Systemic Thiol-Disulfide Homeostasis and Contemporaneous Menstrual Pain Intensity in Primary Dysmenorrhea: A Prospective Observational Study

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ABSTRACT

OBJECTIVE: Primary dysmenorrhea (PD) is associated with prostaglandin-mediated uterine hypercontractility, transient ischemia, inflammation, and oxidative stress. Whether systemic redox biomarkers reflect menstrual pain intensity at a specific clinical time point remains uncertain. This study evaluated the associations of thiol-disulfide homeostasis parameters and ischemia-modified albumin (IMA) with contemporaneous menstrual pain intensity among women with PD.

STUDY DESIGN: This cross-sectional study included 98 women with PD. Pain intensity was assessed immediately before venous blood sampling during menstrual days 1-4 using a 10-cm Visual Analog Scale (VAS). The primary analysis examined continuous contemporaneous VAS scores. A secondary exploratory analysis classified pain as mild (>0 to 3), moderate (>3 to 6), severe (>6 to 8), or very severe (>8 to 10). Native thiol, total thiol, disulfide, thiol-disulfide ratios, and IMA were evaluated.

RESULTS: Spearman correlation coefficients between contemporaneous VAS score and the measured biomarkers ranged from -0.10 to 0.10. All bootstrap 95% confidence intervals included zero, with outermost limits of approximately -0.29 and 0.29. Menstrual sampling-day-adjusted analyses and the sensitivity analysis using the retrospective worst-pain score yielded concordant findings. In the secondary categorical analysis, no significant differences were observed for native thiol (p=0.9913), total thiol (p=0.9805), disulfide (p=0.3098), the thiol-disulfide ratios (p=0.4704-0.5095), or ischemia-modified albumin (p=0.8986). All Holm-adjusted p values were 1.000.

CONCLUSION: Systemic thiol-disulfide homeostasis parameters and unadjusted IMA were not associated with contemporaneous menstrual pain intensity in this cohort. A single systemic measurement may not adequately represent the localized, dynamic, and centrally modulated mechanisms underlying menstrual pain. Future studies are needed to improve diagnostic and therapeutic strategies for PD.

Keywords: Ischemia-modified albumin; Menstrual pain; Oxidative stress; Primary dysmenorrhea; Thiol-disulfide homeostasis; Visual analog scale

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Introduction

Primary dysmenorrhea (PD) is characterized by recurrent menstrual pain in the absence of an identifiable pelvic disorder and is among the most prevalent gynecological conditions in reproductive-age women. Beyond pain itself, the condition is associated with impaired daily functioning, school or work absenteeism, sleep disturbance, and reduced quality of life (1,2).

The pathophysiology of PD is multifactorial. Excess endometrial prostaglandin production promotes uterine hyper-

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contractility, reduced uterine perfusion, transient ischemia, and nociceptor activation. Inflammatory and oxidative pathways may further amplify this process (1-3). Systematic evidence suggests that women with PD may exhibit increased oxidative burden or reduced antioxidant capacity compared with pain-free controls. However, the direction and magnitude of individual biomarkers vary across studies (3). Antioxidant interventions have also been investigated as potential adjunctive approaches for dysmenorrhea-related pain (4).

Thiol groups constitute an important component of the circulating antioxidant system. Under oxidative conditions, thiols are converted to disulfide bonds, while reduction of disulfides restores thiol groups and maintains dynamic redox homeostasis. The automated method developed by Erel and Neselioglu permits simultaneous assessment of native thiol, total thiol, disulfide, and derived thiol-disulfide ratios (5). Prior case-control studies have reported altered thiol-disulfide homeostasis and increased ischemia-modified albumin (IMA) in women with primary dysmenorrhea (6). However, whether these circulating biomarkers track the intensity of pain among women who already have primary dysmenorrhea remains unclear.

This study therefore investigated whether systemic thiol-disulfide homeostasis parameters and IMA were associated with contemporaneous menstrual pain intensity among women with established PD. We hypothesized that increasing pain intensity would be accompanied by a shift toward a more oxidized circulating profile.

Material and Method

Study design and participants: This cross-sectional study was conducted in the Department of Obstetrics and Gynecology at Ankara Bilkent City Hospital, Ankara, Türkiye, between March and December 2024. The Ankara Bilkent City Hospital No. 2 Clinical Research Ethics Committee reviewed and approved the study protocol (approval number: E2-23-3962; approval date: 6 March 2024). We obtained written informed consent from all participants before enrollment. All procedures were conducted in accordance with the Declaration of Helsinki and its subsequent amendments. We reported the study in accordance with the STROBE recommendations for observational studies (Supplementary Appendix S1).

Women aged 18-45 years with regular menstrual cycles and a clinical diagnosis of PD without any identifiable pelvic pathology were eligible. All participants underwent gynecological examination and transvaginal ultrasonography to exclude secondary pelvic pathology (such as endometriosis, adenomyosis, pelvic inflammatory disease, or fibroids). Women with diabetes mellitus, hypertension, autoimmune or other chronic systemic disease, previous abdominal surgery, active smoking, hormonal contraceptive use, daily antioxidant supplementation, or use of analgesic medication, including nonsteroidal anti-inflammatory drugs, during the preceding 48 hours were excluded.

Demographic and menstrual data included age, body mass index (BMI), parity, age at menarche, menstrual cycle length, duration of menstrual bleeding, duration of dysmenorrhea symptoms, and menstrual sampling day. Figure 1 summarizes the participant selection process.

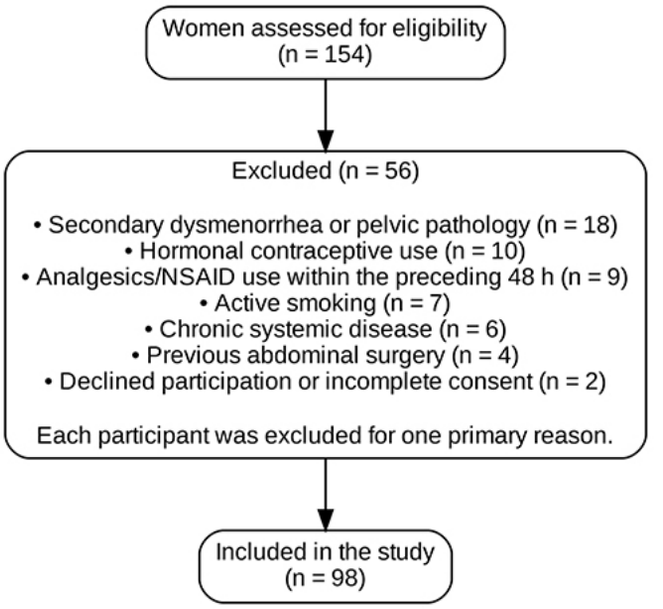

Figure 1: *Flow diagram of participant selection.*

Pain assessment: Pain intensity was assessed immediately before venous blood sampling using a 10-cm Visual Analog Scale (VAS), with 0 representing no pain and 10 representing the worst imaginable pain (7). The contemporaneous VAS score represented menstrual pain at the time of the study visit. We recorded the exact menstrual day of sampling for each participant. We also recorded a retrospective rating of the worst pain experienced during the index menstrual cycle using the same VAS.

The continuous contemporaneous VAS score constituted the primary exposure. For the secondary exploratory analysis, scores were divided into prespecified study-defined categories: mild (>0 to 3), moderate (>3 to 6), severe (>6 to 8), and very severe (>8 to 10). These operational thresholds were not intended to represent an externally validated clinical grading system. No participant had a contemporaneous VAS score of zero.

Blood sampling and biochemical analysis: Fasting venous blood samples were obtained during menstrual days 1-4 after an overnight fast of at least 8 hours. Samples were collected into serum separator tubes, allowed to clot for 30 minutes at room temperature, and centrifuged at 2,000 × g for 10 minutes at 4˚C. Serum was divided into aliquots to avoid repeated freeze-thaw cycles and stored at -80˚C until analysis.

Measurements were completed within three months of collection, and no sample underwent more than one freeze-thaw cycle before analysis.

We measured native thiol and total thiol concentrations using the automated spectrophotometric method described by Erel and Neselioglu on a Cobas c501 chemistry analyzer (Roche Diagnostics, Mannheim, Germany) (5). Disulfide concentration was calculated for each participant as one-half of the difference between total thiol and native thiol. Disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios were calculated at the participant level. IMA was measured using the albumin-cobalt binding assay on the same analytical platform and reported as an unadjusted absorbance value because serum albumin was not measured concurrently.

All measurements were performed in duplicate by laboratory personnel blinded to pain-intensity category. Internal quality-control materials at two concentration levels were analyzed before each analytical run. Intra-assay coefficients of variation were 2.8% for native thiol, 3.1% for total thiol, and 3.6% for IMA; inter-assay coefficients of variation were 3.9%, 4.2%, and 4.8%, respectively.

Statistical analysis

Statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean±standard deviation or median with interquartile range, as appropriate. Categorical variables were summarized as numbers and percentages.

All descriptive statistics were calculated directly from participant-level observations. No mean, standard deviation, or other measure of dispersion was reconstructed, imputed, estimated as a fixed proportion of the mean, or derived from another group-level summary statistic. Native thiol and total thiol were determined separately for each participant. In contrast, disulfide concentration and the corresponding thiol-disulfide ratios were calculated at the participant level before group-level descriptive statistics were obtained.

The primary analysis evaluated pain intensity as a continuous contemporaneous VAS score. Associations between VAS score and native thiol, total thiol, disulfide, the three thiol-disulfide ratios, and IMA were assessed using Spearman rank correlation coefficients. We estimated 95% confidence intervals using 2,000 bootstrap samples.

For the secondary four-category analysis, we evaluated the distribution of one-way analysis of variance (ANOVA) residuals using the Shapiro-Wilk test and graphical inspection of quantile-quantile plots. Homogeneity of variances was assessed using Levene's test. Because we found no material departure from normality or homogeneity of variances, we compared all seven biochemical parameters across pain-intensity categories using one-way ANOVA and present them as mean ± standard deviation. Because subgroup sizes were small and markedly unequal, particularly in the five-participant very severe category, we considered the categorical analyses secondary and exploratory.

We compared age, BMI, age at menarche, menstrual cycle length, and duration of menstrual bleeding across pain categories using one-way ANOVA. We analyzed parity and duration of dysmenorrhea using the Kruskal-Wallis test. Menstrual sampling-day distributions were compared using the Fisher-Freeman-Halton exact test. We evaluated the association between menstrual sampling day and contemporaneous VAS score using Spearman rank correlation. We also evaluated biomarker-VAS associations using partial Spearman correlations calculated from rank-transformed variables while controlling for menstrual sampling day. We assessed associations between the biochemical parameters and retrospective worst-pain VAS scores as a sensitivity analysis using Spearman rank correlation with bootstrap 95% confidence intervals.

We controlled family-wise error using the sequential Holm procedure, applied separately to the seven primary biomarker-VAS correlations and the seven categorical biomarker comparisons. We reported both nominal and Holm-adjusted p values. With n=98, a two-sided alpha level of 0.05, and 80% power, the approximate minimum detectable correlation was $|r|=0.28$. A four-group sensitivity calculation yielded an approximate minimum detectable Cohen's f of 0.34, although this estimate does not fully account for the marked subgroup imbalance. All tests were two-sided, and $p<0.05$ was considered statistically significant.

Results

We assessed 154 women for eligibility. We excluded 56, leaving 98 participants in the final analytical cohort (Figure 1). All 98 participants had complete contemporaneous VAS, sampling-day, and biochemical data. The mean age was 31.34±7.41 years, the mean body mass index was 24.47±4.50 kg/m², and the median parity was 1 (interquartile range [IQR], 0-3).

Contemporaneous pain intensity was categorized as mild in 37 participants (37.7%), moderate in 32 (32.6%), severe in 24 (24.5%), and very severe in five (5.1%). Baseline demographic and menstrual characteristics are presented in Table I. No statistically significant between-group differences were identified in age, body mass index, parity, age at menarche, menstrual cycle length, duration of menstrual bleeding, duration of dysmenorrhea, or menstrual sampling-day distribution (all $p>0.05$).

In the primary continuous analysis, none of the measured systemic redox parameters showed a statistically significant association with contemporaneous VAS score. Spearman cor-

relation coefficients ranged from -0.10 to 0.10, and all bootstrap 95% confidence intervals included zero. Accordingly, although weak associations cannot be excluded, the estimates provided no evidence of a moderate or strong monotonic relationship between the measured circulating biomarkers and contemporaneous menstrual pain intensity (Table II).

The secondary four-category analysis yielded findings consistent with the primary analysis (Table III). Native thiol concentrations were 393.67±59.05, 395.82±59.37, 394.70±59.21, and 402.42±60.36 μmol/L in the mild, moderate, severe, and very severe groups, respectively (p=0.9913). Total thiol concentrations were also comparable across groups (p=0.9805). Likewise, no significant between-group differences were observed in disulfide concentration (p=0.3098), the disulfide/native thiol ratio (p=0.4704), the disulfide/total thiol ratio (p=0.5095), the native thiol/total thiol ratio (p=0.5095), or ischemia-modified albumin (IMA), with mean

Table I: *Baseline demographic and menstrual characteristics according to contemporaneous pain-intensity category*

Variable	Mild (n=37)	Moderate (n=32)	Severe (n=24)	Very severe (n=5)	p
Age, years	30.80 ± 7.13	31.10 ± 7.73	32.00 ± 7.63	33.70 ± 8.03	0.824
Body mass index, kg/m²	24.10 ± 4.35	24.60 ± 4.75	24.80 ± 4.65	24.80 ± 4.45	0.936
Parity	0 (0-2)	1 (0-2)	1 (0-3)	1 (0-3)	0.534
Age at menarche, years	12.6 ± 1.2	12.7 ± 1.3	12.5 ± 1.1	12.8 ± 1.4	0.921
Menstrual cycle length, days	28.5 ± 2.4	28.3 ± 2.7	28.8 ± 2.5	27.9 ± 2.6	0.847
Duration of menstrual bleeding, days	5.2 ± 1.2	5.3 ± 1.1	5.1 ± 1.3	5.4 ± 1.1	0.916
Duration of dysmenorrhea, years	5.0 (3.0-8.0)	6.0 (3.0-9.0)	6.0 (4.0-9.0)	7.0 (4.0-10.0)	0.274
Menstrual sampling day, n (%)					1.000
Day 1	14 (37.8)	13 (40.6)	9 (37.5)	2 (40.0)	
Day 2	12 (32.4)	10 (31.3)	7 (29.2)	2 (40.0)	
Day 3	7 (18.9)	6 (18.8)	5 (20.8)	1 (20.0)	
Day 4	4 (10.8)	3 (9.4)	3 (12.5)	0 (0.0)	

Values are presented as mean ± standard deviation, median (interquartile range), or n (%), as appropriate. Age, body mass index, age at menarche, menstrual cycle length, and duration of menstrual bleeding were compared using one-way analysis of variance. Parity and duration of dysmenorrhea were compared using the Kruskal-Wallis test. Menstrual sampling-day distributions were compared using the Fisher-Freeman-Halton exact test.

Table II: *Correlations between continuous contemporaneous VAS score and systemic oxidative stress parameters*

Biomarker	Spearman Rho	95% CI	Nominal p	Holm-adjusted p
Native thiol	0.03	-0.17 to 0.23	0.772	1.000
Total thiol	0.02	-0.18 to 0.22	0.842	1.000
Disulfide	-0.08	-0.27 to 0.12	0.451	1.000
Disulfide/native thiol	-0.10	-0.29 to 0.10	0.329	1.000
Disulfide/total thiol	-0.10	-0.29 to 0.10	0.329	1.000
Native thiol/total thiol	0.10	-0.10 to 0.29	0.329	1.000
IMA	-0.06	-0.25 to 0.14	0.565	1.000

VAS: Visual analog scale, CI: Confidence interval, IMA: Ischemia-modified albumin. Confidence intervals were estimated using 2,000 bootstrap samples. Holm correction was applied across the seven biomarker-VAS correlations. Disulfide/native thiol and disulfide/total thiol are strictly monotonic transformations of one another and therefore have identical Spearman results; native thiol/total thiol is inversely related and has a coefficient of equal magnitude and opposite sign.

Table III: *Systemic oxidative stress parameters according to contemporaneous pain-intensity category*

Variable	Mild (n=37)	Moderate (n=32)	Severe (n=24)	Very severe (n=5)	Nominal p	Holm-adjusted p
Native thiol, μmol/L	393.67 ± 59.05	395.82 ± 59.37	394.70 ± 59.21	402.42 ± 60.36	0.9913	1.000
Total thiol, μmol/L	435.14 ± 65.27	436.66 ± 65.50	434.35 ± 65.15	447.56 ± 67.13	0.9805	1.000
Disulfide, μmol/L	20.72 ± 3.11	20.42 ± 3.06	19.82 ± 2.97	22.56 ± 3.38	0.3098	1.000
Disulfide/native thiol, %	5.33 ± 0.92	5.21 ± 0.88	5.03 ± 0.84	5.60 ± 1.01	0.4704	1.000
Disulfide/total thiol, %	4.79 ± 0.75	4.70 ± 0.73	4.56 ± 0.70	5.02 ± 0.81	0.5095	1.000
Native thiol/total thiol, %	90.42 ± 1.50	90.60 ± 1.46	90.88 ± 1.40	89.96 ± 1.62	0.5095	1.000
IMA, ABSU	0.79 ± 0.12	0.79 ± 0.12	0.78 ± 0.12	0.75 ± 0.11	0.8986	1.000

Values are presented as mean ± standard deviation. Between-group comparisons were performed using one-way analysis of variance. Distributional assumptions were evaluated using the Shapiro-Wilk test applied to model residuals and graphical inspection of quantile-quantile plots; homogeneity of variances was assessed using Levene's test. Holm correction was applied across the seven biomarker comparisons. IMA:Ischemia-modified albumin. ABSU: Absorbance units. Disulfide/native thiol and disulfide/total thiol are strictly monotonic transformations of one another, and native thiol/total thiol is inversely related; the corresponding rank-based results are therefore mathematically dependent.

values of 0.79±0.12, 0.79±0.12, 0.78±0.12, and 0.75±0.11 absorbance units across the four groups (p=0.8986). Following Holm correction for multiple comparisons, all adjusted p-values were 1.000.

Menstrual sampling day was not significantly associated with contemporaneous VAS score (Spearman ρ=-0.07, p=0.493). Adjustment for sampling day did not materially alter the biomarker -VAS associations: absolute adjusted correlation coefficients remained ≤0.10, none reached statistical significance, and all Holm-adjusted p-values were 1.000 (Supplementary Table S2).

Sensitivity analyses using retrospective worst-pain VAS scores yielded findings consistent with the primary analysis. Correlation coefficients ranged from -0.12 to 0.12, all bootstrap 95% confidence intervals included zero, and all Holm-adjusted p-values were 1.000 (Supplementary Table S3).

Supplementary Table S1: *Distributional assessment and test selection for Table 3 biomarkers*

Variable	Shapiro-Wilk W	Shapiro-Wilk p	Levene p	Selected test
Native thiol	0.987	0.438	0.731	One-way ANOVA
Total thiol	0.985	0.332	0.694	One-way ANOVA
Disulfide	0.979	0.115	0.642	One-way ANOVA
Disulfide/native thiol ratio	0.982	0.188	0.601	One-way ANOVA
Disulfide/total thiol ratio	0.981	0.163	0.588	One-way ANOVA
Native thiol/total thiol ratio	0.983	0.214	0.588	One-way ANOVA
IMA	0.976	0.072	0.557	One-way ANOVA

Shapiro-Wilk testing was applied to one-way ANOVA residuals and interpreted together with quantile-quantile plots. Levene's test assessed homogeneity of variances across the four pain-intensity categories. IMA: Ischemia-modified albumin.

Supplementary Table S2: *Partial Spearman correlations between contemporaneous VAS score and systemic oxidative stress parameters, controlling for menstrual sampling day*

Biomarker	Adjusted Spearman Rho	Nominal p	Holm-adjusted p
Native thiol	0.03	0.771	1.000
Total thiol	0.02	0.846	1.000
Disulfide	-0.08	0.436	1.000
Disulfide/native thiol	-0.10	0.330	1.000
Disulfide/total thiol	-0.10	0.330	1.000
Native thiol/total thiol	0.10	0.330	1.000
IMA	-0.05	0.627	1.000

Partial Spearman correlations were calculated by applying partial correlation to rank-transformed variables while controlling for menstrual sampling day. Holm correction was applied across the seven adjusted associations. VAS: Visual analog scale. IMA: Ischemia-modified albumin.

Supplementary Table S3: *Correlations between retrospective worst pain during the index cycle and systemic oxidative stress parameters*

Biomarker	Spearman Rho	95% CI	Nominal p	Holm-adjusted p
Native thiol	0.04	-0.16 to 0.24	0.696	1.000
Total thiol	0.03	-0.17 to 0.23	0.770	1.000
Disulfide	-0.09	-0.28 to 0.11	0.378	1.000
Disulfide/native thiol	-0.12	-0.31 to 0.08	0.239	1.000
Disulfide/total thiol	-0.12	-0.31 to 0.08	0.239	1.000
Native thiol/total thiol	0.12	-0.08 to 0.31	0.239	1.000
IMA	-0.07	-0.26 to 0.13	0.493	1.000

Confidence intervals were estimated using 2,000 bootstrap samples. Holm correction was applied across the seven worst-pain correlations. VAS, Visual Analog Scale; IMA, ischemia-modified albumin. The two disulfide ratios are strictly monotonic transformations and therefore have identical Spearman results; native thiol/total thiol is inversely related.

Supplementary Appendix S1. STROBE adherence summary

STROBE item	Reporting domain	Location/implementation in the revised manuscript
1	Title and abstract	Design identified; structured abstract reports methods and principal estimates.
2-3	Background and objectives	Scientific rationale and prespecified objective stated in Introduction.
4	Study design	Prospective observational design stated early in Methods.
5	Setting	Clinical department, study period, and sampling window reported.

6	Participants	Eligibility, exclusions, consent, and flow diagram reported.
7	Variables	Pain exposures, biomarkers, covariates, and sensitivity outcomes defined.
8	Data sources and measurement	VAS, ultrasonography, sample processing, and assays described.
9	Bias	Analgesic-related selection and timing limitations discussed.
10	Study size	Final sample and detectable effect estimates reported.
11	Quantitative variables	Continuous VAS primary; study-defined categories secondary.
12	Statistical methods	Primary, secondary, adjusted, sensitivity, bootstrap, and multiplicity procedures reported.
13	Participants	Screened, excluded, and analyzed numbers shown in Figure 1.
14	Descriptive data	Group-stratified demographic and menstrual characteristics reported in Table 1.
15	Outcome data	All biomarker measurements and missing-data status reported.
16	Main results	Effect estimates, confidence intervals, nominal and adjusted p values reported.
17	Other analyses	Sampling-day-adjusted and worst-pain sensitivity analyses reported.
18	Key results	Principal findings summarized relative to study objective.
19	Limitations	Selection, measurement, residual confounding, subgroup imbalance, and generalizability discussed.
20	Interpretation	Null findings interpreted using magnitude and precision without equivalence claims.
21	Generalisability	Single-center scope and external validity addressed.
22	Funding and conflicts	Competing interests and other declarations reported.

This table summarizes manuscript adherence to the STROBE reporting framework. The official journal checklist may be completed separately using final page and line numbers after typesetting.

Discussion

This prospective study examined whether circulating thiol-disulfide homeostasis parameters and IMA tracked contemporaneous menstrual pain intensity among women with PD. Neither the primary continuous analysis nor the secondary categorical comparison demonstrated a detectable association. The observed correlations were small, and their confidence intervals did not support moderate or strong monotonic relationships. Menstrual sampling-day adjustment and the sensitivity analysis based on retrospective worst pain produced concordant findings.

These results address a different question from most previous oxidative-stress studies in PD. Earlier investigations largely used case-control designs to determine whether women with primary dysmenorrhea differ from pain-free controls (3,6). Those studies support the possibility of altered oxidative balance at the disorder level. Still, they do not establish that between-person variation in a circulating biomarker should parallel pain intensity at a specific time point. The present findings therefore should not be interpreted as evidence against oxidative involvement in PD itself. Rather, they suggest that the measured systemic markers may have limited capacity to reflect the momentary severity of a complex clinical symptom within an affected population.

Several biological and methodological mechanisms may explain this lack of association. Menstrual pain is initiated partly by prostaglandin-mediated uterine hypercontractility and ischemia. Still, perceived pain intensity is also shaped by peripheral sensitization, central pain processing, prior recurrent nociceptive exposure, and psychological and behavioral factors (1,8,9). Women with dysmenorrhea may demonstrate increased experimental pain sensitivity both within and outside referred pelvic pain areas (8,9). Moreover, symptom-based research indicates that dysmenorrhea comprises heterogeneous phenotypes, including localized pain and multisystem symptom patterns (10). Similar VAS scores may therefore arise from different combinations of local uterine, systemic inflammatory, central, and psychosocial mechanisms.

The timing and anatomical compartment of biomarker measurement are also relevant. A single serum sample collected on any of the menstrual days 1-4 may combine biologically distinct phases of the painful episode and may not capture transient changes in uterine oxidative activity. Systemic thiol-disulfide homeostasis may not directly reflect localized redox processes within the uterus. Although sampling day was recorded and adjustment did not alter the findings, repeated within-participant measurements before menstruation, at pain onset, at peak pain, and after symptom resolution may provide a more informative assessment of temporal coupling between redox status and pain (11).

The null findings can nevertheless be interpreted quantitatively. With 98 participants, the study had approximately 80% power to detect a correlation of about $|r|=0.28$, and the bootstrap confidence intervals extended no further than approximately ± 0.29 in the primary analysis. Thus, weak associations

remain possible, but the observed data are not compatible with a large or clinically dominant relationship between these circulating biomarkers and contemporaneous pain intensity under the conditions studied.

The IMA result requires specific caution. Serum albumin was not measured concurrently, and an albumin-adjusted IMA index could not be calculated. Because the albumin-cobalt binding assay can be influenced by circulating albumin concentration, unadjusted IMA values may contain variation unrelated to ischemia or oxidative stress (12). Accordingly, the absence of an IMA-pain association should not be considered definitive.

Strengths and Limitations: This study has several strengths. Its prospective design enabled assessment of menstrual pain intensity immediately before venous blood sampling, minimizing the temporal discrepancy between symptom assessment and biomarker measurement. We primarily analyzed contemporaneous pain intensity as a continuous variable, avoiding the information loss associated with categorization. Laboratory measurements were performed in duplicate by personnel blinded to pain-intensity categories and supported by predefined internal quality-control procedures. Bootstrap confidence intervals, adjustment for menstrual sampling day, correction for multiple comparisons, and a sensitivity analysis based on retrospective worst-pain scores further strengthened the statistical evaluation. They demonstrated consistency across complementary analyses.

Several limitations should also be acknowledged. First, the primary pain assessment was based on a single contemporaneous VAS score obtained during menstrual days 1-4 and may not fully represent maximum, average, or typical pain throughout menstruation. Although the sensitivity analysis based on retrospective worst-pain scores yielded similar findings, retrospective assessments may be affected by recall bias. Second, excluding participants who had used analgesics during the preceding 48 hours may have reduced the representation of women with the most severe symptoms and attenuated potential associations. Third, the very severe pain category included only five participants, resulting in marked subgroup imbalance and limited precision; consequently, the categorical comparisons should be regarded as exploratory. Fourth, the absence of a pain-free control group prevented assessment of whether the measured redox biomarkers differed between women with and without primary dysmenorrhea. Fifth, serum albumin was not measured concurrently, and an albumin-adjusted IMA index could not be calculated. Finally, this single-center study was based on a single biomarker measurement. It did not include repeated-cycle assessments, multidimensional pain evaluation, quantitative sensory testing, or objective measurements of uterine perfusion and contractility. These factors may limit the generalizability and mechanistic interpretation of the findings. Future studies should use standardized cycle-day and clock-time sampling, repeated within-participant measurements, concurrent albumin assessment, pain-free controls, and multidimensional phenotyping integrating pain distribution, functional impairment, central sensitization, inflammatory mediators, hormonal variation, and localized uterine physiology.

Conclusion

Systemic thiol-disulfide homeostasis parameters and unadjusted IMA measured once during menstrual days 1-4 were not associated with contemporaneous menstrual pain intensity among women with PD. The observed correlations were small, and their confidence intervals provided no evidence of moderate or strong associations, although weak relationships cannot be excluded. These findings do not refute the potential role of oxidative stress in PD; rather, they indicate that a single circulating redox measurement may not adequately represent the localized, temporally dynamic, and centrally modulated processes that determine subjective menstrual pain. Further studies should adopt a multimodal approach integrating oxidative stress markers with inflammatory, neurobiological, hormonal, and localized uterine parameters to clarify the mechanisms underlying pain variability in PD and identify more targeted therapeutic strategies.

Declarations

Ethics Approval and Consent to Participate: The Ankara Bilkent City Hospital No. 2 Clinical Research Ethics Committee reviewed and approved the study protocol (approval number: E2-23-3962; approval date: 6 March 2024). We obtained written informed consent from all participants before enrollment. All procedures were conducted in accordance with the Declaration of Helsinki and its subsequent amendments.

Consent for Publication: Not applicable. The manuscript does not contain any identifiable personal or clinical information requiring individual consent for publication.

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Authors' Contributions: GTE: Conceptualization, Methodology, Investigation, Data curation, Project administration, Resources, Supervision, and Writing - original draft. GTE also obtained the ethics committee approval and was responsible for custody and management of the study data. MIK, DTE, RT, EIS, and GO: Investigation, Resources, and Writing-review & editing. SN: Methodology, Investigation, Validation,

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References

1. Iacovides S, Avidon I, Baker FC. What we know about primary dysmenorrhea today: a critical review. Hum Reprod Update. 2015;21(6):762-78. Doi: 10.1093/humupd/dmv039. PMID: 26346058.
2. Barcikowska Z, Rajkowska-Labon E, Grzybowska ME, Hansdorfer-Korzon R, Zorena K. Inflammatory markers in dysmenorrhea and therapeutic options. Int J Environ Res Public Health. 2020;17(4):1191. Doi: 10.3390/ijerph17041191. PMID: 32069859, PMCID: PMC7068519..
3. Szmidt MK, Granda D, Sicinska E, Kaluza J. Primary dysmenorrhea in relation to oxidative stress and antioxidant status: a systematic review of case-control studies. Antioxidants (Basel). 2020;9(10):994. Doi: 10.3390/antiox9100994. PMID: 33076228, PMCID: PMC7602455.
4. Baradwan S, Gari A, Sabban H, Alshahrani MS, Khadawardi K, Bukhari IA, et al. The effect of antioxidant supplementation on dysmenorrhea and endometriosis-associated painful symptoms: a systematic review and meta-analysis of randomized clinical trials. Obstet Gynecol Sci. 2024;67(2):186-98. Doi: 10.5468/ogs.23210. PMID: 38221738, PMCID: PMC10948216.
5. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47(18):326-32. Doi: 10.1016/j.clinbiochem.2014.09.026. PMID: 25304913.
6. Eroglu O, Comertpay E, Vural S, Badem ND, Isbasaran P, Neselioglu S, et al. Diagnostic value of oxidative stress markers in patients presenting with primary dysmenorrhea to the emergency department. Niger J Clin Pract. 2022;25(5):636-640. Doi: 10.4103/njcp.njcp_1595_21. PMID: 35593606.
7. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain. 1983;17(1):45-56. Doi: 10.1016/0304-3959(83)90126-4. PMID: 6226917.
8. Iacovides S, Baker FC, Avidon I, Bentley A. Women with dysmenorrhea are hypersensitive to experimental deep muscle pain across the menstrual cycle. J Pain. 2013;14(10):1066-76. Doi: 10.1016/j.jpain.2013.04.010. PMID: 23769507.
9. Payne LA, Rapkin AJ, Seidman LC, Zeltzer LK, Tsao JC. Experimental and procedural pain responses in primary dysmenorrhea: a systematic review. J Pain Res. 2017;10:2233-46. Doi: 10.2147/JPR.S143512. PMID: 29066929, PMCID: PMC5604431.
10. Chen CX, Carpenter JS, Ofner S, LaPradd M, Fortenberry JD. Dysmenorrhea symptom-based phenotypes: a replication and extension study. Nurs Res. 2021;70(1):24-33. Doi: 10.1097/NNR.0000000000000477. PMID: 32956256, PMCID: PMC7736149.
11. Fortún-Rabadán R, Boudreau SA, Bellosta-López P, Herrero P, Graven-Nielsen T, Doménech-García V. Facilitated central pain mechanisms across the menstrual cycle in dysmenorrhea and enlarged pain distribution in women with longer pain history. J Pain. 2023;24(9):1541-54. Doi: 10.1016/j.jpain.2023.04.005. PMID: 37100358.
12. Lippi G, Montagnana M, Salvagno GL, Guidi GC. Standardization of ischemia-modified albumin testing: adjustment for serum albumin. Clin Chem Lab Med. 2007;45(2):261-2. Doi: 10.1515/CCLM.2007.039. PMID: 17311519.