magnesium (mg/day)	PMS (+)	48	244.0	308.88	0.771
	PMS (-)	62	264.5	403.01	
Sodium (mg/day)	PMS (+)	48	2059.2	798.45	0.312
	PMS (-)	62	1921.5	623.75	

CHO: Carbohydrate, PUFA: polyunsaturated fatty acid, PMS: Premenstrual syndrome, SD: Standard deviation

to taking fresh vegetables and fruits with higher antioxidant content in their diet. In addition, the importance of the adequacy of vegetable oils, oil seeds, and oilseeds should be emphasized as they can increase the endogenous antioxidant enzyme activity. Moreover, serum TAC levels are associated with the dietary pattern, and serum antioxidant capacity decreases as the percentages of dietary energy from CHO, protein, and fat increase in individuals with PMS. These findings should be considered in the food patterns of individuals with PMS.

It has been shown that as the percentage of energy derived from CHOs increases, adverse mood and behavioral changes occur more often in healthy women with a regular menstrual cycle (22).

Some micronutrient deficiencies may also be associated with PMS (43). Moreover, it has been indicated that serum Zn levels change during the menstrual cycle and are significantly lower in individuals with PMS than in individuals without PMS (22). This may lead to irregular glucocorticoid production and some neuropsychological symptoms (44).

In the current study, 34.5% of the individuals had Zn deficiency, which was significantly more common in individuals with PMS than in those without PMS. Furthermore, it was found that serum Zn levels were negatively correlated with the dietary percentage of carbohydrate. This finding may be explined thorough the lower zinc content of foods with high carbohydrate content.

In our findings, individuals with PMS also had lower MET values and lower levels of physical activity than individuals without PMS. A study found that 8-week aerobic exercise effectively reduces PMS symptoms (45). The incidence of PMS

Table 4. Multiple linear regression analysis of the effect of nutrient consumption on serum TAC level based on PMS status

Variable	PMS status	Beta	t	p	95% confidence interval	
Energy (kcal/day)	PMS (+)	−2.683E−005	−0.462	0.647	0.000	0.000
	PMS (-)	−2.991E−005	−0.105	0.917	−0.001	0.001
CHO (%)	PMS (+)	−0.029	−2.625	**0.013**	−0.052	−0.007
	PMS (-)	0.004	.299	0.766	−0.023	0.032
Protein (%)	PMS (+)	−0.029	−3.008	**0.005**	−0.049	−0.010
	PMS (-)	−0.011	−0.222	0.825	−0.110	0.088
Fat (%)	PMS (+)	−0.029	−2.047	**0.048**	−0.057	0.000
	PMS (-)	0.004	0.253	0.802	−0.026	0.033
PUFA (g/day)	PMS (+)	0.010	2.488	**0.017**	0.002	0.019
	PMS (-)	−4.620	0.000	1.000	−0.004	0.004
Dietary antioxidant amount (mmol/day)	PMS (+)	0.011	2.060	**0.046**	0.000	0.021
	PMS (-)	0.037	1.750	0.086	−0.005	0.079

CHO: Carbohydrate, PUFA: Polyunsaturated fatty acid, PMS: Premenstrual syndrome, TAC: Total antioxidant capacity

Table 5. Multiple linear regression analysis of the effect of nutrient consumption on serum Zn levels based on PMS status

Variable	PMS status	Beta	t	p	95% confidence interval	
Energy (kcal/day)	PMS (+)	0.004	1.103	0.277	−0.003	0.010
	PMS (-)	−0.41	−3.250	**0.002**	−0.066	−0.016
CHO (%)	PMS (+)	−0.861	−1.255	**0.008**	−1.487	−0.235
	PMS (-)	1.324	1.349	0.183	−0.646	3.295
Protein (%)	PMS (+)	−0.205	−0.540	0.592	−0.972	0.562
	PMS (-)	−2.032	−0.936	0.353	−6.387	2.322
Fat (%)	PMS (+)	−0.693	−1.946	0.059	−1.413	0.028
	PMS (-)	−0.616	−0.836	0.407	−2.094	0.862
Dietary Zn intake (mg/day)	PMS (+)	−0.421	−0.459	0.649	−2.276	1.434
	PMS (-)	−5.972	−2.779	**0.008**	−10.286	−1.659

CHO: Carbohydrate, Zn: Zinc, CHO: Carbohydrate, PMS: Premenstrual syndrome

also increases in individuals who engage in low physical activity levels, suggesting exercise as a potential treatment option (46).

Study Limitations

The limitations of this study include the use of a single daily record of 24-h food consumption on the 21st day of menstruation to evaluate the overall effect of diet on PMS. A longer record of food consumption (3-5 days) could provide more accurate information. Additionally, although ovarian hormones play a crucial role in the pathogenesis of PMS and reduced TAC levels (41), blood hormone measurement was not available in the current study.

Conclusion

This study found lower serum Zn and TAC levels and higher BMI in subjects with PMS. Serum TAC levels were related to dietary antioxidant intake and dietary protein, lipid, and CHO intake. The results suggest that nutritional counseling can be a part of PMS management.

Ethics

Ethics Committee Approval: The study was approved by the Zekai Tahir Burak Clinical Research Ethics Committee (numbered: 38/2019, dated: 14/05/2019).

Informed Consent: A consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: T.K.C., Ö.M., A.G., H.Z., B.Ö., S.A., Concept: T.K.C., Design: T.K.C., Data Collection or Processing: T.K.C., D.A., Ö.M., A.G., H.Z., B.Ö., Analysis or Interpretation: T.K.C., D.A., Literature Search: T.K.C., D.A., Writing: T.K.C., D.A.

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