MARMARA
MEDICAL JOURNAL

Original Article

https://dergipark.org.tr/tr/pub/marumj

The role of dynamic thiol/disulfide homeostasis for the evaluation of oxidative stress in endometriosis patients

Nura Fitnat TOPBAS SELCUKI[1], Pinar YALCIN BAHAT[2], Cihan KAYA[3], Salim NESELIOGLU[4], Kubra BAGCI[5], Mustafa GOKSU[6], Meric KABAKCI[1], Ozcan EREL[4]

[1] Department of Obstetrics and Gynecology, Istanbul Sisli Hamidiye Etfal Training and Research Hospital, University of Health Sciences, Istanbul, Turkey
[2] Department of Obstetrics and Gynecology, Istanbul Kanuni Sultan Suleyman Training and Research Hospital, University of Health Sciences, Istanbul, Turkey
[3] Department of Obstetrics and Gynecology, Acibadem Bakirkoy Hospital, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[4] Department of Biochemistry, School of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkey
[5] Department of Obstetrics and Gynecology, Acibadem Bodrum Medical Center, Mugla, Turkey
[6] Obstetrics and Gynecology Clinic, Sinop Boyabat 75. Years State Hospital, Sinop, Turkey

Corresponding Author: Nura Fitnat TOPBAS SELCUKI
E-mail: fitnat.topbas@gmail.com

Submitted: 20.11.2021 **Accepted:** 21.03.2022

ABSTRACT

Objective: To evaluate the role of oxidative stress in endometriosis patients by determining dynamic thiol/disulfide homeostasis and ischemia modified albumin (IMA) levels.

Patients and Methods: This prospective case-controlled study was conducted at a tertiary gynecology clinic in Istanbul, Turkey. 86 patients previously diagnosed with endometriosis and persistent endometriomas were included in the study group. 60 patients who visited the clinic during the study period for routine gynecological control were included in the control group. Thiol/disulfide parameters and IMA levels were determined from the serum samples.

Results: When the thiol/disulfide parameters were compared between the study and the control group no significant difference was observed (p=0.49). Mean disulfide level in the control group was 18.58 ± 5.73 μmol/L and in the study group was 18.61 ± 7.37 μmol/L. Levels were statistically similar in both groups (p=0.98). In addition, there were no differences between the groups in terms of IMA and albumin levels.

Conclusion: The results of this study revealed no significant difference in the dynamic thiol/disulfide homeostasis among the endometriosis patients. Although, it has been accepted as a potential oxidative stress marker in other chronic inflammatory diseases, its use in determining the systemic oxidative stress level in endometriosis patients is limited.

Keywords: Endometriosis, Endometrioma, Dynamic thiol/disulfide homeostasis, Ischemia modified albumin

1 INTRODUCTION

Endometriosis is a chronic inflammatory disease. It is characterized by the presence of endometrial-like tissue outside of the uterus elsewhere in the body [1]. It affects 10% of women of reproductive ages. Chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility are symptoms that are associated with endometriosis [1]. The presence of endometriosis in the ovaries forming a cystic tumor was first identified by Sampson and termed endometrioma [2]. The etiology of endometriosis is not entirely clear. Sampson's implantation theory, Mayer's coelomic metaplasia theory, and induction theory are among the accepted pathophysiological theories of endometriosis [3,4]. However, none of them sufficiently explain the mechanism of the disease.

It is known that oxidative stress, which occurs due to the imbalance between the production and destruction of reactive oxygen species, plays a role in the pathophysiology of endometriosis. It initiates a generalized inflammatory reaction in the peritoneum [5-8]. Reactive oxygen species are intermediary products of the

How to cite this article: Topbas Selcuki NF, Yalcin Bahat F, Kaya C. The role of dynamic thiol/disulfide homeostasis for the evaluation of oxidative stress in endometriosis patients. Marmara Med J 2022; 2; 35(2):143-147. doi: 10.5472/marumj.1120611

ISSN: 1309-9469

http://doi.org/10.5472/marumj.1120611
Marmara Med J 2022;35(2): 143-147

oxygen metabolism, and they are involved in modulating cell proliferation as a part of an inflammatory response [9]. For the reduction and the inactivation of reactive oxygen species, thiol-containing molecules are essential. During this reaction, thiol groups are oxidized and reactive oxygen species become inactive [10]. Then, the oxidized thiol groups form disulfide bonds. As a result of this reversible reaction, thiol/disulfide homeostasis forms, contributing to the antioxidant mechanism of the organism. Therefore, thiol/disulfhide levels can be used as an indicator of oxidative stress [11,12].

Another potential oxidative stress marker is ischemia-modified albumin (IMA), an important marker in determining myocardial ischemia. Recently, its use in other organs has been reported [13]. Elevated levels of IMA have been observed in systemic sclerosis, diabetic ketoacidosis, acute ischemic stroke, and ectopic pregnancy. Therefore, its use as a biomarker is suitable [14-18].

The role of oxidative stress in endometriosis pathophysiology has already been established. However, data on the utilization of dynamic thiol/disulfide homeostasis and IMA as oxidative stress markers in endometriosis patients are limited.

The objective of this study was to assess the use of dynamic thiol/disulfide homeostasis and IMA levels as oxidative stress biomarkers in endometriosis by measuring the native thiol, total thiol, and IMA levels in serum samples of endometriosis patients and the control group. In addition, an evaluation was carried out to determine whether these parameters showed any changes according to the severity of endometriosis-related pelvic pain and/or endometrioma size.

2. PATIENTS and METHODS

This study was designed as a prospective case-controlled study. It was conducted at a tertiary center of obstetrics and gynecology in Istanbul between March 17 and August 1, 2020. This study was approved by the Clinical Research Ethical Committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital (approval number: 2019/490), and all subjects gave their written informed consent.

A total of 146 women who visited the outpatient gynecological clinic between March 17 2020 and August 1, 2020 were enrolled in the study. Out of these 146 patients, 86 endometriosis patients who were aged between 18 and 45 years and who had endometriomas were included in the study group. All patients had received their endometriosis diagnosis previously either by laparoscopy or by ultrasonography. The control group consisted of 60 patients who visited the outpatient clinic for routine gynecological control. Exclusion criteria were previous ovarian surgeries; endocrinological, rheumatologic and/or metabolic comorbidities; intake of hormonal medications for the previous three months; current antioxidant treatment; smoking; and alcohol abuse.

Following a thorough anamnesis, all patients underwent gynecological examination with transvaginal ultrasound (TVUS). An endometrioma was diagnosed by the presence of a unilocular ovarian cyst with homogeneous ground-glass echogenicity either with TVUS or with magnetic resonance imaging [19]. The volume of the endometrioma volume was calculated using the Orsini formula (length x width x diameter x 0.5235) [20]. TVUS was performed by the same gynecologist using an 8.5-MHz transvaginal transducer (ATL 5000 HDI, Philips, Netherlands). Endometriosis-related pelvic pain severity was self-evaluated by patients with a visual analogue scale (VAS); a score of 0 being no pain and 9 being extreme pain.

Biochemical Analysis

Venous blood samples from the patients were collected before they received any medical treatment. Serum was separated by 1200 x g centrifugation for 10 min. Dynamic thiol/disulfide parameters were measured using an automated methodology with a Cobas c501 chemical analyzer (Roche Diagnostics, Mannheim, Germany) [11]. All results were given in μmol/L. To summarize the methodology, sodium borohydride was first used to reduce disulfide bonds to thiol groups. Then, formaldehyde was added to the reaction to remove excess sodium borohydride. After reacting with 5,5'-dithiobis, reduced total thiol and native thiol groups were measured, and disulfide levels were calculated as described in the methodology. IMA levels were also measured from the same serum samples using the cobalt binding test defined by Bar-Or et al. [13]. IMA levels were recorded as absorbance units (ABSU).

Statistical Analysis

Patients' demographic data were evaluated using descriptive statistics. Comparative statistics was performed using the Mann-Whitney *U* test or Student's t-test depending on the distribution of the variables. Spearman or Pearson correlation analysis was used to evaluate the association between variables. A p-value of <0.05 was accepted as statistically significant. All statistical analyses were done using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA).

3. RESULTS

Patients were compared based on their age, endometrioma volumes, VAS scores, thiol/disulfide parameters, and IMA and albumin levels (Table I). There were no significant age differences between the groups (p= 0.507). The mean endometrioma volume of the study group was calculated to be 7.2 ± 6.05 cm^3. When groups were compared based on pelvic pain severity, a significant difference based on the VAS scores was observed (p< 0.001).

When the thiol/disulfide parameters were compared, the mean native thiol level in the control group was 313.92 ± 70.02 μmol/L and in the study group was 322.52 ± 78.31 μmol/L. No significant difference was observed (p= 0.49). Similarly, the comparison of total thiol levels did not reveal any significant difference (p= 0.48) (control group = 351.11 ± 67.49 μmol/L, study group = 359.71 ± 75.37 μmol/L). The mean disulfide level in the control group was 18.58 ± 5.73 μmol/L and in the study group was 18.61 ± 7.37 μmol/L. The difference was statistically insignificant.

http://doi.org/10.5472/marumj.1120611
Marmara Med J 2022;35(2): 143-147

Also, there were no significant differences between IMA and albumin levels (p= 0.11 and p= 0.62, respectively).

The relationships between thiol/disulfide levels and age, endometrioma volume, and VAS scores in the study group were analyzed with a correlation analysis (Table II). Although, a negative correlation between age and native thiol (r = – 0.07, p= 0.38), total thiol (r = – 0.06, p = 0.44), and IMA levels (r = – 0.01, p = 0.81) and a positive correlation between disulfide (r = 0.11, p = 0.17) and albumin (r = 0.01, p = 0.88) levels were observed, none of these correlations were statistically significant. In the correlation analysis of endometrioma volume, the correlation coefficient with native thiol was r = 0.12, total thiol was r = 0.09, disulfide was r = – 0.06, IMA was r = – 0.015 and albumin r = 0.07. None of these were statistically significant. Similarly, a comparison of VAS scores and thiol/disulfide parameters did not reveal a significant association (Table II).

Table I. *Comparison of clinical, dynamic thiol/disulfide parameters, albumin and IMA levels between the control and the study group*

	Control group (n=60)	Study group (n=86)	p-*value*
Age (years)	32.24±4.56	32.84±4.97	0.507
Endometrioma volume (cm^3)	0	7.2± 6.05	**<0.001**
VAS score	3(1-4)	6(4-9)	**<0.001**
Native thiol (µmol/L)	313.92 ± 70.02	322.52 ± 78.31	0.49
Total thiol (µmol/L)	351.11±67.49	359.71±75.37	0.48
Disulfide (µmol/L)	18.58±5.73	18.61±7.37	0.98
IMA (ABSU)	1.06±0.24	0.98±0.33	0.11
Albumin (g/dl)	2.44±0.79	2.51±0.8	0.62

VAS: visual analogue scale; IMA: ischemia-modified albumin; ABSU: absorbance units

Table II. *Correlation analysis between dynamic thiol/disulfide parameters, IMA, albumin and mean age, mean endometrioma volume and mean VAS score of the study group*

	Age		Endometrioma volume		VAS score	
	r	p	r	p	r	p
Native thiol	-0.07	0.38	0.12	0.146	0.04	0.56
Total thiol	-0.06	0.44	0.09	0.27	0.03	0.71
Disulfide	0.11	0.17	-0.06	0.45	0.03	0.66
IMA	-0.01	0.81	-0.15	0.06	-0.11	0.17
Albumin	0.01	0.88	0.07	0.34	0.03	0.71

IMA: ischemia-modified albumin, VAS: visual analogue scale

4. DISCUSSION

Oxidative stress plays an important role in the mechanism of cell migration and proliferation. This mechanism is also an essential part of endometriosis pathophysiology [21-23]. In this study, we evaluated dynamic thiol/disulfide homeostasis and IMA as potential biomarkers of oxidative stress in endometriosis patients. Since, the levels of these markers were determined in serum samples, oxidative stress was assessed in a systemic manner rather than local. According to our results, a significant difference among the endometriosis patients in terms of dynamic thiol/disulfide homeostasis and IMA was not observed.

Oxidative stress associated with endometriosis, which is observed in the peritoneal tissue or the abdomen, occurs due to the inflammatory response to the ectopic endometrial cells. Furthermore, reactive oxygen species are formed as intermediary products of the metabolic reactions in the erythrocytes, macrophages and apoptotic endometrial tissue accumulating in ectopic implants [24]. In addition, elevated levels of oxidatively modified low-density lipoproteins and nitric oxide radicals, which are also oxidative stress indicators, were reported in the peritoneal fluid of endometriosis patients [25,26]. However, in most studies, oxidative stress has been evaluated in local tissue samples or peritoneal fluid. Systemic evaluation of oxidative stress in endometriosis is limited [27]. In our study, we evaluated oxidative stress by determining the thiol and disulfide levels in serum samples of endometriosis patients. Since, these parameters were measured from serum samples, our results reflected systemic oxidative stress levels of the patients. Although, the data in the literature have pointed out the role of oxidative stress in the pathophysiology of endometriosis, our results did not show any significance. This could be because either oxidative stress observed in endometriosis patients could only be detected at the local ectopic endometriotic loci without altering the systemic oxidative homeostasis of the individuals or thiol/disulfide as biomarkers might not be appropriate in detecting endometriosis-related oxidative stress.

Among the most important nonenzymatic antioxidant molecules that play an essential role in the oxidative stress defense mechanism are glutathione, carotenoids, tocopherols and ascorbates. Glutathione, which contains thiol groups, is an important intracellular antioxidant [28]. When the oxidative-antioxidative balance is disrupted, thiol groups form disulfide bonds in a reversible reaction. The resultant increase in disulfide bonds indicates that the equilibrium in the body has shifted to the side of oxidative stress. Furthermore, the association between the severity of endometriosis and glutathione peroxidase, superoxide dismutase, and lipid peroxidase, which are a part of the enzymatic antioxidative mechanism, has been reported [29]. However, to the best of our knowledge the role of dynamic thiol/disulfide homeostasis in endometriosis has not yet been evaluated.

The prospective design of this study and the evaluation of all the recruits by a single gynecologist who is specialized in endometriosis are among the strengths of this study. Although, the statistically insignificant nature of the results could be seen as a limitation, they could be of importance when designing future studies. As mentioned above, oxidative stress was evaluated at a systemic level in this study. Therefore, the status of local dynamic thiol/disulfide homeostasis in ectopic endometrial implants is still unclear. Therefore, these results can be considered as preliminary outcomes when designing future studies on related topics.

http://doi.org/10.5472/marumj.1120611
Marmara Med J 2022;35(2): 143-147

Conclusion

In this study, the change in the parameters of dynamic thiol/disulfide homeostasis in endometriosis patients was not significant. Although, dynamic thiol/disulfide homeostasis has been shown as a potential oxidative stress marker in several other chronic inflammatory conditions, it does not seem to be suited for evaluating oxidative stress in endometriosis patients on a systemic level. However, local changes in dynamic thiol/disulfide homeostasis should still be evaluated. Therefore, future studies using peritoneal fluid or ectopic endometrial tissue can be meaningful.

Compliance with the Ethical Standards

Ethical Approval: This study was approved by the Clinical Research Ethical Committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital (approval number: 2019/490), and all subjects gave their written informed consent.

Financial Support: The authors have no relevant financial information to close.

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

Author Contributions: NFTS: Study construction and manuscript writing, CK: Manuscript writing and statistical analysis, MK: Data collection, MG: Data collection and interpretation, SN: Laboratory analysis, OE: Laboratory and statistical analysis, KB: Statistical analysis, PYB: Critically revised the article and supervised the study.

REFERENCES

[1] Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med 2020; ;382:1244-56. doi: 10.1056/NEJMra1810764

[2] Sampson JA. Perforating hemorrhagic (chocolate) cysts of the ovary: Their importance and especially their relation to pelvic adenomas of endometrial type (Adenomyoma of the uterus, rectovaginal septum, sigmoid, etc.). Arch Surg 1921;3:245-323. doi:10.1001/archsurg.1921.011.10080003001

[3] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3:93-110.43. PMID: 19969738

[4] Vinatier D, Orazi G, Cosson M, Dufour P. Theories of endometriosis. Eur J Obstet Gynecol Reprod Biol 2001;96:21-34. doi: 10.1016/s0301-2115(00)00405-x

[5] Foyouzi N, Berkkanoglu M, Arici A, Kwintkiewicz J, Izquierdo D, Duleba AJ. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. Fertil Steril 2004;82 Suppl 3:1019-22. doi: 10.1016/j.fertnstert.2004.02.133

[6] Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol 2009;25:75-81. doi: 10.1080/095.135.90802485012

[7] Christodoulakos G, Augoulea A, Lambrinoudaki I, Sioulas V, Creatsas G. Pathogenesis of endometriosis: The role of defective 'immunosurveillance.' Eur J Contracept Reprod Health Care 2007;12:194-202. doi: 10.1080/136.251.80701387266

[8] Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol RBE. 2005 Jul;3:28. doi: 10.1186/1477-7827-3-28

[9] Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. Pathogenic mechanisms in endometriosis-associated infertility. Fertil Steril 2008;90:247-57. doi: 10.1016/j.fertnstert.2008.02.093

[10] Gumusyayla S, Vural G, Bektas H, Deniz O, Neselioglu S, Erel O. A novel oxidative stress marker in patients with Alzheimer's disease: dynamic thiol-disulphide homeostasis. Acta Neuropsychiatr 2016 ;28:315-20. doi: 10.1017/neu.2016.13

[11] Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326-32. doi: 10.1016/j.clinbiochem.2014.09.026

[12] Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. Free Radic Biol Med 2009;47:1329-38. doi: 10.1016/j.freeradbiomed.2009.08.021

[13] Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000;19:311-5. doi: 10.1016/s0736-4679(00)00255-9

[14] Bozkaya G, Karaca I, Fenercioglu O, Yildirim Karaca S, Bilgili S, Uzuncan N. Evaluation of maternal serum ischemia modified albumin and total antioxidant status in ectopic pregnancy. J Matern Fetal Neonatal Med 2019;32:2003-8. doi: 10.1080/14767.058.2017.1422718

[15] Menon B, Ramalingam K, Krishna V. Study of ischemia modified albumin as a biomarker in acute ischaemic stroke. Ann Neurosci 2018;25:187-90. doi: 10.1159/000488188

[16] Borderie D, Allanore Y, Meune C, Devaux JY, Ekindjian OG, Kahan A. High ischemia-modified albumin concentration reflects oxidative stress but not myocardial involvement in systemic sclerosis. Clin Chem 2004;50:2190-3. doi: 10.1373/clinchem.2004.034371

[17] Yazıcı MU, Ayar G, Savas-Erdeve S, et al. Role of ischemia modified albumin serum levels as an oxidative stress marker in children with diabetic ketoacidosis. Comb Chem High Throughput Screen 2019;22:577-81. doi: 10.2174/138.620.7322666.191.008214919

[18] Seneş M, Kazan N, Coşkun O, Zengi O, Inan L, Yücel D. Oxidative and nitrosative stress in acute ischaemic stroke. Ann Clin Biochem 2007;44(Pt 1):43-7. doi: 10.1258/000.456.307779596057

[19] Exacoustos C, Manganaro L, Zupi E. Imaging for the evaluation of endometriosis and adenomyosis. Best Pract Res Clin Obstet Gynaecol 2014;28:655-81. doi: 10.1016/j.bpobgyn.2014.04.010

[20] Vignali M, Belloni GM, Pietropaolo G, et al. Effect of dienogest therapy on the size of the endometrioma. Gynecol Endocrinol 2020;36:723-7. doi: 10.1080/09513.590.2020.1725965

[21] Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril 2002;77:861-70. doi: 10.1016/s0015-0282(02)02959-x

[22] Ngô C, Chéreau C, Nicco C, Weill B, Chapron C, Batteux F. Reactive oxygen species controls endometriosis progression. Am J Pathol 2009;175:225-34. doi: 10.2353/ajpath.2009.080804

[23] Carvalho LFP, Samadder AN, Agarwal A, Fernandes LFC, Abrão MS. Oxidative stress biomarkers in patients with endometriosis: systematic review. Arch Gynecol Obstet 2012 ;286:1033-40. doi: 10.1007/s00404.012.2439-7

[24] Ota H, Igarashi S, Tanaka T. Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril 2001;75:785-90. doi: 10.1016/s0015-0282(01)01670-3

[25] Murphy AA, Santanam N, Parthasarathy S. Endometriosis: a disease of oxidative stress? Semin Reprod Endocrinol 1998;16:263-73. doi: 10.1055/s-2007.101.6286

[26] Kamada Y, Nakatsuka M, Asagiri K, et al. GnRH agonist-suppressed expression of nitric oxide synthases and generation of peroxynitrite in adenomyosis. Hum Reprod Oxf Engl 2000;15:2512-9. doi: 10.1093/humrep/15.12.2512

[27] Lambrinoudaki IV, Augoulea A, Christodoulakos GE, et al. Measurable serum markers of oxidative stress response in women with endometriosis. Fertil Steril 2009;91:46-50. doi: 10.1016/j.fertnstert.2007.11.021.

[28] Fidan F, Alkan BM, Uğurlu FG, et al. Dynamic thiol/disulphide homeostasis in Patients with fibromyalgia. Arch Rheumatol 2017;32:112-7. doi: 10.5606/ArchRheumatol.2017.5931

[29] Amreen S, Kumar P, Gupta P, Rao P. Evaluation of oxidative stress and severity of Endometriosis. J Hum Reprod Sci 2019;12:40-6. doi: 10.4103/jhrs.JHRS_27_17