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GENERAL GYNECOLOGY

Menstrual problems in adolescence: relationship to serum vitamins A and E, and systemic inflammation

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Abstract

Background Vitamin status and inflammatory mechanisms may be related to menstrual cycle abnormalities. We investigated the associations between serum fat soluble vitamin (vitamins A and E) concentrations and biomarkers of inflammation and antioxidant status with menstrual characteristics, primary dysmenorrhea (PD) and premenstrual syndrome (PMS) in healthy adolescents.

Methods A total of 897 adolescent girls either suffering from PMS ($n=134$), PD ($n=322$), PMS and PD ($n=293$) or healthy adolescents ($n=148$) were recruited. Serum vitamin A and E, high-sensitivity C-reactive protein (hs-CRP), antibody titers to Hsp27 (anti-Hsp27), serum prooxidant–antioxidant balance (PAB), WBC, mean platelet volume (MPV), and platelet distribution width (PDW) and RBC distribution width (RDW) were measured. Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and RDW-to-platelet ratio (RPR) were calculated.

Results Girls with long bleeding periods had lower concentrations of serum vitamin E compared to those who reported a normal period duration. There were significantly differences between the groups reporting oligomenorrhea, regular menses and polymenorrhea with respect to NLR, RPR, MPV and PDW. Logistic regression demonstrated that the presence of both PMS and PD was positively related to higher serum hs-CRP, PAB and NLR, while serum vitamin A level was inversely related to the presence of PMS.

Conclusions We found that serum vitamin A, hs-CRP, PAB and NLR are significantly associated with the presence of PMS and PD. Inflammatory processes may contribute to the etiology, symptoms and severity of menstrual disorders. Prospective studies are needed to elucidate the possibility of targeting oxidative stress and inflammatory process for the amelioration of menstrual symptoms.

Keywords Dysmenorrhea · Hs-CRP · Neutrophil · Premenstrual syndrome · Vitamin A

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Introduction

While menstruation is a normal phenomenon, there are some profound psychological changes associated with menarche. Menstrual disorders, premenstrual syndrome (PMS) and primary dysmenorrhea (PD) are prevalent among adolescent girls and adversely affect their quality of life [1]. PMS is one of the most frequent complaints and is seen regularly in most menstrual cycles during the luteal phase of menstruation [2, 3]. PD, defined as painful cramps, occurring with menstruation, is one of the most frequent causes of pelvic pain. Supplements containing combinations of vitamins have been advocated for use in these conditions. However, little is known about the association of vitamin status [vitamins A (retinol) and E (tocopherol)] on the reproductive health of women.

Vitamin A (Vit A) is vital for sustaining multiple physiological actions that include: morphogenesis, night vision, immune response and reproduction [4]. Vitamin E (Vit E) comprises several related lipid soluble compounds (tocopherols and tocotrienols) that have potent antioxidant properties [5]. Excessive and malapropos prostaglandin production has been involved in the pathogenesis of PMS, and there is some document suggesting that Vit E can modulate the production of prostaglandin [5].

It has been reported that menstrual cycle disturbance may be related to inflammatory mechanisms [6]. Furthermore, chronic inflammation has been suggested to be involved in the etiology of the psychiatric and somatic features of PMS [7]. High-sensitivity C-reactive protein (hs-CRP) is an acute-phase reactant secreted by the liver and is a sensitive indicator of chronic low-grade inflammation. Heat shock proteins (Hsps) are stress proteins produced in response to systemic and cellular stress [8]. Hsp27 as a member of the Hsp family regulate in several cell types as well as its antibodies increased in stress diseases [9]. There is normally a balance between the synthesis and the removal of reactive oxygen species. An imbalance between many prooxidant and antioxidant factors leads to oxidative stress (OS). Assessment of prooxidant antioxidant balance (PAB) is one way for measurements of the relative oxidant and the antioxidant levels [9].

The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), red blood cell (RBC) distribution width (RDW), RDW-to-platelet ratio (RPR), mean platelet volume (MPV) and platelet distribution width (PDW) are inexpensive and easily measured variables, which are also associated with pro-inflammatory conditions; they are hematological markers of systemic inflammation, derived from indices of the complete blood count (CBC) [10].

There are few studies that have assessed the role of inflammatory markers in the physiological regulation of reproductive hormones and their relationship with menstrual symptom severity [11, 12]. To clarify the association between menstrual disorders and vitamins levels and also inflammation-like conditions, researches according to a more varied population base appear to be necessary. This study was performed to examine the relationship between serum fat soluble vitamin concentrations, systemic indices of inflammation in serum (hs-CRP, PAB, anti-Hsp27) and in blood (NLR, PLR, RPR, MPV, RDW, PDW) and menstrual cycle features (long and short cycle length, cycle regularity and bleeding pattern) and also PD and PMS in a population of otherwise wholesome girls.

Materials and methods

Target population

This work was carried out among 897 adolescent students habiting in the cities of Mashhad and Sabzevar, Iran, in 2015 [13, 14]. Sample size was determined according to 80% power and $\alpha' = 0.05$, and it was estimated that 850 participants were required for adequate power. Briefly, participants were recruited from six geographic areas in two cities in northeastern Iran (Mashhad and Sabzevar), using a multistage cluster sampling way. Four high schools from each of the six geographic areas were selected, and one class from each grade (3 classrooms from each school) was randomly chosen for inclusion. In each classroom, nearly 15 students were included. Schools, classes and students were recruited using computer-generated random numbers. We excluded girls with any autoimmune abnormalities, carcinoma, metabolic or cardiovascular disorders (CVD), liver or renal failure, periodontal disease and endocrinopathy which were diagnosed by physicians or self-reported. Individuals, who were using anti-inflammatory, anti-depressant, vitamins or any supplement consumption and hormone therapy over the past year, were also elided. Ethical approval was acquired from the university, and informed written consent was completed by all subjects and their parents.

Evaluation of menstrual pattern

Menstrual histories were gathered by questionnaires that included the age of menarche, menstrual cyclicity (cycle length, duration and amount of flow) and the presence of PD as described previously [13]. In brief, menstrual cycle patterns were categorized as follows [15, 16]:

Early menarche age at menarche of ≤ 12 year.
Medium menarche age at menarche between 13 and 14 year.
Delayed menarche age at menarche > 14 year.

Short bleeding times Bleeding time ≤ 4 days.
Long bleeding times Bleeding time ≥ 6 days.
Menorrhagia (heavy menstrual bleeding) utilize of ≥ 4 fully soaked pads a day for protection during the menstrual periods.
Hypomenorrhea (light menstrual loss) < 1 fully soaked pad or utilize of panty liner being suffice for protection.
Oligomenorrhea menstrual cycles of 35–180 days or infrequent menses
Polymenorrhea cycle length time of ≤ 20 days

Participants were instructed to respond to questions about their recurrent experience of 15 specific symptoms within the premenstrual phase that spontaneously resolves at the onset of menstruation: physical symptoms such as leg ache, backache, diarrhea, nausea, vomiting, along with psychological symptoms of appetite changes, irritability, fatigue, palpitation, energy depletion, poor sleep, feeling sad or blue, decrease interest, loss of concentration, and tendency to cry easily. Responders having at least 2 symptoms, 1 physical and 1 psychological symptom, were regarded as having PMS, while those with only 1 symptom or no symptoms were considered as not having PMS, based on reliable instrument developed by Mortola et al. [17]. In addition, the diagnosis of PD was according to attendance of abdominal pain, cramping regarded to menstrual bleeding [18]. Pre-enrollment, secondary causes of dysmenorrhea were omitted through uterine and ovarian sonography.

In sub-analysis, we also classified subjects into 4 distinctive groups (those having only PMS, those having only PD, those having both PMS and PD and normal individuals).

Anthropometric and laboratory analysis

Anthropometric parameters and blood pressure were measured and body mass index (BMI) calculated as previously described [19]. Fasting blood glucose (FBG), serum lipids and hs-CRP were determined by methods that have been previously described [19]. An in-house ELISA assay and a modified PAB assay were used to measure serum antibody titers to Hsp27 (anti-Hsp27) and PAB levels, respectively, according to the previously described methods [20, 21]. White blood cell, lymphocyte, neutrophil, platelet counts, MPV, and PDW, RDW and hemoglobin were measured as part of the automated complete blood using an automated hematology analyzer (Sysmex K-800), and after that, NLR, PLR and RPR were calculated.

Serum concentrations of Vit A (all-trans retinol) and Vit E (measured as α-tocopherol) were simultaneously quantified using isocratic high-performance liquid chromatography (HPLC) by a modification of the method of Driskell et al. [22]. Briefly, 250 µL of ethanol was added to 250 µL of serum and vortexed for 5 s. Then, 500 µL of *n*-hexane was added to the supernatant, vortex-mixed and centrifuged at 1000 g for 1 min. After that, the supernatant was evaporated under nitrogen gas, and the residue was reconstituted in 200 µL of methanol. Then, 20 µL of the solution was injected to the HPLC column at a flow rate of 1.5 mL/min. Methanol was exploited as the mobile phase, and detection was performed at 294 nm using UV detector (Waters 486, Milford, MA, USA).

Statistical analysis

All statistical analyses were carried out using SPSS software version 17 (SPSS Inc., Chicago, Illinois, USA). Normal distribution of variables was judged using the Kolmogorov–Smirnov test. Descriptive data were presented as the mean ± standard deviation (normally distributed parameters) or as the median and interquartile range (none-normally distributed variables). One-way ANOVA (post hoc Tukey) or Kruskal–Wallis (post hoc Mann–Whitney) tests were recruited to contrast the clinical features between the groups. To determine the association of variables and PMS/PD, univariate multinomial logistic regression was firstly used. The variables with a $P < 0.05$ were further analyzed using multivariate multinomial logistic regression. A $P < 0.05$ was set to be significant.

Results

Eight hundred and ninety-seven girls were categorized into four categories: PMS (14.9%), PD (35.9%), both PMS and PD (32.7%) and normal subjects (16.5%). The mean age of the studied populations was 14.66 ± 1.53 (min: 12; max: 18) years. The features of the participants are presented in Table 1. Between the 4 groups, no statistically significant differences were observed in demographic and anthropometric parameters. Among biochemical and hematological measurements, serum Vit A, hs-CRP, PAB and NLR differed between four groups. Vit A was significantly lower in the PMS group in comparison with normal group ($P < 0.05$). Serum hs-CRP, PAB and NLR were higher in the PMS and PD groups versus the normal group ($P < 0.05$).

Association between menstrual pattern with serum Vit A and Vit E

Serum Vit A and Vit E were not different with respect to menarcheal age, cycle length and amount of flow. But, the group with long bleeding periods had lower mean serum Vit E compared to the group with normal periods ($P < 0.05$) (Table 2).

Table 1 Clinical characteristics of the studied population

Variables	Normal $n=148$ (16.5%)	PMS $n=134$ (14.9%)	PD $n=322$ (35.9%)	PMS + PD $n=293$ (32.7%)	P value
Age (years)	14.6 ± 1.7	14.6 ± 1.4	14.6 ± 1.5	14.7 ± 1.5	Ns
WC (cm)	69.3 ± 7.2	69.9 ± 7.3	70.7 ± 8.5	71.9 ± 10.1	Ns
Weight (kg)	52.4 ± 10.2	52.9 ± 9.6	54.3 ± 10.8	54.5 ± 12.7	Ns
Height (cm)	157.5 ± 6.2	158.2 ± 4.7	158.8 ± 5.6	158.1 ± 5.1	Ns
SBP (mmHg)	101.3 ± 12.5	98.2 ± 14.3	98.5 ± 14.3	101.9 ± 14.9	Ns
DBP (mmHg)	67.2 ± 11.0	66.5 ± 12.2	65.6 ± 13.1	66.2 ± 13.2	Ns
Serum biochemical parameters					
FBG (mg/dL)	89.2 ± 12.3	85.0 ± 13.1	86.4 ± 11.5	86.0 ± 11.7	Ns
TG (mg/dL)	82.9 (58.9–96.2)	81.7 (53.0–103)	86.9 (58.4–108.0)	82.3 (56.3–98.3)	Ns
TC (mg/dL)	155.7 ± 25.4	161.7 ± 34.7	162.6 ± 29.1	161.1 ± 28.8	Ns
LDL (mg/dL)	93.7 ± 22.7	100.8 ± 29.9	100.9 ± 26.4	100.1 ± 24.8	Ns
HDL (mg/dL)	47.1 ± 9.1	46.7 ± 8.7	46.8 ± 7.8	47.2 ± 9.2	Ns
Vitamin A (µmol/L)	7.2 (2.66–19.27)	1.12 (0.26–9.30)	6.39 (1.81–14.57)	6.05 (4.14-11.20)	**0.007**$^{\alpha}$
Vitamin E (µmol/L)	4.0 (2.69–5.80)	3.75 (2.87–5.80)	3.82 (2.57–5.80)	3.97 (2.26–5.80)	Ns
hs-CRP (mg/L)	0.8 2(0.41–1.61)	0.98 (0.64–1.72)	0.90 (0.47–1.59)	1.29 (0.63–2.38)	**0.004**$^{\beta,\gamma}$
Anti-Hsp27 (OD)	0.29 ± 0.24	0.26 ± 0.17	0.27 ± 0.17	0.23 ± 0.17	Ns
PAB (AU)	64.6 ± 34.2	72.9 ± 44.7	66.5 ± 34.9	83.4 ± 52.8	**0.007**$^{\beta,\gamma}$
Hematological indices					
Hb (gr/dL)	13.7 ± 1.9	13.5 ± 2.2	13.3 ± 2.3	13.7 ± 2.1	Ns
HCT (%)	43.2 ± 5.9	44.1 ± 6.6	43.4 ± 7.2	43.7 ± 5.8	Ns
WBC (10^9 cells/L)	6.27 ± 1.27	6.30 ± 2.03	6.46 ± 2.02	6.70 ± 3.29	Ns
RBC(10^{12} cells/L)	4.92 ± 0.75	4.99 ± 0.85	4.90 ± 0.83	4.95 ± 0.75	Ns
Neutrophil (%)	55.8 ± 8.9	55.6 ± 9.9	54.9 ± 10.8	55.9 ± 1.4	Ns
Lymphocyte (%)	36.23 ± 7.52	35.18 ± 8.71	36.11 ± 9.16	35.56 ± 8.70	Ns
RDW (%)	12.85 ± 1.12	13.17 ± 1.81	12.84 ± 1.16	12.85 ± 1.12	Ns
NLR	1.56 ± 0.46	1.71 ± 0.65	1.70 ± 0.87	1.87 ± 0.91	**0.039**$^{\beta}$
Platelet (10^6/L)	262.3 ± 70.9	264.1 ± 84.7	254.8 ± 66.5	258.5 ± 60.2	Ns
PLR	7.63 ± 2.99	7.92 ± 3.63	7.74 ± 3.57	7.71 ± 2.60	Ns
RPR	0.053 ± 0.019	0.058 ± 0.03	0.054 ± 0.02	0.053 ± 0.02	Ns
MPV(fL)	10.1 ± 0.95	10.6 ± 3.86	10.3 ± 2.00	10.6 ± 3.77	Ns
PDW (%)	12.81 ± 2.13	12.53 ± 1.88	12.84 ± 1.96	12.84 ± 1.84	Ns

Values expressed as mean ± SD (normally distributed variables) or median and interquartile range (non-normally distributed variables)

Between-group comparisons were assessed by parametric statistical analysis (one-way ANOVA) for normal distributed variables and nonparametric test (Kruskal–Wallis and post hoc Mann–Whitney) for non-normally distributed variables

Significance of bold values are $P < 0.05$

WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; Vit A, vitamin A; Vit E, vitamin E; hs-CRP, high-sensitivity C-reactive protein; HSP27, heat shock protein 27; PAB, prooxidant–antioxidant balance; hemoglobin, Hb; HCT, hematocrit; WBC, white blood cell; RBC, Red blood cell; RDW, red blood cell distribution width; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RPR, red blood cell distribution width-to-platelet ratio; MPV, mean platelet volume; PMS, premenstrual syndrome; PD, primary dysmenorrhea; PDW, platelet distribution width; Ns, non-significant

$^{\alpha}$Significant between normal and PMS

$^{\beta}$Significant between normal and (PMS + PD)

$^{\gamma}$Significant between dysmenorrhea and (PMS + PD)

Table 2 Relation of menstrual pattern and associated symptoms to vitamins

	Serum Vitamin A	Serum Vitamin E	Serum hs-CRP*	Serum Anti-Hsp27	Serum PAB
Early menarche ($n=422$)	6.38 (1.80–13.26)	3.73 (2.67–5.80)	1.01 (0.47–1.85)	0.25 ± 0.16	77.3 ± 49.5
Medium menarche ($n=287$)	5.93 (2.03–11.20)	4.56 (2.90–5.80)	0.93 (0.50–1.83)	0.25 ± 0.18	77.2 ± 52.3
Delayed menarche ($n=188$)	3.63 (0.17–12.35)	5.10 (2.27–5.80)	1.10 (0.68–2.63)	0.23 ± 0.18	81.3 ± 44.7
P value	Ns	Ns	Ns	Ns	Ns
Short bleeding periods ($n=44$)	3.41 (0.18–12.46)	3.78 (2.11–5.80)	0.96 (0.47–2.29)	0.21 ± 0.13	83.4 ± 43.6
Normal periods ($n=727$)	5.01 (1.80–11.20)	4.76 (2.53–5.80)	1.02 (0.51–1.85)	0.27 ± 0.19	73.4 ± 48.5
Long bleeding periods ($n=126$)	1.12 (0.17–10.50)	2.55 (2.15–4.38)	0.86 (0.49–1.92)	0.22 ± 0.13	78.5 ± 49.1
P value	Ns	**0.045**[α]	Ns	Ns	Ns
Hypomenorrhea ($n=204$)	4.55 (0.49–13.08)	3.84 (2.46–5.80)	1.06 (0.57–2.04)	0.27 ± 0.20	70.8 ± 44.9
Normal bleeding ($n=675$)	4.54 (0.83–10.39)	3.84 (2.46–5.61)	0.97 (0.47–1.75)	0.25 ± 0.17	74.6 ± 47.9
Menorrhagia ($n=18$)	3.41 (0.17–11.20)	2.11 (1.72–5.88)	0.91 (0.41–3.53)	0.19 ± 0.12	92.9 ± 54.2
P value	Ns	Ns	Ns	Ns	Ns
Oligomenorrhea ($n=40$)	4.78 (1.12–11.20)	3.76 (2.43–5.80)	0.79 (0.41–1.74)	0.25 ± 0.17	71.8 ± 46.8
Regular menses ($n=662$)	5.58 (1.06–13.31)	4.48 (2.08–5.90)	0.95 (0.51–1.75)	0.24 ± 0.17	80.8 ± 53.5
Polymenorrhea ($n=195$)	3.29 (0.17–9.58)	2.21 (2.11–3.78)	1.14 (0.58–3.33)	0.25 ± 0.11	68.6 ± 42.9
P value	Ns	Ns	Ns	Ns	Ns

Values expressed as mean ± SD or median and interquartile range

Significance of bold values are $P < 0.05$

hs-CRP, high-sensitivity C-reactive protein; HSP27, heat shock protein 27; PAB, prooxidant–antioxidant balance; Ns, non-significant

*Using ANOVA (normally distributed variables) or Kruskal–Wallis (non-normally distributed variables)

[α]Significant between groups 1 and 2 (post hoc Mann–Whitney)

Relationship between menstrual patterns with measures of systemic inflammation

Among inflammatory markers, NLR and PDW were significantly different between the 3 categories of age at which menarche occurred. Individuals with short bleeding periods had significantly higher mean NLR. According to the amount of flow, cases with menorrhagia had increased RDW compared to those with hypomenorrhea. There were substantial differences between the oligomenorrhea, regular menses and polymenorrhea groups with respect to NLR, RPR, MPV and PDW (Table 3).

Multinomial logistic regression analysis showed that the presence of both PMS and PD was associated with higher serum concentrations of serum hs-CRP, PAB and NLR, while Vit A values were negatively associated with the presence of PMS, and this remained significant even after adjusting for potential confounders (age and BMI) (Table 4).

Discussion

This is the first large study in adolescent girls, exploring the possible relationship between serum vitamins A and E status and inflammation status in subjects with PD and PMS. Our data suggest that serum Vit A, PAB and hs-CRP were significantly associated with the presence of PMS and PD. A longer duration of menstruation was associated with lower serum Vit E concentrations.

Retinoic acid (RA), the natural metabolite of Vit A, is involved in the conservation and modulation of differentiation in cycling endometrium. Throughout the menstrual cycle, the intracellular values of RA and serum Vit A concentrations fluctuate [23, 24]. Our study shows that subjects with PMS had a lower serum Vit A compared to normal subjects. Previously, the Centers for Disease Control and Prevention recommended 12,500–25,000 IU dose of Vit A for PMS women of childbearing age [25].

However, we did not find that serum Vit E was related to the presence of PMS or PD. This is consistent with other reports that have shown that serum Vit E in women with PMS is not significantly different than for non-PMS women [5]. Vit E is a potent antioxidant that is protective against OS. Vit E reduces the production of arachidonic acid and the conversion of arachidonic acid to prostaglandin through its effects on the enzymes phospholipase A2 (PA2) and cyclooxygenase (COX). However, we did not find lower serum concentrations of Vit E in patients with PMS, and therefore cannot confirm the reported effects of Vit E supplementation on PMS patients in previous studies [26, 27], although the benefits may be a response to pharmacological doses of Vit E. It is also feasible that peripheral blood concentrations of Vit E do not reflect those in the central nervous system (CNS). It is possible that Vit E affects the

Table 3 Relationship between menstrual pattern and associated symptoms to hematological measures of systemic inflammation

	NLR**	PLR**	RPR**	MPV**	RDW**	PDW**
Early menarche ($n=422$)	1.86 ± 0.83	7.73 ± 3.06	0.055 ± 0.227	10.39 ± 2.89	12.93 ± 1.23	12.79 ± 1.98
Medium menarche ($n=287$)	1.60 ± 0.80	7.75 ± 3.33	0.053 ± 0.018	10.39 ± 2.76	12.81 ± 1.26	12.71 ± 1.83
Delayed menarche ($n=188$)	1.68 ± 0.68	6.83 ± 1.86	0.058 ± 0.014	10.44 ± 0.83	13.73 ± 2.11	13.14 ± 1.53
P value	**0.043**$^{\alpha}$	Ns	Ns	Ns	**0.041**$^{\beta}$	Ns
Short bleeding periods ($n=44$)	1.98 ± 1.39	8.36 ± 4.06	0.050 ± 0.014	10.23 ± 0.84	12.71 ± 1.41	13.03 ± 1.95
Normal periods ($n=727$)	1.64 ± 0.76	7.58 ± 3.17	0.053 ± 0.018	10.31 ± 2.21	12.92 ± 1.19	12.82 ± 1.96
Long bleeding periods ($n=126$)	1.77 ± 0.80	7.72 ± 2.92	0.055 ± 0.024	10.77 ± 4.58	12.91 ± 1.10	12.85 ± 1.99
P value	**0.050**$^{\alpha}$	Ns	Ns	Ns	Ns	Ns
Hypomenorrhea ($n=204$)	1.65 ± 0.74	7.27 ± 2.95	0.056 ± 0.022	10.38 ± 2.18	12.86 ± 0.90	12.92 ± 2.08
Normal bleeding ($n=675$)	1.71 ± 0.85	7.85 ± 3.38	0.053 ± 0.020	10.38 ± 2.78	12.93 ± 1.30	12.81 ± 2.016
Menorrhagia ($n=18$)	1.55 ± 0.70	7.37 ± 2.47	0.056 ± 0.028	9.75 ± 0.71	13.75 ± 2.59	12.22 ± 1.44
P value	Ns	Ns	Ns	Ns	**0.050**$^{\gamma}$	Ns
Oligomenorrhea ($n=40$)	1.75 ± 0.93	7.59 ± 2.87	0.042 ± 0.008	10.71 ± 4.15	12.59 ± 0.64	12.50 ± 1.62
Regular menses ($n=662$)	1.60 ± 0.81	7.79 ± 3.20	0.052 ± 0.019	10.27 ± 1.90	12.92 ± 1.18	12.76 ± 2.00
Polymenorrhea (n = 195)	1.95 ± 0.89	7.32 ± 3.58	0.058 ± 0.023	10.02 ± 0.78	12.98 ± 1.29	13.17 ± 2.27
P value	**0.044**$^{\beta}$	Ns	**0.012**$^{\gamma}$	**0.030**$^{\gamma}$	Ns	0.029$^{\gamma}$

Values expressed as mean ± SD or median and interquartile range

RDW, red blood cell distribution width; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RPR, red blood cell distribution width-to-platelet ratio; MPV, mean platelet volume; PDW, platelet distribution width; Ns, non-significant

Significance of bold values are $P < 0.05$

$^{\alpha}$Significant between groups 1 and 2

$^{\beta}$Significant between groups 2 and 3

$^{\gamma}$Significant between groups 1 and 3 (post hoc Tukey)

**Using ANOVA test

activities of some neurotransmitters in the CNS and may fall within the luteal phase in women with PMS. Another interesting finding of our study was lower serum level of Vit E in cases with long bleeding periods. Ziaei et al. [28] have also reported that Vit E can decreases blood loss during menstruation.

We found a relationship between systemic inflammation and menstrual abnormalities, PMS and PD. Serum hs-CRP, an indicator of low-grade inflammation, may be related to PMS and PD. In the NHANES III study, Mexican–American girls were observed to have higher hs-CRP levels compared to white girls [29]. This finding may be related to the earlier menarche in Mexican–American girls [30]. Recently, Gold and coworkers, in their large, multiethnic study, evaluated the association between serum CRP levels with PMS severity in 2939 premenopausal and early perimenopausal women. The authors reported that a higher serum CRP was related to 26–41% increased risk of four of the five symptom categories of PMS [31]. In another study, it was reported that hs-CRP varied throughout the menstrual cycle and was closely associated with the reported psychological symptoms (e.g., mood and behavior), but less well associated with physical symptoms [11]. But, in mentioned study, women with PMS were omitted, and the relationship between inflammation and severity of menstrual symptoms and PMS was unclear [11]. Although in several studies, CRP levels which were measured in the late luteal phase were found to be increased, decreased, or not significantly varied from other phases [32].

It has been found that serum PAB values are increased in patients with CVD, acute coronary syndrome (ACS) and metabolic syndrome [20]. It has been proposed that PAB is a sensitive and specific index of OS [33, 34]. Our results demonstrated that PAB level was increased in individuals with PMS and dysmenorrhea subjects compared to normal individuals.

We found that NLR was significantly elevated in subjects with both PMS and PD versus to normal cases. In stressful situations, the physiological response of peripheral circulating leukocytes is an elevation in neutrophil count and a fall in lymphocyte counts. The NLR may be increased in several conditions and is a marker of severity of inflammation [35]. Recently, Kalem and co-researchers reported significant association between dysmenorrhea and NLR ($\beta = 0.385$; 95% CI: 0.084–0.687; $P = 0.013$) [36]. PLR has been found to be an independent risk factor for decreased survival in patients with malignancies [37]. RPR appears to be a reliable predictor of mortality in different diseases [38, 39]. RDW is

Table 4 Association of variables and four groups by multinomial logistic regression

	Univariate		Multivariate[a]	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Serum Vitamin A				
PMS	0.84 (0.76–0.93)	**0.001**	0.82 (0.74–0.92)	0.001
PD	0.97 (0.91–1.02)	Ns	0.96 (0.91–1.02)	Ns
PMS + PD	0.96 (0.90–1.02)	Ns	0.95 (0.89-1.01)	Ns
Serum hs-CRP				
PMS	Ns	0.97 (0.78–1.09)	0.97 (0.82–1.16)	Ns
Dysmenorrhea	Ns	0.92 (0.78–1.09)	0.92 (0.77–1.09)	Ns
PMS + PD	**0.048**	1.11 (1.02–1.22)	1.10 (1.01–1.22)	0.050
PAB				
PMS	1.005 (0.99-1.01)	Ns	1.005 (0.99-1.01)	Ns
Dysmenorrhea	1.001 (0.99–1.01)	Ns	1.001 (0.99–1.009)	Ns
PMS + Dysmenorrhea	1.01 (1.003–1.018)	**0.009**	1.01 (1.003–1.02)	0.008
NLR				
PMS	1.08 (0.70–1.66)	Ns	1.16 (0.74–1.81)	Ns
Dysmenorrhea	1.07 (0.77–1.50)	Ns	1.12 (0.783–1.60)	Ns
PMS + Dysmenorrhea	1.33 (1.09–1.63)	**0.035**	1.03 (1.01–1.05)	0.048

The reference category was normal group

Significance of bold values are $P < 0.05$

PD, primary dysmenorrhea; PMS, premenstrual syndrome; PAB, prooxidant–antioxidant balance; NLR, neutrophil-to-lymphocyte ratio; hs-CRP, high-sensitivity C-reactive protein; OR, odds ratio; CI, confidence interval; Ns, non-significant

[a]In present of age and BMI

an index of the heterogeneousness in size of RBCs and has been evaluated as a surrogate marker in many inflammatory conditions. MPV is a biomarker of function and activity of platelet [40]. Reduced MPV levels appear to indicate the activity and inflammatory burden of disease [41]. Low MPV levels are found in pelvic inflammatory disease [42], ovarian cancer [43], ulcerative colitis and rheumatoid arthritis [40]. Kucur et al. [44] have reported that MPV did not differ between adolescents with and without PD. We have found lower MPV levels in participants with polymenorrhea compared to oligomenorrhea, although PMS or PD was not related to MPV levels. Soydinc and colleagues found no significant differences for PLR and NLR in subjects with or without PD [10]. Platelets are involved in hemostasis and regulation of inflammation. The cause for the lower MPV in inflammatory conditions is still not completely understood. Inflammation is associated with a rise in acute-phase reactants and pro-inflammatory cytokines that may also affect megakaryopoiesis, causing the release of smaller platelets [45]. Furthermore, low MPV could occur due to the elevated consumption of larger platelets at the site of inflammation [46]. Larger platelets are more active and may participate more in the inflammatory process [47].

In our study, no statistically significant differences were detected with respect to demographic and anthropometric parameters in relation to the presence of PMS and PD. This is consistent with other studies [18, 48–51]. For instance, in recent study there were no significant differences in anthropometric indices between individuals with PMS and paired normal groups among Iranian nurses [52].

Our study is the first to examine the relationship of Vit A, Vit E and novel low-grade inflammatory markers with menstrual cycle length in a population-based sample of adolescents. This analysis is limited by the use of self-reported questionnaire and by the small numbers of girls with short bleeding time, menorrhagia and irregular cycles. Small numbers of cases with irregular cycles decreased the power to discover an association between the vitamins and irregular cycles. Cross-sectional nature of this work does not allow the inference of causality. Also, participants reporting their menstrual pattern and associated symptoms during the past year at the time onset study.

The present findings suggest that Vit A, Vit E and inflammatory processes may be associated with menstrual disorders, with respect to their symptoms and severity. Although the mechanisms that cause the association between the levels of inflammatory markers during menstrual cycle have not been fully investigated in this study, a high serum hs-CRP, PAB and NLR probably suggest a heightened state of inflammation in subjects with PMS and PD. A better understanding of how chronic inflammation contributes to PMS and PD could provide a great

justification for the prohibition and treatment of PMS. Prospective researches are necessary to define the potential impact of targeting the OS and inflammatory process for therapy and amelioration of menstrual symptoms.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Ethical approval was obtained from the Mashhad University of Medical Sciences and informed written consent was completed by all participants.

Consent for publication Not applicable.

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