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ORIGINAL RESEARCH

Thiol disulfide homeostasis in primary dysmenorrhea

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Abstract

Oxidative stress is defined as a result of the loss of balance between the production of free radical or reactive oxygen species and the antioxidant system. This study aimed to determine the level of thiol-disulfide homeostasis (TDH) in the serum of women with primary dysmenorrhea. The study group consisted of 42 subjects with primary dysmenorrhea, and the control group consisted of 30 volunteer women with demographic characteristics similar to the study group. Native thiol (SH), total thiol (TSH), disulphide (SS), and SH/SS parameters were measured for TDH of the subjects. The SH ($p = 0.038$) and SH/TSH ($p = 0.046$) levels were significantly higher while SS ($p = 0.013$), SS/SH ($p = 0.042$) and SS/TSH ($p = 0.046$) levels were lower in the study group than in the healthy control group. The SS cut-off value was determined as 17.85 in the study group (sensitivity = 61.9%, specificity = 43.3%). Therefore, the probability of dysmenorrhea may increase significantly when SS levels fall below this value. In subjects with dysmenorrhea, there was a decrease in SS levels and an increase in SH levels in order to protect the cells and tissues from the harmful effects of free radicals.

Keywords

Primary dysmenorrhea; Thiol-disulfide; Oxidative stress; ROC analysis; TDH

1. Introduction

Free radicals are defined as high-energy atoms or molecules carrying one or more unpaired electrons in their outer orbitals. Free radicals may originate from oxygen and nitrogen. Oxygen-originated ones are called reactive oxygen species (ROS), while nitrogen-originated ones are called reactive nitrogen species (RNS) [1]. Oxidative stress is defined as a disruption in molecular and cellular functions as a result of the loss of balance between the production of free radicals or ROS and the antioxidant system. When ROS exceed physiological values, they cause oxidative damage. Enzymatic or non-enzymatic antioxidant mechanisms play a role in protecting the organism against the harmful effects of ROS [2]. Thiol-disulfide homeostasis (TDH) is another mechanism that protects organisms from the harmful effects of ROS [2, 3]. Thiols are organic compounds containing—SH group, which play a critical role in preventing the occurrence of any oxidative stress condition in cells. Specifically, thiols protect cells and tissues by reacting with free radicals [3]. Lower thiol and higher disulfide levels lead to reduced clearance of ROS products [4] as the result of changes in the thiol/disulphide balance caused by oxidative stress [5]. Thus, it has been found that TDH disorders are important in the etiology of many diseases [6].

Dysmenorrhea, referring to painful menstrual cramps of uterine origin, is one of the most common causes of pelvic pain and menstrual disorder [7]. Dysmenorrhea and increased or decreased monthly bleeding length and volume are all frequent menstrual problems [8, 9]. Dysmenorrhea is a gynecological condition that affects around half of all women of reproductive age [7]. Painful menstruation without related pelvic illness is called primary dysmenorrhea (PD) [10]. PD refers to menstrual pain without an underlying pathology, while secondary dysmenorrhea refers to painful menstruation associated with the underlying pathology [11]. According to epidemiological research, PD affects roughly 60% of all dysmenorrhea patients, and its symptoms have a significant impact on women's normal working lives, making it critical to discover a safer and more effective treatment [12]. PD is a common gynecological condition causing abdominal pain in women [13]. It is a condition of the female reproductive system that has a negative impact on women's physical and mental health as well as their quality of life [14]. Interventions to decrease pain may be helpful for supporting women with dysmenorrhea [15].

Although its etiology has not been fully elucidated, it is thought that vasospasms develop due to the local release of prostaglandins, and oxidative stress parameters are thus affected as well [16, 17]. Oxidative stress and antioxidant status affect the potential mechanism of primary dysmenorrhea. Antioxidants, either endogenous or exogenous, are used to balance the formation of ROS with the human body's ability to detoxify these products [18]. It has been reported that levels of malic dehydrogenase (MDH) and nitric oxide (NO) among free radicals increased in women with dysmenorrhea [19]. Szmidt *et al.* [20] showed that women with primary dysmenorrhea may have a lack of antioxidant vitamins, which could explain the higher levels of oxidative stress markers they exhibit.

It has been thought that oxidative stress parameters may also be affected by the increase of MDH and NO free radicals in women with primary dysmenorrhea. Therefore, this study aimed to determine TDH levels in the serum of women with primary dysmenorrhea. In addition, the association of TDH with age, BMI, family history, and duration of menstruation was also examined in women with dysmenorrhea.

2. Materials and methods

2.1 Study design

This study was a is a case-control study trial conducted in the emergency department of Ordu University Training and Research Hospital, a tertiary hospital to which approximately 150,000 patients apply annually. This study was conducted in 2018. The data were collected according to the power analysis and were terminated when the required number was reached.

Ethics committee approval (no. 2018-232) was received from Ordu University Ethics Committee for the study. SH, TSH, SS, and SH/SS parameters were the TDH measurements used in the study [3].

The population of the study consisted of 113 patients and 30 control groups. Patients with acute abdomen disease (acute appendicitis, ileus, irritable bowel syndrome), contraceptive use, acute pelvic pathology, ectopic pregnancy, tubal ovarian abscess, ovarian tosion, renal colic, who did not apply on the third day of menstruation, and those who did not want to participate in the study were excluded in the study. Diagnosis and distribution of patients admitted to the Emergency Department with dysmenorrhea are shown in Fig. 1.
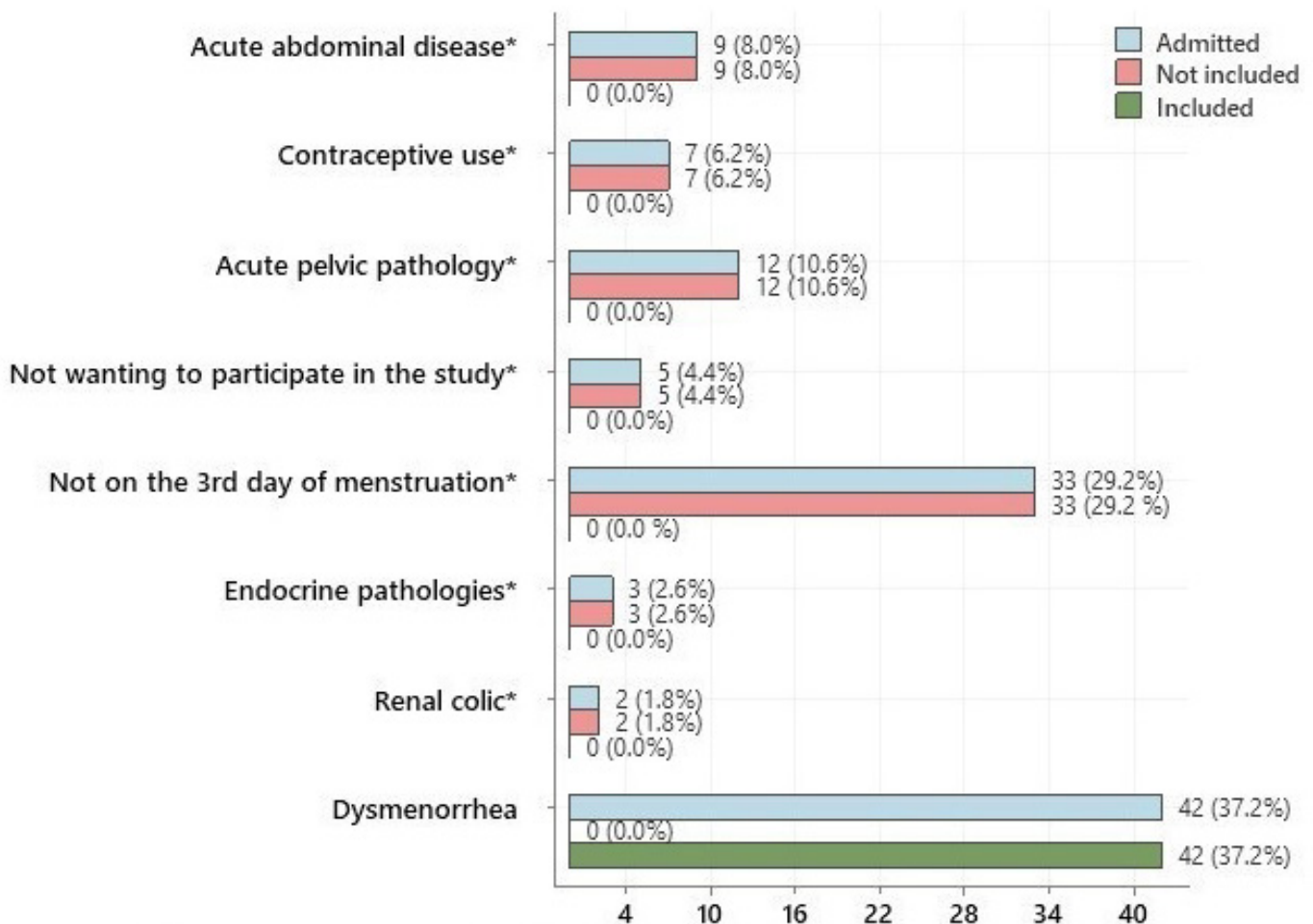

FIGURE 1. Diagnosis and distribution of patients who applied to the emergency department with dysmenorrhea.
*Patients meeting the exclusion criteria in addition to dysmenorrhea.

The study group consisted of 42 subjects with primary dysmenorrhea, and the control group consisted of 30 volunteer women with demographic characteristics similar to the study group who had never had dysmenorrhea. In terms of being similar to the study group, those in the control group, whose first menstrual period is 10–15 years, the number of menstrual days is 25–30 days, the body mass index is between 18.5–25, and the average menstrual period is between 7–10 days, were included in the study. The purpose and method of the study were explained to each of the individuals in the study and control groups, and an approval form indicating their voluntary participation in the study was obtained.

Special attention was paid to the fact that the women with dysmenorrhea included in the study did not take any analgesics in the last 24 hours, and they were in the most painful phase of the menstrual cycle.

For all the women participating in the study, a detailed medical history was taken, and systemic physical and gynecological evaluations were performed. Routine emergency blood biochemical parameters, including a pregnancy test, whole blood cell counts, glucose, liver, and kidney function, were examined. Moreover, women determined to have abdominal pain other than dysmenorrhea by imaging with ultrasonography were excluded from the study.

2.2 Criteria used to define dysmenorrhea

The diagnosis of primary dysmenorrhea was made considering the following criteria:

• Presence of lower abdominal or pelvic pain associated with the onset of menstruation and lasting 8–72 hours;

• Presence of lower abdomen pain during the menstrual period;

• Presence of medial or anterior femur pain; and

• Presence of menstrual pain with associated features like headache, diarrhea, nausea, and vomiting [21].

If the patient met at least one of the criteria above, she was diagnosed with primary dysmenorrhea.

2.3 Exclusion criteria

The researchers excluded from the study the patients with a history of pelvic pathology, inflammatory bowel disease, fibromyalgia, rheumatological, renal, cardiovascular, endocrine or metabolic diseases, women taking intrauterine contraceptive devices or oral contraceptives, and those who did not want to participate in the study. The patients were asked what day of their menstruation was and those who were outside the 3rd day of menarche were not included in the study.

Vital signs, height (centimeter), weight (kilogram), waist circumference (centimeter), body mass index (BMI: kg/m^2), age of starting dysmenorrhea, time of dysmenorrhea (before or during menstruation), menstrual period (days) and other demographic characteristics of the participants were obtained and recorded.

2.4 Power analysis

The sample size for this study was estimated by prior power analysis using G*Power 3.1 (Universität Düsseldorf, Düssel-

dorf) statistical software; the effect size was calculated as d = 0.747 for the SS variable from the old literature [22]. To α = 0.05 and 1-β = 0.80, it was determined that a minimum sample size of 60 (30 in each disease severity group) was required to detect the significance of the statistical test. To reduce statistical error and ensure a more representation of the population, the sample size was increased to 42 patients in the study group.

2.5 Data collection

The pain severity of the subjects participating in the study was calculated using a visual analog scale (Vas) chart and was defined as 0 cm–without pain and 10 cm–most severe pain. The severity was accepted as mild pain between 1 and 3 cm, moderate between 4 and 7 cm and severe pain between 8 and 10 cm in the evaluation [23].

The subjects expressed their experience of living with dysmenorrhea in three different ways: "in every menstruation", "occasionally", and "never".

2.6 Biochemical analysis

After 8–12 hours of fasting on day 3 of menstruation, venous blood samples were taken into tubes (Becton Dickinson and Company, New Jersey, USA) with gel for biochemical test studies and into tubes (Becton Dickinson and Company, New Jersey, USA) with EDTA for TDH testing. After all samples were centrifuged at 1600 × g for 10 minutes, their serum and plasma were separated and stored at –70 °C until the day of testing. Biochemical tests were performed on an AU 2700 auto-analyzer (Beckman Coulter, Inc, USA) using spectrophotometric methods.

For thiol measurement, the evaluation was conducted by spectrometry (Roche, Cobas 501, Mannheim, Germany) using the "Modified Ellman Method" of [3]. Free functional thiol groups (-SH) were formed by breaking disulfide bonds (-S-S) with sodium borohydride (NaBH4). Unused sodium borohydride residues were removed with formaldehyde. Therefore, the reduction of any disulfide bond formed as a result of 5,5'-dithiobis- (2-nitrobenzoic) acid (DTNB) reaction together with DTNB was prevented. The reduced and total native thiol groups were determined based on the reaction with DTNB. After disulfide, SH and TSH were determined and SS, SS/SH + SS%, SH/SH + SS%, and SS/SH% were calculated [3].

2.7 Data analysis

All statistical analyses were performed using SPSS v26 (IBM Inc., Chicago, IL, USA) and Minitab 19 (Minitab, LLC, State College, Pennsylvania) statistical softwares. Prior to the parametric tests, the data were evaluated for homogeneity of variances using Levene's test and for normal distribution using the Kolmogorov–Smirnov test. An independent samples t-test was used to compare the two groups. SS, SS/SH, SS/TSH, SH/TSH and TSH were analyzed with ANCOVA because the serum albumin was determined as the covariate variable. It was also tested whether the age variable as a covariate, but because it was determined that it was not, it was not used as a covariate variable in ANCOVA. Spearman rank correlation analysis was

used to determine the association between the variables, and ROC analysis was used to determine the predictive values and calculate cut-offs. A p-value less than 0.05 was considered statistically significant.

3. Results

The study included a total of 72 women over the age of 18, 42 of whom (study group) presented to the emergency clinic with a complaint of primary dysmenorrhea, and 30 of whom were healthy volunteers (control group). Table 1 and Fig. 2 showed the demographic characteristics of the study and control groups and a comparison of the TDH parameters. In the statistical analysis, a statistically significant differences were found between the groups according to the mean age ($p = 0.000$).

But, no statistically significant differences were found between the groups according to BMI, age of first menstruation, mean duration of menstruation, duration of menstrual period, and serum SH and TSH levels ($p > 0.05$). When the study group's experiences of dysmenorrhea symptoms during previous menstrual periods were examined, dysmenorrhea symptoms were seen in 73.9% (n = 31) of women in each menstrual period, 14.2% (n = 6) occasionally, and 11.9% never.

Of the women in the control group, 50% (n = 15) had a family history of dysmenorrhea, while 50% (n = 15) had no family history. Meanwhile, 66.7% (n = 28) of the women in the study group had a family history of dysmenorrhea, while 33.3% (n = 14) did not. The SH ($p = 0.038$) and SH/TSH ($p = 0.046$) levels were significantly higher while SS ($p = 0.013$), SS/SH ($p = 0.042$) and SS/TSH ($p = 0.046$) levels were
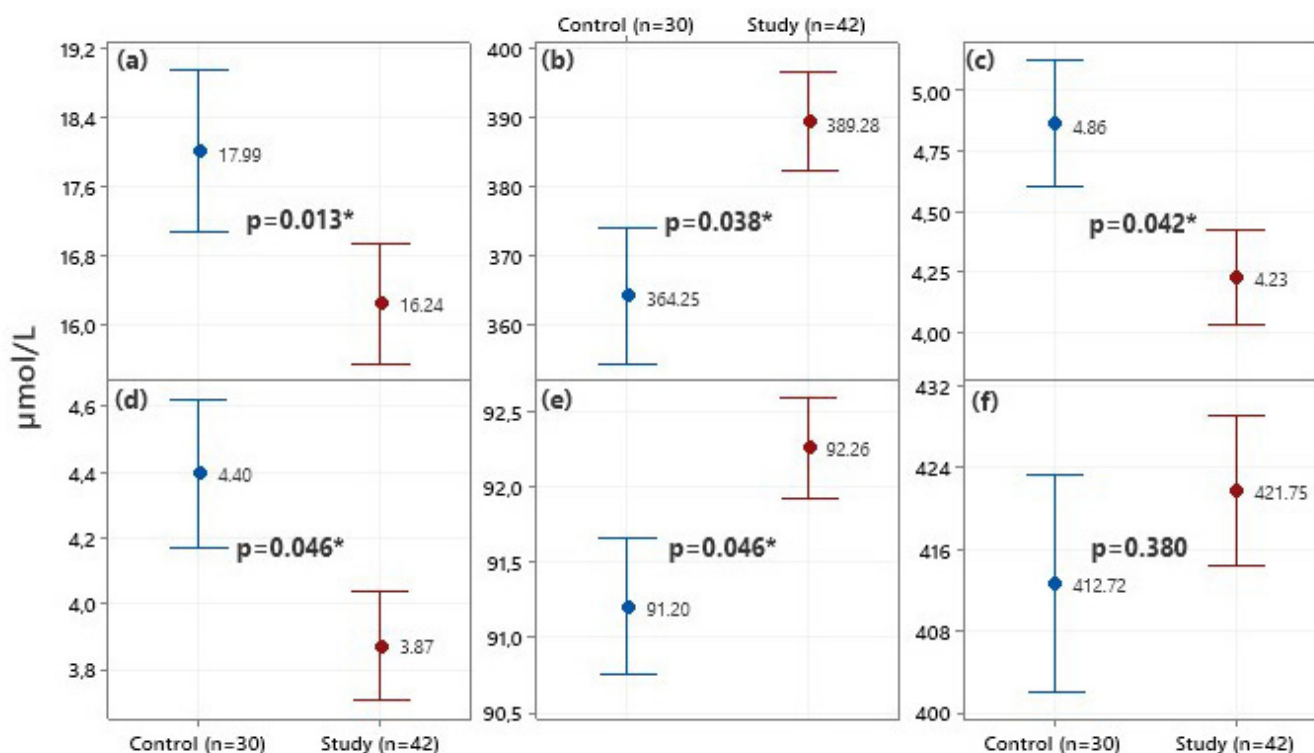

FIGURE 2. The TDH parameters of the study and control groups.
Individual standard errors are used to calculate the intervals. *: <0.05.
(a) SS (μmol/L), (b) SH (μmol/L), (c) SS/SH (μmol/L), (d) SS/TSH (μmol/L), (e) SH/TSH (μmol/L) and (f) TSH (μmol/L).

TABLE 1. The demographic characteristics of the study and control groups.

	Control (n = 30)	Study (n = 42)	p
Age (year)	30.6 ± 8.5	23.5 ± 6.2	0.000***
BMI (kg/m^2)	23.0 ± 4.8	21.6 ± 3.8	0.192
First menstrual age (year)	13.1 ± 1.6	13.3 ± 1.8	0.702
Albumin (g/dL)	4.5 ± 1.5	5.6 ± 0.2	0.001**
Average menstrual period (day)	5.6 ± 1.6	5.9 ± 1.5	0.409
Duration of menstruation period (day)	26.5 ± 3.5	27.6 ± 2.5	0.159
Vas score	-	6.3 ± 3.2	-

*Mean ± Std. Deviation, **: <0.01, ***: <0.001. Student t-test.*
Vas score (visual analog scale).

lower in the study group than in the healthy control group (Fig. 1). The association of TDH parameters with Vas score was investigated by correlation analysis, and no significant relationship was found ($p > 0.05$). While TDH parameters differed significantly depending on the presence or absence of dysmenorrhea, they did not change with the severity of pain. The correlation coefficients between the variables are summarized in Table 2.

The serum albumin, which differed significantly according to the groups ($p = 0.001$, Table 1), was determined as the covariate variable for SS, SS/SH, SS/TSH, SH/TSH and TSH. According to statistical analyzes by removing the covariate effect, SS, SS/SH and SS/TSH in the study group was significantly lower than the control group ($p = 0.013$; $p = 0.042$; $p = 0.046$, respectively). While SH and SH/TSH levels were high in the study group, they were significantly low in the control group ($p = 0.038$; $p = 0.046$, respectively). There was

no stsatistically significant difference between the TSH levels of the groups ($p = 0.380$) (Fig. 2).

ROC curve analysis was performed, and area under the curve (AUC), sensitivity, specificity, and 95% confidence interval (CI) were calculated. Whereas SS was considered to be the predictor of dysmenorrhea ($p = 0.031$), SH was not considered to be the predictor of dysmenorrhea. The results indicated that the AUC of SS was 0.643 (95% CI: 0.512–0.773) for the diagnosis of dysmenorrhea (Table 3). The cut-off SS value was determined as 17.85 in the study group (sensitivity = 61.9%, specificity = 43.3%). Therefore, the probability of dysmenorrhea presence may increase significantly when SS levels fall below this value. ROC curves of SH and SS, which are the indicator variables of dysmenorrhea, are presented in Fig. 3.
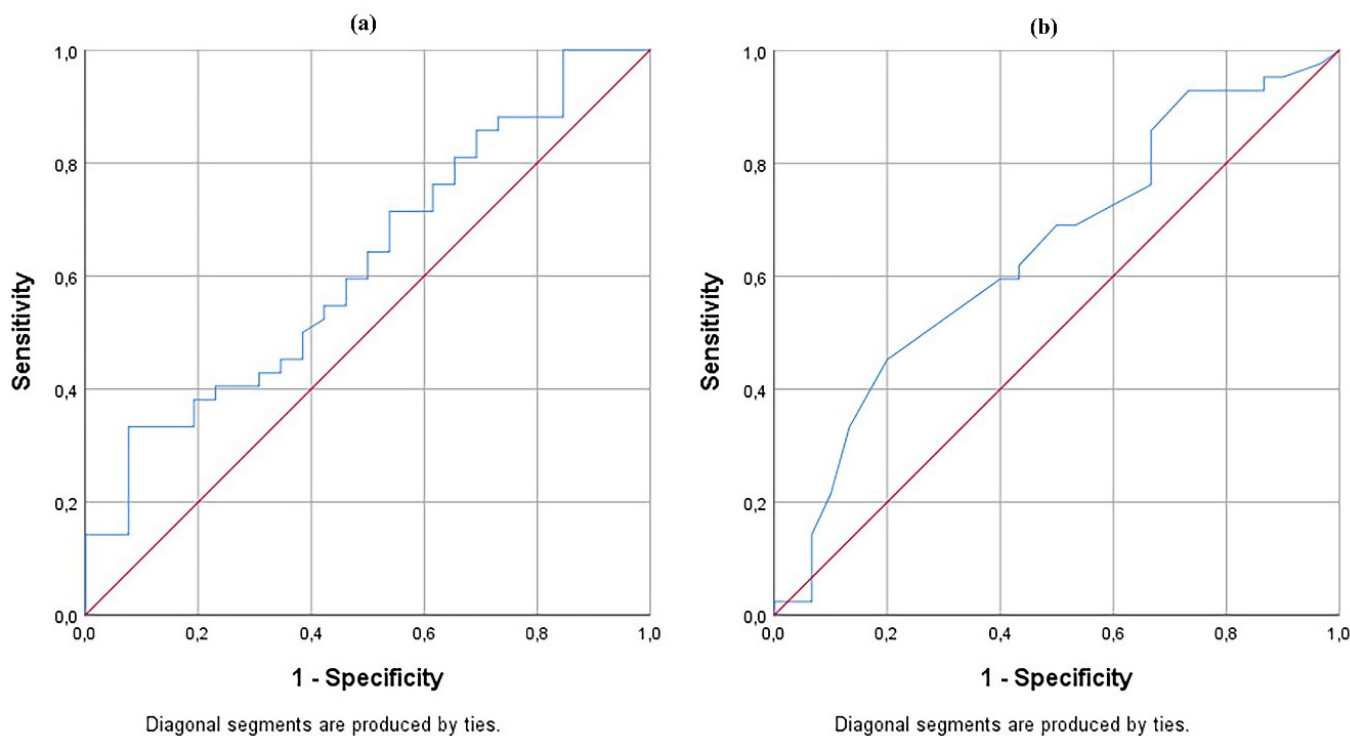

FIGURE 3. The ROC curves of SH (a) and SS (b), which are indicator variables of dysmenorrhea.
SH, Native thiol; SS, disulphide.

TABLE 2. The correlation coefficients between TDH parameters and Vas score.

	SH (μmol/L)	TSH (μmol/L)	SS (μmol/L)	SS/SH (μmol/L)	SS/TSH (μmol/L)	SH/TSH (μmol/L)
r	0.128	0.112	−0.070	−0.118	−0.121	0.118
p	0.426	0.485	0.665	0.461	0.453	0.461

TABLE 3. Area under the curve (AUC) and 95% confidence interval (CI) values of SH and SS for the diagnosis of dysmenorrhea.

	Cut-off value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	p
SH (μmol/L)	-	-	-	0.621 (0.486–0.757)	0.094
SS (μmol/L)	17.85	61.9	43.3	0.643 (0.512–0.773)	0.031*

*: <0.05.

4. Discussion

In this study, TDH levels and their diagnostic value in PD were investigated in women with PD. In addition, the researchers evaluated the association between BMI, age of first menstruation, mean duration of menstruation, menstruation period, and presence of dysmenorrhea in the family and serum SS, SH, and TSH levels. According to the literature analysis, there is no study evaluating women with PD for SS and SH levels of TDH. Accordingly, the present study is believed to be the first research in this area.

Of the women in the control group in the current study, 50% had a family history of dysmenorrhea, while 50% had no family history. Meanwhile, 66.7% of the study group had a family history of dysmenorrhea, while 33.3% did not. No available research on family history was found in the literature. In the current study, the presence of dysmenorrhea in the families of women with dysmenorrhea was taken into consideration, and follow-up research in this direction would be helpful.

The association between TDH parameters and Vas score was also examined by correlation analysis, but no significant relationship was found. However, Akdemir *et al.* [24] observed that asymmetric dimethylarginine (ADMA) levels were significantly higher than VAS scores in patients with severe and moderate dysmenorrhea. This difference may have originated from the method and study group.

TDH parameters did not change with the severity of pain but differed significantly depending on whether or not there was dysmenorrhea. Of the SS, SH, TSH, and SH/SS parameters required for TDH measurement, SH levels were found to be significantly higher in women with dysmenorrhea compared than in the control group ($p = 0.038$). Again, SS was found to be significantly lower in women with dysmenorrhea than in women in the control group ($p = 0.013$). The SS/TSH ratio was also found to be low in the control group ($p = 0.046$). Further, SH/TSH levels were observed to be significantly high in women with dysmenorrhea ($p = 0.046$). Similar to the present study, researchers showed that oxidative stress, which occurs due to the increase in ROS or a decrease in antioxidant levels, plays a role in the etiopathogenesis of many diseases in the human body [25]. It has also been reported that the antioxidant system was activated as a compensatory mechanism in cases where ROS increases, and SH in the organism was affected and transformed into SS in these cases [4, 6]. Plasma thiols are known to have a proxy or antioxidant effect on physiological events. Whether thiols have an antioxidant or pro-oxidant effect is related to oxidant stress, physiological conditions, and the concentration levels of sulfur-containing amino acids in metabolism [22, 26]. It is believed that antioxidants (methionine, N-acetyl cysteine, taurine, homocysteine, etc.) containing thiol groups have an active role in reducing the oxidative condition formed by dysmenorrhea. The main cause of dysmenorrhea-related oxidative stress is the disruption of the pro-oxidant/antioxidant balance in the cell. It is thought that increased oxidative damage in dysmenorrhea could be reduced by giving sulfur-containing antioxidant compounds as preservatives.

Glutathione is the major thiol buffer in cells. SH group sources are converted into metabolites that can stimulate glutathione (GSH) synthesis in the body and promote detoxification, functioning directly as free radical scavengers in metabolism [27]. This occurs because the effect of ROS in tissues or cells can cause high SS and low SH levels. The fact that SS levels were lower in women with dysmenorrhea than in women in the control group as well as the high levels of SH in women with dysmenorrhea indicates that the effect of ROS may be lower in women with dysmenorrhea. This could be because the body's defense mechanism is activated in response to dysmenorrhea and protects the cell or tissue against ROS. With regard to oxidative stress levels, only SH levels were examined in the previous literature [25]. However, all parameters required for TDH were examined in this study. Similar to this study, Erel and Neselioglu [3] reported that both SS and SH levels and the association between them should be measured when evaluating TDH.

In a study, it was determined that free oxygen radicals increase in primary dysmenorrhea [19]. Akdemir *et al.* [23] also found that ADMA, which causes an increase in free oxygen radicals, increases in dysmenorrhea.

Some studies found that serum malondialdehyde (MDA) and interleukin-6 levels were higher in women with dysmenorrhea than in healthy individuals [28, 29]. In addition, Dikensoy *et al.* [19] found that the parameters of MDA, NO, and adrenomedullin (AM) were higher in individuals with PD than in healthy individuals. Moreover, there are studies that showed an increase in proinflammatory cytokines, interleukins, and tumor necrosis factor-α (TNF-α), neutrophil hyperfunction, and excessive ROS production in PD patients [28–30]. It was reported that thiol-disulfide homeostasis, which is an organic compound and can react with free radicals, protects cells and tissues against reactive oxygen products [3, 31]. Similarly, SH levels were found to be higher in patients with PD compared to healthy women in the current study.

Due to the change of SS and SH values in the study, SS could have diagnostic value. Specifically, SS values decreased below 17.85 in patients with dysmenorrhea. In other words, the level of oxidative stress parameters was lower in patients with dysmenorrhea than in the control group. Accordingly, it was considered that diagnosis and follow-up could be facilitated by examining SS levels. However, the inability to follow up with patients is one of the limitations of the study. In addition, the dietary habits of the participants were not recorded, and the average age of the control and study groups differed. As there are no studies in the literature investigating the effects of nutritional habits and age on thiol disulphide homeostasis, there is a need for further research on these subjects.

5. Conclusions

Dysmenorrhea shows a linear association with SH and SS. It was found that SS levels decreased and SH levels increased to protect cells and tissues from the harmful effects of free radicals in the subjects with dysmenorrhea. Therefore, it appears that SS levels could be used in the diagnosis and follow up of dysmenorrhea. Accordingly, controlled and wide-ranging studies should be performed to prove whether SS has diagnostic value.

AUTHOR CONTRIBUTIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICT OF INTEREST

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