Araştırma Sunumu / Research Article

The relationship between Thiol/disulfide homeostasis and endometrial hyperplasia in patients with abnormal uterine bleeding */Anormal Uterin Kanamalı Kadınlarda Endometrial Hiperplazi Ve Thiol Disülfat Homeostazis İlişkisi*

Sezin ERTÜRK AKSAKAL[1], Elif Gülşah DİKTAŞ[2], Ramazan Erda PAY[3], Ömer Lütfi TAPISIZ[4], Burcu TİMUR[5], Vakkas KORKMAZ[6], Özcan EREL[7], Yaprak ENGİN ÜSTÜN[8].

1. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, drsezert@gmail.com

2. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, elifgulsahdiktas@gmail.com,

3. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, drramazanpay@gmail.com

4. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, omertapisiz@yahoo.com.tr

5. Ordu University Training and Research Hospital, drburccu@gmail.com

6. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, korkmazvakkas@gmail.com

7. Yildirim Beyazit University Faculty of Medicine, erelozcan@hotmail.com, orchid no: 0000-0002-2996-3236

8. University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital, ustunyaprak@gmail.com

Gönderim Tarihi | Received: 09.04.2022, Kabul Tarihi | Accepted: 21.04.2022, Yayım Tarihi | Date of Issue: 01.08.2022, DOİ: 10.25279/sak.1101029

Atıf | Reference: ERTÜRK AKSAKAL, S.; DİKTAŞ, E.G.; PAY, R.E.; TAPISIZ, Ö.L.; TİMUR, B; KORKMAZ, V. EREL, Ö.; ENGİN ÜSTÜN, Y. (2022). The Relationship between Thiol/Disulfide Homeostasis and Endometrial Hyperplasia in Patients with Abnormal Uterine Bleeding, Health Academy Kastamonu (HAK) 7 (2), s. 340 -351

Abstract

Introduction: The role of oxidative stress and antioxidant capacity in the development of endometrial hyperplasia (EH) is controversial. Aim: The study aimed to evaluate Thiol/disulfide Homeostasis and ischemia modified albumin (IMA) levels in patients with EH without atypia. Materials and Methods: In this prospective case-control study, patients with EH without atypia (HP group) (n=28), patients with nonhyperplasia (proliferative/secretory/irregular proliferative/irregular secretory endometrium) (non-HP group) (n=28), and 28 healthy women (control group) were included. The patient's clinical characteristics, serum Thiol/disulfide parameters, and IMA levels were compared between groups. Results: A total of 84 patients were included in the study. Patients' mean age, BMI, and mean native thiol (-SH-), total thiol (-SH-+-SS-), disulfide (-SS-), and IMA levels were similar among the three groups. The -SS- /-SH- ratio was higher in the HP group than the non-HP group. -SS- /-SH-+-SS- ratio was higher in the HP group vs. the other two groups. The -SS- /-SH-+-SS ratio was higher in the HP group vs. the non-HP group. -SH-/ -SH-+-SS- ratio was lower in the HP group than in the non-HP group. ET was greater in the HP group than in the non-HP and control groups. ET was also significantly greater in the non-

HP group vs. in the control group. -SS-/-SH- ratio was found to be predictive with 64% sensitivity and 68% specificity for EH (area under curve = 0.672, p = 0.01). Conclusion and Suggestions: The dynamic thiol/disulfide balance shifted to the disulfide side in women with endometrial hyperplasia.

Keywords: Thiol/Disulfide Homeostasis, Oxidative Stress, Endometrial Hyperplasia Without Atypia, Ischemia Modified Albumin.

Öz

Giriş: Endometrial hiperplazi gelişimde oksidatif stres ve antioksidan kapasitenin rolü tartışmalıdır. Amaç: Çalışma, Basit atipisiz endometrial hiperplazili hastalarda thiol-disulfat homeostazis ve iskemi-modifiye albumin (IMA) seviyelerini değerlendirmeyi amaçlamıştır. Gereç ve Yöntemler: Prospektif vaka kontrol dizaynlı çalışmada, atipisiz endometrial hiperplazili (HP grup) (n=28) hastalar, nonhiperplazi (proliferatif/sekretuar/düzensiz proliferatif/düzensiz sekretuar endometrium) saptanan hastalar (non-HP grup) (n=28) ve 28 sağlıklı kadın (kontrol grubu) çalışmaya dahil edildi. Hastaların klinik özellikleri, Thiol-disulfat parametreleri ve IMA seviyeleri gruplar arasında karşılaştırıldı. Bulgular: Çalışmaya toplam 84 hasta dahil edildi. Hastaların ortalama yaş, BMI, mean native thiol (-SH-), total thiol (-SH-+-SS-), disulfide (-SS-) ve IMA seviyeleri 3 grup arasında benzerdi. -SS-/-SH- ve -SS-/-SH-+-SS oranı HP grubunda non-HP grubundan anlamlı yüksekti. -SS-/-SH-+-SS- oranı HP grubunda diğer iki gruptan anlamlı yüksekti. -SH-/-SH-+-SS- oranı Hp grubunda non-HP grubuna göre anlamlı düşüktü. Endometrial kalınlık (ET), HP grubunda, non-HP ve kontrol grubuna göre daha kalındı. ET non-HP grubunda, kontrol grubundan anlamlı kalındı. -SS-/-SH- oranı %64 sensitivite, %68 spesifite ile endometrial hiperplaziyi öngörmektedir. Sonuç ve Öneriler: Endometrial hiperplazili kadınlarda Dinamik thiol-disülfat dengesi disülfat tarafına kaymıştır.

Anahtar Kelimeler: Thiol-Disülfat Homeostazis, Oksidatif Stres, Atipisiz Endometrial Hiperplazi, Iskemi-Modifiye Albumin

1. Introduction

Endometrial hyperplasia is one of the leading causes of abnormal uterine bleeding ; its importance is based on the fact that it may be a precursor lesion for endometrial cancer, or it may accompany cancer (Eppline et. al. 2008; Emons, Beckmann, Schmidt and Mallmann, 2014). Hyperplasia's progression without atypia to cancer is rare, but it is accepted as an endocrine disorder, so follow-up and treatment are recommended (Matsuo et. al. 2015).

Endometrial proliferative activity is influenced by various complex factors, such as cytokines, adhesion molecules, and growth factors that ensure intracellular signal and communication (No, 2016). An impaired antioxidant system could play an essential role in the development of endometrial hyperplasia (Goncharenko et. al. 2013; Lecanda, 2007). Pejic et. al. (2009) found an increase in lipid peroxidation and antioxidant activity in hyperplasia with malignant lesions, compared to benign gynecological disease.

Thiols are organic compounds that contain sulfhydryl groups and can be oxidized to disulfides (-SS-) and create disulfide bonds in case of oxidative stress (Turrel et. al. 2013). The emergent disulfide bonds can again be reduced to thiol groups by several antioxidants. Moreover, in this way, thiol/ disulfide balance is sustained (Cremers and Jakob, 2013). Thiol/disulfide Homeostasis (TDH) plays a significant role in regulating several cellular

functions (Jones and Liang, 2009). Abnormal thiol/disulfide balance may affect various proliferative gynecologic diseases, such as endometrial polyps, myoma uteri, and endometrium cancer (Ozaksit, Tokmak, Kosem and Kuru-Pekcan and Ozcan, 2019; Eroglu et. al. 2017; Sezgin et. al. 2020).

The objective of this study was to assess TDH in patients with endometrial hyperplasia without atypia.

2. Materials and Methods

2.1 Type of Research

This is a prospective, case-control study.

2.2 Place and Time of Research

The study was conducted in Etlik Zübeyde Hanım Training and Research Hospital between August 2020 and January 2021.

2.3 Population, Sample and Sampling Method of Research

Patients with endometrial hyperplasia without atypia (n=28), nonhyperplasia (proliferative/secretory endometrium) (n=28) and 28 healthy controls were enrolled the study. In total, 56 patients between 35-45 years old, with endometrial sampling due to abnormal uterine bleeding and 28 healthy individuals scheduling a routine gynecological examination were enrolled. Hyperplasia was classified according to the system defined by the World Health Organization (WHO) (Emons, Beckmann, Schmidt, Mallmann, 2015). Before data collection, a power analysis was conducted using G Power software. Assuming an alpha of .05 and an effect size of w=.67, power analysis suggested that 86 participants to have 80% power would be required.

Patients with organic pathologies, such as adenomyosis, endometrioma, myoma uteri, Pelvic İnflammatory Disease (PID), Tubo-ovarian Abscess (TOA), endometrial cancer, patients with insufficient clinical data were excluded from the study. Smoking, alcohol and substance addiction, patients using antioxidant vitamins (A, E, and C), and chronic diseases that could affect thiol/disulfide balance were also excluded.

2.4 Data Collection Tools

Patients' age, body mass index (BMI), gravida, parity, endometrial thickness were recorded. Endometrial thickness was measured with a 5.0 MHz transvaginal probe (Voluson E6, GE Medical Systems, Milwaukee, Wisconsin, USA) before endometrial sampling in patients with abnormal uterine bleeding and those in the first 14 days of the menstrual cycle in the control group.

2.5 Data Collection

Blood samples (5 ml) were collected in plain tubes for each participant. Serum samples were separated after centrifugation at 5000 rpm for 10 minutes, then stored at -80° C until analysis. Native thiol (-SH-), total thiol (-SH-+-SS-), disulfide (-SS-) levels were measured using the spectrophotometric method as described by Erel and Neselioglu (2014 s. 326). Dynamic -SS- was calculated as half value of the -SH- and -SH-+-SS- content. -SS- x 100 /-

SH-, -SS- x 100/-SH-+-SS- and -SH-x 100/-SH-+-SS- ratios were calculated. Ischemia modified albumin (IMA) level was detected with the albumin cobalt binding test defined by Bar-Or et al (2008, s. 120).

Differences in dynamic TDH and IMA levels were investigated in women with hyperplasia without atypia (HP), women without hyperplasia (non-HP), and the control group.

2.6 Ethical Considerations

The study was approved by the Ethical Committee of the University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital (2020/82). The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All participants gave a signed written informed consent.

2.7 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) for Windows v. 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The normality of data was tested with the Shapiro-Wilk test. Normally distributed metric variables expressed as mean ±SD and non-normally distributed metric variables were expressed as the median (min-max) for descriptive statics. A one-way analysis of variance and the Tukey posthoc test were used to compare three groups for parametric data. The Kruskal-Wallis test was used for nonparametric data, followed by the Mann-Whitney-U test with Bonferonni correction for pairwise comparisons. Spearman and Pearson correlation analyses could determine the degree of association among variables. Receiver operating characteristic (ROC) analysis determined predictive values and calculated cutoff values. A P-value of < 0.05 was considered statistically significant.

3. Results

A total of 84 patients were included in the study. Patients' mean age, BMI, gravity, and parity were similar among the three groups ($p > 0.05$). When patients were evaluated in terms of TDH, mean -SH-, -SH-+-SS-, -SS- and IMA levels did not differ between groups ($p>0.05$).

In a comparison of the three groups for -SS- /-SH- ratio, it was 4.4 ± 0.8% in the HP group, 3.9 ± 0.7% in the non-HP group, and 3.9 ± 0.6% in the control group ($p = 0.039$). In multiple group comparisons, the -SS- /-SH- ratio was statistically significant and higher in the HP group without atypia compared to the non-HP group.

-SS- /-SH-+-SS- ratio was 3.9 ± 0.6% in the HP group without atypia, 3.6 ± 0.6% in the non-HP group, and 3.6 ± 0.5% in the control group. -SS- /-SH-+-SS- ratio was statistically significant and higher in the HP group without atypia vs. the other two groups ($p = 0.040$). Multiple group comparisons showed the ratio of -SS- /-SH-+-SS- was significantly higher in the HP group without atypia vs. the non-HP group. No difference was observed between HP without atypia and control groups or between non-HP and control groups (Table 1).

Table 1. Demographic Features and TDH Parameters of The Groups

	HP Group n=28	**Non-HP Group** n=28	**Control Group** n= 28	**p**
Age * (years)	42.1 ± 3.8	42.7 ± 4.5	40.3 ± 3.5	0.071
BMI (kg/m^2) *	28 ± 3.8	25.8 ± 3.5	26.8 ± 3.5	0.073
Gravidity **	2 (0-4)	3 (0-7)	2 (0-5)	

Parity **	2 (0-4)	2 (0-7)	2 (0-4)	
-SH-, µmol/L *	398.1 ± 51.1	405.8 ± 44.6	412.1 ± 52.01	0.577
-SH-+-SS-, µmol/L *	432.1 ± 53.5	437.3 ± 46.3	444.4 ± 56.3	0.682
-SS- , µmol/L *	17.1 ± 2.4	15.8 ± 2.7	16.2 ± 3.1	0.194
-SS- /-SH-, % *	4.4 ± 0.8*	3.9 ±0.7	3.9 ± 0.6	**0.039**
-SS- /-SH-+-SS-, % *	3.9± 0.6*	3.6 ± 0.6	3.6 ± 0.5	**0.040**
-SH- /-SH-+-SS, % *	92.1 ± 1.3*	92.8 ±1.1	92.7 ± 0.9	**0.041**
IMA, AbsU *	0.8 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.381
ET (mm) *	15.5 (4-22) *	9 (3-20)	7 (3-12) *,**	**<0.001**

HP: Hyperplasia, BMI: Body mass index, -SH-: Native thiol, -SS-: Disulfide, -SH-+-SS-: Total thiol, IMA: Ischemia modified albumin, ET: Endometrial thickness *Data presented as mean±SD. **Data presented as median (min-max).

-SH- /-SH-+-SS- ratio was 92.1 ± 1.3% in the HP group without atypia, 92.8 ± 1.1% in the non-HP group, and 92.7 ± 0.9% in the control group. -SH-/ -SH-+-SS- ratio was significantly lower in the HP group without atypia than in the non-HP group (p = 0.041). No significant difference was found between the HP without atypia and control groups and between the non-HP and control groups.

Mean endometrial thickness (ET) was 15.5 mm in the HP group without atypia, 9.0 mm in the non-HP group, and 7.0 mm in the control group (Figure 1). ET was greater in the HP group without atypia than in the non-HP and control groups (p < 0.001). ET was also significantly greater in the non-HP group vs. in the control group (Table 1).

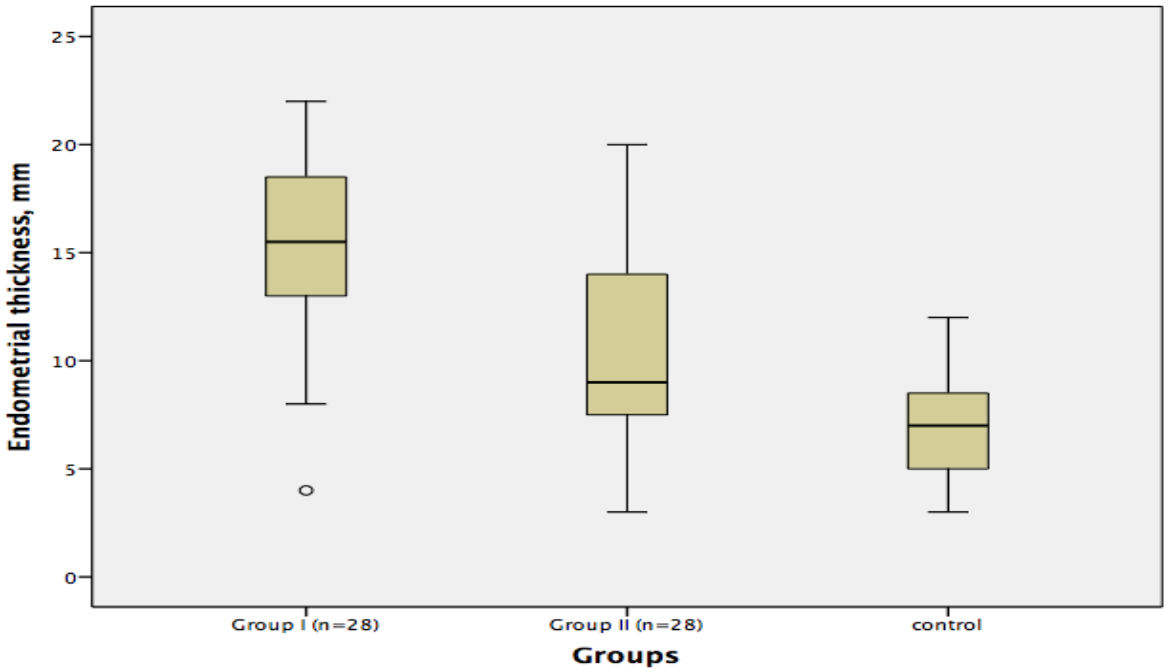

Figure 1. Endometrial Thickness of The Groups

When the correlation between ET and thiol/disulfide parameters was examined, a negative correlation between ET and -SH- (r = -0.239, p = 0.028), -SH-+-SS- levels (r = -0.221, p = 0.043), and -SH- /-SH-+-SS- ratio (r = -0.272, p = 0.012) was observed. A positive correlation was found between ET and -SS- /-SH- (r = 0.274, p = 0.012) and -SS- /-SH-+-SS- ratios (r = 0.272, p = 0.012) (Table 2). -SS-/-SH- ratio was found to be predictive for endometrial hyperplasia (area under curve = 0.672, p = 0.01) (Figure 2). The optimum cutoff value was stated to be 4.15, with 64% sensitivity and 68% specificity.

Table 2. Correlations between TDH Parameters and Endometrial Thickness

	-SH-, µmol/L	-SH-+-SS-, µmol/L	-SS-, µmol/L	-SS-/-SH-, %	-SS-/ -SH-+-SS- %	-SH-/ -SH-+-SS- %	IMA, Absu
ET,mm							
r	**-0.239**	**-0.221**	0.66	**0.274**	**0.272**	**-0.272**	-0.042
p	**0.028**	**0.043**	0.548	**0.012**	**0.012**	**0.012**	0.704

-SH-: Native thiol, -SS-: Disulfide, -SH-+-SS-: Total thiol, IMA: Ischemia modified albumin, ET: Endometrial thickness

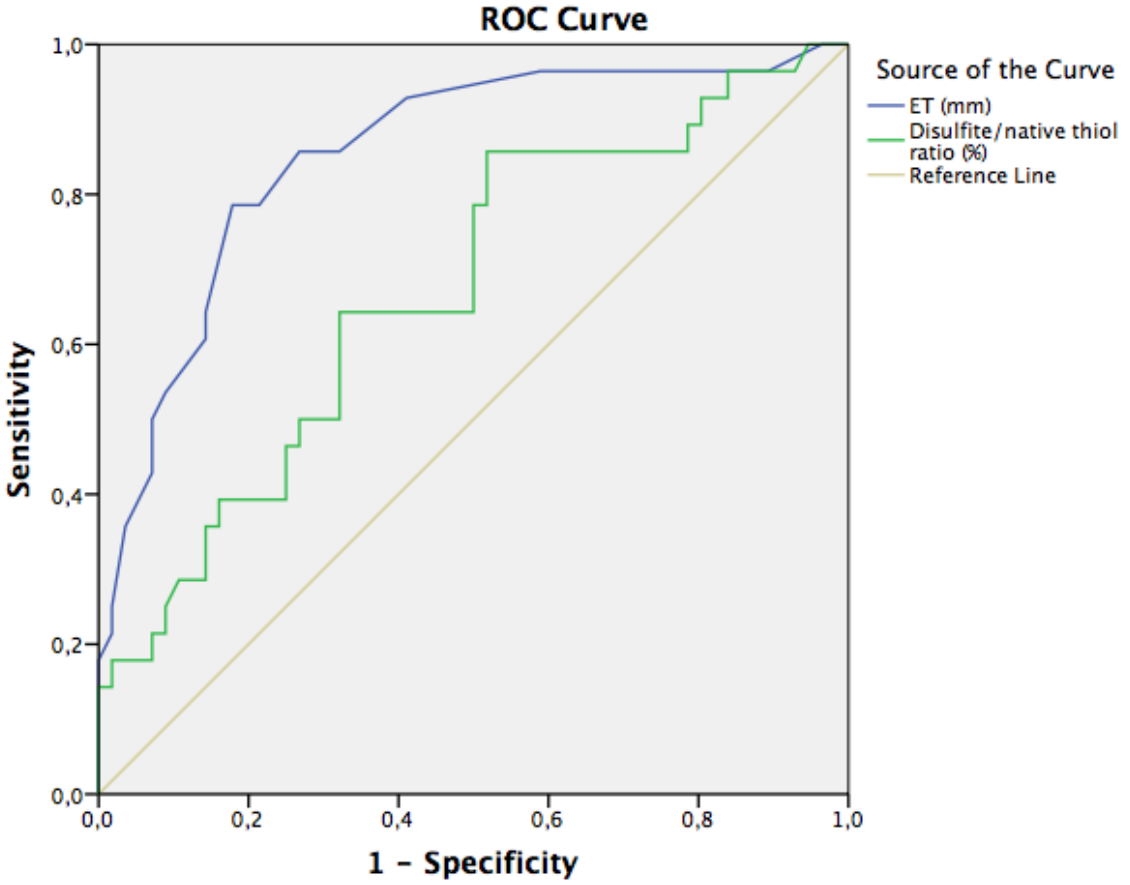

Figure 2. ROC Curve of Disulfide/Native Thiol Ratio To Predict Endometrial Hyperplasia

4. Discussion

Patients who underwent endometrial sampling due to abnormal uterine bleeding or those whose pathology result was HP and non-HP without atypia were compared for endometrial thickness, thiol/disulfide parameters, and IMA. -SS-/-SH-, -SS-/-SH-+-SS-, -SH- /-SH-+-SS- ratios were significantly higher in patients with HP without atypia vs. those with non-HP. This shows a shift of the thiol-disulfide balance to the disulfide side in patients with hyperplasia without atypia. Further, a negative correlation between ET and -SH-, -SS-+-SH- levels, -SH-

/-SH-+-SS- ratio, and a positive correlation between -SS-/-SH- and -SS-/-SH-+-SS- ratios were also identified.

Estrogen and progesterone are the primary hormones to regulate cell proliferation of the endometrium. Oxidative stress and antioxidant mechanisms may have a substantive role in endometrial mechanisms such as menstruation, decidualization, and implantation (Rizk, Badr and Talerico, 2013). Decreased progesterone in the late secretory phase decreases superoxide dismutase enzyme activity (SOD), elevates lipid peroxide level; as lipid peroxide increases, PGF2-α synthesis initiates menstrual bleeding (Sugino et. al. 1996; Sugino, Karube-Harada, Kashida, Takiguchi and Kato, 2001; Sugino et. al. 2000).

Hyperestrogenemia unopposed by progesterone is the main factor in the development of endometrial hyperplasia (*Henderson and Feigelson, 2000). However, the etiopathogenesis of endometrial hyperplasia is not fully understood yet. Oxidative stress and an impaired antioxidant system may have a prominent effect on the pathophysiology of endometrial hyperplasia. Levels of local inflammatory cytokines like IL-1B, IL-6, TNF-*σ, and expression of CD 45^{+} were found to increase in patients with EH (Kubyshkin et. al. 2016). Todorović *et. al. (2008) showed that the level and activity of SOD was decreased, and the level of* lipid hydroperoxides was elevated in patients with EH and adenocarcinoma compared to polyps, myomas, and healthy controls. In addition to this study, SOD activity decreased, while glutathione peroxidase and glutathione reductase activities increased in women with EH (Pejic et. al. 2009; Todorović et. al. 2019) Malondialdehyde levels, defined as lipid peroxidation's final product, were increased in women with EH compared to non-HP patients (Gómez-Zubeldia, Bazo, Gabarre, Nogales and Palomino, 2008). When compared luteal and follicular phase *antioxidant enzymes* in patients with *complex endometrial hyperplasia,* significantly higher SOD activity and *lower glutathione peroxidase and glutathione reductase activity* in the luteal phase and postmenopause than in follicular phase was recorded (Pejic et. al. 2016.)

Thiol groups of proteins or thiol groups of low molecular weight compounds are oxidized by environmental molecules and turn into reversible disulfide bond structures. An increased disulfide ratio is considered an oxidative stress marker (Gómez-Zubeldia, Bazo, Gabarre, Nogales and Palomino, 2008). In our study, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol ratios were found to be significantly higher in patients with hyperplasia without atypia. Considering that endometrial hyperplasia is a precursor lesion of endometrial cancer, Sezgin et al. showed findings consistent with our study. Lower serum native thiol and total thiol levels and higher serum disulfide/native thiol and disulfide/total thiol ratios were recorded. Moreover disulfide/native thiol ratio was strongly, positively correlated with endometrial cancer stage ($r = 0.827$, $p < 0.001$) (Sezgin et. al. 2020). In another study that compared serum paraoxonase and arylesterase activity in patients with endometrial cancer (n=20) and healthy controls (n=23), serum paraoxonase, arylesterase activities, and total free sulphydryl (-sh) groups were found significantly lower in patients with endometrial cancer compared to controls (Arıoz et. al. 2009). Eryilmaz et. al. (2019) investigated one of the main estrogen-dependent cancer and thiol/disulfide balance. They found lower serum native thiol level and higher serum disulfide level in breast cancer patients (Giannella et. al. 2014). These rates changed in favor of disulfide and sulphydryl groups, showing that oxidative stress has an important role in endometrial hyperplasia. Additionally, disulfide/native thiol and disulfide/total thiol ratios could be potential EH markers without atypia.

There were few studies reported regarding Thiol/disulfide Homeostasis and non-malignant gynecologic disease. Ozaksit et al. compared TDH and IMA levels in patients with endometrial polyps and healthy controls. They reported thiol/disulfide homeostasis, and IMA levels were not different between groups (Ozaksit, Tokmak, Kosem, Kuru-Pekcan and Ozcan, 2019; Eroglu et. al. 2017; Sezgin et. al. 2020). Similarly, no statistically significant differences were reported in ratios of the disulfide/native thiol, native thiol/total thiol, and disulfide/total thiol between patients with myoma uteri and healthy controls ($p = 0.096$, 0.092,m0.092, respectively) (Eroglu et. al. 2017) When considering these studies, there was no significant relationship between TDH and benign gynecologic diseases, but these results do not allow us to make an exact decision.

In the literature, endometrial thickness was evaluated to detect malignancy and other pathology in postmenopausal patients (Giannella et. al. 2014; Saatli et. al. 2014; Louie, Canavan and Mansuria, 2016). In the meta-analysis published in 2018, endometrial cancer and EH were reported to be 2.6 times more common when the endometrial thickness was over 11 mm in postmenopausal asymptomatic women (Alcazar et. al. 2019). In a study conducted by Getpook et. al. (2006) for premenopausal women, corresponding sensitivity was 83.9%, specificity 58.8%, and negative predictive value (NPV) 90.4%, cutoff was taken at 8 mm. In another study, sensitivity was 83.6%, specificity 56.4%, and negative predictive value (NPV) 95.6% in predicting endometrial pathology in patients who developed premenopausal abnormal uterine bleeding at the 8 mm cutoff value (Özdemir, Çelik, Gezginç, Kıreşi and Esen, 2010). A study published in 2019 found endometrial HP's risk to increase by 25% in diabetic obese premenopausal women with endometrial thickness > 11 mm (Giannella, Cerami, Setti, Bergamini and Boselli, 2019). In patients with hyperplasia without atypia, taking a cutoff value of 16.5 mm for endometrial thickness could be used to predict treatment success with 64% sensitivity, 72% specificity, and 91% negative predictive value.[39] In our study, as per the literature, measurement of endometrial thickness > 11 mm by ultrasonography predicted HP without atypia with 79% sensitivity and 82% specificity.
The study's limitation includes a low number of patients, and oxidative stress parameters were evaluated only in patients in the hyperplasia group without atypia. Another limitation of our study was the lack of evaluation of hyperplasia with atypia. The study's strength is its prospective case-control study design and the fact that non-HP and healthy control groups were also evaluated. To the best of our knowledge, the status with endometrial hyperplasia without atypia has not been investigated, as ours was the first to report in this field. If these results are supported with further studies, it may be predicted whether hyperplasia is accompanied in patients with abnormal uterine bleeding by evaluating Thiol/disulfide parameters.

5. Conclusion and Suggestions

Endometrial hyperplasia is one of the leading causes of abnormal uterine bleeding and may be a precursor lesion for endometrial cancer. The dynamic thiol/disulfide balance shifted to the disulfide side in women with endometrial hyperplasia. Thiol/disulfide parameters were associated with endometrial thickness in women with abnormal bleeding. The imbalance of Thiol/ disulfide Homeostasis may play a role in endometrial hyperplasia's etiology without atypia. Future studies should assess these results with larger sample sizes to understand Thiol/disulfide Homeostasis's role in endometrial hyperplasia.

Declerations: The authors declare no conflict of interest. The authors guarantee that all 7 authors participated sufficiently in this work to take public responsibility for it, all authors reviewed the final version of the manuscript and the manuscript has not been published; this manuscript has been submitted with the full knowledge and approval of the institution. The

study was approved by the Ethical Committee of the University of Health Sciences Etlik Zubeyde Hanım Women's Health Training and Research Hospital (2020/82). The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Authorship contribution: Ideas: SEA, YEU; Design: SEA, VK, YEU; Inspection: OLT, BT, VK; Resources: YEU, REP, SEA; Materials: SEA, BT, REP; Data collection and/or processing: OLT, SEA, REP; Analysis and/or interpretation: OE, BT; Literature research: SEA, YEU; Writing: SEA, YEU; Critical review: SEA, OLT, YEU.

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