

# Relationship between Dysmenorrhea, Dietary Inflammatory Index, and C-reactive Protein Level

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**Summary.** *Aim:* Primary dysmenorrhea is a common health problem that affects women's quality of life. This study aimed to determine the relationship between dysmenorrhea, dietary inflammatory index (DII), and C-reactive protein (CRP) serum levels. *Study Design:* This cross-sectional study was conducted between March and June 2019 with 106 university students, including 56 with primary dysmenorrhea and 50 without dysmenorrhea. The data were collected via a survey form including questions about the participants' socio-demographic characteristics and obstetric/gynecological histories, the Healthy Lifestyle Behaviors Scale II (HLBS-II), and the Visual Analog Scale (VAS). Anthropometric measurements of the participants were performed, their three-day food consumption record was received, their DII scores were calculated, and their CRP level was measured. The independent samples t-test and Mann-Whitney U test were used to compare the participants' DII scores and CRP levels. A linear regression analysis was performed to determine the relationship between dysmenorrhea, DII, and CRP level. A p-value of less than 0.05 was considered statistically significant. *Results:* The DII 3rd tertile mean score of the dysmenorrhea group ( $5.017 \pm 1.231$ ) was higher than that of the non-dysmenorrhea group ( $3.681 \pm 1.309$ ) ( $t=3.154$ ,  $p=0.003$ ). The median value of CRP was higher in the dysmenorrhea group [ $0.72$  ( $0.37-1.65$ )] than in the non-dysmenorrhea group [ $0.48$  ( $0.23-1.21$ )] ( $z=2.117$ ,  $p=0.034$ ). According to the linear regression analysis, dysmenorrhea significantly increased the CRP value by 0.471 units compared to the non-dysmenorrhea group ( $\beta=0.471$ ;  $p=0.038$ ). *Conclusion:* Participants with dysmenorrhea had higher serum CRP levels and DII scores.

**Key words:** Dysmenorrhea, Diet inflammatory index, C-reactive protein, Healthy lifestyle behaviors

## Introduction

Dysmenorrhea is pain during menstruation. Primary dysmenorrhea affects around three-quarters of all women during their reproductive life (1). There is no pelvic pathology in primary dysmenorrhea. Prostaglandins play an important role in the pathophysiology of dysmenorrhea. Prostaglandins are associated with inflammation, and Prostaglandin F<sub>2a</sub> (PGF<sub>2a</sub>) and Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) have roles in the inflammatory process. Increased secretion of prostaglandins (PGF<sub>2a</sub>, PGE<sub>2</sub>) in the endometrium during the luteal phase increases the frequency and severity of uterine contractions, causing vasoconstriction, ischemia, and pain.

Pain occurs just before or with the onset of menstruation and lasts for the first 1-2 days. It is usually felt as a cramp in the lower abdomen, waist, and thighs (1-5).

The prevalence of dysmenorrhea varies from 28% to 90% across the world (1, 3-9). Dysmenorrhea, which adversely affects the quality of life of women, can cause loss of productivity at work, absenteeism from school, and increased use of painkillers (1, 4, 5, 7), therefore it is also blamed for economic losses (5, 6).

The risk factors that affect the frequency and severity of primary dysmenorrhea are lifestyle (smoking, alcohol consumption, eating habits, and physical activity) (3, 9-13), family history, menstrual characteristics (age of menarche, duration of menstrual bleeding,

amount of the bleeding, and menstrual irregularity) (7,11-14), psychological factors (anxiety, depression, and stress) (12), obesity, age, education level, economic status, and quality of life (5, 6, 9, 10, 14).

It is emphasized that increasing physical activity, decreasing stress factors, developing health awareness, and healthy nutrition are important for coping with the severity of dysmenorrhea (1, 3, 10, 12, 15). Nutritional habits affect the inflammation balance in the body. While some foods increase inflammation (pro-inflammation), other foods decrease it (anti-inflammation). Red meat, high-fat dairy products, processed grains, and simple carbohydrates are pro-inflammatory foods. The Mediterranean diet with a high consumption of whole grains, fish, fruits, green leafy vegetables, and olive oil and diets with a high consumption of fruits and vegetables reduce inflammation (16, 17).

DII is used to evaluate the inflammatory effects of different dietary patterns. Studies have reported a positive linear relationship between diets with high pro-inflammatory potential and diseases such as obesity, diabetes, cardiovascular diseases, metabolic syndrome, cancer types, asthma, and depression (16-21).

CRP, an important inflammatory reaction indicator, is widely used to determine the level of inflammation. CRP is a clinically recognized acute phase protein. CRP concentration increases during the acute phase reaction, a defensive response to inflammation, infection, or injury (2). CRP concentration changes throughout the menstrual cycle, in which CRP decreases (follicular phase) as estrogen increases and CRP increases as progesterone increases (luteal phase) (22, 23).

There was no study in the literature evaluating dysmenorrhea, DII, and CRP together. This study aimed to determine the relationship between dysmenorrhea, DII, and CRP serum levels.

## Material and Methods

### *Design and Study Population*

This cross-sectional study was conducted between March and June 2019 with nursing students in the Health Sciences Faculty of Erciyes University. There were 1085 nursing students, including 839 females, in the 2018-2019 academic spring semester. Students with regular menstruation (every 22-35 days), in the first 5 days of

menstruation, and who never conceived were included in the study. Those who had pelvic pathology (myoma, pelvic tumors, endometriosis, and pelvic infection), a chronic disease, or used medication or antibiotics regularly were excluded from the study. Pelvic pathology was eliminated by ultrasound examination. Participants were divided into two groups according to the presence of primary dysmenorrhea. Students who had menstrual pain and whose daily living activities were affected (school absenteeism, physical activity, personal care, eating and drinking, etc.) during menstruation were included in the dysmenorrhea group, and those who did not have menstrual pain and whose daily life activities were not affected during menstruation were included in the non-dysmenorrhea group.

In this study, the independent variable was the presence of primary dysmenorrhea. Possible confounding factors were socio-demographic characteristics, menstrual characteristics, and anthropometric parameters. For the dependent variable of CRP, the sample size was calculated as 74, taking into account effect size ( $f^2$ )=0.15, type 1 error=0.05, statistical power=0.95, one independent variable, and four confounding factors. To reach the sample size, a poster introducing the study and containing the contact information of the researchers was hung on the notice board of the school between the dates of the study after the necessary permissions were obtained. An appointment was given to students who agreed to participate in the study and met the study inclusion criteria in order to meet during their menstrual phase. The study included 106 students, including 56 with primary dysmenorrhea and 50 without dysmenorrhea. During the statistical analysis of the data, a post power analysis was made for CRP, and the statistical power was calculated as 0.994, considering  $f^2$ =0.169 and type 1 error=0.05. The ethics committee approval was obtained from the Erciyes University Non-Interventional Clinical Research Ethics Committee (Protocol number: 2019/200).

### *Measurements*

The data were collected in the first five days of the menstrual cycle. The data were collected using the face-to-face interview method through a survey form including questions about socio-demographic characteristics, obstetric/gynecological histories, daily life activities, the HLBS-II, and the VAS. Afterward, anthropometric measurements of the students were

performed, and they were asked to record their three-day food consumption. They were trained to use a Food and Nutrition Photo Catalog in order to have them accurately record the types and portions of foods and beverages and were asked to fill out the three-day food consumption record form. Students were asked to keep their food and beverage consumption record for consecutive three days, two on weekdays and one on a weekend. A blood sample of 2 cc was drawn from the participants and their CRP levels were measured.

**Visual analog scale (VAS):** The VAS was used to determine the severity of dysmenorrhea. The scale ranged from 0 to 10, where 0 represented no pain and 10 the worst pain. In this study, the students' VAS mean score was  $6.33 \pm 2.04$  for the dysmenorrhea group and  $0.68 \pm 1.03$  for the non-dysmenorrhea group.

**Healthy lifestyle behaviors scale II (HLBS-II):** The Turkish validity and reliability of HLBS-II was performed by Bahar et al. (15), suggesting a high degree of reliability (Cronbach's Alpha=0.92). The scale measures an individual's healthy lifestyle behaviors. This four-point Likert type scale consists of 52 items and 6 subscales (Self-actualization, Health responsibility, Exercise, Nutrition, Interpersonal relationships, and Stress management). The total scale score indicates the score for healthy lifestyle behaviors. The lowest and highest scale scores are 52 and 208, respectively. A higher score indicates a healthier lifestyle behavior. All scale items are positive, scoring as "1=never", "2=sometimes", "3=often", and "4=regularly" (15).

**Anthropometric measurements:** Students' height, waist circumference, and hip circumference were measured using a tape measure, and their body composition (fat ratio and lean body mass), weight, and basal metabolic rates were measured using a device for the bioelectrical impedance analysis technique (Tanita SC-330, Tokyo, Japan) (24). The measurements were performed in the morning on an empty stomach, without heavy physical activity in the last 48 hours, after defecation and urination, and after removing metal objects, shoes, and excess clothes.

**Dietary Inflammatory Index (DII):** Dietary Inflammatory Index-2013 is used to determine diet-induced inflammation (18). The DII was developed by Cavicchia et al. (16) and revised by Shivappa et al. (18). This index is a literature-based index in which the effect of diet on inflammation is determined according to the

effect of 45 macro and micronutrients as well as the effect of commonly consumed dietary components such as tea and spices on the increase or decrease in serum levels of pro-inflammatory and anti-inflammatory cytokines.

From food consumption records, energy, nutrients, and average daily intakes of nutrients were determined using the Computer Assisted Nutrition Program, Nutritional Information Systems (BeBiS), which has been developed for Turkey (BeBiS, Version 7.2, 2011). Because the database of this software program contains 29 of the 45 nutrients in the calculation of DII, 29 nutrients were used in the DII calculation.

In this study, components that cause high DII and have pro-inflammatory effects are energy, protein, total fat, saturated fatty acids, cholesterol, carbohydrates, vitamin B<sub>12</sub>, and iron. Components that cause low DII and have anti-inflammatory effects are monounsaturated fatty acids, polyunsaturated fatty acids, omega-3 fatty acids, omega-6 fatty acids, fiber, caffeine, vitamin A,  $\beta$ -carotene, vitamin D, vitamin E, thiamine, riboflavin, niacin, vitamin B<sub>6</sub>, folic acid, vitamin C, magnesium, zinc, selenium, alcohol, and green/black tea.

The inflammatory index of each food, nutrient, and energy was calculated using the formula determined by Shivappa et al. (18). Then, each participant's DII score was determined by summing the inflammatory index scores of each nutrient and energy obtained. Subsequently, the DII scores of the groups were divided into tertiles. A higher DII score indicates a more pro-inflammatory diet, whereas a lower DII score indicates a more anti-inflammatory diet.

**C-reactive protein (CRP):** Serum CRP values were measured in Erciyes University Faculty of Medicine Central Laboratory. CRP was measured to examine the systemic inflammation of participants. Blood samples were taken from the vena cubiti, put into a blood collection tube, and vacuum sealed with gel. Serum blood samples were centrifuged at 4000 RPM for 10 minutes. CRP levels were measured using CRPL3 (C-Reactive Protein Gen.3, Cobas C Systems, Roche Diagnostics, made in Germany, Indianapolis, IN, USA). In normal healthy individuals, CRP is a trace protein with a range of up to 5 mg/L.

#### *Statistical analysis*

All statistical analyses were performed by using the IBM SPSS Statistics Standard Concurrent User V

25 (IBM Corp., Armonk, New York, USA). Descriptive statistics are given as unit number (n) and mean  $\pm$  standard deviation (mean  $\pm$  sd). The normal distribution of the data for numerical variables was evaluated using the Shapiro Wilk normality test and Q-Q graphics. The homogeneity of variances was evaluated with the Levene test. Two group comparisons were made using the independent samples t-test for variables with normal distribution and the Mann-Whitney U test for variables without normal distribution. Three group comparisons for numerical variables were evaluated using Kruskal-Wallis analysis and multiple comparisons were evaluated using the Dunn-Bonferroni test. Relationships between numerical variables were evaluated using Spearman correlation analysis. Comparisons between categorical variables were made using Pearson chi-square analysis. A correction for confounding variables was made with linear regression analysis to check the effect of the independent variable on dependent variables. In univariate analyzes, variables with a value of  $p < 0.05$  were included in the regression model. Categorical variables were included in the regression analysis as dummy variables. In the case of a high correlation between numerical variables ( $\rho = 0.500$ ;  $p < 0.001$ ), one of the variables was included in the regression model. Regarding the suitability of the established model for linear regression

analysis, the Shapiro Wilk normality test and Q-Q plot were used for normality of residuals and tolerance and variance inflation factor (VIF) statistics for collinearity. The necessary assumptions were provided for the three regression models established. A value of  $p < 0.05$  was considered statistically significant.

## Results

A total of 115 students were included in this study. During the study, three students did not want to give a blood sample, four students did not bring their food consumption records, and two students were diagnosed with endometriosis in the ultrasound examination, therefore they were excluded from the study. As a result, the data for 106 participants, including 56 with primary dysmenorrhea and 50 without dysmenorrhea, were analyzed in the study.

The group with primary dysmenorrhea had a significantly higher pain mean score ( $6.33 \pm 2.04$ ) was than the group without dysmenorrhea ( $0.68 \pm 1.03$ ) ( $p < 0.001$ ). There was no significant difference between the two groups in terms of age, living place, family structure, income status, smoking, menarche age, menstrual cycle length, menstrual duration, and anthropometric parameters ( $p > 0.05$ , Table 1).

**Table 1.** Demographic and menstrual characteristics and anthropometric parameters of women

Characteristic	Group				Test Statistic	
	Dysmenorrhea		Without dysmenorrhea		$t/\chi^2/z$	$p$ -value
	$n$	%	$n$	%		
<b>Age (Year)</b> <i>Mean<math>\pm</math>sd</i>	21.19 $\pm$ 1.80		20.80 $\pm$ 1.51		1.218	0.226
<b>Family</b>					0.120	0.781
Core	48	85.7	44	88.0		
Large	8	14.3	6	12.0		
<b>Income status</b>					2.570	0.314
Less than expenses	6	10.7	2	4.0		
Equivalent to expenses	44	78.6	39	78.0		
More than expenses	6	10.7	9	18.0		
<b>Living place</b>					2.530	0.312
With family	18	32.1	10	20.0		
In dormitory	31	55.4	35	70.0		
At home	7	12.5	5	10.0		
<b>Smoking</b>						1.000*
Yes	4	7.1	3	6.0		
No	52	92.9	47	94.0		

Characteristic	Group				Test Statistic	
	Dysmenorrhea		Without dysmenorrhea		$t/\chi^2/z$	$p$ -value
	$n$	%	$n$	%		
Menarche age (year) <i>Mean±sd</i>	13.2± 1.2		13.1± 1.0		0.349	0.728
Menstrual cycle length (day) <i>M (Q<sub>1</sub>-Q<sub>3</sub>)</i>	28.0 (25.2-30.0)		28.0 (27.0-29.0)		0.010	0.992
Menstrual duration (day) <i>M (Q<sub>1</sub>-Q<sub>3</sub>)</i>	6.0 (5.0-7.0)		6.0 (5.0-7.0)		0.451	0.652
BMI ( $kg/m^2$ ) <i>Mean±sd</i>	22.79±3.67		22.58±2.70		0.318	0.751
Waist/hip ratio <i>Mean±sd</i>	0.76±0.56		0.76±0.67		0.514	0.608
Body fat ratio (%) <i>Mean±sd</i>	24.79± 5.56		24.77± 7.60		0.018	0.986
Free fat mass (kg) <i>Mean±sd</i>	41.74± 3.95		42.84± 5.71		1.157	0.250
VAS <i>mean ± sd</i>	6.33±2.04		0.68±1.03		-17.619	<b>&lt;0.001</b>

$t$ : Independent samples  $t$ -test;  $\chi^2$ : Chi-square test;  $z$ : Mann-Whitney  $U$  test, VAS: Visual Analogue Scale

\*Fisher's exact test

**Table 2.** C-reactive protein (CRP), Dietary Inflammatory Index (DII), and Healthy lifestyle behaviors of women

Characteristic	Group		Test Statistic	
	Dysmenorrhea	Without dysmenorrhea	$t$	$p$ -value
<b>Normal Variables</b>	<i>Mean±sd</i>	<i>Mean±sd</i>		
DII	1.279 ± 3.41	0.231 ± 3.43	1.573	0.119
DII 1st tertile	-2.746±1.893	-3.509±2.241	1.804	0.286
DII 2nd tertile	1.151±0.904	0.809±0.734	1.196	0.240
DII 3rd tertile	5.017±1.231	3.681±1.309	<b>3.154</b>	<b>0.003</b>
<b>Non-Normal Variables</b>	<i>M (Q<sub>1</sub>-Q<sub>3</sub>)</i>	<i>M (Q<sub>1</sub>-Q<sub>3</sub>)</i>	$z$	$p$ -value
CRP	0.72 (0.37-1.65)	0.48 (0.23-1.21)	<b>2.117</b>	<b>0.034</b>
HLBS Total	128.0 (119.3-136.0)	130.5 (117.3-140.3)	0.896	0.370
Self-actualization	26.0 (23.0-28.0)	28.0 (24.0-30.3)	<b>2.299</b>	<b>0.022</b>
Health responsibility	21.0 (18.0-23.0)	20.0 (16.0-22.3)	1.763	0.078
Exercise	16.0 (13.0-18.0)	15.5 (12.0-20.3)	0.022	0.982
Nutrition	21.0 (18.0-22.0)	20.5 (18.0-22.3)	0.614	0.539
Interpersonal relationships	25.5 (24.0-28.0)	25.5 (24.0-29.0)	0.029	0.977
Stress management	19.0 (17.0-21.0)	20.0 (18.0-22.0)	1.582	0.114

HLBS: Healthy lifestyle behaviors score;  $t$ : Independent samples  $t$ -test;  $z$ : Mann-Whitney  $U$  test

The dysmenorrhea group had a higher DII mean score (1.279 ± 3.41) than the non-dysmenorrhea group (0.231 ± 3.43), but the difference between them was not statistically significant ( $p > 0.05$ ). When their DII scores were divided into tertiles, the dysmenorrhea group had a significantly higher 3rd tertile DII

mean score (5.017 ± 1.231) than the non-dysmenorrhea group (3.681 ± 1.309) ( $p = 0.003$ , Table 2).

The dysmenorrhea group had a significantly higher CRP median value [0.72 (0.37-1.65)] than the non-dysmenorrhea group [0.48 (0.23-1.21)] ( $p = 0.034$ , Table 2). The healthy lifestyle behaviors total mean scores

of the groups with and without dysmenorrhea were similar ( $p = 0.370$ ). However, the self-actualization subscale of the healthy lifestyle behaviors showed a significant difference between the groups ( $p=0.022$ , Table 2).

Table 3 includes the adjusted values for DII and CRP, HLBS according to variables with  $p < 0.05$  in the results. In case of a high correlation between the confounding numeric variables, one of them was included in the model. According to the linear regression analysis, dysmenorrhea significantly increased the CRP value by 0.471 units in the dysmenorrhea group compared to the non-dysmenorrhea group ( $\beta=0.471$ ;  $p=0.038$ ). Dysmenorrhea also increased the DII score by 0.476 units ( $\beta=0.476$ ;  $p=0.353$ ) and decreased the HLBS score by 1.955 units ( $\beta=-1.955$ ;  $p=0.519$ ) compared to the non-dysmenorrhea group. However, this effect was not statistically significant.

## Discussion

The study found that the dysmenorrhea group had significantly higher serum CRP levels than the

non-dysmenorrhea group. This significant difference remained after adjusting for the total dietary fat intake, body fat ratio, and lifestyle. Prostaglandins are over produced in dysmenorrhea and are associated with inflammation. Prostaglandins induce vasoconstriction and ischemia by causing contraction of uterine smooth muscles and lead to pain by lowering the pain threshold. In this study, the higher CRP, an indicator of an inflammatory reaction, in the dysmenorrhea group may be because dysmenorrhea is a health issue associated with inflammation.

One study of healthy women determined a positive correlation between CRP concentration and the severity of menstrual symptoms (mood, behavior, pain, and physical symptoms) (25). Another study on the relationship between premenstrual symptoms and CRP found a significant positive correlation between CRP levels with mood symptoms, abdominal cramps, increased appetite, bloating, and breast pain (26). A separate study about CRP concentration during the menstrual cycle reported that as estrogen level increased, CRP level decreased, whereas as progesterone level increased, CRP level increased. The study

**Table 3.** Linear Regression Analysis Results for DII, CRP, and HLBS

Outcome Variable	Predictor Variable	Regression Coefficients				Collinearity Statistic
		$\beta$	<i>se</i>	<i>t</i>	<i>p</i>	<i>VIF</i>
DII*	<b>Constant</b>	12.919	2.183	5.917	<0.001	1.082
	<b>Groups</b> Without dysmenorrhea ( <i>ref</i> ) Dysmenorrhea	1 0.476				
*Adjusted for beta carotene, energy, tea, body fat ratio, vitamin A, and total body water. Model Summary: $F=10.199$ ; $p<0.001$ ; $R^2=0.518$ ; $Adj R^2=0.467$						
CRP**	<b>Constant</b>	0.818	0.794	1.031	0.305	1.016
	<b>Groups</b> Without dysmenorrhea ( <i>ref</i> ) Dysmenorrhea	1 0.471			<b>0.038</b>	
**Adjusted for total fat, body fat ratio, and lifestyle. Model Summary: $F=4.545$ ; $p<0.001$ ; $R^2=0.185$ ; $Adj R^2=0.145$						
HLBS***	<b>Constant</b>	122.94	21.658	5.676	<0.001	1.117
	<b>Groups</b> Without dysmenorrhea ( <i>ref</i> ) Dysmenorrhea	1 -1.955			0.519	
***Adjusted for niacin, menstrual cycle length, lean muscle mass, MUFA, and total fat. Model Summary: $F=3.765$ ; $p=0.002$ ; $R^2=0.186$ ; $Adj R^2=0.136$						

DII: Dietary Inflammatory Index, CRP: C-reactive protein, HLBS: Healthy Lifestyle Behaviors Score, MUFA: Monounsaturated Fatty Acid

also emphasized that the positive relationship between progesterone and CRP was weaker and the negative relationship between estrogen and CRP was stronger (23).

Chronic inflammation is associated with various chronic diseases such as metabolic syndrome, diabetes, cancer, cardiovascular disease, and asthma. Nutritional habits also play a role in the development of these diseases both directly and indirectly by affecting inflammation pathways. For example, excessive consumption of saturated fat both causes these diseases by increasing energy intake and increases the risk of cardiovascular disease by affecting inflammation and LDL cholesterol (16–21, 27).

DII, which was developed to determine the inflammatory potential of diets in humans, has linear relationships with various inflammatory markers, including CRP, IL-6, and TNF- $\alpha$  in different population and various diseases (21). The National Health and Nutrition Survey of Japan (NHNS-2010) revealed a positive correlation between DII scores and hs-CRP levels (28). Similarly, the present study found a positive correlation between DII score and CRP level. A review study of the nutritional factors affecting dysmenorrhea reports that as the consumption of fruits, vegetables, fish, milk, and dairy products increases, menstrual pain decreases (29).

In the present study, the DII was higher in the dysmenorrhea group. Especially the 3rd tertile DII created a significant difference between the groups. These results suggest that a diet with high pro-inflammatory potential may play a role in dysmenorrhea. In line with these results, those with dysmenorrhea might have consumed more foods with high pro-inflammatory potential in their diets, and this diet may have increased inflammation.

There is no study about the DII of those with dysmenorrhea. However, studies on the relationship between dysmenorrhea symptoms with different food and nutrients have reported that those who consume high fiber foods in their diet have significantly lower menstrual pain scores (30), young women who are fed snacks have a higher risk of dysmenorrhea than those who eat 'lacto-vegetarian' and 'mixed food items' (31), the severity of menstrual pain decreases in women who take supplements such as omega-3 fatty acids (32) or omega-3 and vitamin E (8), and the frequency of

dysmenorrhea decreases in women who take vitamin D supplements (33).

As a strength of this study, there are no studies on the relationship between dysmenorrhea, CRP, and the inflammatory index of the diet together. To our knowledge, this is the first study to determine the relationship between dysmenorrhea, DII, and serum CRP level.

As the most important limitation of this study, only university students were included in the study. Serum CRP levels and DII scores may vary in people who visit the hospital for dysmenorrhea. Therefore, this study can be repeated with people who visit the hospital for dysmenorrhea.

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